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Open-File Report 2015–1094

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Suggested citation:
Luoma, J.A., Weber, K.L., Severson, T.J, Schreier ,T.M., Mayer, D.A., Aloisi, D.B, and Eckert N.L., 2015, Exposure-related effects of formulated *Pseudomonas fluorescens* strain CL145A to glochidia from seven unionid mussel species: U.S. Geological Survey Open-File Report 2015–1094, 474 p., http://dx.doi.org/10.3133/ofr20151094.

ISSN 2331-1258 (online)

Acknowledgments

This study was funded through a grant from the U.S. Environmental Protection Agency Great Lakes Restoration Initiative awarded to the New York State Education Department and U.S. Geological Survey appropriated funds. The authors thank Mark P. Gaikowski of the Upper Midwest Environmental Sciences Center for assisting with study design and statistical analysis as well as Jeremy K. Wise, Samuel M. Stafslien, Pajtshiab L. Moua, and Susan M. Schleis for technical assistance and data collection.

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Conversion Factors

International System of Units to Inch/Pound

Multiply	By	To obtain
	Length	
centimeter (cm)	0.3937	inch (in.)
micrometer (µm)	3.937×10^{-5}	inch (in.)
millimeter (mm)	0.03937	inch (in.)
	Volume	
liter (L)	1.057	quart (qt)
microliter (µL)	0.000033814	ounce, fluid (fl. oz)
milliliter (mL)	0.03382	ounce, fluid (fl. oz)
	Flow rate	
milliliter per minute (mL/min)	0.0002642	gallon per minute (gal/min)
	Mass	
gram (g)	0.03527	ounce, avoirdupois (oz)
milligram (mg)	3.527×10^{-5}	ounce, avoirdupois (oz)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as °F = (1.8 × °C) + 32.
Conductivity is given in microsiemens per centimeter at 25 degrees Celsius (µS/cm at 25 °C).
Concentrations of chemical constituents in water are given in milligrams per liter (mg/L).

Abbreviations

AI	active ingredient
α	alpha
$CaCO_3$	calcium carbonate
DO	dissolved oxygen
FDP	freeze-dried powder
HD	heat deactivated
p	*p*-value
SDP	spray-dried powder
TAN	total ammonia nitrogen
USGS	U.S. Geological Survey

Exposure-Related Effects of Formulated *Pseudomonas fluorescens* Strain CL145A to Glochidia from Seven Unionid Mussel Species

By James A. Luoma[1], Kerry L. Weber[1], Todd J. Severson[1], Theresa M. Schreier[1], Denise A. Mayer[2], Douglas B. Aloisi[3], and Nathan L. Eckert[3]

Abstract

The study was completed to evaluate the exposure-related effects of a biopesticide for dreissenid mussel (*Dreissena polymorpha*, zebra mussel and *Dreissena rostriformis bugensis*, quagga mussel) control on glochidia from unionid mussels endemic to the Great Lakes and Upper Mississippi River Basins. The commercially prepared biopesticide was either a spray-dried powder (SDP) or freeze-dried powder (FDP) formulation of *Pseudomonas fluorescens*, strain CL145A. Glochidia of the unionid mussel species *Lampsilis cardium*, *Lampsilis siliquoidea*, *Lampsilis higginsii*, *Ligumia recta, Obovaria olivaria*, and *Actinonaias ligamentina* were exposed to SDP-formulated *P. fluorescens* and *Lampsilis cardium* and *Megalonaias nervosa* were exposed to FDP-formulated *P. fluorescens*.

All exposures were static, 24 hours in duration, and included six treatment groups. The treatment groups included (1) an untreated control, (2) a positive control which received a nominal target active ingredient (AI) concentration of 300 milligrams per liter (mg/L) of heat-deactivated test article, and (3) treatments that received nominal target AI concentrations of 50, 100, 200, and 300 mg/L of test article. All treatment concentrations are reported based on active ingredient.

Glochidia viability was reduced in two of the six species exposed to 50 mg/L SDP and in four of the six species exposed to 100 mg/L SDP when compared to untreated control groups at 6, 12, and 24 hours. Regardless of sample time, concentrations of 200 and 300 mg/L of SDP and 300 mg/L of heat-deactivated SDP (positive control) substantially reduced glochidia viability in all species except, *L. higginsii*. Glochidia viability was only reduced for *L. cardium* exposed to FDP at concentrations ≥ 200 mg/L. After 24 hours of FDP exposure, differences in glochidia viability were only detected in *M. nervosa* that were exposed to 300 mg/L of heat-deactivated SDP. However, given the low viability in the control group, the results for *M. nervosa* should be interpreted with caution.

Introduction

North America has the greatest diversity of freshwater mussels in the World with historical evidence of approximately 297 taxa consisting of 281 species and 16 subspecies, but because of their sedentary nature, freshwater mussels are particularly vulnerable to anthropogenic influences, such as

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[1] U.S. Geological Survey
[2] New York State Education Department
[3] U.S. Fish and Wildlife Service

habitat alteration; habitat degradation; pollution and overharvest; and more recently, to impacts from dreissenid mussels (*Dreissena polymorpha*, zebra mussel and *Dreissena rostriformis bugensis*, quagga mussel) (Williams and others, 1993, Neves and others, 1997, Strayer and Malcom, 2007). Master and others (1998) listed: 67 percent of freshwater mussels in the United States as extinct or vulnerable to extinction; 95 species of North American freshwater bivalves were listed as vulnerable, endangered, or critically endangered; and 29 were listed as extinct or possibly extinct on the International Union for Conservation of Nature's Red List (http://www.iucnredlist.org/, accessed March 6, 2015). Estimates indicate that 127 mussel species will become extinct in the next 100 years; however, this estimate may be low as it did not factor in impacts from dreissenid mussels (Ricciadi and Rasmussen, 1999). Native mussels in the southeastern United States are in particular danger with only 25 percent of the 269 historical species listed as stable, 13 percent presumed extinct, and 60 percent reported as either endangered, threatened or of special concern (Neves and others, 1997). The negative impact of dreissenid mussels on unionid mussels is well documented in the scientific literature with reports as early as 1937 of extirpation of unionid mussels from European waters due to dreissenid mussel colonization (Sebestyen, 1937; cited in Burlakova and others, 2000). Severe declines in unionid abundance linked to dreissenid colonization in North American waters were reported approximately one decade after dreissenids invaded the United States (Haag and others, 1993; Nalepa, 1994; Ricciardi and others, 1996).

The need to protect and recover imperiled species, such as freshwater mussels, was recognized in the 1973 Endangered Species Act (16 USC 1531-1544, 87 Stat. 884) and the U.S. Fish and Wildlife Service has developed recovery plans for many threatened and endangered unionid mussel species (U.S. Fish and Wildlife Service, 2013; http://www.fws.gov/endangered/species/recovery-plans.html, accessed April 1, 2015). Mitigating the severe impacts of dreissenid mussels on unionid species and the use of population enhancement and reintroduction into historical ranges with propagated animals are commonly cited tasks in unionid recovery plans (U.S. Fish and Wildlife Service, 2004). The U.S. Fish and Wildlife Service mussel propagation program uses wire-mesh rearing cages that are placed in natural waterways for approximately 18 months to rear unionid mussels (Brady and others, 2010). However, some areas previously used for rearing have been abandoned due to dreissenid mussel fouling of the rearing cages. A potential dreissenid mussel management tool for limited, high-value, open-water habitats, such as around unionid mussel rearing cages or mussel beds, is a commercially formulated biopesticide containing a specific strain (CL145A) of the common soil bacterium, *Pseudomonas fluorescens* as the active ingredient. Currently, the spray-dried powder (SDP) formulation of the biopesticide Zequanox® is manufactured by Marrone Bio Innovations, Inc. (Davis, California). It was registered by the U.S. Environmental Protection Agency (registration number 84059–15) for controlling dreissenid mussels in industrial water systems in 2012 and for open-water system use in 2014. The biopesticide containing *P. fluorescens* is readily ingested by dreissenid mussels, which causes necrosis of epithelial cells lining the digestive tract that results in the death of the dreissenid mussel (Molloy, Mayer, Gaylo, Morse, and others, 2013). The component of the *P. fluorescens* biopesticide that causes epithelial cell necrosis has been identified as a heat-liable natural metobilite associated with the bacterium's cell wall (Molloy, Mayer, Gaylo, Burlakova, and others 2013).

Freshwater mussels have a unique reproductive strategy that includes a short parasitic lifestage called glochidia. The ability of mature glochidia to attach to a suitable host (typically a fish) is a critical life-cycle step for most freshwater mussels (ASTM International, 2013). Therefore, evaluating the exposure-related effects of water-borne pesticides on freshwater mussel glochidia is prudent.

The objective of this study was to evaluate the exposure-related effects of a commercially formulated *P. fluorescens,* strain CL145A biopesticide on glochidia from select unionid mussel species present in the Great Lakes and Upper Mississippi River Basins. This report summarizes the methods and results of laboratory trials in which applications of SDP- or FDP-formulated *P. fluorescens* biopesticides were administered for 24 hours to static test chambers containing glochidia extracted from female unionid mussels. During the course of this study, the test article manufacturer, Marrone Bio Innovations, Inc., requested a change to a freeze-dried powder (FDP) formulation. However, the FDP formulation was quickly discontinued; the resulting data for the two species exposed to FDP (*Lampsilis cardium and Megalonaias nervosa*) are included within the report.

Materials and Methods

The protocol and amendments for this study are presented in appendix 1 (items 1–6). All methods and materials followed the written protocol and amendments except those instances that were identified as deviations (appendix 2, items 1–12). Notes to file provide further documentation on the procedures used during the study (appendix 1, items 7 and 8).

Experimental Design

The study was completed at the U.S. Geological Survey's Upper Midwest Environmental Sciences Center (La Crosse, Wisconsin). Seven species of unionid mussel glochidia (table 1) were exposed to either the SDP- (six species) or the FDP-formulation (two species) following procedures outlined in "Standard Guide for Conducting Laboratory Toxicity Tests with Freshwater Mussels" (Annex A1 of the ASTM International, 2013). The six species of unionid mussel glochidia exposed to the SDP-formulation were *L. cardium*, *Lampsilis siliquoidea*, *Lampsilis higginsii*, *Ligumia recta, Obovaria olivaria*, and *Actinonaias ligamentina*. The two species of unionid mussel glochidia exposed to the FDP-formulation were *Lampsilis cardium* and *M. nervosa*. For each species, glochidia were flushed from 3–4 gravid female mussels and viability was estimated to be over 80 percent before the glochidia were used in the study. Consistent with the Standard Guide for Conducting Laboratory Toxicity Tests with Freshwater Mussels, glochidia that closed their valves in response to sodium chloride exposure were considered viable (ASTM International, 2013). Photomicrographs were recorded before and after the addition of the sodium chloride solution and used for definitive determination of initial glochidia viability (table 2). Glochidia were pooled and distributed to test chambers according to a predetermined random distribution scheme (appendix 3, items 4–6). An estimated 843–4,602 glochidia were distributed to each test chamber (table 3).

Each species was assigned to one of three test block locations and treatments were assigned to test chambers using randomized block designs (appendix 3, items 1–3 and 7–9, respectively). Six treatments (n = 3 per treatment) were tested and included (1) an untreated control, (2) nominal target concentrations of 50, 100, 200, and 300 milligrams per liter (mg/L) of test article based on active ingredient (AI), and (3) a nominal target concentration of 300 mg/L AI of heat-deactivated test article group (300 HD, positive control). Exposures were static and lasted 24 hours. Glochidia from each test chamber were assessed for viability at 6, 12 and 24 hours, with the exception of *M. nervosa* glochidia, which were only assessed at 24 hours.

Test Article

The test articles used in the study were commercially prepared SDP or FDP formulated *P. fluorescens* (strain CL145A) biopesticides containing 50 and 100 percent AI (weight-to-weight ratio *P. fluorescens*, strain CL145A cells), respectively. The test articles were produced by Marrone Bio

Innovations, Inc. and test article use was documented in log books (appendix 4, items 4–6). All test article concentrations are reported as active ingredient. Verification of biological activity (the ability of the test article to induce dreissenid mussel mortality) was determined for each test article lot after use in the study by the New York State Museum Field Research Laboratory (Cambridge, New York). Biological activity for each lot of test article was confirmed as indicated by mean zebra mussel mortality ranging from 85.3 to 94.7 percent in the test article treated groups compared to 0.0 to 1.3 percent in the untreated control groups (table 1; appendix 4, items 7–9).

Table 1. Data for test animals, test articles and, exposure dates for studies of selected native freshwater mussel glochidia exposed to two formulations of *Pseudomonas fluorescens*, strain CL145A.

[PPB, plain pocketbook; WAS, washboard; BLS, black sandshell; FAM, fatmucket; HGE, Higgins eye; HIC, hickorynut; MUC, mucket; FDP, freeze-dried powder; SDP, spray-dried powder; MBI, Marrone Bio Innovations, Inc.]

Scientific name	Common name	Abbreviation	Test article: Formulation	Test article: Lot number	Test article: Biological activity[1] (percent)	Exposure date
Lampsilis cardium	Plain pocketbook	PPB	FDP	110607WB-FD-E	94.7 ± 6.1	October 18, 2011
Megalonaias nervosa	Washboard	WAS	FDP	110607WB-FD-E	94.7 ± 6.1	October 18, 2011
Ligumia recta	Black sandshell	BLS	SDP	MBI-401-SDP-4655-12-MIX	85.3 ± 11.5	January 17, 2012
Lampsilis siliquoidea	Fatmucket	FAM	SDP	MBI-401 110308AI-BD-3	86.7 ± 4.6	May 12, 2011
Lampsilis higginsii	Higgins eye	HGE	SDP	MBI-401-110308AI-BD-3	86.7 ± 4.6	May 12, 2011
Obovaria olivaria	Hickorynut	HIC	SDP	MBI-401-SDP-4655-12-MIX	85.3 ± 11.5	January 19, 2012
Actinonaias ligamentina	Mucket	MUC	SDP	MBI-401-SDP-4655-12-MIX	85.3 ± 11.5	January 17, 2012
Lampsilis cardium	Plain pocketbook	PPB	SDP	MBI-401 110308AI-BD-3	86.7 ± 4.6	May 12, 2011

[1] Biological activity is the mean percent zebra mussel mortality in test article treated groups during a bioassay performed by the New York State Museum Field Research Laboratory (Cambridge, New York).

Test system

The test system was a series of glass test chambers (80 × 40 millimeters [mm], width × height; catalog number 89000–286; VWR International, West Chester, Pennsylvania) arranged in three blocks. Each block contained 3 rows with 6 test chambers, for a total of 18 test chambers per species (fig. 1). Test chambers were labelled to allow for identification of treatment type and replicate number. During the exposure, light aeration (1–2 bubbles per second) was provided to each test chamber through a disposable glass pipet. Test water was well water conditioned to ambient temperature (≈20 °C) and dissolved-gas saturation by providing gentle aeration in 19-liter (L) glass jars for a minimum of 24 hours before use. Indirect fluorescent lighting (mean of 108 to 536 lux) was provided on an 18 hours light to 6 hours dark cycle in accordance with ASTM International guidelines (ASTM International, 2013).

Table 2. Data for initial glochidia viability by individual female mussel for each species.

[Viability of glochidia by individual female mussel and species during exposures of selected native freshwater mussel glochidia to two formulations of *Pseudomonas fluorescens*, strain CL145A. SD, standard deviation—number in parentheses; SDP, spray-dried powder; FDP, freeze-dried powder]

Species	Formulation	Mussel number	Total glochidia	Nonviable glochidia[1]	Glochidia viability (percent)	Mean percent glocihidia viability (SD)
Lampsilis cardium	FDP	1	212	16	92.5	84.7 (6.9)
		2	222	39	82.4	
		3	150	31	79.3	
Megalonaias nervosa	FDP	1	124	2	98.4	96.5 (5.5)
		2	155	1	99.4	
		3	51	5	90.2	
		4	104	0	100.0	
Ligumia recta	SDP	1	312	32	89.7	84.5 (6.7)
		2	1,083	250	76.9	
		3	405	53	86.9	
Lampsilis siliquoidea	SDP	1	548	70	87.2	80.3 (7.1)
		2	297	80	73.1	
		3	456	89	80.5	
Lampsilis higginsii	SDP	1	129	49	62.0	80.8 (16.5)
		2	168	20	88.1	
		3	357	27	92.4	
Obovaria olivaria	SDP	1	366	54	85.2	86.0 (1.2)
		2	352	51	85.5	
		3	309	39	87.4	
Actinonaias ligamentina	SDP	1	161	14	91.3	92.7 (1.4)
		2	153	9	94.1	
		3	287	21	92.7	
Lampsilis cardium	SDP	1	467	36	92.3	93.8 (1.4)
		2	537	27	95.0	
		3	927	55	94.1	

[1]Nonviable glochidia are the sum of glochidia closed before the addition of, and open after the addition of, sodium chloride.

Table 3. Estimated number of glochidia distributed to test chambers.
[Distributions to test chambers by species during exposures of selected native freshwater mussel glochidia to two formulations of *Pseudomonas fluorescens*, strain CL145A. SD, standard deviation—number in parentheses; SDP, spray-dried powder; FDP, freeze-dried powder]

Species	Formulation	Estimated number (SD) of glochidia per test chamber
Lampsilis cardium	FDP	4,602 (1,225)
Megalonaias nervosa	FDP	843 (382)
Ligumia recta	SDP	2,021 (500)
Lampsilis siliquoidea	SDP	2,702 (1,094)
Lampsilis higginsii	SDP	3,427 (1,018)
Obovaria olivaria	SDP	4,187 (2,503)
Actinonaias ligamentina	SDP	2,200 (1,061)
Lampsilis cardium	SDP	3,355 (1,077)

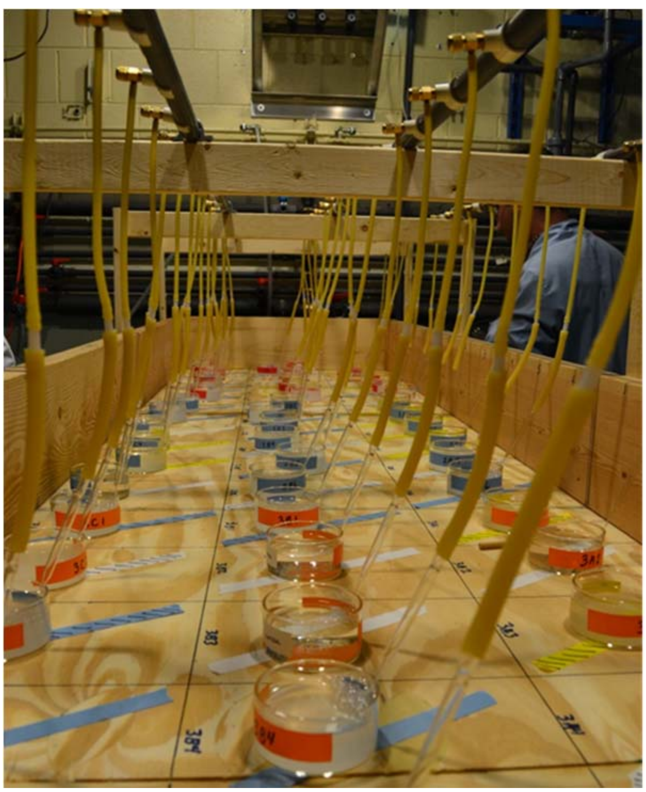

Figure 1. Photograph showing glochidia test system with individual test chambers.

Animal Collection and Distribution

Test animal information is presented in appendix 5, items 1-8. Gravid female mussels were collected from the Upper Mississippi, Chippewa, and St. Croix Rivers and identified to species as described in Cummings and Mayer (1992) and Watters and others (2009) by biologists from the Genoa National Fish Hatchery (Genoa, Wis.). Gravid mussels were maintained at the Genoa National Fish Hatchery on flowing pond water for up to 120 days before being transported to the Upper Midwest Environmental Sciences Center for glochidia extraction. Glochidia were extracted from the gravid female mussels by gently opening the valves and using a syringe to flush the marsupium with water (fig. 2). Glochidia from 6–10 marsupial tubes were flushed into a 500-milliliter (mL) beaker and viability of the glochidia was estimated to be > 80 percent by observation under a microscope before pooling. Glochidia viability was defined as valve closure in response to the addition of a drop of saturated sodium chloride solution (fig. 3). Glochidia from 3–4 mussels of each species were pooled in a 500-mL beaker containing water. Definitive initial viability of glochidia extracted from each female mussel was determined from photomicrographs after exposures were completed (table 2). The beaker containing the pooled glochidia was gently swirled to position the glochidia in a concentrated group at the bottom of the beaker. Glochidia were then indiscriminately collected by placing a 1,000-microliter (µL) wide bore pipet tip (model T-1005-WB-C-R; Axygen Scientific, Union City, California) within the concentrated glochidia and withdrawing a 100-µL sample with an adjustable pipette (model research plus 100–1,000 µL; Eppendorf USA, Hauppauge, N.Y.). The glochidia were then placed into a randomly selected test chamber that contained 96.5 mL of water (fig. 4). After all test chambers had received glochidia, the distribution process was repeated until all test chambers received five independent glochidia distributions (appendix 3, items 4–6). Throughout the distribution process samples (100 µL, n =16 per species) containing glochidia were placed into a 35-mm petri dish and used to estimate the total number of glochidia distributed to the test chambers. Each treatment group contained three test chamber replicates with an estimated range of 843 to 4,602 glochidia per chamber (table 3). The volume of water within the test chambers after glochidia distribution and before test article administration was 97.0 mL.

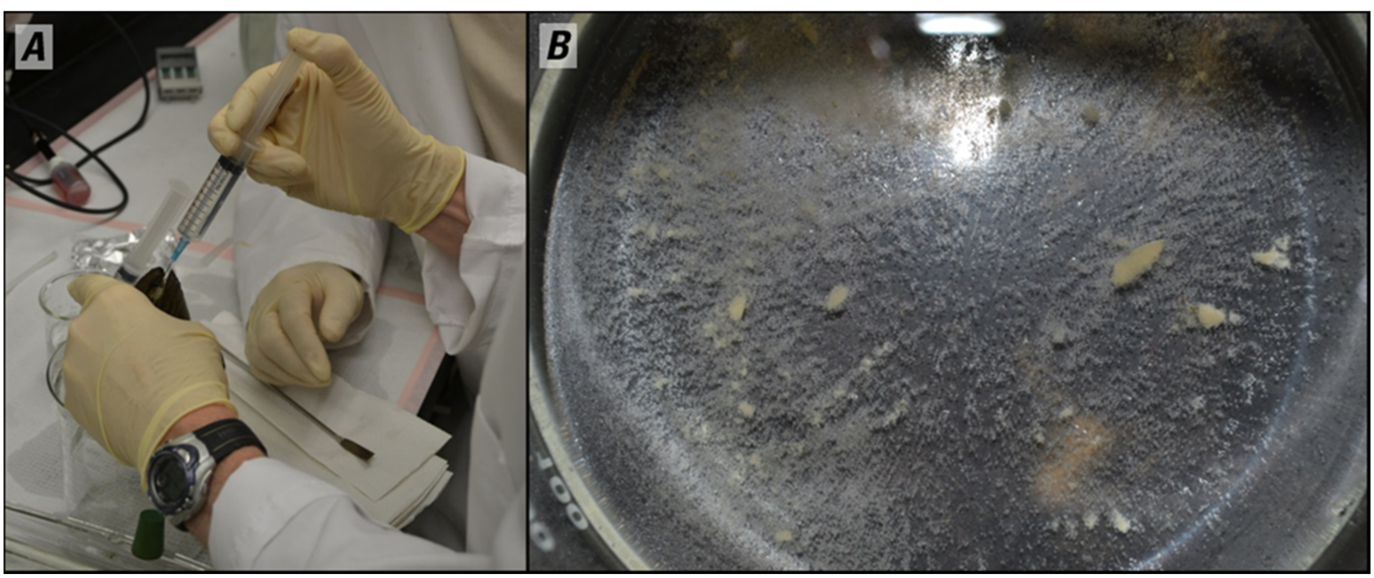

Figure 2. Photographs showing *A*, the extraction of glochidia from a gravid female mussel and, *B*, extracted glochidia.

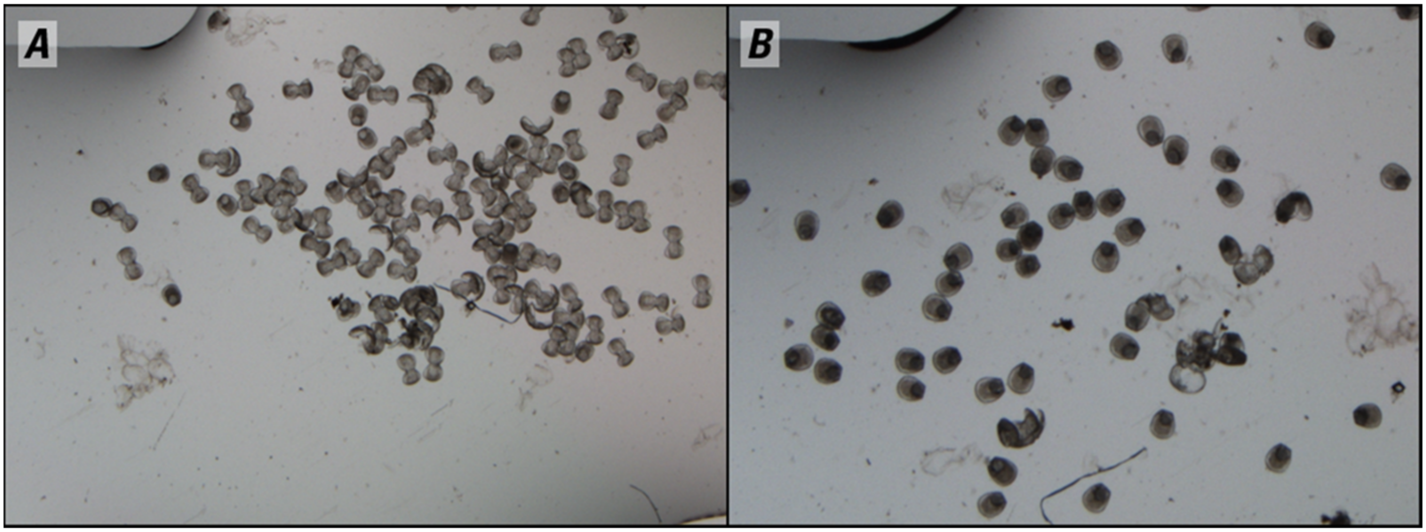

Figure 3. Photomicrographs showing *A*, glochidia before a drop of sodium chloride solution was added to induce valve closure in viable animals and, *B*, glochidia one minute after addition of the sodium chloride solution.

Figure 4. Photograph showing the distribution of glochidia to test chambers.

Test Article Preparation and Application

For each species, a separate test article stock solution was prepared for the 50, 100, 200 and 300 mg/L treatment groups immediately prior to application. A single heat-deactivated test article solution was prepared for each treatment day and used for all applications of heat-deactivated stock solution (positive control) that were completed on the same day. Untreated water was used as the test article stock solution for the untreated control treatment. The stock solutions were prepared by weighing a known amount of test article (1.0 grams FDP; 2.0 grams SDP) on an analytical balance and then placing the test article into a 100-mL flask with water, which resulted in an AI stock solution of 10,000 mg/L. The heat-deactivated stock was prepared by placing the test article into a 100-mL volumetric flask with approximately 50 mL of water, then the flask was placed into a 70 degrees Celsius (°C) water bath for 45 minutes to degrade the activity of the *P. fluorescens* according to methods developed at the New York State Museum Field Research Laboratory (D. Mayer, Director of the New York State Museum Field Research Laboratory, oral commun., 2010). After cooling to ambient temperature, the heat-deactivated stock solution was brought to a final 100 mL volume with water.

Each test chamber received (1) 3.0 mL of untreated water (control), (2) 3.0 mL of heat-deactivated stock solution (positive control), (3) 3.0 mL of stock solution (300 mg/L treatment), or (4) 3.0 mL of the appropriate combination of untreated water and stock solution (50, 100 and 200 mg/L treatments [appendix 1, items 20 and 24]). The final volume in each test chamber after application of the test article was 100 mL.

Water Chemistry

Prior to exposure, temperature, hardness, and alkalinity were measured on the source water for all trials. Conductivity was measured on the source water for all trials, excluding those completed in May 2011 (*L. cardium*, *L. siliquoidea*, and *L .higginsii* SDP exposures; table 1). For the trials completed in May 2011 the water chemistry parameters measured during the exposure included duplicate measurements of dissolved oxygen (DO), pH, and temperature on all control and high test article concentration (300 mg/L) test chambers and on one indiscriminately selected test chamber from the remaining treatment groups. For all other trials, the water chemistry parameters measured during the exposure included duplicate measurements of DO, pH, and temperature in all test chambers. At the termination of the exposure, the contents of each test chamber were pooled by treatment group and the hardness, alkalinity, conductivity, temperature, pH, and total ammonia nitrogen (TAN) were measured on the pooled replicates. The TAN was measured using a YSI 9000 photometer (YSI, Inc., Yellow Springs, Ohio; *L. cardium*, *L. siliquoidea*, and *L. higginsii* SDP exposures) or using the automated phenate method (Standard Method 4500G in American Public Health Association and others, 2012; *L. recta, O. olivaria*, and *A. ligamentina* SDP exposures; *L. cardium* and *M. nervosa* FDP exposures).

Glochidia Viability Assessments

Definitive initial viability determinations for glochidia extracted from each female mussel and the viability of glochidia in samples collected during the exposures were completed from photomicrographs using equation 1:

$$\text{Viability (percent)} = \frac{(\text{N-}[\text{NC}_{\text{pre}}\text{+NO}_{\text{post}}])}{\text{N}} \times 100 \qquad (1)$$

where N is the total number of glochidia in the sample,
NC_{pre} is the number of glochidia closed before adding sodium chloride, and
NO_{post} is the number of glochidia open after adding sodium chloride.

During the exposure, glochidia from each test chamber were assessed for viability at 6, 12, and 24 hours, with the exception of the *M. nerovosa* glochidia, which were assessed only at 24 hours. Water within each test chamber was gently swirled to position the glochidia in a concentrated group centered at the bottom exposure chamber. Then a 100-µL sample was withdrawn from each exposure chamber using a 1,000-µL wide-bore pipet tip attached to an adjustable pipette. For each species, excluding *M.*

nerovosa, the glochidia samples were placed into a 35-mm petri dish, viewed, and photomicrographed using a Nikon model SMZ 1500 compound stereo microscope fitted with a Nikon digital sight DS–Fi1 camera controlled by Nikon Imaging Systems Elements-BR® software, version 3.10 (Nikon Imaging systems, 1991–2010). Photomicrographs were recorded before and after a drop of saturated sodium chloride solution was added to induce valve closure in viable glochidia. The second photomicrograph was recorded one minute after sodium chloride addition to provide a standard valve closure response time. Glochidia on each photomicrograph were enumerated by valve position (either open or closed) and the percentage of viable glochidia was calculated by using equation 1. *Megalonaias nervosa* glochidia are released in a mucoidal matrix, which precluded the ability to obtain an image of the glochidia on a single focal plane. Therefore, *M. nervosa* glochidia were immediately assessed for viability by placing the glochidia under a dissecting microscope (model SMZ745; Nikon Instruments) and enumerating, by valve position, before and after a after a drop of saturated sodium chloride solution was added to induce valve closure in viable glochidia.

Data Analysis

Water chemistry (DO, pH, temperature, hardness, alkalinity, conductivity, and TAN) data analyses were limited to simple descriptive statistics (appendix 6, items 1, 2, 4, and 7). In order to compensate for initial glochidia viabilities of less than 100 percent, the observed viabilities of glochidia during the exposure were adjusted with the initial viability of glochidia using the methods described by Wang and others (2007). Viability adjustments were completed using equation 2:

$$\text{Adjusted viability} = \frac{(V_O)}{(V_I)} \times 100 \qquad (2)$$

where

V_O is the observed (un-adjusted) viability of a treatment replicate (appendix 7, items 5–7), and

V_I is the initial mean viability observed for the species, in percent (table 3).

For example, the mean initial viability of *A. ligamentina* glochidia was 92.7 percent. The observed (unadjusted) viability of *A. ligamentina* glochidia in a control group replicate, at 24 hours, was 90.0 percent, therefore, the adjusted viability of *A. ligamentina* glochidia in the control group replicate, at 24 hours, is 90/92.7 × 100 or 97.1 percent.

Statistical comparisons were completed with the observed (un-adjusted) glochidia viability of each treatment group replicate using SAS® software version 9.3 (SAS, 2010). Significance was declared at $\alpha \leq 0.05$. A generalized linear mixed model was used to analyze the viability of glochidia by treatment group and species (appendix 7, item 2). The proportion of mortalities (number of nonviable glochidia compared to the total number of glochidia present) were modeled with a binomial distribution and a logit link function. A scale parameter was added to the model using the "random_residual_" statement. At each sampling time, glochidia viability in each treatment group was individually compared to the viability in the untreated control group using a two-sided means comparison test.

Results and Discussion

Pre-exposure water chemistry parameters (temperature, alkalinity, hardness, and conductivity) of the source water are summarized in table 4 and are presented in appendix 6 (item 1). Pre-exposure temperature ranged from 18.4 to 19.9 °C; hardness ranged from 171 to 175 mg/L as calcium carbonate ($CaCO_3$); alkalinity ranged from 122 to 130 mg/L as $CaCO_3$; and conductivity ranged from 383 to

422 microsiemens per centimeter (µS/cm). Water chemistry parameters (DO, pH, and temperature) measured during the exposure are presented in table 5 and in appendix 6 (item 2). Dissolved oxygen remained well above the ASTM International criterion of 4 mg/L throughout the exposure period (ASTM International, 2013). The pH of exposure water ranged from 7.52 to 8.56 and the temperature ranged from 17.2 to 20.7 °C. The pooled replicate exposure water samples collected at exposure termination had mean temperatures from 18.2 to 20.3 °C, hardness from 180 to 210 mg/L as $CaCO_3$, alkalinity from 137 to 153 mg/L as $CaCO_3$, and conductivity from 367 to 457 µS/cm (table 4; appendix 6, item 4). The mean TAN from samples collected at the termination of the exposure period are presented in table 6 and appendix 6 (items 5–7). The TAN remained below the 2013 U.S. Environmental Protection Agency criterion for acute exposure (17 mg/L at pH 7 and 20 °C) throughout the entire exposure period for all species and treatment groups (U.S. Environmental Agency, 2013).

The initial viability of glochidia extracted for each female mussel ranged from 62 to 100 percent, with glochidia extracted from four individuals below the 80 percent viability recommended in the "Standard Guide for Conducting Laboratory Toxicity Tests with Freshwater Mussels" (ASTM International, 2013). However, the mean viability of glochidia extracted from the female mussels for each species ranged from 80.3 to 96.5 percent (table 2, appendix 7, item 1). Statistical analyses of the un-adjusted glochidia viability are presented in appendix 7 (item 4) and the un-adjusted-mean glochidia viability of each treatment group are presented by species in table 7. The mean of the adjusted viability of each treatment group are presented by species in table 8 and in appendix 7 (item 5–7).

Regardless of species, the mean of the un-adjusted and adjusted control group viability during the SDP trials exceeded 66 and 82 percent, respectively, throughout the study. After 6 hours of SDP exposure at 50 mg/L, the un-adjusted-mean viabilities of *L. recta* and *A. ligamentina* glochidia were significantly lower (11.7 percent [$p < 0.01$] and 71.7 percent [$p = 0.04$], respectively) than the untreated control groups (85.7 and 88.6 percent, respectively). Concentrations of SDP ≥100 mg/L for 6 hours significantly lowered glochidia viability for all species, except for *L. cardium* and *L. higginsii*, which were impacted at concentrations ≥ 200 and 300 mg/L, respectively. The observed lower viability for *O. olivaria* at 6 hours in the 100 mg/L SDP-treated group is likely an outlier, given no differences were detected at 12 and 24 hours in the viability of glochidia in the 100 mg/L SDP-treated group compared to the control group (p = 0.16 and 0.15, respectively).

The mean for the un-adjusted and adjusted control group viability for *L. cardium* during the FDP trial exceeded 81 and 96 percent, respectively. The un-adjusted- and adjusted-mean control group glochidia viability *for M. nervosa* at 24 hours was 52.1 and 53.9 percent, respectively. During the *L. cardium* FDP-trial, glochidia exposed to FDP concentrations ≥ 200 mg/L were significantly less viable than those in the untreated control group, regardless of sample time. After 24 hours of exposure, no difference was detected between the viability of *M. nervosa* glochidia in the FDP-treated groups compared to the control group, with the exception of the positive control group. However, given the low viability observed in the *M. nervosa* untreated controls (un-adjusted viability of 52.1 percent) the results should be interpreted with caution.

Table 4. Mean temperature, alkalinity, hardness, and conductivity of pre-exposure source water and pooled-exposure termination-water samples.

[Samples were collected before and during exposures of selected native freshwater mussel glochidia to two formulations of *Pseudomonas fluorescens*, strain CL145A. °C, degrees Celsius; mg/L, milligrams per liter; Cond., conductivity; µS/cm, microsiemens per centimeter at 25 degrees Celsius; SDP, spray-dried powder; FDP, freeze-dried powder; number in parentheses, standard deviation; --, no data collected or cannot calculate]

		Preexposure				Exposure termination			
Species	Formulation	Temperature (°C)	Alkalinity (mg/L)[1]	Hardness (mg/L)[1]	Cond. (µS/cm)	Temperature (°C)	Alkalinity (mg/L)[1]	Hardness (mg/L)[1]	Cond. (µS/cm)
Lampsilis cardium	FDP	18.4 (0.0)	126 (1)	174 (0)	391 (--)	18.2 (0.2)	144 (3)	196 (4)	431 (12)
Megalonaias nervosa	FDP	18.4 (0.0)	126 (1)	174 (0)	391 (--)	18.7 (0.3)	148 (5)	201 (2)	440 (18)
Ligumia recta	SDP	19.9 (0.0)	124 (1)	171 (1)	422 (9)	20.3 (0.5)	153 (8)	199 (6)	433 (21)
Lampsilis siliquoidea	SDP	19.3 (0.1)	130 (0)	171 (1)	--	19.9 (0.1)	137 (2)	181 (2)	367 (13)
Lampsilis higginsii	SDP	19.3 (0.1)	130 (0)	171 (1)	--	19.5 (0.1)	142 (4)	185 (3)	387 (17)
Obovaria olivaria	SDP	19.9 (0.2)	122 (1)	175 (1)	383 (2)	19.7 (0.7)	147 (7)	210 (4)	457 (27)
Actinonaias ligamentina	SDP	19.9 (0.0)	124 (1)	171 (1)	422 (9)	20.0 (0.4)	153 (5)	198 (2)	440 (25)
Lampsilis cardium	SDP	19.3 (0.1)	130 (0)	171 (1)	--	19.1 (0.1)	137 (3)	180 (1)	391 (14)

[1] Alkalinity and hardness reported as milligrams per liter of calcium carbonate.

Table 5. Mean dissolved oxygen, mean temperature, and pH range of exposure water.

[Values are by treatment group, during exposures of selected native freshwater mussel glochidia to two formulations of *Pseudomonas fluorescens*, strain CL145A. mg/L, milligrams per liter; pH, reported as standard units; °C, degrees Celsius; SDP, spray-dried powder; number in parentheses, standard deviation; HD, heat-deactivated test article, FDP, freeze-dried powder]

Treatment group	Dissolved oxygen (mg/L)	Temperature (°C)	pH
Lampsilis cardium, FDP formulation			
Control	8.34 (0.51)	17.8 (0.4)	8.12–8.32
50	8.13 (0.54)	17.2 (0.5)	8.26–8.31
100	8.08 (0.81)	17.5 (0.3)	8.31–8.34
200	7.92 (0.63)	17.4 (0.50	8.31–8.34
300	7.81 (0.62)	17.6 (0.5)	8.29–8.33
300 HD	7.19 (0.89)	17.7 (0.4)	8.31–8.36
Megalonaias nervosa, FDP formulation			
Control	7.43 (0.19)	18.6 (0.2)	8.39–8.41
50	7.64 (0.64)	17.8 (0.4)	8.39–8.40
100	8.39 (0.79)	18.3 (0.4)	8.39–8.41
200	8.12 (0.69)	18.5 (0.3)	8.35–8.49

Treatment group	Dissolved oxygen (mg/L)	Temperature (°C)	pH
300	7.87 (0.53)	18.1 (0.3)	8.35–8.39
300 HD	7.84 (0.68)	18.4 (0.2)	8.37–8.40
Ligumia recta, SDP formulation			
Control	9.17 (0.31)	20.4 (0.4)	8.27–8.56
50	9.36 (0.48)	20.7 (0.7)	8.34–8.44
100	9.59 (0.94)	20.5 (0.4)	8.32–8.38
200	8.72 (0.57)	20.2 (0.3)	8.21–8.37
300	8.75 (1.09)	20.6 (0.4)	8.15–8.31
300 HD	9.29 (0.88)	19.6 (0.9)	8.27–8.50
Lampsilis siliquoidea, SDP formulation			
Control	8.59 (0.28)	19.4 (0.4)	8.39–8.48
50	7.71 (0.57)	19.3 (0.4)	8.05–8.36
100	7.58 (0.81)	19.5 (0.8)	8.09–8.35
200	7.31 (1.82)	19.5 (0.6)	7.73–8.26
300	6.92 (2.09)	19.7 (0.7)	7.52–8.37
300 HD	6.78 (2.24)	19.1 (0.1)	7.68–8.31
Lampsilis higginsii, SDP formulation			
Control	8.05 (0.20)	19.3 (0.6)	8.38–8.46
50	7.59 (0.26)	19.3 (0.2)	8.08–8.38
100	8.02 (0.76)	19.0 (1.1)	8.00–8.29
200	7.49 (0.68)	18.7 (0.9)	8.03–8.30
300	7.21 (1.54)	19.3 (0.8)	7.58–8.41
300 HD	7.02 (1.34)	19.5 (0.7)	7.85–8.34
Obovaria olivaria, SDP formulation			
Control	9.82 (0.78)	19.9 (0.2)	8.36–8.51
50	10.41 (0.32)	19.7 (0.5)	8.32–8.48
100	10.16 (0.42)	19.6 (0.5)	8.30–8.45
200	9.67 (0.34)	19.7 (0.5)	8.10–8.35
300	9.66 (0.93)	19.7 (0.2)	8.10–8.30
300 HD	10.57 (0.30)	19.3 (1.0)	8.11–8.36
Actinonaias ligamentina, SDP formulation			
Control	8.40 (0.63)	20.1 (0.1)	8.34–8.53
50	9.20 (0.47)	20.1 (0.1)	8.33–8.43
100	8.65 (0.18)	19.8 (0.5)	8.31–8.43
200	8.96 (0.50)	19.7 (0.2)	8.26–8.40

Treatment group	Dissolved oxygen (mg/L)	Temperature (°C)	pH
300	8.74 (0.25)	19.8 (0.3)	8.29–8.38
300 HD	8.63 (0.31)	19.7 (0.6)	8.23–8.42
	Lampsilis cardium, SDP formulation		
Control	8.50 (0.11)	19.5 (0.4)	8.23–8.42
50	8.15 (0.11)	19.7 (0.7)	8.25–8.39
100	8.04 (0.91)	19.3 (0.4)	8.03–8.39
200	7.66 (0.87)	19.3 (0.4)	8.09–8.36
300	7.21 (1.63)	19.6 (0.5)	7.74–8.29
300 HD	7.46 (1.17)	19.6 (0.6)	8.07–8.37

Table 6. Mean total ammonia nitrogen of exposure water by treatment group at 24 hours.

[Total ammonia nitrogen in milligrams per liter measured at the termination of 24 hours exposures of selected native freshwater mussel glochidia to two formulations of *Pseudomonas fluorescens*, strain CL145A. FDP, freeze-dried powder; SDP, spray-dried powder; --, cannot calculate data; number in parentheses, standard deviation; HD, heat-deactivated product]

	FDP formulation		SDP formulation					
Treatment group	*Lampsilis cardium*	*Megalonaias nervosa*	*Ligumia recta*	*Lampsilis siliquoidea*	*Lampsilis higginsii*	*Obovaria olivaria*	*Actinonaias ligamentina*	*Lampsilis cardium*
Control	0.058 (0.016)	0.026 (0.004)	0.080 (0.002)	0.10 (--)	0.09 (--)	0.066 (0.029)	0.066 (0.039)	0.06 (--)
50	0.107 (0.013)	0.050 (0.010)	0.297 (0.121)	0.25 (--)	0.17 (--)	0.210 (0.097)	0.268 (0.234)	0.08 (--)
100	0.137 (0.011)	0.056 (0.019)	0.714 (0.014)	0.24 (--)	0.31 (--)	0.367 (0.238)	0.445 (0.241)	0.13 (--)
200	0.225 (0.014)	0.047 (0.013)	1.054 (0.127)	0.44 (--)	0.53 (--)	0.243 (0.027)	0.853 (0.072)	0.24 (--)
300	0.296 (0.012)	0.123 (0.057)	1.452 (0.242)	0.63 (--)	0.45 (--)	0.302 (0.157)	1.047 (0.116)	0.44 (--)
300 HD	0.193 (0.012)	0.086 (0.020)	0.230 (0.028)	0.55 (--)	0.50 (--)	0.489 (0.389)	0.581 (0.197)	0.51 (--)

Table 7. Mean un-adjusted glochidia viability from selected native freshwater mussels at 6, 12 and 24 hours.

[Selected native freshwater mussel glochidia exposed for 6, 12 and 24 hours to two formulations of *Pseudomonas fluorescens*, strain CL145A. FDP, freeze dried powder; SDP, spray dried powder; number in parentheses, standard deviation; --, no data collected; HD, heat-deactivated test article]

	FDP formulation		SDP Formulation					
Treatment group	*Lampsilis cardium*	*Megalonaias nervosa*	*Ligumia recta*	*Lampsilis siliquoidea*	*Lampsilis higginsii*	*Obovaria olivaria*	*Actinonaias ligamentina*	*Lampsilis cardium*
6 hours								
Control	82.3 (4.7)	--	85.7 (1.1)	72.3 (4.0)	72.3 (9.5)	80.0 (11.5)	88.6 (4.4)	92.3 (1.0)
50	69.6 (11.5)	--	11.7[a] (9.5)	56.8 (2.7)	77.7 (3.7)	85.2 (1.3)	71.7[a] (6.6)	77.9 (8.9)
100	65.7 (3.4)	--	0.5[a] (0.8)	29.0[a] (14.8)	63.2 (1.0)	61.0[a] (13.8)	72.2[a] (9.7)	75.3 (6.1)
200	48.7[a] (11.4)	--	1.7[a] (1.6)	19.3[a] (17.1)	56.7 (5.5)	40.6[a] (11.5)	40.5[a] (16.3)	52.2[a] (21.2)
300	40.7[a] (14.7)	--	1.1[a] (1.6)	11.9[a] (16.7)	29.1[a] (21.7)	15.7[a] (12.7)	11.8[a] (7.3)	9.9[a] (10.4)
300 HD	20.4[a] (23.0)	--	0.8[a] (1.5)	2.3[a] (2.4)	13.3[a] (12.3)	13.8[a] (6.3)	3.6[a] (2.1)	3.4[a] (3.1)
12 hours								
Control	81.8 (3.4)	--	89.5 (0.5)	66.6 (6.5)	68.7 (6.1)	86.4 (6.3)	94.2 (1.5)	86.1 (2.0)
50	76.7 (6.2)	--	16.2[a] (5.6)	59.1 (4.1)	68.7 (6.5)	84.5 (2.9)	66.6[a] (17.8)	78.6 (6.6)
100	70.6 (8.4)	--	8.8[a] (3.3)	43.6[a] (3.4)	65.5 (3.1)	77.2 (12.4)	64.4[a] (17.7)	52.7[a] (45.6)
200	34.9[a] (30.6)	--	1.2[a] (0.3)	19.6[a] (13.2)	46.2 (17.9)	50.3[a] (16.5)	40.0[a] (4.5)	46.7[a] (22.3)
300	9.1[a] (8.8)	--	0.0[a] (0.0)	4.5[a] (1.9)	38.8[a] (8.1)	39.2[a] (3.0)	10.2[a] (2.5)	6.5[a] (4.2)
300 HD	4.8[a] (1.2)	--	0.9[a] (0.8)	2.1[a] (2.8)	9.5[a] (0.9)	1.2[a] (0.4)	8.2[a] (5.0)	5.8[a] (2.6)
24 hours								
Control	81.9 (1.4)	52.1 (11.3)	84.5 (1.9)	69.4 (8.2)	69.3 (7.3)	87.0 (1.8)	92.6 (2.2)	92.1 (0.9)
50	74.1 (9.3)	55.6 (9.5)	14.7[a] (1.8)	56.8 (6.0)	74.6 (3.4)	89.2 (2.9)	75.8[a] (13.0)	79.8 (5.6)
100	67.7 (3.8)	48.1 (17.0)	7.0[a] (4.9)	41.6[a] (6.5)	65.5 (4.9)	78.3 (6.8)	63.5[a] (11.2)	66.9[a] (15.8)
200	29.5[a] (23.8)	49.5 (18.7)	3.3[a] (2.2)	11.6[a] (4.7)	42.5[a] (14.8)	60.7[a] (4.5)	30.0[a] (7.2)	59.2[a] (12.8)
300	12.0[a] (1.8)	40.1 (20.8)	0.7[a] (0.6)	6.0[a] (1.7)	22.3[a] (26.6)	26.5[a] (3.2)	6.0[a] (3.5)	6.1[a] (7.2)
300 HD	4.9[a] (2.3)	26.8[a] (10.6)	0.7[a] (0.8)	1.7[a] (0.9)	13.9[a] (10.4)	0.5[a] (0.3)	5.2[a] (3.4)	4.7[a] (1.3)

[a]At each sample time, the mean un-adjusted treatment group glochidia viability (in percent) within the same column that have a letter superscript are significantly different ($p > 0.05$) from the control group.

Table 8. Mean adjusted glochidia viability from selected native freshwater mussels at 6, 12 and 24 hours.

[Selected native freshwater mussel glochidia exposed for 6, 12 and 24 hours to two formulations of *Pseudomonas fluorescens*, strain CL145A. FDP, freeze-dried powder; SDP, spray-dried powder; number in parentheses, standard deviation; --, no data collected; HD, heat-deactivated test article]

Treatment group	FDP formulation		SDP Formulation					
	Lampsilis cardium	*Megalonaias nervosa*	*Ligumia recta*	*Lampsilis siliquoidea*	*Lampsilis higginsii*	*Obovaria olivaria*	*Actinonaias ligamentina*	*Lampsilis cardium*
6 hours								
Control	97.1 (5.6)	--	101.4 (1.3)	90.1 (5.0)	89.5 (11.8)	93.0 (13.4)	95.6 (4.8)	98.4 (1.1)
50	82.1 (13.6)	--	13.9 (11.3)	70.7 (3.4)	96.2 (4.6)	99.1 (1.5)	77.3 (7.1)	83.1 (9.5)
100	77.6 (4.1)	--	0.6 (1.0)	36.1 (18.4)	78.2 (1.3)	70.9 (16.1)	77.9 (10.5)	80.2 (6.5)
200	57.5 (13.5)	--	2.1 (1.8)	24.0 (21.4)	70.1 (6.8)	47.3 (13.3)	43.7 (17.6)	55.7 (22.6)
300	48.0 (17.3)	--	1.3 (1.8)	14.8 (20.8)	36.0 (26.9)	18.3 (14.8)	12.7 (7.9)	10.6 (11.1)
300 HD	24.1 (27.2)	--	1.0 (1.7)	2.9 (2.9)	16.4 (15.2)	16.1 (7.3)	3.9 (2.2)	3.7 (3.3)
12 hours								
Control	96.6 (4.0)	--	105.9 (0.6)	82.9 (8.0)	85.0 (7.6)	100.5 (7.4)	101.7 (1.6)	91.8 (2.2)
50	90.5 (7.4)	--	19.1 (6.6)	73.6 (5.1)	85.0 (8.0)	98.2 (3.4)	71.8 (19.1)	83.8 (7.0)
100	83.3 (10.0)	--	10.5 (3.9)	54.3 (4.2)	81.1 (3.9)	89.7 (14.5)	69.4 (19.1)	56.1 (48.7)
200	41.2 (36.1)	--	1.4 (0.4)	24.4 (16.4)	57.2 (22.2)	58.4 (19.2)	43.2 (4.9)	49.8 (23.8)
300	10.8 (10.4)	--	0.0 (0.0)	5.6 (2.4)	48.0 (10.0)	45.6 (3.5)	11.0 (2.7)	6.9 (4.5)
300 HD	5.7 (1.4)	--	1.1 (1.0)	2.7 (3.5)	11.8 (1.1)	1.4 (0.4)	8.8 (5.4)	6.2 (2.8)
24 hours								
Control	96.7 (1.7)	53.9 (11.7)	100.0 (2.3)	86.5 (10.2)	85.8 (9.0)	101.2 (2.0)	99.9 (2.4)	98.2 (1.0)
50	87.5 (11.0)	57.6 (9.8)	17.4 (2.1)	70.8 (7.4)	92.3 (4.2)	103.7 (3.3)	81.7 (14.1)	85.1 (6.0)
100	80.0 (4.5)	49.8 (17.6)	8.2 (5.8)	51.9 (8.0)	81.0 (6.0)	91.0 (8.0)	68.5 (12.1)	71.3 (16.8)
200	34.9 (28.0)	51.3 (19.3)	3.8 (2.6)	14.5 (5.8)	52.6 (18.3)	70.6 (5.3)	32.4 (7.8)	63.2 (13.6)
300	14.2 (2.2)	41.6 (21.5)	0.9 (0.8)	7.5 (2.2)	27.6 (32.9)	30.9 (3.8)	6.5 (3.8)	6.5 (7.7)
300 HD	5.8 (2.7)	27.7 (11.0)	0.9 (0.9)	2.1 (1.1)	17.2 (12.9)	0.6 (0.4)	5.6 (3.7)	5.1 (3.3)

Conclusions

The mean adjusted-viability of control group glochidia in trials with spray-dried powder (SDP) formulation of *Pseudomonas fluorescens* strain CL145A exceeded 82 percent. Differences in glochidia viability were detected in two of six species exposed to 50 milligrams per liter (mg/L) SDP and in four of six species exposed to 100 mg/L SDP at 6, 12, and 24 hours when compared to untreated controls. Regardless of sample time, viability of glochidia in the 200 and 300 mg/L SDP-treated groups and the 300 mg/L heat-deactivated SDP positive control groups were significantly lower than glochidia in the untreated control for all species except *Lampsilis higginsii*. The significant impact of the SDP positive control treatments on glochidia viability indicate that the decreases in glochidia viability may not be caused by the same mode of action that causes dreissenid mussel (zebra mussel, *Dreissena polymorpha* and quagga mussel, *Dreissena rostriformis bugensis*) mortality.

The mean adjusted-viability of control group glochidia in trials with freeze-dried powder (FDP) formulation of *P. fluorescens* (strain CL145A) exceeded 96 percent in the *Lampsilis cardium* trial and was 53.9 percent in the *Megalonaias nervosa* at 24 hours. Regardless of sample time, differences in glochidia viability were only detected in the *L. cardium* FDP-trial when the concentration was ≥ 200 mg/L. In the *M. nervosa* FDP exposures, differences in glochidia viability were only detected between the 300 mg/L heat-deactivated FDP-treated group (positive control) and the untreated control group.

However, given the low viability observed in the *M. nervosa* control group (52.1 percent un-adjusted viability), the results for *M. nervosa* should be interpreted with caution. Similar to the SDP-trials, the impact of the FDP positive control treatments indicate that the observed decreases in glochidia viability may not be caused from the same mode of action that causes dreissenid mussel mortality. The results of these exposures indicate that applications of a biopesticide formulated with *P. fluorescens* as the active ingredient may impact the viability of native freshwater mussel glochidia if they are present in the water column during an application. However, freshwater mussel glochidia are only present in the water column for a relatively short period which could be avoided by timing *P. fluorescens* biopesticide applications. Additionally, applications of a *P. fluorescens* biopesticide for dreissenid mussel control would be of short duration and the biological activity of *P. fluorescens* degrades rapidly; further reducing the risk of exposure to *P. fluorescens* to native mussel glochidia.

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Appendix 1. Study Protocol, Amendments, and Datasheets

Item number	Item description	Number of pages	Report page number
1	Protocol: "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"	21	20
2	Amendment 1: Revision of Study Protocol, Study # AEH-11-PSEUDO-01	6	41
3	Amendment 2: Revision of Study Protocol, Study # AEH-11-PSEUDO-01	8	47
4	Amendment 3: Revision of Study Protocol, Study # AEH-11-PSEUDO-01	5	55
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7	Note to File 1: Study # AEH-11-PSEUDO-01	6	69
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9	Version 1.1 "Donor Mussel UMESC Lot Number Assignment Form" Datasheet	1	82
10	Version 1.1 "Daily Care Worksheet Donor Mussel Holding" Datasheet	1	83
11	"Adult Mussel Holding Daily Algal Diet 1/13/2012" Datasheet	1	84
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14	Version 1.1 "Glochidia Photomicrograph Counting Record" Datasheet	1	87
15	Version 1.1 "Initial Viability and Concentration Determination of Glochidia" Datasheet	1	88
16	Version 1.1 "Water Quality – Form 1 Initial (Dilution water Hardness, Alkalinity, and Temperature)" Datasheet	1	89
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Protocol Title:

Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species

Study Number: AEH-11-PSEUDO-01

Item No. 1

FOLDER NO. 3

Test Facilities

Upper Midwest Environmental Sciences Center (UMESC)
US Geological Survey
2630 Fanta Reed Rd.
La Crosse, Wisconsin 54603

Proposed Experimental Start Date: May 2011
Proposed Experimental Termination Date: February 2012

Protocol Approval

Reviewed by:

[redacted] 25Apr2011
Mark P. Gaikowski, M.A. Date
Supervisory Biologist

[redacted] 26Apr2011
Jane E. Rivera Date
Acting Quality Assurance Officer

[redacted] 5/2/2011
Michael D. Jawson, Ph.D. Date
Center Director

Approved by:

[redacted] 03MAY2011
James A. Luoma, B.A. Date
Study Director

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PROOFED BY
Initials: JMS Date 1/27/12
REVIEWED BY
Initials: ____ Date: ____

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ORIGINAL

1. INTRODUCTION:

Historical native freshwater mussel populations of North America were considered the most diverse in the world with about 297 recognized taxa consisting of 281 species and 16 subspecies (Williams et al., 1993). Mussels are largely sedentary in nature, relying on movement of host fish during glochidial attachment as means of transport. Due to these facts, mussels are particularly vulnerable to a variety of anthropogenic influences including habitat degradation and alteration, pollution and over harvest. Master (1990) reported a survey conducted by the Nature Conservancy which showed 55% of North America's mussels as extinct or imperiled compared to 7% of terrestrial species which traditionally have received far greater attention. Ricciardi and Rasmussen (1999) projected that at least 127 imperiled mussel species will be lost in the next 100 years. The extinction rate was calculated to be 6.4% per decade and should be considered conservative because it did not take into account the extirpations caused by the invasive dreissenid mussels (Ricciardi and Rasmussen, 1999).

Neves et al (1997) reported the ominous status of native mussels in the Southeast with only 25 percent of the 269 species historically present reported as stable. Thirteen percent were reported as presumed extinct, 28 percent as endangered, followed by 14 percent as threatened and 18 percent listed as species of special concern. Many unionid mussel species in North America were imperiled prior to epizoic colonization by zebra mussels (*Dreissena polymorpha*), which has dramatically heightened concerns for their continued survival. Zebra mussels have been deemed responsible for the extirpation of unionids from waters in Europe as early as 1937 (Sebestyen, 1937). Severe declines in unionid abundance in Europe (Karatayev and Burlakova, 1995; Burlakova, 1998) and North America (Haag et al, 1993; Nalepa, 1994; Ricciardi et al., 1996) have since been well documented in the literature.

The 1973 Endangered Species Act (ESA) brought forth the need to recognize, protect and recover rare mussels in the United States. The United States Fish and Wildlife Service (USFWS) develops recovery plans for threatened and endangered species which utilize a range of tools to promote recovery of the species including restoring and acquiring critical habitat, removing introduced or invasive species and captive propagation and release into historic ranges.

As of 2004, mussel propagation work was being conducted in several different facilities in 7 states as well as in Ontario, Canada (Neves, 2004). The Genoa National Fish Hatchery (GNFH) in Wisconsin has been involved in mussel recovery since 2000, releasing tens of thousands of propagated subadult Higgins eye pearlymussel (*Lampsilis higginsii*) for recovery efforts. The GNFH produces subadult mussels using cage culture techniques. This technique involves placing glochidia laden host fish into submerged cages within natural water bodies such as the Upper Mississippi and St. Croix Rivers. The fish are released from the cages after mussel excystment and the mussels are allowed to grow on the cage bottom for an additional 6-18 months before being harvested. Areas that were previously successful in rearing mussels using this technique have been abandoned due to the proliferation and colonization by zebra mussels.

Biologists at the New York State Museum (NYSM) Field Research Laboratory have been researching dreissenid mussel control since 1991 and they discovered that a bacterium isolated from soils (*Pseudomonas fluorescens* [*Pf*-CL145A]) is efficacious for controlling zebra mussels. Marrone Bio Innovations (MBI; Davis, CA) is currently developing a commercial sprayed dried formulation of this bacterium called MBI-401 SDP. The current commercial applications of this product are for use with closed systems such as power generating plant cooling systems. The NYSM has partnered with the USFWS (Genoa NFH) and United States Geological Survey's (USGS) Upper Midwest Environmental Sciences Center (UMESC) to determine the suitability of this product for open water zebra mussel control applications such as treatment of native mussel propagation cages or native mussel beds.

The Glochidia life stage of unionid mussels has been identified as a critical life stage and has been shown to be highly sensitive to various contaminants (ASTM E2455-06). This research will determine the animal effects of various concentrations of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussels species present in the Great Lakes and Upper Mississippi River basins.

2. PROTOCOL OBJECTIVE:

This study will determine the animal effects of various concentrations of MBI-401 SDP (*Pseudomonas fluorescens* [*Pf*-CL145A]) to the glochidia lifestage of seven unionid mussels species present in the Great Lakes and Upper Mississippi River basins.

3. STUDY SCHEDULE:

3.1 Proposed initiation: May 2011
3.2 Schedule of events: A proposed schedule of events is provided in Table 1.
3.3 Proposed completion date: February 2012

ORIGINAL

Table 1. Proposed Schedule of Events

Date	Activity
May 2011-November 2011	Glochidia exposures
November 2011-January 2012	Data analysis
February 2012	Final Report submission

4. STUDY DESIGN:

4.1 General Description:
Glochidia from 7 unionid mussel species (Table 2) endemic in the Great Lakes and Mississippi River basins will be evaluated for effects from exposure to varying concentrations of MBI-401 SDP (*Pseudomonas fluorescens* [*Pf*-CL145A]). Glochidia will be exposed in static exposures for 24 h as described in ASTM E-2455-06 and evaluated at 6, 12 and 24 h for viability as determined by valve closure response after addition of a saturated sodium chloride solution.

Table 2. Mussel species to be evaluated for MBI-401 SDP (*Pseudomonas fluorescens* [*Pf*-CL145A]) exposure effects.

Common name	Scientific name
Black sandshell	*Ligumia recta*
Fatmucket	*Lampsilis siliquoidea*
Hickorynut	*Obovaria olivaria*
Higgins eye	*Lampsilis higginsii*
Mucket	*Actinonaias ligamentina*
Plain pocketbook	*Lampsilis cardium*
Washboard	*Megalonaias nervosa*

4.2 Experimental Design:
Gravid donor mussels will be collected by biologists from the Genoa National Fish Hatchery from the Upper Iowa, Upper Mississippi or St. Croix Rivers. Mussels will be identified to species as described in Cummings and Mayer (1992) and Watters et al. (2009) and transported to the Genoa National Fish Hatchery. Glochidia from at least three gravid female mussels of each species will be extracted and evaluated for viability and if acceptable (≥80%) glochidia groups will pooled for use by the Genoa NFH according to the procedures outlined in ASTM E2455-06 and immediately transferred to the UMESC in a

cooler at ~20°C. Glochidia will be acclimated to test water by the addition of 50% UMESC well water at the Genoa NFH and upon arrival at UMESC. Prior to exposure initiation, viability will be assessed in a subsample of glochidia by the addition of a saturated sodium chloride solution (12 g NaCl/ 50 mL of D.I. H_2O [ASTM E2455-06]). Viability will be assessed ~1 minute after addition of sodium chloride and must exhibit > 80% viability for use in exposures. An appropriate amount of glochidia suspension (ie: 2 mL) will be randomly aliquoted to a exposure chamber (80 mm x 40 mm crystallizing dish) containing acclimated well water to achieve a final volume of 100 mL. Approximately 500 glochidia will be transferred to each exposure chamber in a minimum of two aliquots per chamber (ie: two 1 mL aliquots that contain ~250 glochidia each). Once all of the exposure chambers receive test animals the exposures will be initiated by the addition of the appropriate amount of MBI-401 SDP, deactivated MBI-401 SDP (positive control) or plain water (control) in a completely randomized design to achieve the desired test concentrations of 0 (control), 300 mg/L deactivated (positive control), or 50, 100, 200, and 300 mg/L of active MBI-401 SDP. The exposure chambers will be gently mixed with a glass rod or stainless steel spatula to ensure uniform distribution of the test chemical. All concentrations will be tested in triplicate for a total of 18 exposure chambers per species. exposure chambers will be held at 20°C in a water bath or controlled environment room and maintained on an 18:6 h light/dark cycle. Viability of a subsample of approximately 100 glochidia from each chamber will be assessed at 6, 12 and 24 h post exposure by enumeration through a dissecting microscope. Photomicrographs of subsamples may be recorded. The study will be terminated after 24 h post exposure evaluations are completed.

5. STUDY PROCEDURES

5.1 Test Animals

5.1.1 Description:

5.1.1.1 Age – <24 h extracted glochidia.

5.1.1.2 Sex – glochidia are an immature lifestage and they cannot be identification sexually.

5.1.1.3 Species – See Table 2

5.1.2 Number of animals: Approximately 9,000 glochidia (500 per replicate x 3 replicates x 6 concentrations) of each species. This design uses the fewest number of glochidia possible, consistent with the objective of the study, contemporary scientific standards and ASTM guide E2455-06.

5.1.3 Source of animals: All glochidia will be obtained from donor mussels located at the Genoa NFH.

5.1.4 Inclusion criterion: Glochidia will be used if they meet or exceed the following percentage of valve closure requirements after addition of a saturated sodium chloride solution (12 g NaCl/ 50 mL of D.I. H_2O). Glochidia must exhibit ≥ 80% valve closure to be pooled for use at the Genoa NFH and glochidia must exhibit ≥ 80% valve closure immediately prior study initiation at the UMESC.

5.1.5 Acclimation: Glochidia will be acclimated to UMESC well water and temperature (20 °C) by the addition of up to 50% UMESC well water at the Genoa NFH prior to transport to UMESC and again after arrival at the UMESC.

5.1.6 Feeding: In compliance with contemporary scientific standards, glochidia will not be fed during the exposures.

5.2 Water Chemistry

5.2.1 Dissolved oxygen: Dissolved oxygen will be measured and recorded at least twice during the exposure period in the control and high concentration. (UMESC SOP AEH 394 or equivalent).

5.2.2 Temperature: Temperature will be measured and recorded at least twice during the exposure period.

5.2.3 pH: The pH will be measured and recorded at least twice during the exposure period in the control and high concentration (UMESC SOP AEH 310, 334 or equivalent).

5.2.4 Hardness: The hardness will be measured and recorded on dilution water prior to test initiation and upon test termination from pooled replicate samples from the control and high concentrations (UMESC SOP AEH 712 or equivalent).

5.2.5 Alkalinity: The alkalinity will be measured and recorded on dilution water prior to test initiation and upon test termination from pooled replicate samples from the control and high concentrations (UMESC SOP AEH 706 or equivalent).

5.2.6 Conductivity: The conductivity will be measured and recorded upon test termination from pooled replicate samples of each test concentration (UMESC SOP AEH 188 or equivalent).

5.2.7 Ammonia: The ammonia will be measured and recorded at the termination of the exposure from pooled replicate samples from the controls and all test concentrations (UMESC SOP AEH 301 or equivalent).

5.3 Disposal: Upon study termination the glochidia in each test vessel will be euthanized by MS-222 overdose (UMESC SOP GEN 132) then disposed of by incineration.

5.4 Study facilities:

5.4.1 Test Facility

U.S. Geological Survey, Upper Midwest Environmental Sciences Center
2630 Fanta Reed Rd
La Crosse, Wisconsin 54603

5.4.1.1 Exposure system: The test system is a series of eighteen static 80 x 40 mm crystallizing dishes (100 mL of test water) for each species, placed in a recirculating water bath system or on a bench top of a controlled environment room. Each replicate will be uniquely identified (eg: 1A2) to allow for identification of species treatment type and replicate number. Coding identification procedures will be documented in the laboratory notebook.

5.4.1.2 Aeration: Supplemental aeration will be supplied during exposures by gently bubbling in laboratory air through a pasteur pipet at a rate of approximately 1 bubble/second.

5.4.1.3 Water supply: UMESC well water will be temperature acclimated (20°C) and aerated for at least 24 h prior to use for test water.

5.4.1.4 Lighting: Direct light may adversely affect test results, thus indirect lighting(~18 h L:6 h D; 100-1000 lux) will be provided; light intensity will be recorded at the initiation and completion of the exposure period (UMESC SOP AEH 308).

5.4.1.5 Exposure chamber dimensions: The exposure chambers are 80 x 40 mm crystallizing dishes. Each exposure chamber will be filled with 100 mL of test water (ASTM E2455-06).

5.4.1.6 Water discharge: All water will be discharged into the UMESC invasive species isolation facility.

5.5 Observations:

5.5.1 Behavioral Observations: Behavioral observations will be limited to viability assessments. Viability is determined by the ability of the glochidia to constrict their adductor mussel thereby causing valve closure in response to saturated sodium chloride (12 g NaCl/ 50 mL of D.I. H_2O) exposure. Viability will be determined on a subsample of approximately 100 glochidia at 6, 12 and 24 h post exposure for each test chamber.

5.5.2 Water chemistry: Dissolved oxygen, pH, and temperature will be monitored after the addition of the specified treatment. Alkalinity, hardness, conductivity and ammonia will be measured on a sample of dilution water at test initiation and on pooled replicate samples upon test termination. Alkalinity, hardness, conductivity and ammonia will not be measured during the exposures due to the quantity of test water required and probe submersion requirements to complete the analysis.

5.6 Treatment administration:

5.6.1 Treatment: Each species of mussel glochidia will be exposed to three replicates of either 0 (control), 50, 100, 200 or 300 mg/L (active ingredient) and a 300 mg/L heat deactivated (70°C/45 minutes) control as a one time single dose exposure.

5.6.2 Route of administration: To assure uniform exposure, an appropriate amount of freshly prepared stock solution will added to each test chamber from an agitated stock suspension with a pipet.

6. SPECIFICATION OF VARIABLES

6.1 Distribution to experimental units: The glochidia concentration (glochidia/mL) will be determined for each species prior to test initiation by enumeration of replicate subsamples of glochidial suspension through a dissecting microscope. Appropriate volumes (mL) will be determined that would contain approximately 500 glochidia. Test chambers will then be filled with appropriate amounts of dilution water to obtain a final volume of 100 mL. Distribution to test chambers will then proceed by the addition of the calculated volume of suspension in at least two separate aliquots. Aliquots will be randomly assigned to a test chamber until all receive one aliquot. This procedure will then be repeated until all chambers receive approximately 500 glochidia in two or more aliquots. The test chambers will be randomly assigned treatment concentration.

6.2 Determination of viability: Viability will determined by the ability of the glochidia to constrict their adductor mussel thereby causing valve closure in response to saturated sodium chloride (12 g NaCl/ 50 mL of D.I. H_2O) exposure. Viability will be determined on a subsample of approximately 100 glochidia for each species prior to test initiation and at 6, 12 and 24 h post exposure for each test replicate. A volume of water containing approximately 100 glochidia (eg: 1-2 mL) will be placed on a 35 mm mini petri dish and examined under a dissecting scope to enumerate the percentage of glochidia with closed valves. Then approximately 1 drop of a saturated sodium chloride solution will be added to the sample and the glochidia will then be reassessed for valve closure after approximately one minute. Subsample photomicrographs of glochidia pre and post sodium chloride addition may be recorded and uniquely identified for documentation and optional later enumeration and/or validation of closure percentages. Only glochidia that are open and responsive to sodium chloride addition will be considered viable.

7. DATA ANALYSIS

7.1 Experimental unit: The experiment unit will be the exposure chamber.

7.2 Number of exposures and replicates: There will be a total of 6 treatment levels (control, positive control (heat treated *Pf*-CL145A) 50, 100, 200 and 300 mg *Pf*-CL145A/L) for each replicate and three replicate exposure chambers per treatment level for a total of 18 exposure chambers for each mussel species.

7.3 Statistical methodology:

Viability data will be analyzed using a generalized linear mixed model (SAS PROC GLIMMIX). In every analysis, the exposure chamber will be treated as the experimental unit. The change in proportion of viability will be analyzed using a generalized linear mixed model where the distribution is binomial and the link used is the logit function.

If a significant effect of treatment is identified then pairwise comparison tests will be completed to compare each treatment group to the control group using unadjusted least squares means.

7.4 Statistical significance: Statistical significance will be declared at $p < 0.05$.

7.5 Other data analyses: Statistical methods for other study data collected will include calculation of means, standard deviations and coefficients of variation.

8. PERSONNEL

8.1 Study Director: James A. Luoma, B.A.

8.1.1 Address: Upper Midwest Environmental Sciences Center, US Geological Survey, 2630 Fanta Reed Rd., La Crosse, Wisconsin 54603

8.1.2 Contact: Tel: (608) 781-6391, Fax: (608) 783-6066; jluoma@usgs.gov

8.1.3 Training and experience: CV on file at UMESC.

8.2 Other personnel involved in study: Technical staff involved in the study will be identified in the study raw data to include study function. UMESC technical staff training and experience will be documented in CVs included in the study raw data.

9. DISPOSITION/STORAGE

9.1 Study Records: All data generated in the study at UMESC will be recorded in bound laboratory notebooks or kept in file folders (SOP No. GEN 008). All data sheets, file folders, laboratory notebooks and computer disks will be encoded with the study number when the data are generated and stored in secure files (SOP No. GEN 008). Raw data, laboratory notebooks and electronic files (including a CD-ROM containing the annotated SAS program used for the statistical analysis, the data files, SAS log and SAS output files) generated by UMESC and contract laboratory reports will be filed in the UMESC archives (SOP No. GEN 007) of the Upper Midwest Environmental Sciences Center, LaCrosse Wisconsin, before the final report is signed by the Study Director. The final report will then be signed and archived.

10. GOOD LABORATORY PRACTICES

Data collection, storage and retrieval procedures for the study will be conducted in compliance with FDA regulations for Good Laboratory Practices (GLP; 21 CFR, Part 58). The study protocol and progress of the study will be reviewed at the start of the study and periodically throughout the study by the Quality Assurance Unit (QAU). The Study Director has the responsibility of ensuring that all procedures used in conjunction with the study conform with Good Laboratory Practices.

11. AMENDMENT/DEVIATIONS TO THE PROTOCOL

11.1 Protocol amendments: A signed copy of the Study Protocol will be retained on-site. Proposed amendments to the protocol shall be brought to the attention of UMESC Management. When the Study Director and Management agree verbally, the study can proceed with the change. As soon as possible, the Study Director will then prepare a written protocol amendment that is signed by the Study Director, Branch Chief, UMESC Center Director, and UMESC-QA. The UMESC statistician or UMESC Animal Care and Use Chair may also sign as needed. The amendment then becomes an official part of the protocol.

11.2 Protocol deviations: All deviations from this approved protocol will be documented and reviewed by the Study Director. The Study Director will notify Management, UMESC-QA, as soon as possible, in writing, of any deviations to the protocol, including their impact on the study.

12. INVESTIGATIONAL TEST ARTICLE

12.1 Test Substance(s): MBI-401 SDP, *Pseudomonas fluorescens* (*Pf*-CL145A)

12.1.1 Chemical name: *Pseudomonas fluorescens* (*Pf*-CL145A)

12.1.2 Trade name: MBI-401 SDP

12.1.3 Active ingredients: *Pseudomonas fluorescens* (*Pf*-CL145A) is the sole active ingredient, 50% active by weight.

12.1.4 Source: Marrone Bio Innovations (MBI); Davis, CA

12.1.5 Lot number: None established

12.1.6 Expiration date: None established

12.1.7 Storage during study: test chemical will be stored at refrigerated in a locked container within a restrictive entry laboratory. A subsample of the test chemical will be archived in the UMESC Chemical Archive (UMESC SOP GEN 011).

12.1.8 A NIOSH approved respirator will be used when preparing stock solutions to avoid inhalation. Protective eyewear, gloves and lab coats will be worn at all times when working with the test substance.

13. ADVERSE EVENTS: Any adverse event will be recorded in the study logbook and the Study Director will be notified.

14. BIOSECURITY PROCEDURES

14.1 General Procedures: All personnel involved in the study will review the UMESC biosecurity (UMESC SOP APP 075) and project HACCP plans. Testing will be conducted in a laboratory with controlled access. All effluent and sample water will be iodine or chlorine disinfected prior to discharge.
Biosecurity procedures outlined in UMESC SOP APP 075.0 will be followed for samples and equipment.

14.2 HACCP Plan: See Appendix 1 for the HACCP plan for this project.

15. STANDARD OPERATING PROCEDURES

UMESC SOP GEN 007 – Archives Management for Regulated Studies
UMESC SOP GEN 008 – Maintenance of Data Recording of Raw Data for Regulated Studies
UMESC SOP GEN 012 – Receipt, Identification, Storage, Handling, Checkout and Documentation of Use of Test Chemicals
UMESC SOP APP 075 – Procedures to Minimize the Risk of Transfer of Pathogens and Invasive Species
UMESC SOP GEN 132 – Care, Maintenance & Disposal of Aquatic Vertebrates
UMESC SOP AEH 011 – Procedures for Labeling Chemicals and Specimens.
UMESC SOP AEH 213 – Sartorius Balance, Model LC34000P, Serial 30303922
UMESC SOP AEH 188 – Accumet Portable Waterproof Conductivity meter Model # AP75
UMESC SOP AEH 301 – Instrument Operating Procedure: YSI Photometer Model # 9000 Serial # 3638017
UMESC SOP AEH 308 – Instrument Operating Procedure: Milwaukee Light Meter Model # SM7000 Serial # 727298
UMESC SOP AEH 310 – Hanna pH Meter, Model HI991001, Serial Number 370973
UMESC SOP AEH 334 – Beckman Portable pH/mV Meter, Model F210, Serials 330167 & 330168
UMESC SOP AEH 338 – Sartorius Model BP 3100S, Serial Number 12907582
UMESC SOP AEH 394 – YSI Handheld Dissolved Oxygen Meter, Model 55/12FT, Serials 94C17261 & 97F0837AG
UMESC SOP AEH 606 – Methods Used to Weigh, Measure & Mark Test Animals
UMESC SOP AEH 706 – Determination of Total Alkalinity by the Titrimetric (pH 4.5) Method
UMESC SOP AEH 712 – Determination of Total Hardness
UMESC SOP AEH 903 – Verification of Thermometer & Temperature Recorder Calibration UMESC

16. REFERENCES.

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17. APPENDIX.

17.1 Appendix 1. HACCP PLAN for the study Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species

Step 1 – Activity Description

Facility: US Geo ogical Survey-Upper Midwest Environmental Sciences Center	Site: Lower B, rooms 15, 16 and 17
Site Coordinator: Jim Luoma Site Manager: Mark Gaikowski Address: 2630 Fanta Reed Road La Crosse WI, 54601 Phone: 608-781-6322	Activity: Determine the effects of various concentrations of *Pseudomonas fluorescens* (*Pf*-CL145A) to the glochidia lifestage of seven unionid mussels species present in the Great Lakes and Upper Mississippi River basins.

Project Description
The objective of this study is to determine effects of various concentrations of *Pseudomonas fluorescens* (*Pf*-CL145A) to the glochidia lifestage of seven unionid mussels species present in the Great Lakes and Upper Mississippi River basins.

Step 2 – Potential Hazards: Species which may potentially be moved/introduced

Vertebrates: None
Invertebrates: Zebra mussel (*Dreissena polymorpha*)
Plants: None
Other biologicals (disease, pathogen, parasite): Largemouth Bass Virus Spring Viremia of Carp Virus Bluegill Virus Infectious Pancreatic Necrosis Virus Viral Hemorrhagic Septicemia Furunculosis *Aeromonas salmonicida* Enteric Redmouth Disease *Yersinia ruckeri* Bacterial Kidney Disease *Renibacterium salmoninarum* Other Assorted parasites/pathogens commonly found in the upper Mississippi River Basin
Other (construction materials): NA

Step 3 – Flow Diagram

Flow diagram outlining sequential tasks to complete activity/project

Task 1	Mussel glochidia are obtained from the Genoa NFH and transferred to UMESC.
Task 2	Mussel glochidia distributed to test chambers in the invasive species laboratory
Task 3	Mussel glochidia are removed for enumeration
Task 4	Water samples collected and analyzed for chemical parameters
Task 5	After exposure, the glochidia will be euthanized using a lethal dose of MS-222 and discarded according to UMESC SOPs GEN 132.

1 Tasks (from HACCP Step 3 - Flow Diagram)	2 Potential hazards identified in HACCP Step 2	3 Are any potential hazards probable? (yes/no)	4 Justify evaluation for column 3	5 What control measures can be applied to prevent undesirable results?	6 Is this task a critical control point? (yes/no)
Task 1 Mussel glochidia are obtained from the Genoa NFH and transferred to UMESC.	Vertebrates	No	Mussel glochidia will be transported in clean well water and the will not be vertebrates present	N/A	no
	Invertebrates	yes	Mussel glochidia will be collected from donor mussels collected from wild sources.	Assure donor mussels have been cleaned and rinsed with well water prior to use. Effluent water is treated and equipment will be disinfected. Equipment remains in the laboratory.	yes
	Plants	No	The donor mussels are cleaned upon collection and held in clean well water prior to use.	Any plant material will be removed from donor mussels and equipment before transporting Genoa NFH.	no
	Others	No	The potential transfer of fish diseases from glochidia harvested from donor mussels is extremely low.	Effluent water is treated and equipment will be disinfected. Equipment remains in the laboratory.	no
Task 2 Mussel glochidia distributed to test chambers in the invasive species laboratory	Vertebrates	no	Risk eliminated in Task 1	N/A	no
	Invertebrates	no	Risk eliminated in Task 1	Effluent water is treated and equipment will be disinfected. Equipment remains in the laboratory.	no
	Plants	no	Risk eliminated in Task 1	N/A	no
	Others	no	The potential transfer of fish diseases from glochidia has never been documented.	Effluent water is treated and equipment will be disinfected. Equipment remains in the laboratory.	no

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Task 3 Mussel glochidia are removed for enumeration	Vertebrates	No	Risk eliminated in Task 1	N/A	no
	Invertebrates	No	Risk eliminated in Task 1	Effluent water is treated and equipment will be disinfected. Equipment remains in the laboratory.	no
	Plants	No	Risk eliminated in Task 1	N/A	no
	Others	No	The potential transfer of fish diseases from glochidia has never been documented.	Effluent water is treated and equipment will be disinfected. Equipment remains in the laboratory.	no

Task 4 Water samples collected and analyzed for chemical parameters	Vertebrates	No	Risk eliminated in Task 1	N/A	no
	Invertebrates	No	Risk eliminated in Task 1	Effluent water is treated and equipment will be disinfected. Equipment remains in the laboratory.	no
	Plants	No	Risk eliminated in Task 1	N/A	no
	Others	No	The potential transfer of fish diseases from glochidia has never been documented.	Effluent water is treated and equipment will be disinfected. Equipment remains in the laboratory.	no

Task 5 After exposure, the glochidia will be euthanized using a lethal dose of MS-222 and discarded according to UMESC SOPs GEN 132.	Vertebrates	No	Risk eliminated in Task 1	N/A	no
	Invertebrates	No	Risk eliminated in Task 1	N/A	no
	Plants	No	Risk eliminated in Task 1	N/A	no

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	Others	No	The potential transfer of fish diseases from glochidia has never been documented.	Effluent water is treated and equipment will be disinfected. Equipment remains in the laboratory.	no

HACCP Plan Form
(all CCP's or "yes's" from column 6 of HACCP Step 4 – Hazard Analysis Worksheet)

			Monitoring					
Critical Control Point (CCP)	**Significant Hazard(s)**	**Limits for each Control Measure**	**What**	**How**	**Frequency**	**Who**	**Evaluation & Corrective Action(s) (If needed)**	**Supporting Documentation (If any)**
Task 1 Invertebrates	Invertebrate transfer	Invertebrates cannot be transferred. Donor mussels must be cleaned and rinsed Effluent water must be captured and treated.	Macro/Micro scopic Invertebrates	Manually remove inverts Clean and rinse donor mussels effluent water disinfection	Daily inspections Immediately upon animal or sample transfer	All Staff	Supervisor and staff are responsible for careful attention to detail-Disinfection of equipment	Records in log book

Facility: Upper Midwest Environmental Sciences Center	**Activity:** Determine the effects of various concentrations of *Pseudomonas fluorescens* (*Pf*-CL145A) to the glochidia lifestage of seven unionid mussels.
Address: 2630 Fanta Reed Road, La Crosse, WI 54601	
Signature: HACCP Plan was followed.	**Date:**

FOLDER NO. 3

PROOFED BY
Initials: TMS Date: 7/27/12
REVIEWED BY
Initials: ____ Date: ____

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Item No. 2

United States Department of the Interior

U.S. GEOLOGICAL SURVEY
Biological Resources Division
Upper Midwest Environmental Sciences Center
2630 Fanta Reed Road
La Crosse, Wisconsin 54603

FOLDER NO. 3

Date: May 9, 2011
To: The Record Study Number AEH-11PSEUDO-01

Subject: Amendment 1- Amendment to the study AEH-11-PSEUDO-01 "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"

Revision of Study Protocol, Study # AEH-11PSEUDO-01 is proposed as detailed on pages 2-6 of this amendment. Revised text is indicated **in bold**.

This amendment 1) changes the physical location where glochidia are extracted from the gravid female mussels and adds a description of the acclimation procedures applied to the female mussels and glochidia; 2) adds the use of photomicrographs to enumerate glochidia; 3) changes the glochidia distribution and sampling methods; 4) adds the requirements to determine stock and test solution absorbance; and 5) includes the test material lot number and post-testing activity determination.

The amendment adjusts the glochidia collection methods to minimize the time period between collection and testing and maximizes acclimation to test conditions. The amendment adjusts the procedures used to enumerate, distribute and sample glochidia based on preliminary observations. The amendment also adds methods to assess the stock solution concentration. Last, the amendment adds the test material lot number and post-testing activity assessment.

Reviewed by:

[redacted] 09 May 11
Mark P. Gaikowski, M.A. Date
Supervisory Biologist
Aquatic Ecosystem Health,
UMESC[1]

[redacted] 09 May 11
Jane E. Rivera, B.A. Date
Acting Quality Assurance Officer,
UMESC

[redacted] 5/10/2011
Michael Jawson, Ph.D. Date
Center Director, UMESC

Approved by:

[redacted] 5/10/2011
James A. Luoma, B.A. Date
Study Director, UMESC

[1] UMESC: U.S. Geological Survey, Upper Midwest Environmental Sciences Center

PROOFED BY
Initials: TMS Date: 2/6/12
REVIEWED BY
Initials: ____ Date: ____

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Current text:

4.2 Experimental Design:

Gravid donor mussels will be collected by biologists from the Genoa National Fish Hatchery from the Upper Iowa, Upper Mississippi or St. Croix Rivers. Mussels will be identified to species as described in Cummings and Mayer (1992) and Watters et al. (2009) and transported to the Genoa National Fish Hatchery. Glochidia from at least three gravid female mussels of each species will be extracted and evaluated for viability and if acceptable (≥80%) glochidia groups will pooled for use by the Genoa NFH according to the procedures outlined in ASTM E2455-06 and immediately transferred to the UMESC in a cooler at ~20°C. Glochidia will be acclimated to test water by the addition of 50% UMESC well water at the Genoa NFH and upon arrival at UMESC. Prior to exposure initiation, viability will be assessed in a subsample of glochidia by the addition of a saturated sodium chloride solution (12 g NaCl/ 50 mL of D.I. H_2O [ASTM E2455-06]). Viability will be assessed ~1 minute after addition of sodium chloride and must exhibit > 80% viability for use in exposures. An appropriate amount of glochidia suspension (ie: 2 mL) will be randomly aliquoted to a exposure chamber (80 mm x 40 mm crystallizing dish) containing acclimated well water to achieve a final volume of 100 mL. Approximately 500 glochidia will be transferred to each exposure chamber in a minimum of two aliquots per chamber (ie: two 1 mL aliquots that contain ~250 glochidia each). Once all of the exposure chambers receive test animals the exposures will be initiated by the addition of the appropriate amount of MBI-401 SDP, deactivated MBI-401 SDP (positive control) or plain water (control) in a completely randomized design to achieve the desired test concentrations of 0 (control), 300 mg/L deactivated (positive control), or 50, 100, 200, and 300 mg/L of active MBI-401 SDP. The exposure chambers will be gently mixed with a glass rod or stainless steel spatula to ensure uniform distribution of the test chemical. All concentrations will be tested in triplicate for a total of 18 exposure chambers per species. exposure chambers will be held at 20°C in a water bath or controlled environment room and maintained on an 18:6 h light/dark cycle. Viability of a subsample of approximately 100 glochidia from each chamber will be assessed at 6, 12 and 24 h post exposure by enumeration through a dissecting microscope. Photomicrographs of subsamples may be recorded. The study will be terminated after 24 h post exposure evaluations are completed.

Revised text (in bold):

4.2 Experimental Design:

Gravid donor mussels will be collected by biologists from the Genoa National Fish Hatchery from the Upper Iowa, Upper Mississippi or St. Croix Rivers. Mussels will be identified to species as described in Cummings and Mayer (1992) and Watters et al. (2009) and transported to the Genoa National Fish Hatchery. **Approximately 72 h prior to test initiation the donor mussels will be transferred from the Genoa NFH to the UMESC and acclimated to test temperature. Glochidia from at least three gravid female mussels of each species will be extracted by personnel from the Genoa NFH. The viability of glochidia from each mussel will be assessed by drawing aliquots (e.g. 100 µL) of settled glochidia from the stock container with an adjustable manual pipet fitted with a wide bore tip and placing it on a pre-labeled 35mm petri dish. The sample will be diluted with a known and consistent volume (e.g. 200 µL) of acclimated well water to enhance dispersion of glochidia for more accurate enumeration. The sample will be viewed under a dissecting microscope, adjusted for maximum clarity and magnification, and a photomicrograph will be recorded. Approximately one drop of a saturated sodium chloride solution (12 g NaCl/ 50 mL of D.I. H_2O [ASTM E2455-06]) will be added to the sample and after one minute a second photomicrograph will be recorded. Enumerations will be conducted for the total number of glochidia within the sample and the number of glochidia closed or open before and after sodium chloride addition. The percentage of viable glochidia (glochidia that respond to sodium chloride exposure by valve closure) will be calculated by the following method:**

Percent viable = ([Total number of glochidia in sample – {total number of glochidia

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closed before addition of sodium chloride + number of glochidia open after sodium chloride addition)]/Total number of glochidia in sample) * 100

If viability is acceptable (≥80%) for a mussel, the glochidia from that mussel will be pooled with glochidia from other mussels of that species. An appropriate amount of settled glochidia (e.g.: 500 µL) will be randomly drawn from the pooled glochidia stock and aliquoted to an exposure chamber (80 mm x 40 mm crystallizing dish) containing exposure water to achieve a final volume of 100 mL. Between 500-1,000 glochidia will be transferred to each exposure chamber in a minimum of two aliquots (dependent on number of glochidia per aliquot and the estimated number of glochidia available) per chamber (e.g. two 100 µL aliquots that contain ~250 glochidia each). Once all of the exposure chambers receive test animals the exposures will be initiated by the addition of the appropriate amount of MBI-401 SDP, deactivated MBI-401 SDP (positive control) or plain water (control) in a completely randomized design to achieve the desired test concentrations of 0 (control), 300 mg/L deactivated (positive control), or 50, 100, 200, and 300 mg/L of active MBI-401 SDP. The exposure chambers will be gently mixed with a glass rod or stainless steel spatula to ensure uniform distribution of the test chemical. All concentrations will be tested in triplicate for a total of 18 exposure chambers per species. Exposure chambers will be held at 20°C in a water bath or controlled environment room and maintained on an 18:6 h light/dark cycle. Viability of a subsample of approximately 100 glochidia from each chamber will be assessed at 6, 12 and 24 h post exposure by enumeration **from photomicrograph records pre- and post-sodium chloride addition.** The study will be terminated after 24 h post exposure evaluations are completed.

Current text:

5. STUDY PROCEDURES

5.1 Test Animals

5.1.2 Number of animals: Approximately 9,000 glochidia (500 per replicate x 3 replicates x 6 concentrations) of each species. This design uses the fewest number of glochidia possible, consistent with the objective of the study, contemporary scientific standards and ASTM guide E2455-06.

5.1.3 Source of animals: All glochidia will be obtained from donor mussels located at the Genoa NFH.

5.1.5 Acclimation: Glochidia will be acclimated to UMESC well water and temperature (20 °C) by the addition of up to 50% UMESC well water at the Genoa NFH prior to transport to UMESC and again after arrival at the UMESC.

5.6 Treatment administration:

5.6.1 Treatment: Each species of mussel glochidia will be exposed to three replicates of either 0 (control), 50, 100, 200 or 300 mg/L (active ingredient) and a 300 mg/L heat deactivated (70°C/45 minutes) control as a one time single dose exposure.

5.6.2 Route of administration: To assure uniform exposure, an appropriate amount of freshly prepared stock solution will added to each test chamber from an agitated stock suspension with a pipet.

Revised text (in bold):

5. STUDY PROCEDURES

5.1 Test Animals

5.1.2 Number of animals: Approximately 9,000-**18,000** glochidia (500-**1,000** per replicate x 3 replicates x 6 concentrations) of each species. This design uses the fewest number of glochidia possible, consistent with the objective of the study, contemporary scientific standards and ASTM guide E2455-06.

5.1.3 Source of animals: All glochidia will be obtained from donor mussels **collected and temporarily held** at the Genoa NFH. **The donor mussels will be transported to the**

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UMESC for acclimation to test conditions. Glochidia extraction will occur at UMESC; glochidia will be extracted by trained GNFH personnel.

5.1.5 Acclimation: **Donor mussels will be acclimated from approximately 12 °C to the exposure temperature (20°C) over a period of approximately 72 h upon arrival at UMESC.**

5.6 Treatment administration:

5.6.1 Treatment: Each species of mussel glochidia will be exposed to three replicates of either 0 (control), 50, 100, 200 or 300 mg/L (active ingredient) and a 300 mg/L heat deactivated (70°C/45 minutes) control as a one time single dose exposure.

5.6.2 Route of administration: To assure uniform exposure, an appropriate amount of freshly prepared stock solution will added to each test chamber from an agitated stock suspension with a pipet.

5.6.3 **Dose verification: The activity of MBI-401 SDP (*Pseudomonas fluorescens* [*Pf*-CL145A]) cannot be assessed by current analytical techniques as the chemical nature of the active agent in the bacterium has not been determined or characterized. Dosage verification of MBI-401 SDP will be assessed by measuring and recording the absorbance of the prepared stock dosing solution and the high treatment concentrations. The collected absorbance information will be used to determine inter- and intra-test variability in the preparation of stock and test solutions.**

Current text:

6.1 Distribution to experimental units:

The glochidia concentration (glochidia/mL) will be determined for each species prior to test initiation by enumeration of replicate subsamples of glochidial suspension through a dissecting microscope. Appropriate volumes (mL) will be determined that would contain approximately 500 glochidia. Test chambers will then be filled with appropriate amounts of dilution water to obtain a final volume of 100 mL. Distribution to test chambers will then proceed by the addition of the calculated volume of suspension in at least two separate aliquots. Aliquots will be randomly assigned to a test chamber until all receive one aliquot. This procedure will then be repeated until all chambers receive approximately 500 glochidia in two or more aliquots. The test chambers will be randomly assigned treatment concentration.

6.2 Determination of viability:

Viability will determined by the ability of the glochidia to constrict their adductor mussel thereby causing valve closure in response to saturated sodium chloride (12 g NaCl/ 50 mL of D.I. H_2O) exposure. Viability will be determined on a subsample of approximately 100 glochidia for each species prior to test initiation and at 6, 12 and 24 h post exposure for each test replicate. A volume of water containing approximately 100 glochidia (eg: 1-2 mL) will be placed on a 35 mm mini petri dish and examined under a dissecting scope to enumerate the percentage of glochidia with closed valves. Then approximately 1 drop of a saturated sodium chloride solution will be added to the sample and the glochidia will then be reassessed for valve closure after approximately one minute. Subsample photomicrographs of glochidia pre and post sodium chloride addition may be recorded and uniquely identified for documentation and optional later enumeration and/or validation of closure percentages. Only glochidia that are open and responsive to sodium chloride addition will be considered viable.

Revised text (in bold)

6.1 Distribution to experimental units:

The number of glochidia per 100 µL aliquot will be estimated from samples taken to determine initial viability for each species prior to test initiation. Appropriate volumes (µL) will be determined that would contain at least 500 glochidia. Test chambers will then be filled with appropriate amounts of dilution water to obtain a final volume of 100 mL. Distribution to **exposure** chambers will then proceed by the addition of the calculated volume of **settled glochidia** in at least two separate aliquots. Aliquots will be randomly assigned to **an exposure**

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chamber until all receive one aliquot. This procedure will then be repeated until all chambers receive approximately 500 glochidia in two or more aliquots. The test chambers will be randomly assigned treatment concentration.

6.2 Determination of viability:

Viability will determined by the ability of the glochidia to constrict their adductor mussel thereby causing valve closure in response to saturated sodium chloride (12 g NaCl/ 50 mL of D.I. H_2O) exposure. Viability will be determined on a subsample of approximately 100 glochidia **from each mussel prior** to test initiation and at 6, 12 and 24 h post exposure for each test replicate. **A subsample of at least 100 glochidia will be removed by drawing an aliquot (e.g. 100 µL) of settled glochidia from the exposure chamber with an adjustable manual pipet fitted with a wide bore tip and placing it on a pre-labeled 35 mm petri dish. The samples will be diluted with a known and consistent volume (e.g. 200 µL) of acclimated well water to enhance dispersion of glochidia for more accurate enumeration. The sample will be viewed under a dissecting microscope, adjusted for maximum clarity and magnification, and a photomicrograph will be recorded. Approximately one drop of a saturated sodium chloride solution (12 g NaCl/ 50 mL of D.I. H_2O [ASTM E2455-06]) will be added to the sample and after one minute a second photomicrograph will be recorded. Enumerations will be conducted for the total number of glochidia within the sample and the number of glochidia closed or open before and after sodium chloride addition. The percentage of viable glochidia (glochidia that respond to sodium chloride exposure by valve closure) will be calculated by the following method:**

Percent viable = ([Total number of glochidia in sample – {total number of glochidia closed before addition of sodium chloride + number of glochidia open after sodium chloride addition}]/Total number of glochidia in sample) * 100

Current text:

12. INVESTIGATIONAL TEST ARTICLE

12.1 Test Substance(s): MBI-401 SDP, *Pseudomonas fluorescens* (*Pf*-CL145A)

12.1.1 Chemical name: *Pseudomonas fluorescens* (*Pf*-CL145A)

12.1.2 Trade name: MBI-401 SDP

12.1.3 Active ingredients: *Pseudomonas fluorescens* (*Pf*-CL145A) is the sole active ingredient, 50% active by weight.

12.1.4 Source: Marrone Bio Innovations (MBI); Davis, CA

12.1.5 Lot number: None established

12.1.6 Expiration date: None established

12.1.7 Storage during study: test chemical will be stored at refrigerated in a locked container within a restrictive entry laboratory. A subsample of the test chemical will be archived in the UMESC Chemical Archive (UMESC SOP GEN 011).

12.1.8 A NIOSH approved respirator will be used when preparing stock solutions to avoid inhalation. Protective eyewear, gloves and lab coats will be worn at all times when working with the test substance.

Revised text (in bold)

12. INVESTIGATIONAL TEST ARTICLE

12.1 Test Substance(s): MBI-401 SDP, *Pseudomonas fluorescens* (*Pf*-CL145A)

12.1.1 Chemical name: *Pseudomonas fluorescens* (*Pf*-CL145A)

12.1.2 Trade name: MBI-401 SDP

12.1.3 Active ingredients: *Pseudomonas fluorescens* (*Pf*-CL145A) is the sole active ingredient, 50% active by weight.

12.1.4 Source: Marrone Bio Innovations (MBI); Davis, CA

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12.1.5 Lot number: **MBI-401-110308AI-BD-3**

12.1.6 Expiration date: None established**, an aliquot of test material will be returned to the NYSM or MBI at the conclusion of exposures at UMESC for confirmatory post-test activity comparative zebra mussel bioassay tests (the standard testing protocol to assess *Pseudomonas fluorescens* [*Pf*-CL145A] formulation activity). Results of these confirmation bioassays will be used to validate the activity retention of the MBI-401 SDP, *Pseudomonas fluorescens* (*Pf*-CL145A).**

12.1.7 Storage during study: test chemical will be stored refrigerated **(≤ 4°C)** in a locked container within a restrictive entry laboratory. A subsample of the test chemical will be archived in the UMESC Chemical Archive (UMESC SOP GEN 011).

12.1.8 A NIOSH approved respirator will be used when preparing stock solutions to avoid inhalation. Protective eyewear, gloves and lab coats will be worn at all times when working with the test substance.

Item No. 2

FOLDER NO. 3

PROOFED BY
Initials: TMS Date: 2/6/12
REVIEWED BY
Initials: ____ Date: ____

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Item No. 3

FOLDER NO. 3

United States Department of the Interior

U.S. GEOLOGICAL SURVEY
Biological Resources Division
Upper Midwest Environmental Sciences Center
2630 Fanta Reed Road
La Crosse, Wisconsin 54603

Date: October 19, 2011
To: The Record Study Number AEH-11-PSEUDO-01

Subject: Amendment 2 to the study AEH-11-PSEUDO-01 "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"

Revision of amended study protocol, study # AEH-11-PSEUDO-01 is proposed as detailed on pages 2-8 of this amendment. Revised text is indicated **in bold**.

This amendment changes 1) the test material from a spray dried (SDP) to a freeze dried (FDP) formulation; 2) the procedures for acclimation of the donor mussels and 3) specifies the procedures for ammonia sample collection and analysis.

The FDP *Pseudomonas fluorescens* is a new manufacturer-preferred formulation that is considered to be ~100% active compared to the spray-dried product (SDP) which was considered ~50% active. The exposures will be conducted in an identical fashion except for the change in product type. Mussel species previously exposed to SDP will be exposed to the new FDP formulation; data collected during SDP exposures will be retained in the study data management system. Acclimation procedures previously outlined may induce glochidial abortion in some species that are sensitive to handling (ie: washboard *Megalonaias nervosa*). The proposed acclimation procedures provide flexibility to address acclimation at the direction of the Genoa NFH mussel biologist. The amendment specifies the procedures to determine ammonia concentration in exposure chamber water at the end of the 24-h exposure period.

Reviewed by:

[redacted] — 19 Oct 2011 — Date
Mark P. Gaikowski, M.A.
Supervisory Biologist
Aquatic Ecosystem Health,
UMESC[1]

for [redacted] — 19 Oct 2011 — Date
Michael Jawson, Ph.D.
Center Director, UMESC

Approved by:

[redacted] — 19 Oct 11 — Date
James A. Luoma, B.A.
Study Director, UMESC

PROOFED BY
Initials: TMS Date: 2/6/12
REVIEWED BY
Initials: ____ Date: ____

[1] UMESC: U.S. Geological Survey, Upper Midwest Environmental Sciences Center

ORIGINAL

Current text:

1. INTRODUCTION: (paragraph 4 of Section 1 is the only paragraph affected by this amendment)

Biologists at the New York State Museum (NYSM) Field Research Laboratory have been researching dreissenid mussel control since 1991 and they discovered that a bacterium isolated from soils (*Pseudomonas fluorescens* [*Pf*-CL145A]) is efficacious for controlling zebra mussels. Marrone Bio Innovations (MBI; Davis, CA) is currently developing a commercial sprayed dried formulation of this bacterium called MBI-401 SDP. The current commercial applications of this product are for use with closed systems such as power generating plant cooling systems. The NYSM has partnered with the USFWS (Genoa NFH) and United States Geological Survey's (USGS) Upper Midwest Environmental Sciences Center (UMESC) to determine the suitability of this product for open water zebra mussel control applications such as treatment of native mussel propagation cages or native mussel beds.

2. PROTOCOL OBJECTIVE:

This study will determine the animal effects of various concentrations of MBI-401 SDP (*Pseudomonas fluorescens* [*Pf*-CL145A]) to the glochidia lifestage of seven unionid mussels species present in the Great Lakes and Upper Mississippi River basins.

4.1 General Description:

Glochidia from 7 unionid mussel species (Table 2) endemic in the Great Lakes and Mississippi River basins will be evaluated for effects from exposure to varying concentrations of MBI-401 SDP (*Pseudomonas fluorescens* [*Pf*-CL145A]). Glochidia will be exposed in static exposures for 24 h as described in ASTM E-2455-06 and evaluated at 6, 12 and 24 h for viability as determined by valve closure response after addition of a saturated sodium chloride solution.

4.2 Experimental Design:

Gravid donor mussels will be collected by biologists from the Genoa National Fish Hatchery from the Upper Iowa, Upper Mississippi or St. Croix Rivers. Mussels will be identified to species as described in Cummings and Mayer (1992) and Watters et al. (2009) and transported to the Genoa National Fish Hatchery. Approximately 72 h prior to test initiation the donor mussels will be transferred from the Genoa NFH to the UMESC and acclimated to test temperature. Glochidia from at least three gravid female mussels of each species will be extracted by personnel from the Genoa NFH. The viability of glochidia from each mussel will be assessed by drawing aliquots (e.g. 100 µL) of settled glochidia from the stock container with an adjustable manual pipet fitted with a wide bore tip and placing it on a pre-labeled 35mm petri dish. The sample will be diluted with a known and consistent volume (e.g. 200 µL) of acclimated well water to enhance dispersion of glochidia for more accurate enumeration. The sample will be viewed under a dissecting microscope, adjusted for maximum clarity and magnification, and a photomicrograph will be recorded. Approximately one drop of a saturated sodium chloride solution (12 g NaCl/ 50 mL of D.I. H_2O [ASTM E2455-06]) will be added to the sample and after one minute a second photomicrograph will be recorded. Enumerations will be conducted for the total number of glochidia within the sample and the number of glochidia closed or open before and after sodium chloride addition. The percentage of

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viable glochidia (glochidia that respond to sodium chloride exposure by valve closure) will be calculated by the following method:

Percent viable = ([Total number of glochidia in sample – {total number of glochidia closed before addition of sodium chloride + number of glochidia open after sodium chloride addition}]/Total number of glochidia in sample) * 100

If viability is acceptable (≥80%) for a mussel, the glochidia from that mussel will be pooled with glochidia from other mussels of that species. An appropriate amount of settled glochidia (e.g.: 500 µL) will be randomly drawn from the pooled glochidia stock and aliquoted to an exposure chamber (80 mm x 40 mm crystallizing dish) containing exposure water to achieve a final volume of 100 mL. Between 500-1,000 glochidia will be transferred to each exposure chamber in a minimum of two aliquots (dependent on number of glochidia per aliquot and the estimated number of glochidia available) per chamber (e.g. two 100 µL aliquots that contain ~250 glochidia each). Once all of the exposure chambers receive test animals the exposures will be initiated by the addition of the appropriate amount of MBI-401 SDP, deactivated MBI-401 SDP (positive control) or plain water (control) in a completely randomized design to achieve the desired test concentrations of 0 (control), 300 mg/L deactivated (positive control), or 50, 100, 200, and 300 mg/L of active MBI-401 SDP. The exposure chambers will be gently mixed with a glass rod or stainless steel spatula to ensure uniform distribution of the test chemical. All concentrations will be tested in triplicate for a total of 18 exposure chambers per species. Exposure chambers will be held at 20°C in a water bath or controlled environment room and maintained on an 18:6 h light/dark cycle. Viability of a subsample of approximately 100 glochidia from each chamber will be assessed at 6, 12 and 24 h post exposure by enumeration from photomicrograph records pre- and post-sodium chloride addition. The study will be terminated after 24 h post exposure evaluations are completed.

5. STUDY PROCEDURES

5.1 Test Animals

5.1.2 Number of animals: Approximately 9,000-18,000 glochidia (500-1,000 per replicate x 3 replicates x 6 concentrations) of each species. This design uses the fewest number of glochidia possible, consistent with the objective of the study, contemporary scientific standards and ASTM guide E2455-06.

5.1.3 Source of animals: All glochidia will be obtained from donor mussels collected and temporarily held at the Genoa NFH. The donor mussels will be transported to the UMESC for acclimation to test conditions. Glochidia extraction will occur at UMESC; glochidia will be extracted by trained GNFH personnel.

5.1.4 Inclusion criterion: Glochidia will be used if they meet or exceed the following percentage of valve closure requirements after addition of a saturated sodium chloride solution (12 g NaCl/ 50 mL of D.I. H_2O). Glochidia must exhibit ≥ 80% valve closure to be pooled for use at the Genoa NFH and glochidia must exhibit ≥ 80% valve closure immediately prior study initiation at the UMESC.

5.1.5 Acclimation: Donor mussels will be acclimated from approximately 12 °C to the exposure temperature (20°C) over a period of approximately 72 h upon arrival at UMESC.

5.2 Water Chemistry

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5.2.7 Ammonia: The ammonia will be measured and recorded at the termination of the exposure from pooled replicate samples from the controls and all test concentrations (UMESC SOP AEH 301 or equivalent).

5.5 Observations:

5.5.2 Water chemistry: Dissolved oxygen, pH, and temperature will be monitored after the addition of the specified treatment. Alkalinity, hardness, conductivity and ammonia will be measured on a sample of dilution water at test initiation and on pooled replicate samples upon test termination. Alkalinity, hardness, conductivity and ammonia will not be measured during the exposures due to the quantity of test water required and probe submersion requirements to complete the analysis.

5.6 Treatment administration:

5.6.1 Treatment: Each species of mussel glochidia will be exposed to three replicates of either 0 (control), 50, 100, 200 or 300 mg/L (active ingredient) and a 300 mg/L heat deactivated (70°C/45 minutes) control as a one time single dose exposure.

5.6.2 Route of administration: To assure uniform exposure, an appropriate amount of freshly prepared stock solution will added to each test chamber from an agitated stock suspension with a pipet.

5.6.3 Dose verification: The activity of MBI-401 SDP (*Pseudomonas fluorescens* [*Pf*-CL145A]) cannot be assessed by current analytical techniques as the chemical nature of the active agent in the bacterium has not been determined or characterized. Dosage verification of MBI-401 SDP will be assessed by measuring and recording the absorbance of the prepared stock dosing solution and the high treatment concentrations. The collected absorbance information will be used to determine inter- and intra-test variability in the preparation of stock and test solutions.

12. INVESTIGATIONAL TEST ARTICLE

12.1 Test Substance(s): MBI-401 SDP, *Pseudomonas fluorescens* (*Pf*-CL145A)

12.1.1 Chemical name: *Pseudomonas fluorescens* (*Pf*-CL145A)

12.1.2 Trade name: MBI-401 SDP

12.1.3 Active ingredients: *Pseudomonas fluorescens* (*Pf*-CL145A) is the sole active ingredient, 50% active by weight.

12.1.4 Source: Marrone Bio Innovations (MBI); Davis, CA

12.1.5 Lot number: MBI-401-110308AI-BD-3

12.1.6 Expiration date: None established, an aliquot of test material will be returned to the NYSM or MBI at the conclusion of exposures at UMESC for confirmatory post-test activity comparative zebra mussel bioassay tests (the standard testing protocol to assess *Pseudomonas fluorescens* [*Pf*-CL145A] formulation activity). Results of these confirmation bioassays will be used to validate the activity retention of the MBI-401 SDP, *Pseudomonas fluorescens* (*Pf*-CL145A).

Revised text (in bold):

1. INTRODUCTION: (paragraph 4 of Section 1 is the only paragraph affected by this amendment)

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Biologists at the New York State Museum (NYSM) Field Research Laboratory have been researching dreissenid mussel control since 1991 and they discovered that a bacterium isolated from soils (*Pseudomonas fluorescens* [*Pf*-CL145A]) is efficacious for controlling zebra mussels. Marrone Bio Innovations (MBI; Davis, CA) is currently developing a commercial **freeze dried formulation of this bacterium referred to as MBI-401 FDP.** The current commercial applications of this product are for use with closed systems such as power generating plant cooling systems. The NYSM has partnered with the USFWS (Genoa NFH) and United States Geological Survey's (USGS) Upper Midwest Environmental Sciences Center (UMESC) to determine the suitability of this product for open water zebra mussel control applications such as treatment of native mussel propagation cages or native mussel beds.

2. PROTOCOL OBJECTIVE:

This study will determine the animal effects of various concentrations of MBI-401 **FDP** (*Pseudomonas fluorescens* [*Pf*-CL145A]) to the glochidia lifestage of seven unionid mussels species present in the Great Lakes and Upper Mississippi River basins.

4.1 General Description:

Glochidia from 7 unionid mussel species (Table 2) endemic in the Great Lakes and Mississippi River basins will be evaluated for effects from exposure to varying concentrations of MBI-401 **FDP** (*Pseudomonas fluorescens* [*Pf*-CL145A]). Glochidia will be exposed in static exposures for 24 h as described in ASTM E-2455-06 and evaluated at 6, 12 and 24 h for viability as determined by valve closure response after addition of a saturated sodium chloride solution.

4.2 Experimental Design:

Gravid donor mussels will be collected by biologists from the Genoa National Fish Hatchery from the Upper Iowa, Upper Mississippi or St. Croix Rivers. Mussels will be identified to species as described in Cummings and Mayer (1992) and Watters et al. (2009) and transported to the Genoa National Fish Hatchery. **Prior to test initiation the donor mussels will be acclimated to the test temperature (see Section 5.1).** Glochidia from at least three gravid female mussels of each species will be extracted by personnel from the Genoa NFH **or UMESC**. The viability of glochidia from each mussel will be assessed by drawing aliquots (e.g. 100 µL) of settled glochidia from the stock container with an adjustable manual pipet fitted with a wide bore tip and placing it on a pre-labeled 35 mm petri dish. The sample will be diluted with a known and consistent volume (e.g. 200 µL) of acclimated well water to enhance dispersion of glochidia for more accurate enumeration. The sample will be viewed under a dissecting microscope, adjusted for maximum clarity and magnification, and a photomicrograph will be recorded. Approximately one drop of a saturated sodium chloride solution (12 g NaCl/ 50 mL of D.I. H_2O [ASTM E2455-06]) will be added to the sample and after one minute a second photomicrograph will be recorded. Enumerations will be conducted for the total number of glochidia within the sample and the number of glochidia closed or open before and after sodium chloride addition. The percentage of viable glochidia (glochidia that respond to sodium chloride exposure by valve closure) will be calculated by the following

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method:

Percent viable = ([Total number of glochidia in sample – {total number of glochidia closed before addition of sodium chloride + number of glochidia open after sodium chloride addition}]/Total number of glochidia in sample) * 100

If viability is acceptable (≥80%) for a mussel, the glochidia from that mussel will be pooled with glochidia from other mussels of that species. An appropriate amount of settled glochidia (e.g.: 500 µL) will be randomly drawn from the pooled glochidia stock and **distributed** to an exposure chamber (80 mm x 40 mm crystallizing dish) containing exposure water to achieve a final volume of 100 mL. Between 500-1,000 glochidia will be transferred to each exposure chamber in a minimum of two **distributions** (dependent on number of glochidia per aliquot and the estimated number of glochidia available) per chamber (e.g. two 100 µL aliquots that contain ~250 glochidia each). Once all of the exposure chambers receive test animals the exposures will be initiated by the addition of the appropriate amount of MBI-401 **FDP**, deactivated MBI-401 **FDP** (positive control) or plain water (control) in a completely randomized design to achieve the desired test concentrations of 0 (control), 300 mg/L deactivated (positive control), or 50, 100, 200, and 300 mg/L of active MBI-401 **FDP**. The exposure chambers will be gently mixed with a glass rod or stainless steel spatula to ensure uniform distribution of the test chemical. All concentrations will be tested in triplicate for a total of 18 exposure chambers per species. Exposure chambers will be held at 20°C in a water bath or controlled environment room and maintained on an 18:6 h light/dark cycle. Viability of a subsample of approximately 100 glochidia from each chamber will be assessed at 6, 12 and 24 h post exposure by enumeration from photomicrograph records pre- and post-sodium chloride addition. The study will be terminated after 24 h post exposure evaluations are completed.

5. STUDY PROCEDURES

5.1 Test Animals

5.1.2 Number of animals: Approximately 9,000-18,000 glochidia (500-1,000 per replicate x 3 replicates x 6 concentrations) of each species. This design uses the fewest number of glochidia possible, consistent with the objective of the study, contemporary scientific standards and ASTM guide E2455-06.

5.1.3 Source of animals: All glochidia will be obtained from donor mussels collected and temporarily held at the Genoa NFH. **Donor mussel holding procedures (from collection to glochidia extraction) will be described in the study data management system**. Glochidia extraction will occur at UMESC; glochidia will be extracted by trained GNFH **or UMESC** personnel.

5.1.5 Acclimation: **Donor mussels will be acclimated from the collection/holding temperature to the exposure temperature (20±2°C) before glochidia extraction at a rate specified by the Genoa NFH mussel biologist. The acclimation procedures will be described in the study data management system.**

5.2 Water Chemistry

5.2.7 **Ammonia: Total ammonia-nitrogen will be measured in a sample collected at the end of the 24-h exposure period from each exposure chamber. Samples (~3 mL) will be filtered (0.45 µm syringe filter), acidified (pH ~2.5**

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with H_2SO_4) and then stored at ~4°C. Total ammonia-nitrogen in samples will be determined by the UMESC Long Term Resources Monitoring Program (LTRMP) Water Quality Laboratory using the automated phenate method. Unionized ammonia will be calculated from the ammonia pKa (based on the pH and temperature recorded in the respective exposure chamber at the end of the 24-h exposure period) and the total ammonia-nitrogen concentration. The UMESC-LTRMP laboratory does not comply with Good Laboratory Practices (GLP) and thus data generated by the UMESC-LTRMP will be so annotated in the final report.

5.5 Observations:

5.5.2 **Water chemistry: Dissolved oxygen, pH, and temperature will be monitored after the addition of the specified treatment. Alkalinity, hardness, and conductivity will be measured using separate samples of dilution water collected before exposure initiation and from separate samples collected at the end of the 24-h exposure period. Water from replicate exposure chambers will be pooled to provide sufficient volume for these tests. Total ammonia-nitrogen will be measured in samples collected at the end of the 24-h exposure period (see Section 5.2.7). Alkalinity, hardness, conductivity and ammonia will not be measured during the exposures due to the quantity of test water required and probe submersion requirements to complete the analysis.**

5.6 Treatment administration:

5.6.1 Treatment: Each species of mussel glochidia will be exposed to three replicates of either 0 (control), 50, 100, 200 or 300 mg/L (active ingredient) and a 300 mg/L heat deactivated (70°C/45 minutes) control as a one time single dose exposure.

5.6.2 Route of administration: To assure uniform exposure, an appropriate amount of freshly prepared stock solution will added to each test chamber from an agitated stock suspension with a pipet.

5.6.3 Dose verification: The activity of MBI-401 **FDP** (*Pseudomonas fluorescens* [*Pf*-CL145A]) cannot be assessed by current analytical techniques as the chemical nature of the active agent in the bacterium has not been determined or characterized. Dosage verification of MBI-401 **FDP** will be assessed by measuring and recording the absorbance of the prepared stock dosing solution and the high treatment concentrations. The collected absorbance information will be used to determine inter- and intra-test variability in the preparation of stock and test solutions.

12. INVESTIGATIONAL TEST ARTICLE

12.1 Test Substance(s): MBI-401 **FDP**, *Pseudomonas fluorescens* (*Pf*-CL145A)

12.1.1 Chemical name: *Pseudomonas fluorescens* (*Pf*-CL145A)

12.1.2 Trade name: MBI-401 **FDP**

12.1.3 Active ingredients: *Pseudomonas fluorescens* (*Pf*-CL145A) is the sole active ingredient, **100%** active by weight.

12.1.4 Source: Marrone Bio Innovations (MBI); Davis, CA

12.1.5 Lot number: **More than one lot will be used. Test article lot numbers for each species will be recorded in the study files.**

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12.1.6 Expiration date: ***Pseudomonas fluorescens* (*Pf*-CL145A) FDP is susceptible to degradation if not properly handled. Therefore an aliquot of test material will be returned to the NYSM or MBI after each set of glochidia exposures at UMESC for post-exposure activity confirmation through completion of comparative zebra mussel bioassay tests (the standard testing protocol to assess *Pseudomonas fluorescens* [*Pf*-CL145A] formulation activity). Results of the zebra mussel bioassays will be used to validate the retention of the activity of the MBI-401 FDP lot used for a given mussel species.**

Item No. 3

FOLDER NO. 3

PROOFED BY
Initials: TMS Date: 2/6/12
REVIEWED BY
Initials: ______ Date: ______

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Item No. 4

FOLDER NO. 3

United States Department of the Interior

U.S. GEOLOGICAL SURVEY
Biological Resources Division
Upper Midwest Environmental Sciences Center
2630 Fanta Reed Road
La Crosse, Wisconsin 54603

Date: October 19, 2011
To: The Record Study Number AEH-11-PSEUDO-01

Subject: Amendment 3 to the study AEH-11-PSEUDO-01 "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"

Revision of amended study protocol, study # AEH-11-PSEUDO-01 is proposed as detailed on pages 2-5 of this amendment. Revised text is indicated **in bold**.

This amendment changes 1) the procedures for the viability assessment for mussels which cannot be reliably assessed from photomicrographic records (e.g. *Megalonaias nervosa*). *M. nervosa* glochidia are released from the donor mussel in a mucoidal matrix. Viability assessment from photomicrographic records is unreliable because glochidia within the mucus occupy several different focal planes, making it nearly impossible to bring all glochidia into focus. Therefore, this amendment specifies the procedures used to determine viability of species of donor mussels whose glochidia are released in a mucoidal matrix and when those assessments will be completed. The Study Director will determine the appropriate glochidia viability determination procedure to use for each mussel species after collecting glochidia from donor mussels. That decision will be recorded in the study data management system. This amendment serves as the record that the viability of glochidia of *M. nervosa* was determined using direct microscopic enumeration, not enumeration from photomicrographic recordings.

Reviewed by:

[redacted] 19 Oct 2011
Mark P. Gaikowski, M.A. — Date
Supervisory Biologist
Aquatic Ecosystem Health,
UMESC[1]

[redacted] 19 Oct 2011
for Michael Jawson, Ph.D. — Date
Center Director, UMESC

Approved by:

[redacted] 19 Oct 11
James A. Luoma, B.A. — Date
Study Director, UMESC

PROOFED BY
Initials: TMS Date 2/6/12
REVIEWED BY
Initials: ____ Date: ____

[1] UMESC: U.S. Geological Survey, Upper Midwest Environmental Sciences Center

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Current text:

4.2 Experimental Design:

Gravid donor mussels will be collected by biologists from the Genoa National Fish Hatchery from the Upper Iowa, Upper Mississippi or St. Croix Rivers. Mussels will be identified to species as described in Cummings and Mayer (1992) and Watters et al. (2009) and transported to the Genoa National Fish Hatchery. Prior to test initiation the donor mussels will be acclimated to the test temperature (see Section 5.1). Glochidia from at least three gravid female mussels of each species will be extracted by personnel from the Genoa NFH or UMESC. The viability of glochidia from each mussel will be assessed by drawing aliquots (e.g. 100 µL) of settled glochidia from the stock container with an adjustable manual pipet fitted with a wide bore tip and placing it on a pre-labeled 35 mm petri dish. The sample will be diluted with a known and consistent volume (e.g. 200 µL) of acclimated well water to enhance dispersion of glochidia for more accurate enumeration. The sample will be viewed under a dissecting microscope, adjusted for maximum clarity and magnification, and a photomicrograph will be recorded. Approximately one drop of a saturated sodium chloride solution (12 g NaCl/ 50 mL of D.I. H_2O [ASTM E2455-06]) will be added to the sample and after one minute a second photomicrograph will be recorded. Enumerations will be conducted for the total number of glochidia within the sample and the number of glochidia closed or open before and after sodium chloride addition. The percentage of viable glochidia (glochidia that respond to sodium chloride exposure by valve closure) will be calculated by the following method:

Percent viable = ([Total number of glochidia in sample – {total number of glochidia closed before addition of sodium chloride + number of glochidia open after sodium chloride addition}]/Total number of glochidia in sample) * 100

If viability is acceptable (≥80%) for a mussel, the glochidia from that mussel will be pooled with glochidia from other mussels of that species. An appropriate amount of settled glochidia (e.g.: 500 µL) will be randomly drawn from the pooled glochidia stock and distributed to an exposure chamber (80 mm x 40 mm crystallizing dish) containing exposure water to achieve a final volume of 100 mL. Between 500-1,000 glochidia will be transferred to each exposure chamber in a minimum of two distributions (dependent on number of glochidia per aliquot and the estimated number of glochidia available) per chamber (e.g. two 100 µL aliquots that contain ~250 glochidia each). Once all of the exposure chambers receive test animals the exposures will be initiated by the addition of the appropriate amount of MBI-401 FDP, deactivated MBI-401 FDP (positive control) or plain water (control) in a completely randomized design to achieve the desired test concentrations of 0 (control), 300 mg/L deactivated (positive control), or 50, 100, 200, and 300 mg/L of active MBI-401 FDP. The exposure chambers will be gently mixed with a glass rod or stainless steel spatula to ensure uniform distribution of the test chemical. All concentrations will be tested in triplicate for a total of 18 exposure chambers per species. Exposure chambers will be held at 20°C in a water bath or controlled environment room and maintained on an 18:6 h light/dark cycle. Viability of a subsample of approximately 100 glochidia from each chamber will be assessed at 6, 12 and 24 h post exposure by enumeration from photomicrograph records pre- and post-sodium chloride

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addition. The study will be terminated after 24 h post exposure evaluations are completed.

5.5 Observations:

5.5.1 Behavioral Observations: Behavioral observations will be limited to viability assessments. Viability is determined by the ability of the glochidia to constrict their adductor mussel thereby causing valve closure in response to saturated sodium chloride (12 g NaCl/ 50 mL of D.I. H_2O) exposure. Viability will be determined on a subsample of approximately 100 glochidia at 6, 12 and 24 h post exposure for each test chamber.

Revised text (in bold):

4.2 Experimental Design:

Gravid donor mussels will be collected by biologists from the Genoa National Fish Hatchery from the Upper Iowa, Upper Mississippi or St. Croix Rivers. Mussels will be identified to species as described in Cummings and Mayer (1992) and Watters et al. (2009) and transported to the Genoa National Fish Hatchery. Prior to test initiation the donor mussels will be acclimated to the test temperature (see Section 5.1). Glochidia from at least three gravid female mussels of each species will be extracted by personnel from the Genoa NFH or UMESC. The viability of glochidia from each mussel will be assessed by drawing aliquots (e.g. 100 µL) of settled glochidia from the stock container with an adjustable manual pipet fitted with a wide bore tip and placing it on a pre-labeled 35 mm petri dish. The sample will be diluted with a known and consistent volume (e.g. 200 µL) of acclimated well water to enhance dispersion of glochidia for more accurate enumeration.

The Study Director will determine which method of glochidia viability assessment will be used (enumeration from photomicrographic recording or direct enumeration) after glochidia are collected from donor mussels. That decision will be recorded in the study data management system. If glochidia can be enumerated and viability status (i.e. open or closed) reliably determined through photomicrographic records (i.e. the glochidia remain on a single focal plane) then the following procedures will be used for viability assessment. The sample will be viewed under a dissecting microscope, adjusted for maximum clarity and magnification, and a photomicrograph will be recorded. Approximately one drop of a saturated sodium chloride solution (12 g NaCl/ 50 mL of D.I. H_2O [ASTM E2455-06]) will be added to the sample and after one minute a second photomicrograph will be recorded. The total number of glochidia within the sample and the number of glochidia closed or open before and after sodium chloride addition will be determined. The percentage of viable glochidia (glochidia that respond to sodium chloride exposure by valve closure) will be calculated by the following method:

Percent viable = ([Total number of glochidia in sample – {total number of glochidia closed before addition of sodium chloride + number of glochidia open after sodium chloride addition}]/Total number of glochidia in sample) * 100

If glochidia enumeration and viability cannot be reliably determined through

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photomicrographic records because the glochidia do not occupy a single focal plane (e.g. *Megalonaias nervosa* and other mussels that release glochidia in a mucoidal matrix) the following procedures will be used to assess viability.

The sample will be viewed under a dissecting microscope, adjusted for maximum clarity and magnification, and the total number of glochidia and the number of closed or open glochidia (discretion of the individual performing the count) will be directly enumerated. Approximately one drop of a saturated sodium chloride solution (12 g NaCl/ 50 mL of D.I. H_2O [ASTM E2455-06]) will be added to the sample. After one minute, the number of glochidia that remain open after sodium chloride addition will be directly enumerated. The percentage of viable glochidia (glochidia that respond to sodium chloride exposure by valve closure) will be calculated by the following method:

Percent viable = ([Total number of glochidia in sample – {total number of glochidia closed before addition of sodium chloride + number of glochidia open after sodium chloride addition}]/Total number of glochidia in sample) * 100

If **glochidia** viability is acceptable (≥80%) for a **donor** mussel, the glochidia from that **donor** mussel will be pooled with glochidia from other **donor** mussels of that species. An appropriate amount of settled glochidia (e.g.: 500 µL) will be randomly drawn from the pooled glochidia stock and distributed to an exposure chamber (80 mm x 40 mm crystallizing dish) containing exposure water to achieve a final volume of 100 mL. Between 500-1,000 glochidia will be transferred to each exposure chamber in a minimum of two distributions (dependent on number of glochidia per aliquot and the estimated number of glochidia available) per chamber (e.g. two 100 µL aliquots that contain ~250 glochidia each). Once all of the exposure chambers receive test animals the exposures will be initiated by the addition of the appropriate amount of MBI-401 FDP, deactivated MBI-401 FDP (positive control) or plain water (control) in a completely randomized design to achieve the desired test concentrations of 0 (control), 300 mg/L deactivated (positive control), or 50, 100, 200, and 300 mg/L of active MBI-401 FDP. The exposure chambers will be gently mixed with a glass rod or stainless steel spatula to ensure uniform distribution of the test chemical. All concentrations will be tested in triplicate for a total of 18 exposure chambers per species. Exposure chambers will be held at 20°C in a water bath or controlled environment room and maintained on an 18:6 h light/dark cycle. **For mussel species for which the viability of glochidia can be assessed from photomicrographic records, the viability of a subsample of approximately 100 glochidia from each chamber will be assessed at 6, 12 and 24 h post exposure by enumeration from photomicrograph records pre- and post-sodium chloride addition. For mussel species for which the viability of glochidia cannot be assessed from photomicrographic records (e.g. *M. nervosa*), the viability of a subsample of approximately 100 glochidia from each chamber will be assessed only at 24 h post exposure using the methods described for initial glochidia viability assessment.**
The study will be terminated after 24 h post exposure evaluations are completed.

5.5 Observations:

5.5.1 Behavioral Observations: Behavioral observations will be limited to

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viability assessments. Viability is determined by the ability of the glochidia to constrict their adductor mussel thereby causing valve closure in response to saturated sodium chloride (12 g NaCl/ 50 mL of D.I. H_2O) exposure. **For mussel species for which the viability of glochidia can be assessed from photomicrographic records, the viability of a subsample of approximately 100 glochidia from each chamber will be assessed at 6, 12 and 24 h post exposure by enumeration from photomicrograph records pre- and post-sodium chloride addition. For mussel species for which the viability of glochidia cannot be assessed from photomicrographic records (e.g. *M. nervosa*), the viability of a subsample of approximately 100 glochidia from each chamber will be assessed only at 24 h post exposure using the methods described for initial glochidia viability assessment.**
The study will be terminated after 24 h post exposure evaluations are completed.

Item No. 4

FOLDER NO. 3

PROOFED BY
Initials: TMS Date: 2/6/12
REVIEWED BY
Initials: ____ Date: ____

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United States Department of the Interior

U.S. GEOLOGICAL SURVEY
Biological Resources Division
Upper Midwest Environmental Sciences Center
2630 Fanta Reed Road
La Crosse, Wisconsin 54603

Item No. 5

FOLDER NO. 3

Date: December 20, 2011
To: The Record Study Number AEH-11-PSEUDO-01

Subject: Amendment 4 to the study AEH-11-PSEUDO-01 "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"

Revision of amended study protocol, study # AEH-11-PSEUDO-01 is proposed as detailed on pages 2-7 of this amendment. Revised text is indicated **in bold**.

This amendment changes the test material from a Freeze dried (FDP) to a spray dried (SDP) formulation.

The initially manufacturer-preferred freeze dried formulation (FDP) of *Pseudomonas fluorescens* has recently been discontinued and the manufacturer has indicated support for a spray-dried (SDP) formulation.
The exposures will be conducted in an identical fashion except for the change in product formulation. Mussel species previously exposed to FDP will not be re-evaluated with the SDP formulation; data collected during FDP exposures will be retained in the study data management system.

Reviewed by:

[redacted] 20Dec2011
Mark P. Gaikowski, M.A. Date
Supervisory Biologist
Aquatic Ecosystem Health,
UMESC[1]

[redacted] 12/22/2011
Michael Jawson, Ph.D. Date
Center Director, UMESC

Approved by:

[redacted] 12/22/11
James A. Luoma, B.A. Date
Study Director, UMESC

PROOFED BY
Initials: JmS Date: 2/6/12
REVIEWED BY
Initials: ____ Date: ____

[1] UMESC: U.S. Geological Survey, Upper Midwest Environmental Sciences Center

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Current text:

1. INTRODUCTION: (paragraph 4 of Section 1 is the only paragraph affected by this amendment)

Biologists at the New York State Museum (NYSM) Field Research Laboratory have been researching dreissenid mussel control since 1991 and they discovered that a bacterium isolated from soils (*Pseudomonas fluorescens* [*Pf*-CL145A]) is efficacious for controlling zebra mussels. Marrone Bio Innovations (MBI; Davis, CA) is currently developing a commercial freeze dried formulation of this bacterium referred to as MBI-401 FDP. The current commercial applications of this product are for use with closed systems such as power generating plant cooling systems. The NYSM has partnered with the USFWS (Genoa NFH) and United States Geological Survey's (USGS) Upper Midwest Environmental Sciences Center (UMESC) to determine the suitability of this product for open water zebra mussel control applications such as treatment of native mussel propagation cages or native mussel beds.

2. PROTOCOL OBJECTIVE:

This study will determine the animal effects of various concentrations of MBI-401 FDP (*Pseudomonas fluorescens* [*Pf*-CL145A]) to the glochidia lifestage of seven unionid mussels species present in the Great Lakes and Upper Mississippi River basins.

4.1 General Description:

Glochidia from 7 unionid mussel species (Table 2) endemic in the Great Lakes and Mississippi River basins will be evaluated for effects from exposure to varying concentrations of MBI-401 FDP (*Pseudomonas fluorescens* [*Pf*-CL145A]). Glochidia will be exposed in static exposures for 24 h as described in ASTM E-2455-06 and evaluated at 6, 12 and 24 h for viability as determined by valve closure response after addition of a saturated sodium chloride solution.

4.2 Experimental Design:

4.2 Experimental Design:

Gravid donor mussels will be collected by biologists from the Genoa National Fish Hatchery from the Upper Iowa, Upper Mississippi or St. Croix Rivers. Mussels will be identified to species as described in Cummings and Mayer (1992) and Watters et al. (2009) and transported to the Genoa National Fish Hatchery. Prior to test initiation the donor mussels will be acclimated to the test temperature (see Section 5.1). Glochidia from at least three gravid female mussels of each species will be extracted by personnel from the Genoa NFH or UMESC. The viability of glochidia from each mussel will be assessed by drawing aliquots (e.g. 100 µL) of settled glochidia from the stock container with an adjustable manual pipet fitted with a wide bore tip and placing it on a pre-labeled 35 mm petri dish. The sample will be diluted with a known and consistent volume (e.g. 200 µL) of acclimated well water to enhance dispersion of glochidia for more accurate enumeration.

The Study Director will determine which method of glochidia viability assessment will be used (enumeration from photomicrographic recording or direct enumeration) after glochidia are collected from donor mussels. That decision will be recorded in the study data management system. If glochidia can be enumerated and viability status (i.e. open or closed) reliably determined through photomicrographic records (i.e. the

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glochidia remain on a single focal plane) then the following procedures will be used for viability assessment. The sample will be viewed under a dissecting microscope, adjusted for maximum clarity and magnification, and a photomicrograph will be recorded. Approximately one drop of a saturated sodium chloride solution (12 g NaCl/ 50 mL of D.I. H_2O [ASTM E2455-06]) will be added to the sample and after one minute a second photomicrograph will be recorded. The total number of glochidia within the sample and the number of glochidia closed or open before and after sodium chloride addition will be determined. The percentage of viable glochidia (glochidia that respond to sodium chloride exposure by valve closure) will be calculated by the following method:

Percent viable = ([Total number of glochidia in sample – {total number of glochidia closed before addition of sodium chloride + number of glochidia open after sodium chloride addition}]/Total number of glochidia in sample) * 100

If glochidia enumeration and viability cannot be reliably determined through photomicrographic records because the glochidia do not occupy a single focal plane (e.g. *Megalonaias nervosa* and other mussels that release glochidia in a mucoidal matrix) the following procedures will be used to assess viability.

The sample will be viewed under a dissecting microscope, adjusted for maximum clarity and magnification, and the total number of glochidia and the number of closed or open glochidia (discretion of the individual performing the count) will be directly enumerated. Approximately one drop of a saturated sodium chloride solution (12 g NaCl/ 50 mL of D.I. H_2O [ASTM E2455-06]) will be added to the sample. After one minute, the number of glochidia that remain open after sodium chloride addition will be directly enumerated. The percentage of viable glochidia (glochidia that respond to sodium chloride exposure by valve closure) will be calculated by the following method:

Percent viable = ([Total number of glochidia in sample – {total number of glochidia closed before addition of sodium chloride + number of glochidia open after sodium chloride addition}]/Total number of glochidia in sample) * 100

If glochidia viability is acceptable (≥80%) for a donor mussel, the glochidia from that donor mussel will be pooled with glochidia from other donor mussels of that species. An appropriate amount of settled glochidia (e.g.: 500 µL) will be randomly drawn from the pooled glochidia stock and distributed to an exposure chamber (80 mm x 40 mm crystallizing dish) containing exposure water to achieve a final volume of 100 mL. Between 500-1,000 glochidia will be transferred to each exposure chamber in a minimum of two distributions (dependent on number of glochidia per aliquot and the estimated number of glochidia available) per chamber (e.g. two 100 µL aliquots that contain ~250 glochidia each). Once all of the exposure chambers receive test animals the exposures will be initiated by the addition of the appropriate amount of MBI-401 FDP, deactivated MBI-401 FDP (positive control) or plain water (control) in a completely randomized design to achieve the desired test concentrations of 0 (control), 300 mg/L deactivated (positive control), or 50, 100, 200, and 300 mg/L of active MBI-401 FDP. The exposure chambers will be gently mixed with a glass rod or stainless steel spatula to ensure uniform distribution of the test chemical. All concentrations will be tested in triplicate for a total of 18 exposure chambers per species. Exposure chambers will be held at 20°C in a water bath or controlled

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environment room and maintained on an 18:6 h light/dark cycle. For mussel species for which the viability of glochidia can be assessed from photomicrographic records, the viability of a subsample of approximately 100 glochidia from each chamber will be assessed at 6, 12 and 24 h post exposure by enumeration from photomicrograph records pre- and post-sodium chloride addition. For mussel species for which the viability of glochidia cannot be assessed from photomicrographic records (e.g. *M. nervosa*), the viability of a subsample of approximately 100 glochidia from each chamber will be assessed only at 24 h post exposure using the methods described for initial glochidia viability assessment.
The study will be terminated after 24 h post exposure evaluations are completed.

5. STUDY PROCEDURES
5.6 Treatment administration:
5.6.1 Treatment: Each species of mussel glochidia will be exposed to three replicates of either 0 (control), 50, 100, 200 or 300 mg/L (active ingredient) and a 300 mg/L heat deactivated (70°C/45 minutes) control as a one time single dose exposure.
5.6.2 Route of administration: To assure uniform exposure, an appropriate amount of freshly prepared stock solution will added to each test chamber from an agitated stock suspension with a pipet.
5.6.3 Dose verification: The activity of MBI-401 FDP (*Pseudomonas fluorescens* [*Pf*-CL145A]) cannot be assessed by current analytical techniques as the chemical nature of the active agent in the bacterium has not been determined or characterized. Dosage verification of MBI-401 FDP will be assessed by measuring and recording the absorbance of the prepared stock dosing solution and the high treatment concentrations. The collected absorbance information will be used to determine inter- and intra-test variability in the preparation of stock and test solutions.

12. INVESTIGATIONAL TEST ARTICLE
12.1 Test Substance(s): MBI-401 FDP, *Pseudomonas fluorescens* (*Pf*-CL145A)
12.1.1 Chemical name: *Pseudomonas fluorescens* (*Pf*-CL145A)
12.1.2 Trade name: MBI-401 FDP
12.1.3 Active ingredients: *Pseudomonas fluorescens* (*Pf*-CL145A) is the sole active ingredient, 100% active by weight.
12.1.4 Source: Marrone Bio Innovations (MBI); Davis, CA
12.1.5 Lot number: More than one lot will be used. Test article lot numbers for each species will be recorded in the study files.

Revised text:

1. INTRODUCTION: (paragraph 4 of Section 1 is the only paragraph affected by this amendment)

Biologists at the New York State Museum (NYSM) Field Research Laboratory have been researching dreissenid mussel control since 1991 and they discovered that a bacterium isolated from soils (*Pseudomonas fluorescens* [*Pf*-CL145A]) is efficacious for controlling zebra mussels. Marrone Bio Innovations (MBI; Davis, CA) is currently developing a commercial **spray** dried formulation of this bacterium referred to as MBI-401 **SDP**. The current commercial applications

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of this product are for use with closed systems such as power generating plant cooling systems. The NYSM has partnered with the USFWS (Genoa NFH) and United States Geological Survey's (USGS) Upper Midwest Environmental Sciences Center (UMESC) to determine the suitability of this product for open water zebra mussel control applications such as treatment of native mussel propagation cages or native mussel beds.

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This study will determine the animal effects of various concentrations of MBI-401 **SDP** (*Pseudomonas fluorescens* [*Pf*-CL145A]) to the glochidia lifestage of seven unionid mussels species present in the Great Lakes and Upper Mississippi River basins.

4.1 General Description:

Glochidia from 7 unionid mussel species (Table 2) endemic in the Great Lakes and Mississippi River basins will be evaluated for effects from exposure to varying concentrations of MBI-401 **SDP** (*Pseudomonas fluorescens* [*Pf*-CL145A]). Glochidia will be exposed in static exposures for 24 h as described in ASTM E-2455-06 and evaluated at 6, 12 and 24 h for viability as determined by valve closure response after addition of a saturated sodium chloride solution.

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The study will be terminated after 24 h post exposure evaluations are completed.

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5. STUDY PROCEDURES

5.6 Treatment administration:

5.6.1 Treatment: Each species of mussel glochidia will be exposed to three replicates of either 0 (control), 50, 100, 200 or 300 mg/L (active ingredient) and a 300 mg/L heat deactivated (70°C/45 minutes) control as a one time single dose exposure.

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12. INVESTIGATIONAL TEST ARTICLE

12.1 Test Substance(s): MBI-401 **SDP**, *Pseudomonas fluorescens* (*Pf*-CL145A)

12.1.1 Chemical name: *Pseudomonas fluorescens* (*Pf*-CL145A)

12.1.2 Trade name: MBI-401 **SDP**

12.1.3 Active ingredients: *Pseudomonas fluorescens* (*Pf*-CL145A) is the sole active ingredient, **50%** active by weight.

12.1.4 Source: Marrone Bio Innovations (MBI); Davis, CA

12.1.5 Lot number: More than one lot will be used. Test article lot numbers for each species will be recorded in the study files.

United States Department of the Interior

U.S. GEOLOGICAL SURVEY
Biological Resources Division
Upper Midwest Environmental Sciences Center
2630 Fanta Reed Road
La Crosse, Wisconsin 54603

Date: May 12, 2014
To: The Record Study Number AEH-11-PSEUDO-01

5 typo 5/15/14 JAL
Subject: Amendment ~~1~~- Amendment to the study AEH-11-PSEUDO-01 "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"

Revision of Study Protocol, Study # AEH-11PSEUDO-01 as detailed on page 2 of this amendment. Revised text is in **bold and underlined**, deleted text has a ~~strike through~~.

This amendment documents the change of status of study number AEH-11-PSEUDO-01 titled "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species" from a Good Laboratory Practices (GLP) regulated study to a non-GLP regulated study. The data resulting from this study will not be used to support a product registration, therefore, a verbal decision with UMESC management was agreed to on April 22, 2014 to change the status of the study to non-regulated.

Reviewed by:

[redacted] 13May2014
Date

Mark P. Gaikowski, M.A.
Supervisory Biologist
Aquatic Ecosystem Health,
UMESC[1]

[redacted] 5/13/2014
Date

Jane E. Rivera, B.A.
Acting Quality Assurance
Officer, UMESC

[redacted] 05/14/2014
Date

For Kevin D. Richards, Ph.D.
Acting Center Director, UMESC

Approved by:

[redacted] 15 MAY 2014
Date

James A. Luoma, B.A.
Study Director, UMESC

[1] UMESC: U.S. Geological Survey, Upper Midwest Environmental Sciences Center

Current text:

10. GOOD LABORATORY PRACTICES

Data collection, storage and retrieval procedures for the study will be conducted in compliance with FDA regulations for Good Laboratory Practices (GLP; 21 CFR, Part 58). The study protocol and progress of the study will be reviewed at the start of the study and periodically throughout the study by the Quality Assurance Unit (QAU). The Study Director has the responsibility of ensuring that all procedures used in conjunction with the study conform with Good Laboratory Practices.

Revised text (<u>in bold and underlined</u>):

10. GOOD LABORATORY PRACTICES

Data collection, storage and retrieval procedures for the study will be **<u>not be</u>** conducted in compliance with FDA regulations for Good Laboratory Practices (GLP; 21 CFR, Part 58). The study protocol and progress of the study **<u>may</u>** ~~will~~ be reviewed at the start of the study and periodically throughout the study by the Quality Assurance Unit (QAU). The Study Director has the responsibility of ensuring that all procedures used in conjunction with the study **<u>conforms to</u>** ~~with~~ Good Scientific ~~Laboratory~~ Practices.

United States Department of the Interior

U.S. GEOLOGICAL SURVEY
Biological Resources Division
Upper Midwest Environmental Sciences Center
2630 Fanta Reed Road
La Crosse, Wisconsin 54603

MEMORANDUM

Date: February 4, 2013
To: The Record study Number AEH-11-PSEUDO-01

Subject: Note To File #1 to the study AEH-11-PSEUDO-01 "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"

This note to file is to document and clarify the lab notebook entries for the study events and procedures used in the *Lampsilis cardium, L. Higginsii* and *L. Siliquoidea* glochidia exposures conducted in May of 2011. This document was prepared on May 31, 2011 and signed on February 4, 2013.

Pre-exposure period:

Test Chemical: (Lab notebook pages 2, 3 & 7)
Approximately 96 g of *Pseudomonas fluorescens* (*Pf*-CL145A) test material (lot# MBI-401-110308A1-BD-3; SDP formulation) was received on 28 April, 2011 from the New York State Museum Field Research Laboratory (NYSM-FRL) located in Cambridge, NY. The test material was shipped from Denise Mayer (NYSM-FRL) on 27 April, 2011. The test material was received with a thermometer inside the cooler which indicated a temperature of 9.3°C above the ice packs in the cooler. A clean glass thermometer inserted into the test chemical indicated a temperature of 8.5°C. The temperature observed was greater than the recommend storage temperature of 4°C. Therefore, an aliquot of test material was returned to the NYSM-FRL after the conclusion of testing at UMESC (shipped May 17, 2011) to confirm the activity of the product. A chemical lab notebook was prepared for the test material upon arrival and the material was placed in a lock box located in a refrigerator in Room 122. On 29 April, 2011, 1.00147 g of test material was placed into a pre-cleaned glass archive vial for storage in the UMESC ultra-cold freezer chemical archive. All weights and documentation were recorded in the chemical lab notebook.

Donor Mussels: (Lab notebook pages 3-6)
On 09 May 2011, four gravid adult mussels of 3 species [*Lampsilis cardium* (lot # 111100), *L. higginsii* (lot # 111300) and *L. siliquoidea* (lot# 111200)] were received from the Genoa NFH at approximately 1130 h. The mussels were received at approximately 11.7°C and they were placed directly into holding tank 1 (compartments 1A, 1B and 1C) in room 11 which contained cold (~13°C) UMESC well water flowing at approximately 1.1 LPM (1 tank exchange/hr). A temperature ramp was set to acclimate the donor mussels to test temperature (20°C) over a period of 60 h. The donor mussels were feed by mixing the following Reed Mariculture (Campbell, CA) instant algae with 14,400 mL of UMESC well water: 44.0 g of shellfish diet, 35.3 g of tetraselmis and 92.4 g of Thalassiorsira weissflogii. This recipe approximates a 1:1:1 dry weight ratio of ~10 mg/L total dry weight of food in the culture system when delivered to the holding tank at ~ 10.0 mL/min. The donor mussels were feed this diet on a continuous basis throughout the entire holding period.

Note: Separate Daily Care Worksheets were prepared for each section of the tank that held mussels, however, water chemistry analysis were only completed on a single compartment, farthest from the incoming water. The measurements were recorded for all 3 sections as the water is not separated between compartments. The data sheets were labeled (5/12/11) with a note (dated 5/12/11) detailing this procedure (File Folder 7A).

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UMESC lot numbers were obtained from the UMESC fish culture for the donor mussels. The information regarding the assignment of lot numbers was placed into File Folder 7B. The following lot numbers were assigned: *L. Cardium* (plain pocketbook; PPB) = Lot # 11110; *L. Siliquoidea* (fat mucket; FAM) = Lot# 111200; *L. Higginsii* (Higgins eye; HGE) = Lot# 111300.

On May 10, 2011 the temperature acclimation was found to have failed after running 660 minutes. The temperature then increased to the 20°C study temperature. A screen capture of the acclimation ramp setting and curve were recorded in the electronic study record (I:\AEH-11-PSEUDO-01\Donor mussels) and was saved to disk. The temperatures were then set to remain at the test temperature until the use. The temperature increase would likely have no impacts to the study as the temperature increase was buffered from the existing tank water and it likely took several hours to raise the temperature to 20°C in the holding tank.

Daily observations for donor mussels included pH, temperature and dissolved oxygen (D.O.) in addition to daily preparation of food.

Equipment calibration: (Lab notebook pages 4-5)
Adjustable pipettors were calibrated on 09 and 10 May, 2011 for the tips and volumes to be used in the study. Results from the calibrations were placed into File Folder 13.

Test Water preparation: (Lab notebook pages 6-7)
Three 20 L glass jars were filled with UMESC well water on 10 May 2011 and supplied with air from an air pump and the water was allowed to equilibrate to room temperature for use in exposures of glochidia. The test water prepared on 10 May 2011 was discarded and replaced with fresh well water on 11 May 2011 due to over aeration of the water. A smaller air pump was used to lightly bubble air into the water acclimation jars. Two jars were placed on bench top in room 15 for use in all aspects of the study (glochidia extraction, stock preparation and dilution water). Siphons were placed into the jars for easy removal of water as required.

Randomization Procedures: (Lab notebook pages 4)
Randomizations were prepared by Mark Gaikowski on 10 May 2011 to perform the following:

1) Assignment of each mussel species to a treatment block (1, 2, or 3)
2) The distribution order of glochidia to exposure chambers for each species
    a. Ten separate rounds prepared for each species by exposure chamber
    b. Only 5 rounds of prepared distribution rounds were used during the distribution
3) The assignment of treatment to each exposure vessel
    a. One of 6 treatment types to each vessel
        i. 0, 50, 100, 200 or 300 mg/L active ingredient
        ii. 300 mg/L heat deactivated product
    b. Three replicates per species

Exposure Period: (initiated 12 May 2011)

Pipette calibration verification: (Lab notebook pages 7-8)
Pipette calibrations were verified for pipettes 1-4 by weighing 20°C distilled water using a calibrated analytical balance (BAL1/WTS2). The pipette setting and target volume (µl) were recorded along with the verification reading (g). Additionally, a summary table was prepared for each pipette indicating the delivery volume, delivery setting and tip type to use for each pipette assigned to the study. The pipette verifications were performed by Todd Severson. The data was placed into File Folder 13.

Initial water Chemistry: (Lab notebook pages 7, 13)
The water quality parameters were measured on the initial water dilution water (from the 3 20 L glass jars) and the donor mussel culture water between 0645 and 0730 by Jeremy Wise on 12 May 2011. The initial parameters included hardness, alkalinity and temperature. The measurements were recorded on Water Quality Form 1 and were filed in File Folder 10a. The initial measurements conducted by Jeremy Wise

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were found to be conducted in error as he mixed water from the dilution vessels with the donor mussel culture tanks and then performed the water chemistry analysis. Once the mistake was realized, new water chemistry parameters were conducted on remaining dilution water and donor mussel culture tank water (separately) at ~1900 h by Todd Severson on 12 May 2011. The water chemistry values were found to be nearly identical to the earlier mixed samples. This was explained on the lab notebook page 13 and the original data sheets that contained the values from the mixed water were noted as such and retained. All of the data sheets were placed into File Folder 10a.

Test Chemical Stock Preparations: (Lab notebook pages 8-10)
In order to preserve efficacy and to maintain similar times between stock preparation and dosing, one stock solution was prepared for each species active dosing (i.e., 50, 100, 200 and 300 mg/L). A common stock solution was prepared for dosing the heat deactivated controls for use in all 3 species tested on 12 May 2011. Individual 50 mL beakers were labeled and tared on analytical balance BAL1. Then (~0645h) approximately 2.0 g of test material was weighed into each beaker. The exact weight of test material was recorded on each beaker and the beaker was then covered with parafilm and refrigerated until used in making a test material solution. The exact weights for each stock solution were as follows: 1.99990 g used for heat deactivated stock; 2.00062 g used to make *L. cardium* active stock; 2.00008 g used to make *L. higginsii* active stock and 2.00062 g used to make *L. siliquoidea* active stock. Each stock solution was prepared in the following fashion: the test material was poured through a funnel into a 100 mL volumetric flask. The 50 mL beaker and funnel were rinsed with well water and the flask filled approximately 25% with well water and shaken to suspend the test material. The flask was then filled to ~ 75% and was shaken for ~15 minutes to suspend the test material. The resulting foam was allowed to dissipate and the volumetric flask was brought to volume with well water. The heat deactivated stock was placed in a 70°C water bath for 45 minutes for deactivation at approximately 0725 h. The heat deactivated stock was allowed to cool to room temperature prior to use in deactivated control dosing. Other stocks were prepared ~ 30 minutes prior to use. Test material was weighed by Kerry Weber and the information regarding the stock preparation was recorded on page 8 of the laboratory notebook, File Folder 8 and in the test material lab notebook.

Glochidia extraction: (Lab notebook pages 8-10)
Glochidia were extracted in the order of block assignment (Block 1 = *L. cardium*, Block 2 = *L. Higginsii* and Block 3 = *L. siliquoidea*). The glochidia were all extracted by Nathan Eckert (Genoa NFH) following the same procedures. The glochidia were extracted by irrigating donor gills with well water from a 10 cc syringe fitted with a 1.5 inch 25 gauge needle. The gill water tubes containing glochidia were pierced with the needle and the gill was gently irrigated to express the glochidia. The glochidia were captured in a pre-wetted 500 mL beaker. From 6-10 water tubes from each mussel were extracted. For more detailed information regarding glochidia extraction and the donor mussels see the laboratory notebook pages 8-10. Glochidia from three mussels of each species were extracted for use in the exposures. The glochidia from each mussel were assessed visually for viability prior to acceptance for use. After acceptable viability was determined (>80%), the glochidia were pooled into a common 500 mL beaker for distribution. Glochidia of each species were extracted at the following times: *L. cardium* = 0900, 0910 and 0922 h; *L. higginsii* = 1033, 1041 and 1045h; *L. siliquoidea* = 1215, 1220 and 1225h.

Glochidia viability assessment and pooling: (Lab notebook pages 9-10)
Glochidia from each mussel were assessed for viability immediately after extraction to determine acceptable viability (>80%) for use in exposure trials. Assessments were conducted by Kerry Weber and Theresa Schreier for all the mussels. The procedures used for all mussels were as follows: A 100 µl sample of settle glochidia was placed on a 35 mm petri dish. 200 µl of well water was added to the sample and the sample was placed under a dissecting scope (SCO 1). The scope was adjusted for maximum clarity and a photomicrograph was recorded using Image-Pro Express software (SOFT1) and stored in the AEH-11-PSEUDO-01 electronic study folder at the following path I:\AEH-11-PSEUDO-01\Pictures\12May11\Initial Viability. A drop of saturated sodium chloride solution (12g NaCl/50mL well water, page 7 of lab notebook) was then added to the sample and allowed to sit for 1 minute. A second photomicrograph was recorded for each sample and stored in the same location and path as the previous. The samples were coded for species, mussel number, and before or after salt addition. The samples were visually assessed for viability and if they appeared to have greater than 80% viability then

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the mussels were used for the study. No mussels that were assessed were rejected due to initial glochidia viability. Two of the HGE mussels visually appeared to have near 80% viability so a preliminary hand count was done to determine that they were indeed greater than 80% viable. The method used was: (total number of glochidia in the sample – [number of glochidia closed prior to addition of the salt solution + number of glochidia open after the addition of the salt solution])/total number of glochidia in the sample *100. The initial counts revealed that the glochidia viability exceeded the minimum threshold of 80% viability so they were used in the study. The photomicrograph records will be used to determine and document the exact initial viability for each mussel used in the study.
After the glochidia were verified to be of acceptable viability they were pooled into a common 500 mL beaker and then distributed to the exposure chambers.

Glochidia Lot number assignments: (Lab notebook page 11)
The glochidia from each species were assigned a UMESC lot number by Steve Redman after they were pooled and an estimate of the number of glochidia was available. Glochidia lot number assignment data sheets were completed and filed in File Folder 7C. The lot numbers assigned were as follows: *L. cardium* #111400, *L. higginsii* #111500, and *L. siliquoidea* #111600.

Glochidia distribution to the exposure chambers: (Lab notebook pages 9-10, 13-14)
The glochidia from all three species were distributed to the exposure chambers using identical procedures. The glochidia were distributed in 5 separate randomized rounds for each species with each exposure chamber receiving a 100 µL aliquot of settled glochidia during each round. The order of the aliquots to each exposure chambered followed the randomization order produced by SAS and prepared by Mark Gaikowski (File Folder 14b). Prior to the start of each distribution round and every sixth aliquot, a separate 100 µL aliquot was placed on a petri dish for enumeration. The enumeration aliquots will be used to estimate the total number of glochidia that were placed in each exposure chamber. The distribution to the exposure chambers followed these procedures:

1) The exposure chambers were filled with 97 mL of acclimated test water using a 100 mL graduated cylinder. Then 500 µL was removed from each chamber using a Biohit pipette (PIP3) fitted with a 5000µL tip. The starting volume was then 96.5 mL; which when added to the 500 µL of glochidia aliquots and the 3.0 mL of dosing aliquots the resulting final volume will be 100 mL.
2) The exposure chambers were placed in order in the bench top in room 15.
3) The distributions were performed by James Luoma and were conducted by drawing 100 µl of settled glochidia from the source 1000 mL beaker of pooled glochidia using an Eppendorf Research Plus pipette (PIP1) fitted with 1000 µL wide bore pipette tip. The glochidia were then released below the surface of the water in the exposure chamber identified in the randomization order or placed on a petri dish for distribution enumeration. A watch glass was then placed over the exposure chamber to denote that the chamber had received an aliquot of glochidia for that distribution round. After completion of the distribution round the watch glasses were removed and the procedure was repeated until a total of 5 rounds were completed.

Exposure chamber Dosing: (Lab notebook Pages 9-11)

After the glochidia were distributed to the exposure chambers the chambers were dosed with the appropriate amount of stock solutions to attain the desired concentration of test chemical. The exposure chambers were previously labeled with color coded tape to denote the assigned test concentration that was determined in the dosing randomization procedures (File Folder 14c). The color coding was as follows: white = control; white with stripes = 300 mg/L heat deactivated control; yellow = 50 mg/L active ingredient (A.I.); yellow with stripes = 100 mg/L A.I.; blue = 200 mg/L A.I.; and blue with stripes = 300 mg/L A.I. The exposure chambers for each species were arranged on the bench top in room 15 by concentration for the dosing.

All exposure chambers received 3 mL of the appropriate amount of stock solution(s) required to attain the desired concentration. Chambers that received a combination of control (well water) stock and active ingredient stock to attain the desired concentration received the control water stock first followed by the active ingredient stock. This procedure allowed the final volume of all chambers to be 100 mL and all

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chambers received 3 mL of stock(s). The amount of each stock that each chamber received was documented on the "Chemical Stock Solution Determination" sheet and the sheets were filed in File Folder 16 along with the "Glochidia Exposure Dosing Form" that was prepared for each species. Dosing for each species was initiated and completed at the following times (File Folder 16): *L. cardium*: 1036-1040 h; *L. higginsii*: 1155-1204 h and *L. siliquoidea*: 1317-1325.

After the dosing was completed for each species the exposure chambers were placed into their appropriate position within the test system (room 16) as determined by the randomization procedures (File Folder 14a). The exposure chambers were then provided light aeration via a disposable glass pipet that was suspended from a PVC air supply line with a length of latex tubing. Air was bubbled into the chambers at a rate to provide approximate 1-5 bubbles/sec.

Water Chemistry - Exposure Period Measurements: (Lab notebook Pages 11 &15)

Water chemistry parameters including dissolved oxygen, pH and temperature were measured and recorded twice during the exposure period, after all dosings were completed on May 12, 2011 and prior to completion of the exposure period on May 13, 2011. Measurements were recorded for all test block replicates for the control and high (300 mg/L active ingredient) concentrations and one replicate of each of the other concentrations (50, 100, 200 mg/L active ingredient and the 300 mg/L heat deactivated). Data for the water quality measurements including dissolved oxygen, pH and temperature were recorded on Water Quality – Form 2 and placed into study File Folder 10b. Additionally, light intensity was measured at the center of each species block and recorded on the bottom of Form 2. The measurements were conducted by Jeremy Wise and Pajtshiab Moua.

Glochidia Viability Assessments: (Lab notebook pages 14 & 15)

Samples of glochidia from each exposure replicate from all species were removed at 6, 12 and 24 h post exposure to determine the viability. The exposure chambers were slightly stirred to bring the glochidia to the center of the chamber. A 100 µl sample of settled glochidia was removed from each exposure chamber using an adjustable 1000 µl pipette (PIP1) fitted with a wide bore pipet tip. The sample was placed onto a pre-labeled (chamber code + sample time) 35 mm petri dish. 200 µl of 20°C well water was added to each sample to increase dispersion for easier enumeration from photomicrograph records. A photomicrograph was recorded for each sample and then 1 drop of saturated sodium chloride was added to each petri dish to induce closure of viable glochidia. A second photomicrograph was recorded after allowing the sample to stand for 1 minute. During the 1 minute closure time the sample was removed from the stage and a new sample petri dish was placed on the stage and a pre-sodium chloride photomicrograph was recorded. Immediately after the 1 minute had elapsed for valve closure the sample was returned to the stage and the after sodium chloride addition photomicrograph was recorded. All photomicrograph records were recorded in the studies electronic file log and were coded for exposure chamber, sampling time, and before (bs) or after (as) sodium chloride addition. The photomicrograph records were recorded by Kerry Weber and Theresa Schreier; the samples were collected by Jim Luoma and Todd Severson.

Note: No 24h data was collected from exposure chamber 2B1 (HGE control) as it was inadvertently pooled with the control replicates for Block 1 during the pooling for final water quality analysis. When this was discovered, additional 24h viability samples were removed and processed as described above for the 2 remaining HGE control chambers, 2C3 and 2C6. This is further explained in study deviations # 1 and 2, dated 6/3/2011. The study deviation is located in the electronic study file as well as File Folder 3.

Water Chemistry – Experimental Termination Measurements: (Lab notebook Page16)

After completion of the 24h viability sampling and the second exposure period water quality measurements (DO, pH, temp) the replicate concentrations from each species block were pooled for final hardness, alkalinity, conductivity and ammonia analysis.

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Initially, hardness and alkalinity data analysis were performed on the control and high concentrations as described in the study protocol. Due to a slight variance in the observed measurements, hardness and alkalinity measurements were performed for all pooled concentrations.

Conductivity and total ammonia were recorded for all pooled replicate samples. pH and temperature were not initially determined for the pooled replicate samples (Block 1, PPB) however, due to what appeared to be high ammonia concentrations, the termination water quality (Form 3) was modified and pH and temperature data were recorded for the pooled replicate samples for Blocks 2 and 3 (HGE and FAM).

Un-ionized ammonia concentrations for the pooled replicate samples from Blocks 2 and 3 can be determined using the collected pH and temperature data. Un-ionized ammonia concentrations for Block 1 will have to be estimated using the second exposure period temperature and pH measurements collected prior to termination. The meters used to collect the terminal water quality data were calibrated according to their respective SOP's and are denoted in the study as pH2, Therm 3, CON1 and PHO1. All data sheets for the terminal water quality were placed into study File Folder 10C.

Final Termination: (Lab notebook Page17)

The glochidia were euthanized by MS-222 overdose and discarded. All sample analysis was completed by 1530 h on 05/13/2011.

Prepared by:

[redacted] 2/4/2013

James A. Luoma, B.A. Date
Study Director, UMESC

cc: UMESC QAU

United States Department of the Interior

U.S. GEOLOGICAL SURVEY
Biological Resources Division
Upper Midwest Environmental Sciences Center
2630 Fanta Reed Road
La Crosse, Wisconsin 54603

MEMORANDUM

Date: October 31, 2012
To: The Record study Number AEH-11-PSEUDO-01

Subject: Note To File #2 to the study AEH-11-PSEUDO-01 "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"

This note to file is to document and clarify the path, folder and file names for glochidia photomicrographs for study number AEH-11-PSEUDO-01.

Pictures of glochidia (photomicrographs) were used to: 1) determine the viability of extracted glochidia that were used in the exposures; 2) estimate the number of glochidia distributed to each exposure replicate and; 3) to assess the treatment effects on the viability of glochidia at three time points (6, 12 and 24 hour) during the exposure period.

The original and enumerated photomicrograph files are saved as described below. Additionally, how the files were enumerated is also described.

ORIGINAL PHOTOMICROGRAPHS

Initial Viability

Photomicrographs were recorded to document the viability of glochidia extracted from each donor mussel. The glochidia were photographed before and after a concentrated salt solution was added to the sample to induce valve closure as a valve closure response indicates viability. Glochidia that visually appeared to have suitable viability (>80%) were pooled for use prior to determining the initial viability. Examples of path and filenames for the original initial viability photomicrographs are shown in Table 1.

Table 1. Initial Viability Path and Filenames

Path	I://AEH-11-PSEUDO-01/Pictures/12May11/Initial Viability/FAM-SDP-2-I-bs Photomicrograph Filename Sample Type Folder (Initial Viability, Distribution, 6h, 12h or 24h) Exposure Date Folder (12May11, 18OCT11, 17JAN12 or 19JAN12) Picture Folder (Location of all study photomicrographs) Study Folder (Location of all study specific data) I:// Drive (Location of all study data)

Table 1 (con.) Initial Viability Path and Filenames

Filename	FAM-SDP-2-I-bs bs or as (before or after salt addition) I (Initial Viability) Mussel # (1, 2 or 3) Formulation Type (FDP or SDP) Species 3 Letter Code

Distribution

Photomicrographs were recorded on 100 μL glochidia samples collected during each round of glochidia distribution to the exposure chambers. Glochidia distribution was completed in 5 randomized rounds with 18 100 μL glochidia aliquots (1 aliquot per exposure chamber) per round. Photomicrographs were recorded on the initial aliquot and then after every 6 aliquots for a total of 16 distribution samples per species. Distribution samples that had very dense quantities of glochidia were subdivided into more manageable counts before being photomicrographed. Plain pocketbook (PPB) was the only species exposed to both SDP and FDP formulations. Therefore, only the distribution file names for PPB were modified to include the formulation type. Examples of path and filenames for the original distribution photomicrographs are shown in Table 2.

Table 2. Distribution Path and Filenames

Path	I://AEH-11-PSEUDO-01/Pictures/18OCT11/Distribution/PPB-R2-2.*1* Photomicrograph Filename Sample Type Folder (Initial Viability, Distribution, 6h, 12h or 24h) Exposure Date Folder (12May11, 18OCT11, 17JAN12 or 19JAN12) Picture Folder (Location of all study photomicrographs) Study Folder (Location of all study specific data) I:// Drive (Location of all study data)	
Filename	HIC-R3-1.*1* *Subdivided (.1, .2, .3, etc.)* *(only for dense distribution samples)* Sample Number (1-5) Distribution Round (R1-R5) Species 3 Letter Code	PPB-SDP-R2-2.*1* *Subdivided (.1, .2, .3, etc.)* *(only for dense distribution samples)* Sample Number (1-5) Distribution Round (R1-R5) Formulation Type (FDP or SDP) Species 3 Letter Code

Viability Samples (6, 12 and 24 hour)

Two photomicrographs were recorded for each sample collected for exposure period viability assessment. The first photomicrograph was recorded prior to the addition of a salt solution to induce valve closure and the second after induced closure. Photomicrographs were recorded on 100 μL glochidia samples collected from each exposure chamber at 6, 12 and 24 hours post-dose initiation.

Examples of path and filenames for the original viability samples (6, 12 and 24 hour) photomicrographs are shown in Table 3.

Table 3. Viability Samples (6, 12 and 24 hour) Path and Filenames

Path	I://AEH-11-PSEUDO-01/Pictures/17JAN12/6h/BLS-SDP-100-1B2-6h-as Photomicrograph Filename Sample Type Folder (Initial Viability, Distribution, 6h, 12h or 24h) Exposure Date Folder (12May11, 18OCT11, 17JAN12 or 19JAN12) Picture Folder (Location of all study photomicrographs) Study Folder (Location of all study specific data) I:// Drive (Location of all study data)
Filename	BLS-SDP-100-1B2-6h-as bs or as (before or after salt addition) Sample Time (6, 12 or 24h) 3 Letter Sample ID 1 = Test System Section (1, 2 or 3) B = Row (A, B or C) 2 = Position within row (1 – 6) Concentration ctrl = Control (0 mg/L) 50 = 50 mg/L active ingredient 100 = 100 mg/L active ingredient 200 = 200 mg/L active ingredient 300AI = 300 mg/L active ingredient 300HD = 300 mg/L heat deactivated Formulation Type (FDP or SDP) Species 3 Letter Code

ENUMERATED PHOTOMICROGRAPHS

Enumerated data files are saved in different electronic folders, depending on when they were enumerated. Some photomicrograph files were inadvertently over-written during file conversion and during the enumeration process (see Deviations 8 and 9). To prevent reoccurrence, the folders containing the raw data files were locked to prevent additional file overwrites. All of the initial viability photomicrographs were enumerated prior to the locking of the electronic file folders. All of distribution photomicrographs were enumerated after the locking of the electronic file folders. Some of the viability samples were enumerated prior to the locking of the electronic file folders (FAM, PPB and HGE from 12May11 at 12 and 24 hour; PPB from 18OCT11 at 6, 12 and 24 hour) while other viability samples were enumerated after the locking of the electronic file folders (FAM, PPB and HGE from 12May11 at 6h; BLS and MUC from 17JAN12 at 6, 12 and 24 hour; HIC from 19JAN12 at 6, 12, and 24 hour). Those files that were enumerated prior to the locking of the electronic file folders were saved in the raw data file folders in a separate electronic folder entitled "Enumerated" (e.g., I://AEH-11-PSEUDO-01/Pictures/18OCT11/6h/Enumerated/PPB-FDP-ctrl-3B6-6h-bs-C). Those files that were enumerated after the locking of the electronic file folders were saved in a separate enumerated file folder within the study folder (e.g., I://AEH-11-PSEUDO-01/Pictures/Enumerated/18OCT11/6h/PPB-FDP-ctrl-3B6-6h-bs-C).

Initial Viability

All of the initial viability photomicrographs were enumerated prior to the locking of the raw data electronic file folders. The photomicrographs were enumerated for total number of glochidia within the sample and the number of glochidia open or closed before and after salt addition. The percentage of viable glochidia (i.e., glochidia that respond to salt exposure by valve closure) was calculated by the following method

$$Percent\ Viability =$$
$$\left(\frac{[Total\ number\ of\ glochidia\ in\ sample - (Number\ of\ glochidia\ closed\ before\ salt + Number\ of\ glochidia\ open\ after\ salt)]}{Total\ number\ of\ glochidia\ in\ sample}\right) * 100$$

For more specific details regarding the enumeration process, refer to Amendment 1 of the protocol. Examples of path and filenames for the enumerated initial viability photomicrographs are shown in Table 4.

Table 4. Enumerated Initial Viability Path and Filename

Path	I://AEH-11-PSEUDO-01/Pictures/12May11/Initial Viability/Enumerated/FAM-SDP-2-I-bs-T Photomicrograph Filename Enumerated Folder (Includes all enumerated initial viability photomicrographs) Sample Type Folder (Initial Viability, Distribution, 6h, 12h or 24h) Exposure Date Folder (12May11, 18OCT11, 17JAN12 or 19JAN12) Picture Folder (Location of all study photomicrographs) Study Folder (Location of all study specific data) I:// Drive (Location of all study data)
Filename	FAM-SDP-2-I-bs-T Count Type (T, O, or C: total count, open count or closed count, respectively) bs or as (before or after salt addition) I (Initial Viability) Mussel # (1, 2 or 3) Formulation Type (FDP or SDP) Species 3 Letter Code

Distribution
All of the distribution photomicrographs were enumerated after the locking of the raw data electronic file folders containing the original photomicrograph files. The photomicrographs were recorded and enumerated to estimate the number of animals distributed to each chamber. Distribution samples in which the glochidia were too dense to enumerate from a single photomicrograph were subdivided and multiple photomicrographs were recorded for each sample. Plain pocketbook (PPB) was the only species exposed to both SDP and FDP formulations, therefore, the distribution file names for PPB were the only files renamed to include the formulation type used. Distribution photomicrographs were enumerated for total counts only. All glochidia, regardless of shell position (open or closed) were counted for each sample. Enumerated files were saved with a "T" to designate that they had been counted. Examples of path and filenames for the enumerated distribution photomicrographs are shown in Table 5.

Table 5. Enumerated Distribution Path and Filename

Path	I://AEH-11-PSEUDO-01/Pictures/Enumerated/18OCT11/Distribution/PPB-R2-2.1-T Photomicrograph Filename Sample Type Folder (Initial Viability, Distribution, 6h, 12h or 24h) Exposure Date Folder (12May11, 18OCT11, 17JAN12 or 19JAN12) Enumerated Folder (Includes all enumerated distribution photomicrographs) Picture Folder (Location of all study photomicrographs) Study Folder (Location of all study specific data) I:// Drive (Location of all study data)	
Filename	HIC-R3-1.*1*-T Count Type (T = total count) *Subdivided (.1, .2, .3, etc.)* *(only for dense distribution samples)* Sample Number (1-5) Distribution Round (R1-R5) Species 3 Letter Code	PPB-SDP-R2-2.*1*-T Count Type (T = total count) *Subdivided (.1, .2, .3, etc.)* *(only for dense distribution samples)* Sample Number (1-5) Distribution Round (R1-R5) Formulation Type (FDP or SDP) *only for PPB* Species 3 Letter Code

Viability Samples (6, 12 and 24 hour)
Photomicrographs were recorded on glochidia samples collected from each exposure chamber at 6, 12 and 24 hours post-dose initiation for viability assessment. Two photomicrographs were recorded for each sample. The first photomicrograph was recorded prior to the addition of a salt solution to induce valve closure (a closure response indicates viability) and the second after induced closure. To determine viability the photomicrographs were examined to 1) determine the total number of glochidia with the sample, 2) to determine the number of glochidia in an open state prior to induced valve closure and 3) the number of open glochidia (prior to the addition of salt solution) that responded to the addition of the salt solution by valve closure. The photomicrographs were enumerated for total number of glochidia within the sample (T) and the number of glochidia open (O) or closed (C) before and after salt addition. The percentage of viable glochidia (i.e., glochidia that respond to salt exposure by valve closure) was

calculated by the following method:

$$Percent\ Viability = \left(\frac{[Total\ number\ of\ glochidia\ in\ sample - (Number\ of\ glochidia\ closed\ before\ salt + Number\ of\ glochidia\ open\ after\ salt)}{Total\ number\ of\ glochidia\ in\ sample}\right) * 100$$

For more specific details regarding the enumeration process, refer to Amendment 1 of the protocol.

Enumerated photomicrograph files were saved in one of two locations. Viability samples enumerated prior to the locking of the electronic file folders (FAM, PPB and HGE from 12May11 at 12 and 24 hour; PPB from 18OCT11 at 6, 12 and 24 hour) are saved within the raw data file folders in a separate electronic folder entitled enumerated. Viability samples enumerated after the locking of the electronic file folders (FAM, PPB and HGE from 12May11 at 6h; BLS and MUC from 17JAN12 at 6, 12 and 24 hour; HIC from 19JAN12 at 6, 12, and 24 hour) are saved in a separated enumerated file folder within the study folder. Examples of path and filenames for the enumerated viability samples (6, 12 and 24 hour) photomicrographs are shown in Table 6.

Table 6. Enumerated 6, 12 and 24 hour Viability Samples Path and Filenames

Path prior to locking	I://AEH-11-PSEUDO-01/Pictures/18OCT11/6h/Enumerated/PPB-FDP-ctrl-3B6-6h-bs-C Photomicrograph Filename Enumerated Folder Includes: 6h – PPB from 18OCT11 12h – FAM, PPB, HGE from 12May11 PPB from 18OCT11 24 h – FAM, PPB, HGE from 12May11 PPB from 18OCT11 Sample Type Folder (Initial Viability, Distribution, 6h, 12h or 24h) Exposure Date Folder (12May11, 18OCT11, 17JAN12 or 19JAN12) Picture Folder (Location of all study photomicrographs) Study Folder (Location of all study specific data) I:// Drive (Location of all study data)

Table 6 (con.) Enumerated 6, 12 and 24 hour Viability Samples Path and Filenames

Path after locking	I://AEH-11-PSEUDO-01/Pictures/Enumerated/17JAN12/24h/MUC-SDP-200-2A6-24h-as-O Photomicrograph Filename Sample Type Folder (Initial Viability, Distribution, 6h, 12h or 24h) Exposure Date Folder (12May11, 18OCT11, 17JAN12 or 19JAN12) Enumerated Folder Includes: 6h – FAM, PPB, HGE from 12May11 BLS, MUC from 17JAN12 HIC from 19JAN12 12h – BLS, MUC from 17JAN12 HIC from 19JAN12 24 h – BLS, MUC from 17JAN12 HIC from 19JAN12 Picture Folder (Location of all study photomicrographs) Study Folder (Location of all study specific data) I:// Drive (Location of all study data)
Filename	BLS-SDP-100-1B12-6h-as-O Count Type (T, O, or C: total count, open count or closed count, respectively) bs or as (before or after salt addition) Sample Time (6, 12 or 24h) 3 Letter Sample ID 1 = Test System Section (1, 2 or 3) A = Row (A, B or C) 2 = Position within row (1 – 6) Concentration ctrl = Control (0 mg/L) 50 = 50 mg/L active ingredient 100 = 100 mg/L active ingredient 200 = 200 mg/L active ingredient 300AI = 300 mg/L active ingredient 300HD = 300 mg/L heat deactivated Formulation Type (FDP or SDP) Species 3 Letter Code

The electronic log can be used to also determine where the raw and enumerated files are located in the electronic study folder.

[redacted] 31OCT12
Written by — Date
Kerry L. Weber, M.S.
Principal Investigator, UMESC

[redacted] 10-31-12
Approved by — Date
James A. Luoma, B.A.
Study Director, UMESC

cc: UMESC QAU

Study Title: "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"

Study number: AEH-11-PSEUDO-01

File Folder:______ Lab book/pgs:______ Reviewed:_______ Verified:________

Donor Mussel UMESC Lot Number Assignment Form

DONOR MUSSEL INFORMATION:

Species:____

Number of Donor Mussels:________ **Collection Date:**_______________________

Collection Location:____

Species Identification performed by:_______________________________________

Title/affiliation of identifier:_______________________________________

UMESC Arrival Date:____

Receiving UMESC tank/room #_______________________________________

Additional information:

____

____

UMESC LOT NUMBER DESIGNATION:_______________________________

Witness and form recorded by:

Printed Name	**Signature**	UMESC **Affiliation**	**Date**

This datasheet was approved by [redacted]____________ on 12 JAN 2012.

11JAN2012/Version 1.1

Study Number AEH-11-PSEUDO-01

File Folder: __________ Lab book/pgs: __________ Reviewed: __________ Verified: __________

Daily Care Worksheet

Donor Mussel Holding

Tank #: ______ **Section #:** ______ **Room #:** __________ **Month/Year:** __________

Species: ______________ **Lot Number:** ______________

Instruments: ______________________________

Day	Feed Time (military)	Flow Rate (mL/min)		Temperature (°C)	Dissolved Oxygen (mg/L)	pH	Initials
		Inflow	Pump				
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							
15							
16							
17							
18							
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22							
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26							
27							
28							
29							
30							
31							

This datasheet was approved b[redacted]__________ on 12JAN2012.
11JAN2012/version1.1

AEH-11-PSEUDO-01

Adult Mussel Holding Daily Algal Diet
1/13/2012

Tank Dimensions and volume
48"x 14" x 6" (121.92 cm x 35.56 cm x 15.24 cm) = 66,073 cm^3
6" (15.24 cm) standpipe: 66.073 L

Flow rate (1 turnover per hour): 1.1 L/m)

Daily water volume: 1.1 L/m X 60 m/h X 24 h/d = 1584 L/d

Diet weights (50% *Tetraselmis* and 50% *Nannochloropsis*)
to achieve 10 mg/L by dry weight:

Tetraselmis: Target 5.0 mg/L (17.93% Dry Wt); *Nannochloropsis*: Target 5.0 mg/L (20.48% Dry Wt)

Tetraselmis: 27.89 mg/L; *Nannochloropsis*: 24.41mg/L

X 1584 L/d

Tetraselmis: 44.2 g/d; *Nannochloropsis*: 38.7g/d

Diet Inflow Rate: 10 mL/min X 60 m/h X 24 h/d = 14400 mL/d

Daily Care:
Mix 44.2g *Tetraselmis* and 38.7g *Nannochloropsis* with 14,400 mL well water each day, to be delivered by peristaltic pump at 10 mL per minute. Prepare fresh diet daily.

Approved by: [redacted] Date: 13 Jan 2012

Study Title: "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"

Study number: AEH-11-PSEUDO-01

File Folder:______ Lab book/pgs:______ Reviewed:_______ Verified:________

Glochidia Test Organism UMESC Lot Number Assignment Form

Species:____

Number of Donor Mussels:________ **Donor Mussel Lot #:**____________________

Extraction Date:____/____/_____ **Extraction Time (military)**____________________

Extraction Location:________________ **Container ID:**____________________

Approximate Number of glochidia:________________

Glochidia extracted by: ____________________ **Affiliation:**________________

Additional information:

____

____

____

UMESC GLOCHIDIA LOT NUMBER DESIGNATION:____________________

Witness and form recorded by:

____________	____________	UMESC	____
Printed Name	**Signature**	**Affiliation**	**Date**

This datasheet was approved by [redacted]__________ on 12 JAN 2012.

11JAN2012/Version 1.1

Study Title: "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"

Study number: AEH-11-PSEUDO-01

File Folder:______ Lab book/pgs:______ Reviewed:_______ Verified:________

Glochidia Aliquot Distribution Form

Species:________________________________ **UMESC LOT NUMBER:**____________

Test Block Assignment (circle one): 1 2 3

Aliquot Volume (µl):____________ **Estimated # glochidia/aliquot**____________

Initial Distribution aliquot date/time (military):________________________________

Final Distribution Aliquot date/time (military):________________________________

Number of Aliquot rounds:__________ **Estimated # glochidia/chamber**________________

Additional information:

____

____

____

____

____

____

____

Witness and form recorded by:

		UMESC	
Printed Name	**Signature**	**Affiliation**	**Date**

This datasheet was approved by [redacted] on 11 JAN 2012.

11JAN2012/version 1.1

Study Number AEH-11-PSEUDO-01

File Folder: ______________ Lab book/pgs: ______________ Reviewed: ______________ Verified: ______________

Glochidia Photomicrograph Counting Record

Mussel Species: ______________ **Sample Period (h post exposure):** ______________

Dosing Date: ______________ **Formulation and lot #:** ______________

Photomicrograph Record ID	Photomicrograph Capture Time (military)	Total Glochidia Counted	Glochidia Count Before NaCl Solution			Glochidia Count After NaCl Solution			Date	Initials
			No. Counted	Circle One[1]		No. Counted	Circle One[1]			
				Open	Closed		Open	Closed		
				Open	Closed		Open	Closed		
				Open	Closed		Open	Closed		
				Open	Closed		Open	Closed		
				Open	Closed		Open	Closed		
				Open	Closed		Open	Closed		
				Open	Closed		Open	Closed		
				Open	Closed		Open	Closed		
				Open	Closed		Open	Closed		
				Open	Closed		Open	Closed		
				Open	Closed		Open	Closed		
				Open	Closed		Open	Closed		
				Open	Closed		Open	Closed		
				Open	Closed		Open	Closed		
				Open	Closed		Open	Closed		
				Open	Closed		Open	Closed		
				Open	Closed		Open	Closed		
				Open	Closed		Open	Closed		

[1] Technician counting glochidia photomicrograph will select either open or closed glochidia, whichever is less, and circle the corresponding selection for each sample.

This datasheet was approved by [redacted] ______________ on 12 JAN 2012.
11JAN2012/version1.1

Study Number AEH-11-PSEUDO-01

File Folder: ______ Lab book/pgs: ______ Reviewed: ______ Verified: ______

Initial Viability and Concentration Determination of Glochidia

Mussel Species: ______ **File/Folder Path:** ______

Dosing Date: ______ **Formulation and lot #:** ______

A	B	C	D	E	F	G	H	I		
Mussel #	Sample Volume (μL)	Photomicrograph Record ID	Total Number Glochidia Counted	Closed Glochidia Counted (Before NaCl)	Open Glochidia Counted (After NaCl)	Number Glochidia per mL (D/B*1000)	Percent Viability ([D-(E+F)]/D) *100	Date	Time (military)	Initials

This datasheet was approved by [redacted] on 12 JAN 2012.
11JAN2012/version1.1

Study Number AEH-11-PSEUDO-01

File Folder: __________ Lab book/pgs: __________ Reviewed: __________ Verified: __________

Water Quality – Form 1

Initial (Dilution Water Hardness, Alkalinity, and Temperature)

Date/Time Containers Filled: ______________________ **Initials:** __________

Mussel Species: ______________ **Block ID:** ____________

Dosing Date: ______________ **Formulation and lot #:** ______________

Instruments: ______________________

Rep #	Hardness (mg/L $CaCO_3$)	Alkalinity (mg/L $CaCO_3$)	Temperature (°C)	Conductivity (µS)	Date	Time (military)	Initials
1							
2							
3							
Mean							

This datasheet was approved by [redacted] on 17 JAN 2012
11JAN2012/Version 1.1

Study Number AEH-11-PSEUDO-01

File Folder: ___________ Lab book/pgs: ___________ Reviewed: ___________ Verified: ___________

Water Quality – Form 2

During Exposure (Dissolved Oxygen, pH, Temperature)

Mussel Species: ______________________ **Block ID:** ______________________

Dosing Date: ____________________ **Formulation and lot #:** ______________________

Instruments: ____

Chamber ID	Dissolved Oxygen (mg/L)	pH	Temperature (°C)	Date	Time (military)	Initials

Light Measurement	Block Number	Date	Time (military)	Initials

Note: Dissolved oxygen, pH, temperature, and light intensity will be measured at least twice during the exposure period in the controls and high concentrations.

11JAN2012/vs1.2
This datasheet was approved by [redacted] on 12 JAN 2012.

Study Number AEH-11-PSEUDO-01

File Folder: ___________ Lab book/pgs: ___________ Reviewed: ___________ Verified: ___________

Water Quality – Form 3

Upon Termination (Hardness, Alkalinity, Conductivity, Temperature, and pH)

Mussel Species: ________________ **Block ID:** ________________

Dosing Date: ________________ **Formulation and lot #:** ________________

Instruments: ________________________________

Pooled Sample Concentration	Chamber IDs	Hardness (mg/L $CaCO_3$)	Alkalinity (mg/L $CaCO_3$)	Conductivity (µS/cm)	Date	Time (military)	Initials

Note: Hardness, alkalinity, and conductivity will be measured upon termination of trial from pooled replicate samples.

Chamber ID	Temperature (°C)	pH	Date	Time (military)	Initials

Note: Temperature and pH will be measured upon termination of trial from each individual test chamber. These values will be used for ammonia analysis.

13JAN2012/vs1.2

This datasheet was approved by [redacted] on 17JAN2012.

Study Title: "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven different unionid species"

Study number: AEH-11-PSEUDO-01

File Folder:______ Lab book/pgs:______ Reviewed:_______ Verified:________

Test Chemical Stock Preparation Data Form

Test Chemical: *Pseudomonas fluorescens* strain 145A

Test Chemical Lot #____________Date Rec'd_____________ Exp. Date______________

Mussel Species __________________ Block ID _______________

Instruments: ____

Chemical Weighing:

Sample I.D.	Sample wt. (g)	Date/Time	Initials

*Chemical samples to be stored refrigerated until used for stock preparation.

Stock Preparation:

Sample I.D.	Dilution Vol. (ml)	Dilution time	Use (ie: Active stock for HGE)	Date/ Time	Initials

*Stocks to be prepared immediately before use, except for heat deactivated stock which will be prepared prior to use to allow for deactivation and cooling.

This datasheet was approved by [redacted]________ on 12 JAN 2012.
11JAN2012/version 1.1

Study Number AEH-11-PSEUDO-01
Reviewed: ________________
Verified: ________________

File Folder: ________________
Lab book/pgs: ________________
Species: ________________
Date of Dosing: ________________

Chemical Stock Solution Determination and Preparation

Formulation and lot #: ________________

Stock A = Control (20 °C well water)

Stock B = 10,000 mg/L active material (2.0 g/100 mL product = 1.0 g/100 mL active ingredient)

Stock C = 10,000 mg/L detoxified active material (2.0 g/100 mL product = 1.0 g/100 mL active ingredient - Heat deactivated for 45 minutes at 70°C/cooled to 20 °C prior to use)

Dosage Level (mg/L)	Exposure Volume (mL)	Deactivated mg Required	Active mg required	*Well Water* Aliquot Stock A (mL)	*Active Stock* Aliqot Stock B (mL)	*Deactive Stock* Aliquot Stock C (mL)	Color Code Assignment
0	100	0	0	3.0	0.0	0.0	White
300 HD	100	30	0	0.0	0.0	3.0	White/black stripes
50	100	0	5	2.5	0.5	0.0	Yellow
100	100	0	10	2.0	1.0	0.0	Yellow/black stripes
200	100	0	20	1.0	2.0	0.0	Blue
300 Active	100	0	30	0.0	3.0	0.0	Blue/black stripes
Total per rep					8.5	6.5	3.0
Total per species					25.5	19.5	9.0
Total per 3 species					76.5	58.5	27.0

Concentration	Stock Preperation Time	Time Dosing Started	Time Dosing Completed	Date	Initials
0 mg/L					
300 mg/L Deactive					
50 mg/L					
100 mg/L					
200 mg/L					
300 mg/L Active					

13JAN2012/vs1.2

This datasheet was approved by [redacted] on 17 JAN 2012.

Study Number AEH-11-PSEUDO-01

File Folder: ___________ Lab book/pgs: ___________ Reviewed: ___________ Verified: ___________

Pipette Calibration

Pipette Brand/Model/SN: ______________________________ **Tip Type:** __________________

Balance: ____________________________ **Thermometer:** ____________________________

Temperature: ____________________ **Water Density[1] (g/mL):** ____________________________

Pipette Setting (µL)	Rep #	Water Mass (g)	Date	Time (military)	Initials
	1				
	2				
	3				
	4				
	5				
	6				
	7				
	8				
	9				
	10				
	Mean				
	Standard Deviation				

Relative Standard Deviation (Standard Deviation / Mean *100): ______________________________

Pass or Fail (Pass = Relative Standard Deviation ≤ 1%): ______________________________

Pipette Volume (Mean mass / Water Density[1]): ______________________________

Note: Pipette calibration must be performed with deionized water left to adjust to ambient room temperature for at least 24 hours prior to calibration.

[1] Water density (g/mL) at varying temperatures: 18°C: 0.9985976; 19°C: 0.9984073; 20°C: 0.9982063; 21°C: 0.9979948; 22°C:0.9977730.

This datasheet was approved by [redacted] on 12 JAN 2012.

11JAN2012/version1.1

Study Title: "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven different unionid species"

Study number: AEH-11-PSEUDO-01

Approved by [redacted] Date 10-18-11

File Folder:_____ Lab book/pgs:_____ Proofed:_____ Reviewed:_____

Test Chemical Stock Preparation Data Form

Test Chemical: *Pseudomonas fluorescens* strain 145A

Test Chemical Lot #________ Date Rec'd________ Exp. Date________

Chemical Weighing:

Sample I.D.	Sample wt. (g)	Date/Time	Initials

*Chemical samples to be stored refrigerated until used for stock preparation.

These forms were revised from 12 JAN 2012 to 18 JAN 2012 and will no longer be used. KLW 23 JAN 2012

Stock Preparation:

Sample I.D.	Dilution Vol. (ml)	Dilution time	Use (ie: Active stock for HGE)	Date/ Time	Initials

*Stocks to be prepared immediately before use, except for heat deactivated stock which will be prepared at least 1 hr prior to use to allow for deactivation and cooling.

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Study Number AEH-11-PSEUDO-01

File Folder: ___________ Lab book/pgs: ___________ Proofed: ___________ Reviewed: ___________

Water Quality – Form 2

During Exposure (Dissolved Oxygen, pH, Temperature)

Mussel Species: ______________________ **Block ID:** ______________________

Sample ID	Dissolved Oxygen (mg/L)	pH	Temperature (°C)	Date	Time (military)	Initials

Light Measurement	Block Number	Date	Time (military)	Initials

Note: Dissolved oxygen, pH, and temperature will be measured at least twice during the exposure period in the controls and high concentrations.

Approved by: [redacted] Date: 10/17/11

17OCT2011/vs1.1

Pg 2 **of** 8

Study Number AEH-11-PSEUDO-01
Approved by: [redacted] Date: 10/17/11

Species Dosed: ____________
Date of Dosing: ____________

Chemical Stock Solution Determination

Stock A = Control (20 °C well water); **Stock B** = 10,000 mg/L active material (1.0g/100 mL product = 10.0mg/mL active ingredient);
Stock C = 10,000 mg/L detoxified active material (1.0g/100 mL - Heat deactivated for 45 minutes at 70°C/cooled to 20 °C prior to use

Dosage Level (mg/L)	Dose concentration	Exposure Volume (mL)	Deactivated mg Required	Active mg required	*Well Water* Aliquot Stock A (mL)	*Active Stock* Aliqot Stock B (mL)	*Deactive Stock* Aliquot Stock C (mL)	Color Code Assignment
0	control (well water blank)	100	0	0	3.0	0.0	0.0	White
300	mg/L (Deactivated)	100	30	0	0.0	0.0	3.0	White/black stripes
50	mg/L	100	0	5	2.5	0.5	0.0	Yellow
100	mg/L	100	0	10	2.0	1.0	0.0	Yellow/black stripes
200	mg/L	100	0	20	1.0	2.0	0.0	Blue
300	mg/L	100	0	30	0.0	3.0	0.0	Blue/black stripes
Total					8.5	6.5	3.0	
Total per species					25.5	19.5	9.0	
Total per 3 species					76.5	58.5	27.0	

Concentration	Time Dosing Started	Time Dosing Completed	Date	Initials
0 mg/L				
300 mg/L Deactive				
50 mg/L				
100 mg/L				
200 mg/L				
300 mg/L Active				

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Study Number AEH-11-PSEUDO-01

File Folder: ___________ Lab book/pgs: ___________ Proofed: ___________ Reviewed: ___________

Water Quality – Form 1

Initial (Dilution Water Hardness, Alkalinity, and Temperature)

Date/Time Containers Filled: ______________________ **Initials:** ___________

Rep #	Hardness (mg/L $CaCO_3$)	Alkalinity (mg/L $CaCO_3$)	Temperature (°C)	Date	Time (military)	Initials
1						
2						
3						
Mean						

Note: Hardness, alkalinity, and temperature will be measured prior to test initiation from well water aerated and allowed to acclimate to room temperature for at least 24 hours prior to use.

Approved by: [redacted] Date: 5-11-11

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Study Number AEH-11-PSEUDO-01

File Folder: ___________ Lab book/pgs: ___________ Proofed: ___________ Reviewed: ___________

Water Quality – Form 3

Upon Termination (Hardness, Alkalinity, Conductivity, Temperature, and pH)

Mussel Species: _______________ **Block ID:** _______________

Pooled Sample Concentration	Sample IDs	Hardness (mg/L $CaCO_3$)	Alkalinity (mg/L $CaCO_3$)	Conductivity (µS/cm)	Date	Time (military)	Initials

Note: Hardness, alkalinity, and conductivity will be measured upon termination of trial from pooled replicate samples.

Sample ID	Dissolved Oxygen (mg/L)	Temperature (°C)	pH	Date	Time (military)	Initials

Note: D.O., temperature and pH will be measured upon termination of trial from each individual test chamber for ammonia analysis.

Approved by: [redacted] Date: 10/17/11

17OCT2011/vs1.1

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Study Number AEH-11-PSEUDO-01

File Folder: ___________ Lab book/pgs: ___________ Proofed: ___________ Reviewed: ___________

Pipette Calibration

Pipette Brand/Model/SN: ______________________________ **Tip Type:** ________________

Balance: ____________________________ **Thermometer:** ____________________________

Temperature: ____________________ **Water Density[1] (g/mL):** ____________________________

Pipette Setting (μL)	Rep #	Water Mass (g)	Date	Time (military)	Initials
	1				
	2				
	3				
	4				
	5				
	6				
	7				
	8				
	9				
	10				
	Mean				
	Standard Deviation				

Relative Standard Deviation (Standard Deviation / Mean *100): ______________________________

Pass or Fail (Pass = Relative Standard Deviation ≤ 1%): ______________________________

Pipette Volume (Mean mass / Water Density[1]): ______________________________

Note: Pipette calibration must be performed with deionized water left to adjust to ambient room temperature for at least 24 hours prior to calibration.

[1] Water density (g/mL) at varying temperatures: 18°C: 0.9985976; 19°C: 0.9984073; 20°C: 0.9982063; 21°C: 0.9979948; 22°C:0.9977730.

Approved by: [redacted]__________ Date: 5-9-11

Pg 6 of 18

Study Title: "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"

Study number: AEH-11-PSEUDO-01

Approved by [redacted]____________ Date 5-5-11

File Folder:______ Lab book/pgs:______ Proofed:______ Reviewed:________

Donor Mussel UMESC Lot Number Assignment Form

DONOR MUSSEL INFORMATION:

Species:____

Number of Donor Mussels:________ **Collection Date:**______________________

Collection Location:______________________________________

Species Identification performed by:_______________________

Title/affiliation of identifier:______________________________

UMESC Arrival Date:_______________________________________

Receiving UMESC tank/room #_______________________________

Additional information:

____

____

UMESC LOT NUMBER DESIGNATION:________________________

Witness and form recorded by:

		UMESC	
Printed Name	**Signature**	**Affiliation**	**Date**

Pg 7 **of** 18

Study Title: "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"

Study number: AEH-11-PSEUDO-01

Approved by [redacted]____________ Date 5-5-11________

File Folder:______ Lab book/pgs:______ Proofed:_______ Reviewed:________

Glochidia Test Organism UMESC Lot Number Assignment Form

Species:____

Number of Donor Mussels:________ **Donor Mussel Lot #:**__________________________

Extraction Date:____/_____/201__ **Extraction Time (military)**______________________

Extraction Location:____UMESC rm 15_____ **Container ID:**_________________________

Approximate Number of glochidia:___________________

Glochidia extracted by:________________________ **Affiliation:**______________________

Additional information:

____

____

____

UMESC GLOCHIDIA LOT NUMBER DESIGNATION:___________________________

Witness and form recorded by:

		UMESC	
Printed Name	**Signature**	**Affiliation**	**Date**

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Study Title: "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"

Study number: AEH-11-PSEUDO-01

Approved by [redacted]________ Date 5-5-11

File Folder:______ Lab book/pgs:______ Proofed:______ Reviewed:________

Glochidia Exposure Dosing Form

Species:_________________________________ **UMESC lot number:**___________

Test Block Assignment (circle one):__1____2____3____

Estimated # glochidia/Chamber____________

Date/time (military) of dosing initiation:_________________________________

Date/time (military) of dosing completion:_________________________________

Additional information:

____

____

____

____

____

____

____

Witness and form recorded by:

		UMESC	
Printed Name	**Signature**	**Affiliation**	**Date**

Pg 9 **of** 18

Study Title: "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"

Study number: AEH-11-PSEUDO-01

Approved by [redacted] Date 5-5-11

File Folder:______ Lab book/pgs:______ Proofed:______ Reviewed:_______

Glochidia Aliquot Distribution Form

Species:______________________________ **UMESC LOT NUMBER:**____________

Test Block Assignment (circle one): __1____2____3____

Aliquot Volume (µl):____________ **Estimated # glochidia/aliquot**____________

Initial Distribution aliquot date/time (military):______________________________

Final Distribution Aliquot date/time (military):______________________________

Number of Aliquot rounds:__________ **Estimated # glochidia/chamber**________________

Additional information:

____

____

____

____

____

____

____

Witness and form recorded by:

		UMESC	
Printed Name	**Signature**	**Affiliation**	**Date**

Pg 10 **of** 18

Study Number AEH-11-PSEUDO-01

File Folder: ___________ Lab book/pgs: ___________ Proofed: ___________ Reviewed: ___________

Daily Care Worksheet

Tank #: ___________ **Room #:** ___________ **Month/Year:** ___________
Species: ___________
Lot Number: ___________

Day	Feed Time (military)	Flow Rate (mL/min)		Temperature (°C)	Dissolved Oxygen (mg/L)	pH	Initials
		Inflow	Pump				
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							
15							
16							
17							
18							
19							
20							
21							
22							
23							
24							
25							
26							
27							
28							
29							
30							
31							

Daily Food Prep: Mix 44.0g Shellfish diet, 35.3g *Tetraselmis*, and 92.4g *Thalassiosira weissflogii* with 14,400 mL well water each day, to be delivered by peristaltic pump at 10 mL per minute. Target water inflow rate is 1,100 mL/min. Prepare fresh diet daily.

Approved by: [redacted] Date: 5-9-11 **Pg** 11 **of** 18

Study Number AEH-11-PSEUDO-01

File Folder: __________ Lab book/pgs: __________ Proofed: __________ Reviewed: __________

Water Quality – Form 2

During Exposure (Dissolved Oxygen, pH, Temperature)

Mussel Species: ______________________________ **Block ID:** ____________________

Sample ID	Dissolved Oxygen (mg/L)	pH	Temperature (°C)	Date	Time (military)	Initials

Note: Dissolved oxygen, pH, and temperature will be measured at least twice during the exposure period in the controls and high concentrations.

Approved by: [redacted] Date: 5-11-11

Pg 12 **of** 18

Study Number AEH-11-PSEUDO-01

File Folder: ______ Lab book/pgs: ______ Proofed: ______ Reviewed: ______

Water Quality – Form 3

Upon Termination (Hardness, Alkalinity, Conductivity, and Ammonia)

Mussel Species: ______ **Block ID:** ______

Pooled Sample Concentration	Sample IDs	Hardness[1] (mg/L $CaCO_3$)	Alkalinity[1] (mg/L $CaCO_3$)	Conductivity[2] (µS/cm)	Ammonia[2] (mg/L)	Date	Time (military)	Initials

Note: Water quality parameters will be measured upon termination of trial from pooled replicate samples.
1 Hardness and alkalinity will be taken from the control and high concentration.
2 Conductivity and ammonia will be taken from all concentrations.

Approved by: [redacted] Date: 5-11-11

Pg 13 of 18

Study Number AEH-11-PSEUDO-01

File Folder: ______________ Lab book/pgs: ______________ Proofed: ______________ Reviewed: ______________

Initial Viability and Concentration Determination of Glochidia

Mussel Species: ______________ **File/Folder Path:** ______________

A	B	C	D	E	F	G	H	I		
Mussel #	Sample Volume (µL)	Photomicrograph Record ID	Total Number Glochidia Counted	Closed Glochidia Counted (Before NaCl)	Open Glochidia Counted (After NaCl)	Number Glochidia per mL (D/B*1000)	Percent Viability ([D-(E+F)]/D) *100	Date	Time (military)	Initials

Approved by: [redacted] Date: 5-11-4

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Study Number AEH-11-PSEUDO-01

File Folder: ________ Lab book/pgs: ________ Proofed: ________ Reviewed: ________

Glochidia Photomicrograph Counting Record

Mussel Species: ________ **Sample Period (h post exposure):** ________

Photomicrograph Record ID	Photomicrograph Capture Time (military)	Total Glochidia Counted	Glochidia Count Before NaCl Solution			Glochidia Count After NaCl Solution			Date	Initials
			No. Counted	Circle One[1]		No. Counted	Circle One[1]			
				Open	Closed		Open	Closed		
				Open	Closed		Open	Closed		
				Open	Closed		Open	Closed		
				Open	Closed		Open	Closed		
				Open	Closed		Open	Closed		
				Open	Closed		Open	Closed		
				Open	Closed		Open	Closed		
				Open	Closed		Open	Closed		
				Open	Closed		Open	Closed		
				Open	Closed		Open	Closed		
				Open	Closed		Open	Closed		
				Open	Closed		Open	Closed		
				Open	Closed		Open	Closed		
				Open	Closed		Open	Closed		
				Open	Closed		Open	Closed		
				Open	Closed		Open	Closed		
				Open	Closed		Open	Closed		
				Open	Closed		Open	Closed		

[1] Technician counting glochidia photomicrograph will select either open or closed glochidia, whichever is less, and circle the corresponding selection for each sample.

Approved by: [redacted] Date: 5-11-11

Pg 15 of 18

Study Number AEH-11-PSEUDO-01

File Folder: ____________ Lab book/pgs: ____________ Proofed: ____________ Reviewed: ____________

Water Quality – Form 3

Upon Termination (Hardness, Alkalinity, Conductivity, Temperature, pH, %T, and Total Ammonia Nitrogen)

Mussel Species: ____________ **Block ID:** ____________

Pooled Sample Concentration	Sample IDs	Hardness[1] (mg/L $CaCO_3$)	Alkalinity[1] (mg/L $CaCO_3$)	Conductivity[2] (µS/cm)	Temperature[2] (°C)	pH[2]	Percent Transmittance[2] (%T)	Total Ammonia Nitrogen[3] (mg/L)	Date	Time (military)	Initials

Note: Water quality parameters will be measured upon termination of trial from pooled replicate samples.
1 Hardness and alkalinity will be taken from the control and high concentration.
2 Conductivity, temperature, pH, and percent transmittance will be taken from all concentrations.
3 Follow procedure in SOP AEH 301.0 to calculate total ammonia nitrogen from %T. Additionally, SOP AEH 301.0 contains a procedure for calculating un-ionized ammonia from temperature, pH, and total ammonia nitrogen.

Approved by: [redacted] Date: 5-13-11

Pg 16 **of** 18

Study Title: "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"

Study number: AEH-11-PSEUDO-01

Approved by [redacted]______________ Date 5-5-11

File Folder:______ Lab book/pgs:______ Proofed:______ Reviewed:______

Donor Mussel UMESC Lot Number Assignment Form

DONOR MUSSEL INFORMATION:

Species:____

Number of Donor Mussels:________ **Collection Date:**________________________

Collection Location:____

Species Identification performed by:________________________________

Title/affiliation of identifier:________________________________

UMESC Arrival Date:____

Receiving UMESC tank/room #________________________________

Additional information:

____

____

UMESC LOT NUMBER DESIGNATION:________________________________

Witness and form recorded by:

		UMESC	
Printed Name	**Signature**	**Affiliation**	**Date**

Pg 17 of 18

Study Title: "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"

Study number: AEH-11-PSEUDO-01

Approved by [redacted] Date 5-5-11

File Folder:_____ Lab book/pgs:_____ Proofed:_____ Reviewed:_____

Glochidia Test Organism UMESC Lot Number Assignment Form

Species:_____

Number of Donor Mussels:_____ **Donor Mussel Lot #:**_____

Extraction Date:___/___/201__ **Extraction Time (military)**_____

Extraction Location:___UMESC rm 15___ **Container ID:**_____

Approximate Number of glochidia:_____

Glochidia extracted by:_____ **Affiliation:**_____

These forms were revised 12 JAN 2012 to 18 JAN 2012 and will no longer be used. KLW 23 JAN 2012

Additional information:

UMESC GLOCHIDIA LOT NUMBER DESIGNATION:_____

Witness and form recorded by:

Printed Name	Signature	Affiliation	Date
		UMESC	

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Appendix 2. Deviations from the Study Protocol

United States Department of the Interior

U.S. GEOLOGICAL SURVEY
Biological Resources Division
Upper Midwest Environmental Sciences Center
2630 Fanta Reed Road
La Crosse, Wisconsin 54603

ORIGINAL

Item No. 6

FOLDER NO. 3

MEMORANDUM

Date: June 3, 2011
To: The Record Study Number AEH-11-PSEUDO-01

Subject: Deviations 1 and 2 to study AEH-11-PSEUDO-01 "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"

Deviation #1 – Sections 4.2 and 6.2 (Experimental Design and Determination of viability) state that the viability will determined on subsamples of approximately 100 glochidia taken from each exposure chamber at 6, 12 and 24 h post exposure. After completing the 24-h viability assessments for Block 1 (*Lampsilis cardium*) the test replicates for each concentration were pooled for the final water quality measurements. A control chamber (2B1) from Block 2 (*L. higginsii*) was inadvertently pooled with the control chambers from Block 1. Because chamber 2B1 was pooled with the control chambers from Block 1, a glochida sample was not taken for this one control replicate at 24-h post exposure to assess *L. higginsii* viability in that one control replicate. The remaining two *L. higginsii* controls (chambers 2C3 and 2C6) were sampled to assess glochida viability at 24-h post exposure according to the study protocol except that two ~100-µL aliquots were collected per replicate and the glochida viability recorded in each aliquot (two separate glochida viability determinations per control replicate).

Deviation #2 – Section 5.2 (Water Chemistry) of the study protocol specifies that water remaining in the replicates will be pooled after glochidia aliquots are taken to determine glochidia viability at 24-h post exposure. *Lampsilis higginsii* control replicate chamber 3B1 ① was inadvertently combined with the *L. cardium* control chambers. Therefore, the water quality measurements at 24-h post exposure for *L. cardium* were determined in a water sample of which a portion was derived from the L. higginsii control. The water quality measurements for the *L. higginsii* controls were made in a water sample which only contained water from control replicate chambers 2C3 and 2C6.

The impact of these deviations to the outcome of the study will not be fully known until the glochidia viability data are analyzed. Therefore, an assessment of the impact of this deviation will be rendered in the final study report.

[redacted]
James A. Luoma, B.A.
Study Director, UMESC

6/3/11
Date

① Typographical error - the chamber is 2B1 3-16-15 JAL

PROOFED BY
Initials: JMS Date: 2/6/12
REVIEWED BY
Initials: ____ Date: ____

cc: UMESC QAU

United States Department of the Interior

U.S. GEOLOGICAL SURVEY
Biological Resources Division
Upper Midwest Environmental Sciences Center
2630 Fanta Reed Road
La Crosse, Wisconsin 54603

ORIGINAL

Item No. 7

FOLDER NO. 3

MEMORANDUM

Date: June 3, 2011
To: The Record Study Number AEH-11-PSEUDO-01

Subject: Deviation 3 to study AEH-11-PSEUDO-01 "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"

Deviation #3 – Sections 4.2 and 6.2 (Experimental Design and Determination of Viability) of the amended protocol states that the viability of glochidia from each mussel will be assessed by "drawing aliquots (e.g. 100 µL) of settled glochidia from the stock container with an adjustable manual pipet fitted with a wide bore tip and placing it on a pre-labeled 35mm petri dish. The sample will be diluted with a known and consistent volume (e.g. 200 µL) of acclimated well water to enhance dispersion of glochidia for more accurate enumeration. The sample will be viewed under a dissecting microscope, adjusted for maximum clarity and magnification, and a photomicrograph will be recorded. Approximately one drop of a saturated sodium chloride solution (12 g NaCl/ 50 mL of D.I. H_2O [ASTM E2455-06]) will be added to the sample and after one minute a second photomicrograph will be recorded. Enumerations will be conducted for the total number of glochidia within the sample and the number of glochidia closed or open before and after sodium chloride addition. The percentage of viable glochidia (glochidia that respond to sodium chloride exposure by valve closure) will be calculated by the following method:

> Percent viable = ([Total number of glochidia in sample – {total number of glochidia closed before addition of sodium chloride + number of glochidia open after sodium chloride addition}]/Total number of glochidia in sample) * 100

If viability is acceptable (≥80%) for a mussel, the glochidia from that mussel will be pooled with glochidia from other mussels of that species."

The procedures as described in the protocol were followed except mussels that were observed to have high viability, the exact viability percentage was not determined prior to allowing the glochidia into the study. For these mussels a preliminary visual observation was used to determine acceptable viability. Only two L. higginsii mussels appeared to have viability near the threshold limit of 80% viability. For these mussels a preliminary estimation of the viability using the formula in the protocol showed that the viability was greater than the minimum 80% for acceptance.

The methods described were used to substantially reduce the period from glochidia extraction until use in the study. No negative impacts to the study are anticipated as the exact viability of the glochidia will be determined from the photomicrograph records, viability ≥ 80% for all but two mussels was easily identified by visual observation and the two higginsii mussels with somewhat reduce viability were determined to have acceptable viability by using the formula described in the protocol. The calculations were preliminary and not recorded, however they did indicate greater than 80% viability.

[redacted] 6/3/11

James A. Luoma, B.A. — Date
Study Director, UMESC

cc: UMESC QAU

PROOFED BY
Initials: JMS Date: 2/6/12
REVIEWED BY
Initials: ____ Date: ____

ORIGINAL

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U.S. GEOLOGICAL SURVEY
Biological Resources Division
Upper Midwest Environmental Sciences Center
2630 Fanta Reed Road
La Crosse, Wisconsin 54603

Item No. 8

FOLDER NO. 3

MEMORANDUM

Date: June 7, 2011
To: The Record Study Number AEH-11-PSEUDO-01

Subject: Deviation 4 to study AEH-11-PSEUDO-01 "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"

Deviation #4 – UMESC SOP GEN 012.2 section C, 3 & 4, specify that " the chemical removed from the test chemical container will be weighed into a tared vessel and the weight of the chemical mass recorded in the Test Chemical Use Log (Form GEN 012.2b)." and "After the chemical has been removed from the test chemical container (including the cap or lid) re-weigh the container and its contents and record the weight in the use log".

When the test chemical was removed from the container, four separate aliquots of ~ 2.0 grams each were weighed into separate tared 50-mL beakers. The exact weight of test material weighed into each beaker was recorded on a piece of laboratory tape fixed to the beaker. At the conclusion of the all samples being removed the container was re-weighed and a single entry was placed into the chemical use logbook. The original tapes (containing the exact chemical weights) affixed to the beakers were retained and were filed in file folder 8 of the study records along with a description and time of their use. Additionally, an entry explaining the procedures used were recorded in the chemical logbook and laboratory notebook.

No adverse impacts to the study are anticipated as a result of this deviation.

[redacted]

James A. Luoma, B.A.
Study Director, UMESC

6/7/11
Date

PROOFED BY
Initials: TMS Date: 2/6/12
REVIEWED BY
Initials ____ Date: ____

cc: UMESC QAU

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U.S. GEOLOGICAL SURVEY
Upper Midwest Environmental Sciences Center
2630 Fanta Reed Road
La Crosse, Wisconsin 54603

Item No. 9

FOLDER NO. 3

MEMORANDUM

Date: January 11, 2012

To: The Record Study Number AEH-11-PSEUDO-01

Subject: Deviation 5 to study AEH-11-PSEUDO-01 "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"

Deviation #5 – Sections 4.2 and 6.2 (Experimental Design and Determination of viability) of the amended protocol (see Amendment 3) state that "approximately one drop of saturated sodium chloride solution (12g NaCl/50 mL of D.I. H_2O [ASTM E2455-06]) will be added to the sample and after one minute a second photomicrograph will be recorded. The total number of glochidia within the sample and the number of glochidia closed or open before and after sodium chloride addition will be determined". Due to the large quantity of samples to be processed, samples were placed on the microscope stage and photographed to enumerate for viable glochidia (i.e., those with open valves). One drop of a saturated sodium chloride solution was added to the sample and a timer was started. The sample was then removed from the microscope stage and replaced with the next sample. Once the minute was over, the original sample exposed to the saturated sodium chloride solution was again placed on the microscope staged and photographed to enumerate for viable glochidia (i.e., those with closed valves). This process was used for the initial viability, distribution, 6, 12, and 24 hour viability determinations.

The method described was only used for samples processed on May 12-13, 2011. No negative impacts to the study are anticipated as each sample was re-photographed at the one minute post-exposure to saturated sodium chloride. The photographs are not aligned in the same way from before salt to after salt, but will have no impact on enumeration.

[redacted] 11 JAN 2012
Written by / Date
Kerry L. Weber, M.S.
Principal Investigator, UMESC

[redacted] 11 JAN 2012
Approved by / Date
James A. Luoma, B.A.
Study Director, UMESC

PROOFED BY
Initials: JMS Date 2/6/12
REVIEWED BY
Initials: ____ Date: ____

cc: UMESC QAU

Page 1 of 1

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ORIGINAL

U.S. GEOLOGICAL SURVEY
Upper Midwest Environmental Sciences Center
2630 Fanta Reed Road
La Crosse, Wisconsin 54603

Item No. 10

FOLDER NO. 3

MEMORANDUM

Date: January 11, 2012

To: The Record Study Number AEH-11-PSEUDO-01

Subject: Deviation 6 to study AEH-11-PSEUDO-01 "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"

Deviation #6 – Sections 4.2 and 6.2 (Experimental Design and Determination of viability) of the amended protocol (see Amendment 3) state that "if glochidia can be enumerated and viability statues (i.e. open or closed) reliably determined through photomicrograph records (i.e. the glochidia remain on a single focal plane)" then the viability of the subsample of approximately 100 glochidia from each chamber will be assessed at 6, 12, and 24 h post exposure by enumeration through a dissection microscope. The 24 h control dosing (0 mg/L) photographs taken on May 12, 2011 for Plain pocketbook (*Lampsilis cardium*), Higgins eye (*Lampsilis higginsii*) and Fatmucket (*Lampsilis siliquoidea*) were enumerated on May 16, 2011 using Image Pro Software. The new Nikon NIS-Elements BR imaging software was received and installed after this enumeration occurred. Therefore, the samples were recounted on August 10-16, 2011 using the new software. The original enumerations from Image Pro were retained as .jpg images. The enumerations using the Nikon NIS-Elements BR software were saved as .tif files. All count data from both enumerations were recorded on the Glochidia Photomicrograph Counting Record datasheet (File Folder 12C) and within the electronic log (pages 1 to 24 of I://AEH-11-PSEUDO-01/Pictures/12May11/24h). The count data from the Nikon NIS-Elements BR software were used during analysis.

Samples that were re-counted using the Nikon NIS-Elements BR software include:

Plain Pocketbook	Higgins Eye	Fatmucket
1A3-24h-1	2C3-24h	3A2-24h
1A3-24h-2	2C3-24h-R2	3B2-24h
1B1-24h	2C6-24h	3B3-24h
1C3-24h	2C6-24-R2	

No negative impacts to the study are anticipated. The images analyzed with Nikon BR Elements [redacted] which glochidia are included in the dataset.

[redacted] 11 JAN 2012
Written by — Date
Kerry L. Weber, M.S.
Principal Investigator, UMESC

[redacted] 11 JAN 2012
Approved by — Date
James A. Luoma, B.A.
Study Director, UMESC

PROOFED BY
Initials: JMS Date: 2/6/12
REVIEWED BY
Initials: ____ Date: ____

cc: UMESC QAU

Page 1 of 1

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U.S. GEOLOGICAL SURVEY
Upper Midwest Environmental Sciences Center
2630 Fanta Reed Road
La Crosse, Wisconsin 54603

Item No. 11

FOLDER NO. 3

MEMORANDUM

Date: January 11, 2012

To: The Record Study Number AEH-11-PSEUDO-01

Subject: Deviation 7 to study AEH-11-PSEUDO-01 "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"

Deviation #7 – Section 6.2 (Determination of viability) of the amended protocol (see Amendment 3) states that "the total number of glochidia within the sample and the number of glochidia closed or open before and after sodium chloride addition will be determined." Samples taken on May 12, 2011 for Plain pocketbook (*Lampsilis cardium*), Higgins eye (*Lampsilis higginsii*) and Fatmucket (*Lampsilis siliquoidea*) were enumerated from August 10 to 22, 2011 in this manner, which is prior to the written amendment. The samples analyzed following this procedure can be found in Table 1 on page 2 of this deviation.

No negative impacts to the study are anticipated as an official amendment was approved for this procedure after the fact.

[redacted] 11 JAN 2012

Written by
Kerry L. Weber, M.S.
Principal Investigator, UMESC

Date

[redacted] 11 JAN 2012

Approved by
James A. Luoma, B.A.
Study Director, UMESC

Date

PROOFED BY
Initials: TMS Date: 3/6/12
REVIEWED BY
Initials ____ Date: ____

cc: UMESC QAU

Page 1 of 2

ORIGINAL

AEH-11-PSEUDO-01

Table 1. Samples analyzed using modified enumeration procedure outlined in amended protocol (see Amendment 3)

	Plain Pocketbook	***Higgins Eye***	***Fatmucket***
12 hour samples	1A2-12h	2A5-12h	3A4-12h
	1A5-12h	2A6-12h	3A6-12h
	1A6-12h	2B4-12h	3B1-12h
	1B3-12h	2B6-12h	3B4-12h
	1B6-12h		3C1-12h
	1C2-12h		3C2-12h
	1C5-12h		3C3-12h
	1C6-12h		3C4-12h
24 hour samples	1A5-24h	2A5-24h	3A4-24h
	1A6-24h	2B3-24h	3A5-24h
	1B3-24h	2B5-24h	3A6-24h
	1B6-24h	2B6-24h	3B1-24h
	1C2-24h	2C1-24h	3B4-24h
	1C5-24h		3B6-24h
			3C1-24h
			3C2-24h
			3C4-24h

Item No. 11

FOLDER NO. 3

Approved [redacted] 11JAN2012

PROOFED BY
Initials: JMS Date: 2/6/12
REVIEWED BY
Initials ____ Date: ____

cc: UMESC QAU

Page **2** of **2**

ORIGINAL

United States Department of the Interior

U.S. GEOLOGICAL SURVEY
Upper Midwest Environmental Sciences Center
2630 Fanta Reed Road
La Crosse, Wisconsin 54603

Item No. 12

FOLDER NO. 3

MEMORANDUM

Date: January 11, 2012

To: The Record Study Number AEH-11-PSEUDO-01

Subject: Deviation 8 to study AEH-11-PSEUDO-01 "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"

Deviation #8 – Sections 4.2 and 6.2 (Experimental Design and Determination of viability) of the amended protocol (see Amendments 2 and 3) state that "if glochidia can be enumerated and viability status (i.e. open or closed) reliably determined through photomicrographic records (i.e. the glochidia remain on a single focal plane" then "the sample will be viewed under a dissecting microscope, adjusted for maximum clarity and magnification, and a photomicrograph will be recorded". Photographs taken on May 13, 2011 were saved as .jpg files. During a training session, it was brought to our attention that compression inherent to .jpg files actually discards information associated with images. Over time, the loading and saving of .jpg images can cause enough information to be lost as to actually degrade the image. Therefore, the .jpg photographs taken on May 13, 2011 were converted to a .tif file on August 3, 2011, which were then enumerated from August 9-22, 2011. During the conversion process and/or enumeration, 5 of the photographs were inadvertently over-written. Two of the over-written photographs were restored by Martin Tagesen using the network back-up while three of the over-written photographs were converted back to a .tif file from the original .jpg file. See the Table 1 on page 2 of this deviation for detailed information.

No negative impacts to the study are anticipated as the .tif files are have greater integrity and each photograph was restored to its original file before analysis was conducted.

[redacted] 11JAN2012
Written by — Date
Kerry L. Weber, M.S.
Principal Investigator, UMESC

[redacted] 11JAN2012
Approved by — Date
James A. Luoma, B.A.
Study Director, UMESC

PROOFED BY
Initials: JMS Date: 2/6/12
REVIEWED BY
Initials ____ Date ____

cc: UMESC QAU

Page 1 of 2

ORIGINAL

AEH-11-PSEUDO-01

Table 1. Detailed information regarding restoration for over-written files:

Sample ID	Data Restoration Information
2B4-12h-as.jpg	Over-written on: August 3, 2011 Restored on: August 4, 2011 by Martin Tagesen Restored using: Network back-up from 13 May 2011
3B1-12h-as.jpg	Over-written on: August 3, 2011 Restored on: August 4, 2011 by Martin Tagesen Restored using: Network back-up from 13 May 2011
PPB-3-1-bs.tif	Over-written on: August 9, 2011 Restored on: August 11, 2011 by Kerry Weber Restored using original picture file: PPB-3-1-bs.jpg
2A5-24h-bs.tif	Over-written on: August 12, 2011 Restored on: August 12, 2011 by Kerry Weber Restored using original picture file: 2A5-24h-bs.jpg
1B6-12h-bs.tif	Over-written on: August 18, 2011 Restored on: August 18, 2011 by Kerry Weber Restored using original picture file: 1B6-12h-bs.jpg

Item No. 12

FOLDER NO. 3

Approval [redacted] 11 JAN 2012

PROOFED BY
Initials: JMS Date: 2/6/12
REVIEWED BY
Initials: ____ Date: ____

cc: UMESC QAU

United States Department of the Interior

U.S. GEOLOGICAL SURVEY
Upper Midwest Environmental Sciences Center
2630 Fanta Reed Road
La Crosse, Wisconsin 54603

MEMORANDUM

Item No. 13

File Folder: 3

Date: February 7, 2012

To: The Record Study Number AEH-11-PSEUDO-01

Subject: Deviation 9 to study AEH-11-PSEUDO-01 "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"

Deviation #9 – Sections 4.2 and 6.2 (Experimental Design and Determination of viability) of the amended protocol (see Amendments 2 and 3) state that "if glochidia can be enumerated and viability status (i.e. open or closed) reliably determined through photomicrographic records (i.e. the glochidia remain on a single focal plane" then "the sample will be viewed under a dissecting microscope, adjusted for maximum clarity and magnification, and a photomicrograph will be recorded". During enumeration of Hickorynut glochidia (dosed on January 19, 2012) for initial viability, the following photograph was inadvertently over-written: HIC-3-I-bs.tif. The original file was restored by Martin Tagesen using the network back-up from January 19, 2012.

No adverse impacts to the study will result from this error as the over-written file was restored from a backup of the original file.

[redacted] 07 FEB12

Written by | Date
Kerry L. Weber, M.S.
Principal Investigator, UMESC

[redacted] 07 Feb 12

Approved by | Date
James A. Luoma, B.A.
Study Director, UMESC

PROOFED BY
Initials: JMS Date: 4/10/12
REVIEWED BY
Initials: ____ Date: ____

cc: UMESC QAU

United States Department of the Interior

U.S. GEOLOGICAL SURVEY
Upper Midwest Environmental Sciences Center
2630 Fanta Reed Road
La Crosse, Wisconsin 54603

MEMORANDUM

Date: October 31, 2012

To: The Record Study Number AEH-11-PSEUDO-01

Subject: Deviation 10 to study AEH-11-PSEUDO-01 "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"

Deviation #10 – Filenames for the photomicrographs of glochidia samples were changed to allow for unique file identification. The original photomicrograph codes used were not unique as the test system was used for multiple species and formulations and the original photomicrograph codes did not identify the species or product formulation. The filenames for each photomicrograph were renamed to include unique identifiers for each time point at which photomicrographs were recorded. Codes were used within the filenames to make the file identifiable to species, formulation, concentration, sample, exposure time and before or after salt addition, as applicable. The resulting codes allow for unambiguous photomicrograph tracking.

INITIAL VIABILITY

Photomicrographs were used to determine the suitability of glochidia for inclusion in the exposures. Photomicrographs were recorded for samples of glochidia flushed from each gravid mussel. The glochidia were photographed before and after a concentrated salt solution was added to the sample to induce valve closure. The photomicrographs were enumerated according to the procedures described in Amendment 1 of the protocol.

The original codes used in the original filenames were not unique as two formulations were used in exposures. The formulation type was incorporated into the filename. Additionally, once files were enumerated, they were then saved with a new filename that had the addition of T, O or C (Total count, Open count or Closed count) at the end of the filename which designate the type of count performed. See Table 1 for the original filenames, the new filenames and enumerated filenames for the initial viability samples.

Table 1. Initial Viability Filenames

Original Filename	**PPB-1-I-bs** **bs or as (before or after salt addition)** **I (Initial Viability)** **Mussel # (1, 2 or 3)** **Species 3 Letter Code**
New Filename	**PPB-SDP-1-I-bs** **bs or as (before or after salt addition)** **I (Initial Viability)** **Mussel # (1, 2 or 3)** **Formulation Type (FDP or SDP)** **Species 3 Letter Code**
Enumerated Filename	**PPB-SDP-1-I-bs-T** **Count Type** **(T, O or C: total count, open count or closed count)** **bs or as (before or after salt addition)** **I (Initial Viability)** **Mussel # (1, 2 or 3)** **Formulation Type (FDP or SDP)** **Species 3 Letter Code**

DISTRIBUTION

Photomicrographs were recorded on samples collected during each round of distribution to the exposure chambers. Distributions were completed in 5 rounds with 18 aliquots per round. Photomicrographs were recorded on the initial aliquot and then after every 6 aliquots for a total of 16 distribution samples for each species. The photomicrographs were recorded and enumerated to estimate the number of animals distributed to each chamber. Distribution samples that had very dense quantities of glochidia were subdivided into more manageable counts before being photomicrographed. The photomicrographs were enumerated according to the procedures described in Amendment 1 of the protocol.

The codes used in the original filenames were unique and were retained with the exception of the plain pocketbook (PPB) photomicrograph files. Plain pocketbook was the only species that was exposed to both formulations. Therefore, the formulation type was added only to the PPB distribution photomicrographs. Additionally, once files were enumerated, they were then saved with a new filename that had the addition of T (Total count) at the end of the filename which designate the type of count performed. See Table 2 for the original filenames, the new filenames and enumerated filenames for the distribution samples.

Table 2. Distribution Filenames

Original Filename	**PPB-R2-2.*1*** ***Subdivided (.1, .2, .3, etc.)*** ***(only for dense distribution samples)*** **Sample Number (1-5)** **Distribution Round (R1-R5)** **Species 3 Letter Code**
New Filename	**PPB-SDP-R2-2.*1*** ***Subdivided (.1, .2, .3, etc.)*** ***(only for dense distribution samples)*** **Aliquot Number (1-5)** **Distribution Round (R1-R5)** **Formulation Type (FDP or SDP)** **Species 3 Letter Code**
Enumerated Filename	**PPB-SDP-R2-2.*1*-T** **Total Count ONLY** ***Subdivided (.1, .2, .3, etc.)*** ***(only for dense distribution samples)*** **Aliquot Number (1-5)** **Distribution Round (R1-R5)** **Formulation Type (FDP or SDP)** **Species 3 Letter Code**

VIABILITY SAMPLES (6, 12 AND 24 HOUR)

Photomicrographs were recorded on glochidia samples collected from each exposure chamber at 6, 12 and 24 hours post-dose initiation to determine viability. Two photomicrographs were recorded for each sample collected for viability assessment. The first photomicrograph was recorded prior to the addition of a salt solution to induce valve closure and the second after induced closure. The photomicrographs were enumerated according to the procedures described in Amendment 1 of the protocol.

The codes used in the original filenames were not unique as multiple species were exposed using the same test system and two formulations were used in the exposures. Therefore, the species ID, formulation type and concentration were added to the filenames. Additionally, once files were enumerated, they were then saved with a new filename that had the addition of T, O or C (Total count, Open count or Closed count) at the end of the filename which designate the type of count performed. See Table 3 for the original filenames, the new filenames and enumerated filenames for the viability samples at 6, 12 and 24 hours.

Table 3. Viability Samples (6, 12 and 24 hour)

Original Filename	**1A2-6h-bs** **bs or as** (before or after salt addition) **Sample Time** (6, 12 or 24h) **3 Letter Sample ID** 1 = Test System Section (1, 2 or 3) A = Row (A, B or C) 2 = Position within row (1 – 6)
New Filename	**PPB-SDP-100-1A2-6h-bs** **bs or as** (before or after salt addition) **Sample Time** (6, 12 or 24h) **3 Letter Sample ID** 1 = Test System Section (1, 2 or 3) A = Row (A, B or C) 2 = Position within row (1 – 6) **Concentration** ctrl = Control (0 mg/L) 50 = 50 mg/L active ingredient 100 = 100 mg/L active ingredient 200 = 200 mg/L active ingredient 300AI = 300 mg/L active ingredient 300HD = 300 mg/L heat deactivated **Formulation Type** (FDP or SDP) **Species 3 Letter Code**

Table 3 (con.) 6, 12 and 24 Hour Viability Samples

Enumerated Filename	**PPB-SDP-100-1A2-6h-bs-T** **T, O or C** (Total Count, Open Count or Closed Count) **bs or as** (before or after salt) **Sample Time** (6, 12 or 24h) **3 Letter Sample ID** 1 = Test System Section (1, 2 or 3) A = Row (A, B or C) 2 = Position within row (1 – 6) **Concentration** ctrl = Control (0 mg/L) 50 = 50 mg/L active ingredient 100 = 100 mg/L active ingredient 200 = 200 mg/L active ingredient 300AI = 300 mg/L active ingredient 300HD = 300 mg/L heat deactivated **Formulation Type** (FDP or SDP) **Species 3 Letter Code**

No adverse impacts to the study will result from this change.

[redacted]	31 OCT 12	[redacted]	10-31-12
Written by Kerry L. Weber, M.S. Principal Investigator, UMESC	Date	Approved by James A. Luoma, B.A. Study Director, UMESC	Date

cc: UMESC QAU

United States Department of the Interior

U.S. GEOLOGICAL SURVEY
Upper Midwest Environmental Sciences Center
2630 Fanta Reed Road
La Crosse, Wisconsin 54603

MEMORANDUM

Date: November 27, 2012

To: The Record Study Number AEH-11-PSEUDO-01

Subject: Deviation 11 to study AEH-11-PSEUDO-01 "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"

Deviation #11 – Sections 4.2 (Experimental Design) and 5.1.4 (Test Animal: Inclusion criterion) of the amendment 1 of the protocol entitled "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species" states that "if viability [of glochidia] is acceptable (≥ 80%) for a mussel, the glochidia from that mussel will be pooled with glochidia from other mussels of that species". Deviation 3 further states that "mussels that were observed to have high viability, the exact viability percentage was not determined prior to allowing the glochidia into the study. For these mussels a preliminary visual observation was used to determine acceptable viability… exact viability of the glochidia will be determined from the photomicrograph records…"

The initial viability of three individual mussels [Fatmucket (FAM 2), Higgins eye (HGE 1) and Plain pocketbook (PPB 3)] appeared (by visual observation) to be greater than 80%. Upon conducting the final counts of the photomicrograph records, it was discovered that the viability of the glochidia from these individual mussels were slightly below 80%. See Table 1 for the mussel ID and the percent viability.

The initial viability of one individual mussel [Black sandshell (BLS 2)] did not appear (by visual observation) to be greater than 80% (photomicrograph enumeration = 54.4% viable). Two additional glochidia samples (BLS 2.2 and BLS 2.3) from this mussel were observed for initial viability and they appeared to have near or greater than 80% viability (photomicrograph enumeration = 79.4% and 85.4%, respectively). Upon visual observation of the two subsequent samples, the glochidia from this mussel were pooled for use in the study.

The results obtained from the three viability samples (BLS 2, BLS 2.2 and BLS 2.3) photomicrograph record counts were averaged to determine the viability of glochidia extracted from BLS 2. Additionally, the mean initial viability from BLS 2 was used to determine the pooled viability of all glochidia extracted from the BLS donor mussels. See Table 1 for the

cc: UMESC QAU

mussel ID and the percent viabilities.

No negative impacts to the study are anticipated as the mean glochidia viability for all pooled samples were greater than 80% criteria identified in the study protocol.

[redacted]

Written by Kerry L. Weber, M.S. Principal Investigator, UMESC	27 NOV 12 Date	[redacted] Approved by James A. Luoma, B.A. Study Director, UMESC	27 Nov 12 Date

Table 1. Photomicrograph record determined glochidia viability from individual mussels.

Mussel ID	Individual Percent Viability (%)	Pooled Viability (%)
FAM 1	87.2	
FAM 2	73.1	80.3
FAM 3	80.5	
HGE 1	62.0	
HGE 2	88.1	80.8
HGE 3	92.4	
PPB 1	92.5	
PPB 2	82.4	84.7
PPB 3	79.3	
BLS 1	89.7	
BLS 2	73.1[a]	83.2
BLS 3	86.9	

[a] This value is the mean percent initial viability (%) of the 3 glochidia samples (BLS 2, BLS 2.2, BLS 2.3) from BLS mussel 2. This value was used to determine the pooled initial viability (%) from the BLS donor mussels.

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United States Department of the Interior

U.S. GEOLOGICAL SURVEY
Upper Midwest Environmental Sciences Center
2630 Fanta Reed Road
La Crosse, Wisconsin 54603

MEMORANDUM

Date: December 17, 2012

To: The Record Study Number AEH-11-PSEUDO-01

Subject: Deviation 12 to study AEH-11-PSEUDO-01 "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"

Deviation #12 – Sections 4.2 (Experimental Design) and 6.2 (Determination of viability) of the amendment 3 of the protocol entitled "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species" states that "if glochidia can be enumerated and viability status (i.e. open or closed) reliably determined through photomicrograph records (i.e. the glochidia remain on a single focal plane)" then the viability of the subsample of approximately 100 glochidia from each chamber will be assessed at 6, 12, and 24 h post exposure by enumeration through a dissection microscope. As outlined in Note to File #2, the glochidia photomicrograph records were used to: 1) determine the viability of extracted glochidia that were used in the exposures; 2) estimate the number of glochidia distributed to each exposure replicate and; 3) assess the treatment effects on the viability of glochidia at three time points (6, 12 and 24 h) during the exposure period. Note to File #2 provides clarification to the path and unique folder and filenames for the photomicrograph records. Enumerated photomicrographs each contain a text box legend containing the study number, species, formulation type (SDP vs. FDP), concentration, filename and the glochidia count (i.e. Total Number Glochidia = the total number of glochidia present before salt; Glochidia Open or Glochidia Closed = the number of glochidia open or closed before or after salt). The legend allows the photomicrograph to be uniquely identified. Legend data were typed in by the analyst upon completion of the enumerations and the legend was then burned as an overlay onto the image.

During data proofing, the hard copy data was verified against the legend information on the enumerated photomicrographs. Typographical errors were noted and corrected on 6 photomicrographs. The individual images were opened in the Nikon NIS-Elements BR software. A new legend was created with the correct information. The new legend was burned as an overlay on the image before it was saved. See Table 1 for the photomicrograph filename, the typographical error and the correction.

No negative impacts to the study are anticipated as the data in the legend reflects the original hard copy data. Enumeration data was immediately recorded on the "Glochidia Photomicrograph Counting Record" datasheet (File Folders 11, 12A, 12B, 12C and 12D).

Table 1. Typographical errors in AEH-11-PSEUDO-01 enumerated photomicrograph records.

Photomicrograph Filename	Typographical error and correction
HGE-SDP-300HD-2A5-6h-bs-T.tif	Error: HGE-SDP-300HD-2A5**0**6h-bs-T.tif Correction: HGE-SDP-300HD-2A5-6h-bs-T.tif[a]
HIC-SDP-100-3C5-6h-bs-T.tif	Error: HIC-SDP-100-3C**6**-6h-bs-T.tif Correction: HIC-SDP-100-3C**5**-6h-bs-T.tif[a]
BLS-SDP-50-1A1-6h-bs-T.tif	Error: BLS-SDP-50-1A1-6h-**as**-T.tif Correction: BLS-SDP-50-1A1-6h-**bs**-T.tif[a]
MUC-SDP-100-2A2-12h-bs-C.tif	Error: Glochidia Closed = **0** Correction: Glochidia Closed = **91**[b]
HIC-SDP-300AI-3B5-12h-bs-T.tif	Error: HIC-SDP-300AI-3B5**0**12h-bs-T.tif Correction: HIC-SDP-300AI-3B5-12h-bs-T.tif[a]
HGE-SDP-300HD-2A5-24h-as-O.tif	Error: Glochidia Open = **137** Correction: Glochidia Open = **42**[b]

[a] – Typographical errors identified by comparison to the filename.
[b] – Typographical errors identified by comparison of the data in the text box legend to the original hard copy data.

[redacted] 17 DEC 12
Written by
Kerry L. Weber, M.S.
Principal Investigator, UMESC
Date

[redacted] 17 Dec 12
Approved by
James A. Luoma, B.A.
Study Director, UMESC
Date

cc: UMESC QAU

United States Department of the Interior

U.S. GEOLOGICAL SURVEY
Upper Midwest Environmental Sciences Center
2630 Fanta Reed Road
La Crosse, Wisconsin 54603

MEMORANDUM

Date: March 16, 2015

To: The Record Study Number AEH-11-PSEUDO-01

Subject: Deviation 13 to study AEH-11-PSEUDO-01 "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"

Deviation #13 – Sections 4.2 (Experimental Design) and 5.1.4 (Test Animal: Inclusion criterion) of the amendment 1 of the protocol entitled "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species" states that "if viability [of glochidia] is acceptable (≥ 80%) for a mussel, the glochidia from that mussel will be pooled with glochidia from other mussels of that species".

Deviation 11 calculated black sandshell donor mussel 2 (BLS 2) percent viability by taking the mean of all three BLS 2 sample percent viabilities (BLS 2, BLS 2.2, and BLS 2.3). See deviation 11 for clarification. Deviation 13 calculates percent viability of BLS 2 using the sum total of all glochidia and non-viable glochidia (those closed before the addition, and open after the addition of salt) from all three BLS 2 samples.

See Table 1 for viable and non-viable glochidia counts and percent viability calculations.

The mean percent viability of the glochidia from all black sandshell donor mussels was 84.5 percent. No negative impacts to the study were observed from the viability of glochidia from donor mussel BLS 2 being 76.9 percent.

[redacted] 3/16/15
Written by / Date
Todd J. Severson, B.S.
Biologist, UMESC

[redacted] 3/16/15
Approved by / Date
James A. Luoma, B.A.
Study Director, UMESC

cc: UMESC QAU

Table 1. Black sandshell donor mussel 2 initial glochidia viability samples and calculations.

Sample ID	Total glochidia	Non-viable glochidia[1]	Percent viability
BLS 2	237	108	54.4[2]
BLS 2.2	310	64	79.4[2]
BLS 2.3	536	78	85.4[2]
BLS 2 sum total	1083	250	76.9[3]

[1]Non-viable glochidia are glochidia closed prior to the addition of, and open after the addition of salt.

[2]Percent viability calculation: (total glochidia – non-viable glochidia) / total glochidia * 100.

[3]Percent viability calculation: (sum total glochidia – sum total non-viable glochidia) / sum total glochidia * 100.

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Appendix 3. Randomization Assignments

Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel species 1
AEH-11-PSUEDO-01
Random assignment of mussel species to block

File Folder 14A
Book 1 p. 4

Obs	block	x	blocka	sps
1	1	0.93721	Block 1	Pocketbook
2	2	0.64024	Block 2	Higgins Eye
3	3	0.95216	Block 3	Fatmucket

AEH-11-PSEUDO-01

Item No. 1

PROOFED BY
Initials: TMS Date: 5/14/12
REVIEWED BY
Initials: ____ Date: ____

Page 1 of 4

MM
Analysis performed by M. Gaikowski SAS version 9.2 08:56 10MAY11

```
/*******************************************************************
*  Study Number  : AEH-11-PSUEDO-01
*  Study Director: Jim Luoma
*  date created  : 22Apr11 - MPG MM
*  Verified by:  _______ (Date:_______)                page ____ of ____
*  Random allocation of mussel species to block.sas
*******************************************************************/
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

FOOTNOTE1 'Analysis performed by M. Gaikowski SAS version ' &SYSVER &SYSTIME &SYSDATE;

options /*ls=85 ps=40 formdlim='-' */ pageno = 1 nocenter nodate nosource2;

/*Random assignment of mussel species to block*/

data sps;
 do block = 1 to 3 by 1;
   x = ranuni(-1);
   output;
 end;
run;
proc sort data=sps;
 by x;
run;
data block; set sps;
if block = 1 then blocka = 'Block 1';
if block = 2 then blocka = 'Block 2';
if block = 3 then blocka = 'Block 3'; run;
data assign_sps; set block;
 if _n_ = 1 then sps = 'Higgins Eye';
  if _n_ = 2 then sps = 'Pocketbook';
   if _n_ = 3 then sps = 'Fatmucket';
 run;
 proc sort data=assign_sps;
 by block;
run;
proc print data= assign_sps;
title1 h=2 'Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel
title2 h=1.5 'AEH-11-PSUEDO-01';
title3 h=1 'Random assignment of mussel species to block';
run;
```

AEH-11-PSEUDO-01

```
*  date created  : 22Apr11 - MPG
*  Verified by:  _______ (Date:_______)                  page ____ of ____
*  Random allocation of mussel species to block.sas
**********************************************************************/
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

FOOTNOTE1 'Analysis performed by M. Gaikowski SAS version ' &SYSVER &SYSTIME &SYSDATE;
WARNING: The FOOTNOTE statement is ambiguous due to invalid options or unquoted text.

options /*ls=85 ps=40 formdlim='-' */ pageno = 1 nocenter nodate nosource2;

/*Random assignment of mussel species to block*/

data sps;
 do block = 1 to 3 by 1;
   x = ranuni(-1);
   output;
 end;
run;

NOTE: The data set WORK.SPS has 3 observations and 2 variables.
NOTE: DATA statement used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds


proc sort data=sps;
 by x;
run;

NOTE: There were 3 observations read from the data set WORK.SPS.
NOTE: The data set WORK.SPS has 3 observations and 2 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds


data block; set sps;
if block = 1 then blocka = 'Block 1';
if block = 2 then blocka = 'Block 2';
if block = 3 then blocka = 'Block 3'; run;

NOTE: There were 3 observations read from the data set WORK.SPS.
NOTE: The data set WORK.BLOCK has 3 observations and 3 variables.
NOTE: DATA statement used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds


data assign_sps; set block;
 if _n_ = 1 then sps = 'Higgins Eye';
  if _n_ = 2 then sps = 'Pocketbook';
   if _n_ = 3 then sps = 'Fatmucket';
 run;

NOTE: There were 3 observations read from the data set WORK.BLOCK.
NOTE: The data set WORK.ASSIGN_SPS has 3 observations and 4 variables.
```

AEH-11-PSEUDO-01

```
NOTE: DATA statement used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds


proc sort data=assign_sps;
by block;
run;

NOTE: There were 3 observations read from the data set WORK.ASSIGN_SPS.
NOTE: The data set WORK.ASSIGN_SPS has 3 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds


proc print data= assign_sps;
title1 h=2 'Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid
mussel species';
title2 h=1.5 'AEH-11-PSUEDO-01';
title3 h=1 'Random assignment of mussel species to block';
run;

NOTE: There were 3 observations read from the data set WORK.ASSIGN_SPS.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds
```

File Folder 14a

PROOFED BY
Initials: TMS Date: 5/14/12
REVIEWED BY
Initials: ____ Date: ____

Page 4 of 4

MM 10May 11

```
/*******************************************************************
*  Study Number  : AEH-11-PSUEDO-01
*  Study Director: Jim Luoma
*  date created  : 14Oct11 - MPG
*  Verified by:  _______ (Date:_______)                page ____ of ____
*  Random allocation of mussel species to block.sas
*******************************************************************/
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

FOOTNOTE1 'Analysis performed by M. Gaikowski SAS version ' &SYSVER &SYSTIME &SYSDATE;

options /*ls=85 ps=40 formdlim='-' */ pageno = 1 nocenter nodate nosource2;

/*Random assignment of mussel species to block*/

data sps;
 do block = 1 to 2 by 1;
   x = ranuni(-1);
   output;
 end;
run;
proc sort data=sps;
 by x;
run;
data block; set sps;
if block = 1 then blocka = 'Block 1';
if block = 2 then blocka = 'Block 3'; run; /*Note: only two mussel species tested therefore Block
data assign_sps; set block;
 if _n_ = 1 then sps = 'Washboard';
  if _n_ = 2 then sps = 'Fatmucket';
 run;
 proc sort data=assign_sps;
 by block;
run;
proc print data= assign_sps;
title1 h=2 'Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel
title2 h=1.5 'AEH-11-PSUEDO-01';
title3 h=1 'Random assignment of mussel species to block';
run;
```

AEH-11-PSEUDO-01

Item No. 2

File Folder 14A
Book 1 p. 21-22

PROOFED BY
Initials: TMS Date: 5/14/12
REVIEWED BY
Initials: ____ Date: ____

Page 1 of 4

```
*  date created  : 14Oct11 - MPG
*  Verified by:  ______ (Date:_______)                 page ____ of ____
*  Random allocation of mussel species to block.sas
*******************************************************************/
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;
```

AEH-11-PSEUDO-01

```

FOOTNOTE1 'Analysis performed by M. Gaikowski SAS version ' &SYSVER &SYSTIME &SYSDATE;
WARNING: The FOOTNOTE statement is ambiguous due to invalid options or unquoted text.

options /*ls=85 ps=40 formdlim='-' */ pageno = 1 nocenter nodate nosource2;

/*Random assignment of mussel species to block*/

data sps;
 do block = 1 to 2 by 1;
   x = ranuni(-1);
   output;
 end;
run;

NOTE: The data set WORK.SPS has 2 observations and 2 variables.
NOTE: DATA statement used (Total process time):
      real time           0.36 seconds
      cpu time            0.09 seconds


proc sort data=sps;
 by x;
run;

NOTE: There were 2 observations read from the data set WORK.SPS.
NOTE: The data set WORK.SPS has 2 observations and 2 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.05 seconds
      cpu time            0.03 seconds


data block; set sps;
if block = 1 then blocka = 'Block 1';
if block = 2 then blocka = 'Block 3'; run;

NOTE: There were 2 observations read from the data set WORK.SPS.
NOTE: The data set WORK.BLOCK has 2 observations and 3 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


!                                              /*Note: only two mussel species tested therefore
! Block 2 was not used. MPG Oct 14, 2011*/
data assign_sps; set block;
 if _n_ = 1 then sps = 'Washboard';
  if _n_ = 2 then sps = 'Fatmucket';
 run;

NOTE: There were 2 observations read from the data set WORK.BLOCK.
NOTE: The data set WORK.ASSIGN_SPS has 2 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.03 seconds
```

Page 2 of 4

```
proc sort data=assign_sps;
by block;
run;

NOTE: There were 2 observations read from the data set WORK.ASSIGN_SPS.
NOTE: The data set WORK.ASSIGN_SPS has 2 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc print data= assign_sps;
title1 h=2 'Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid
! mussel species';
title2 h=1.5 'AEH-11-PSUEDO-01';
title3 h=1 'Random assignment of mussel species to block';
run;

NOTE: There were 2 observations read from the data set WORK.ASSIGN_SPS.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.33 seconds
      cpu time            0.06 seconds
```

Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel species 1
AEH-11-PSUEDO-01
Random assignment of mussel species to block

AEH-11-PSEUDO-01

File Folder 14A

Obs	block	x	blocka	sps
1	1	0.75861	Block 1	Washboard
2	2	0.78587	Block 3	~~Fatmucket~~ ①

① Species changed to plain pocketbook due to availability. 10-18-11 KLW

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Initials: TMS Date: 5/14/12
REVIEWED BY
Initials: ____ Date: ____

Page 4 of 4

Analysis performed by M. Gaikowski SAS version 9.2 11:03 14OCT11 MPL

Effects of Pseudomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel species 1
AEH-11-PSUEDO-01
Random assignment of mussel species to block JL

File folder 14A
Lab Notebook 1 / p. 30-31

bs	block	x	blocka	sps
1	1	0.03703	Block 1	Black Sandshell
2	2	0.53285	Block 2	Mucket
3	3	0.18011	Block 3	Hickory Nut

Item No. 3

AEH-11-PSEUDO-01

PROOFED BY
Initials: JMS Date: 5/14/12
REVIEWED BY
Initials: ______ Date: ______

Page 1 of 5

JL
Analysis performed by J. Luoma SAS version 9.2 10:50 09JAN12

```
  Study Number  : AEH-11-PSUEDO-01
  Study Director: Jim Luoma
  date created  : 09Jan12 - JAL
  Verified by:  _______ (Date:_______)                    page ____ of ____
  Random allocation of mussel species to block.sas
*******************************************************************/
```

AEH-11-PSEUDO-01

```
M 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

OOTNOTE1 'Analysis performed by J. Luoma SAS version ' &SYSVER &SYSTIME &SYSDATE;

ptions /*ls=85 ps=40 formdlim='-' */ pageno = 1 nocenter nodate nosource2;

*Random assignment of mussel species to block*/

ata sps;
do block = 1 to 3 by 1;
  x = ranuni(-1);
  output;
end;
un;
roc sort data=sps;
by x;
un;
ata block; set sps;
f block = 1 then blocka = 'Block 1';
f block = 2 then blocka = 'Block 2';
f block = 3 then blocka = 'Block 3'; run;
ata assign_sps; set block;
if _n_ = 1 then sps = 'Black Sandshell';
 if _n_ = 2 then sps = 'Hickory Nut';
  if _n_ = 3 then sps = 'Mucket';
run;
proc sort data=assign_sps;
by block;
un;
roc print data= assign_sps;
itle1 h=2 'Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel spe
itle2 h=1.5 'AEH-11-PSUEDO-01';
itle3 h=1 'Random assignment of mussel species to block';
un;
*******************************************************************
  Study Number  : AEH-11-PSUEDO-01
  Study Director: Jim Luoma
  date created  : 09Jan12 - JAL
  Verified by:  _______ (Date:_______)                    page ____ of ____
  Random allocation of mussel species to block.sas
*******************************************************************/
M 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

OOTNOTE1 'Analysis performed by J. Luoma SAS version ' &SYSVER &SYSTIME &SYSDATE;

ptions /*ls=85 ps=40 formdlim='-' */ pageno = 1 nocenter nodate nosource2;

*Random assignment of mussel species to block*/
```

Page 2 of 5

```
ata sps;
do block = 1 to 3 by 1;
  x = ranuni(-1);
  output;
end;
un;
roc sort data=sps;
by x;
un;
ata block; set sps;
f block = 1 then blocka = 'Block 1';
f block = 2 then blocka = 'Block 2';
f block = 3 then blocka = 'Block 3'; run;
ata assign_sps; set block;
if _n_ = 1 then sps = 'Black Sandshell';
 if _n_ = 2 then sps = 'Hickory Nut';
  if _n_ = 3 then sps = 'Mucket';
run;
proc sort data=assign_sps;
by block;
un;
roc print data= assign_sps;
itle1 h=2 'Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel spe(
itle2 h=1.5 'AEH-11-PSUEDO-01';
itle3 h=1 'Random assignment of mussel species to block';
un;
```

AEH-11-PSEUDO-01

```
*  date created  : 09Jan12 - JAL
*  Verified by:  _____ (Date:_______)                     page ____ of ____
*  Random allocation of mussel species to block.sas
*****************************************************************/
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

FOOTNOTE1 'Analysis performed by J. Luoma SAS version ' &SYSVER &SYSTIME &SYSDATE;
ARNING: The FOOTNOTE statement is ambiguous due to invalid options or unquoted text.

options /*ls=85 ps=40 formdlim='-' */ pageno = 1 nocenter nodate nosource2;

/*Random assignment of mussel species to block*/

data sps;
 do block = 1 to 3 by 1;
   x = ranuni(-1);
   output;
 end;
run;

OTE: The data set WORK.SPS has 3 observations and 2 variables.
OTE: DATA statement used (Total process time):
     real time           0.01 seconds
     cpu time            0.01 seconds


proc sort data=sps;
 by x;
run;

OTE: There were 3 observations read from the data set WORK.SPS.
OTE: The data set WORK.SPS has 3 observations and 2 variables.
OTE: PROCEDURE SORT used (Total process time):
     real time           0.01 seconds
     cpu time            0.01 seconds


data block; set sps;
if block = 1 then blocka = 'Block 1';
if block = 2 then blocka = 'Block 2';
if block = 3 then blocka = 'Block 3'; run;

OTE: There were 3 observations read from the data set WORK.SPS.
OTE: The data set WORK.BLOCK has 3 observations and 3 variables.
OTE: DATA statement used (Total process time):
     real time           0.01 seconds
     cpu time            0.01 seconds


data assign_sps; set block;
 if _n_ = 1 then sps = 'Black Sandshell';
  if _n_ = 2 then sps = 'Hickory Nut';
   if _n_ = 3 then sps = 'Mucket';
 run;

OTE: There were 3 observations read from the data set WORK.BLOCK.
```

Page 4 of 5

```
OTE: The data set WORK.ASSIGN_SPS has 3 observations and 4 variables.
OTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds
```

AEH-11-PSEUDO-01

File Folder 14A

```
proc sort data=assign_sps;
by block;
run;

OTE: There were 3 observations read from the data set WORK.ASSIGN_SPS.
OTE: The data set WORK.ASSIGN_SPS has 3 observations and 4 variables.
OTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc print data= assign_sps;
title1 h=2 'Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid
mussel species';
title2 h=1.5 'AEH-11-PSUEDO-01';
title3 h=1 'Random assignment of mussel species to block';
run;

OTE: There were 3 observations read from the data set WORK.ASSIGN_SPS.
OTE: PROCEDURE PRINT used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds
```

PROOFED BY
Initials: TMS Date: 5/14/12
REVIEWED BY
Initials: ______ Date: ______

Page 5 of 5

JAL 1-9-12

Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel species 1
AEH-11-PSUEDO-01
Random assignment of glochidia to test tanks
Mussel species: Plain pocketbook

AEH-11-PSEUDO-01

write over 5
9:53am aliquot start
PLM 12MAY11

File Folder 14B
Book 1 p.4

Item No. 1

Obs	round	row	tank	x	_row_	tankn	
1	1	1	1	0.01879	A	1A1	*
2	1	2	3	0.02945	B	1B3	
3	1	1	2	0.08175	A	1A2	
4	1	1	3	0.21417	A	1A3	
5	1	3	2	0.38756	C	1C2	
6	1	2	4	0.40940	B	1B4	*
7	1	3	4	0.48443	C	1C4	
8	1	3	1	0.52590	C	1C1	
9	1	3	5	0.56059	C	1C5	
10	1	2	5	0.58232	B	1B5	
11	1	3	3	0.74320	C	1C3	
12	1	1	6	0.77304	A	1A6	*
13	1	2	1	0.80460	B	1B1	
14	1	2	2	0.80583	B	1B2	
15	1	2	6	0.85455	B	1B6	
16	1	1	5	0.97248	A	1A5	
17	1	3	6	0.99067	C	1C6	
18	1	1	4	0.99498	A	1A4	*
19	2	2	1	0.01131	B	1B1	
20	2	3	6	0.04127	C	1C6	
21	2	1	2	0.05369	A	1A2	
22	2	2	4	0.27941	B	1B4	
23	2	1	3	0.29343	A	1A3	
24	2	1	4	0.36830	A	1A4	*
25	2	3	2	0.39506	C	1C2	
26	2	3	1	0.43877	C	1C1	
27	2	2	2	0.55963	B	1B2	
28	2	2	3	0.58457	B	1B3	
29	2	2	5	0.63270	B	1B5	
30	2	1	6	0.69204	A	1A6	*
31	2	1	1	0.74570	A	1A1	
32	2	3	3	0.74910	C	1C3	
33	2	2	6	0.77992	B	1B6	
34	2	3	4	0.85247	C	1C4	
35	2	3	5	0.87033	C	1C5	
36	2	1	5	0.98962	A	1A5	*
37	3	3	6	0.02285	C	1C6	
38	3	2	4	0.10895	B	1B4	
39	3	3	1	0.17771	C	1C1	
40	3	1	3	0.18393	A	1A3	
41	3	1	6	0.29164	A	1A6	
42	3	2	2	0.38015	B	1B2	*
43	3	2	5	0.59635	B	1B5	
44	3	1	5	0.60414	A	1A5	
45	3	2	3	0.62901	B	1B3	
46	3	1	2	0.64482	A	1A2	
47	3	2	1	0.65972	B	1B1	
48	3	3	5	0.66020	C	1C5	*

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Initials: TMS Date: 5/14/12
REVIEWED BY
Initials: ____ Date: ____

MM
Analysis performed by M. Gaikowski SAS version 9.2 07:41 12MAY11

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* Sample for distribution aliquot
5-12-11
JNL

Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel species 2
AEH-11-PSUEDO-01
Random assignment of glochidia to test tanks
Mussel species: Plain pocketbook

AEH-11-PSEUDO-01

Obs	round	row	tank	x	_row_	tankn
49	3	1	4	0.69985	A	1A4
50	3	3	2	0.72392	C	1C2
51	3	2	6	0.80850	B	1B6
52	3	3	4	0.83134	C	1C4
53	3	1	1	0.94910	A	1A1
54	3	3	3	0.97114	C	1C3 *
55	4	3	1	0.05000	C	1C1
56	4	3	5	0.05937	C	1C5
57	4	1	4	0.09591	A	1A4
58	4	3	4	0.14079	C	1C4
59	4	2	5	0.15159	B	1B5
60	4	1	5	0.21245	A	1A5 *
61	4	1	2	0.23873	A	1A2
62	4	3	3	0.27039	C	1C3
63	4	3	2	0.31746	C	1C2
64	4	1	1	0.39248	A	1A1
65	4	2	4	0.41132	B	1B4
66	4	2	6	0.56963	B	1B6 *
67	4	1	6	0.60171	A	1A6
68	4	2	3	0.74600	B	1B3
69	4	2	2	0.79595	B	1B2
70	4	3	6	0.80073	C	1C6
71	4	2	1	0.94103	B	1B1
72	4	1	3	0.95186	A	1A3 *
73	5	3	3	0.04548	C	1C3
74	5	1	2	0.13121	A	1A2
75	5	1	5	0.15073	A	1A5
76	5	3	4	0.21676	C	1C4
77	5	2	4	0.31224	B	1B4
78	5	3	6	0.31484	C	1C6 *
79	5	2	5	0.51845	B	1B5
80	5	1	1	0.58270	A	1A1
81	5	3	5	0.58642	C	1C5
82	5	3	1	0.67026	C	1C1
83	5	1	6	0.68606	A	1A6
84	5	1	3	0.68973	A	1A3 *
85	5	3	2	0.69908	C	1C2
86	5	2	2	0.80909	B	1B2
87	5	2	3	0.86182	B	1B3
88	5	1	4	0.87902	A	1A4
89	5	2	6	0.96993	B	1B6
90	5	2	1	0.99264	B	1B1 *
91	6	1	3	0.04500	A	1A3
92	6	1	1	0.05454	A	1A1
93	6	1	2	0.08718	A	1A2
94	6	2	6	0.11644	B	1B6
95	6	3	5	0.17687	C	1C5
96	6	2	4	0.29001	B	1B4

Five rounds of glochidia were added to exposure chambers. Rounds 6-10 of the random assignment were not used.
statement added for clarification
TMS 5/14/12

Analysis performed by M. Gaikowski SAS version 9.2 07:41 12MAY11

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* sample for distribution
5-12-11
JJ

AEH-11-PSUEDO-01
Random assignment of glochidia to test tanks
Mussel species: Plain pocketbook

AEH-11-PSEUDO-01

Obs	round	row	tank	x	_row_	tankn
97	6	3	2	0.29738	C	1C2
98	6	1	5	0.40275	A	1A5
99	6	1	6	0.43727	A	1A6
100	6	3	3	0.48861	C	1C3
101	6	2	2	0.50353	B	1B2
102	6	3	6	0.56958	C	1C6
103	6	2	1	0.62141	B	1B1
104	6	2	3	0.76177	B	1B3
105	6	1	4	0.89170	A	1A4
106	6	3	1	0.89421	C	1C1
107	6	2	5	0.94943	B	1B5
108	6	3	4	0.95587	C	1C4
109	7	1	6	0.01753	A	1A6
110	7	1	3	0.05265	A	1A3
111	7	3	1	0.06021	C	1C1
112	7	2	3	0.13135	B	1B3
113	7	1	4	0.15031	A	1A4
114	7	3	2	0.16166	C	1C2
115	7	1	2	0.17852	A	1A2
116	7	1	1	0.18945	A	1A1
117	7	1	5	0.21711	A	1A5
118	7	2	2	0.30167	B	1B2
119	7	3	6	0.34314	C	1C6
120	7	2	6	0.48469	B	1B6
121	7	3	4	0.75785	C	1C4
122	7	3	3	0.76057	C	1C3
123	7	3	5	0.79406	C	1C5
124	7	2	4	0.81814	B	1B4
125	7	2	1	0.84270	B	1B1
126	7	2	5	0.97628	B	1B5
127	8	3	4	0.01800	C	1C4
128	8	1	3	0.05110	A	1A3
129	8	1	4	0.06923	A	1A4
130	8	1	2	0.08503	A	1A2
131	8	3	5	0.14368	C	1C5
132	8	1	6	0.14720	A	1A6
133	8	1	5	0.18610	A	1A5
134	8	3	6	0.31005	C	1C6
135	8	3	2	0.47913	C	1C2
136	8	2	1	0.55774	B	1B1
137	8	2	6	0.61696	B	1B6
138	8	2	5	0.63578	B	1B5
139	8	3	1	0.75157	C	1C1
140	8	3	3	0.76715	C	1C3
141	8	2	2	0.77851	B	1B2
142	8	2	4	0.78177	B	1B4
143	8	2	3	0.80188	B	1B3
144	8	1	1	0.83057	A	1A1

Analysis performed by M. Gaikowski SAS version 9.2 07:41 12MAY11

AEH-11-PSUEDO-01
Random assignment of glochidia to test tanks
Mussel species: Plain pocketbook

AEH-11-PSEUDO-01

Obs	round	row	tank	x	_row_	tankn
145	9	3	1	0.00567	C	1C1
146	9	1	4	0.04799	A	1A4
147	9	3	4	0.09070	C	1C4
148	9	1	2	0.13436	A	1A2
149	9	1	5	0.17031	A	1A5
150	9	2	2	0.18916	B	1B2
151	9	1	3	0.28699	A	1A3
152	9	3	5	0.41896	C	1C5
153	9	2	6	0.45724	B	1B6
154	9	1	1	0.47143	A	1A1
155	9	3	3	0.50109	C	1C3
156	9	1	6	0.51486	A	1A6
157	9	2	3	0.53944	B	1B3
158	9	2	5	0.62377	B	1B5
159	9	3	2	0.63384	C	1C2
160	9	3	6	0.91619	C	1C6
161	9	2	4	0.97574	B	1B4
162	9	2	1	0.98193	B	1B1
163	10	1	5	0.13894	A	1A5
164	10	3	1	0.16112	C	1C1
165	10	3	5	0.18016	C	1C5
166	10	2	1	0.21049	B	1B1
167	10	1	4	0.28226	A	1A4
168	10	3	4	0.28484	C	1C4
169	10	2	5	0.39719	B	1B5
170	10	2	4	0.45883	B	1B4
171	10	1	2	0.47575	A	1A2
172	10	2	6	0.55730	B	1B6
173	10	1	1	0.71916	A	1A1
174	10	2	3	0.75737	B	1B3
175	10	3	3	0.80742	C	1C3
176	10	3	6	0.84725	C	1C6
177	10	1	6	0.86038	A	1A6
178	10	2	2	0.86791	B	1B2
179	10	3	2	0.95113	C	1C2
180	10	1	3	0.96901	A	1A3

Analysis performed by M. Gaikowski SAS version 9.2 07:41 12MAY11

Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel species
AEH-11-PSUEDO-01
Random assignment of glochidia to test tank
Mussel species: Higgins eye

AEH-11-PSEUDO-01

Start: 11:32AM
PLM 12MAY11

Obs	round	row	tank	x	_row_	tankn	
1	1	2	5	0.00353	B	2B5	
2	1	3	1	0.01828	C	2C1	
3	1	3	3	0.06547	C	2C3	
4	1	1	1	0.09979	A	2A1	
5	1	2	2	0.11238	B	2B2	
6	1	1	6	0.27072	A	2A6	*
7	1	2	1	0.28549	B	2B1	
8	1	3	2	0.30041	C	2C2	
9	1	3	6	0.34286	C	2C6	
10	1	2	3	0.44640	B	2B3	
11	1	2	6	0.54274	B	2B6	
12	1	2	4	0.54557	B	2B4	*
13	1	1	2	0.59140	A	2A2	
14	1	1	4	0.63600	A	2A4	
15	1	1	3	0.65528	A	2A3	
16	1	1	5	0.65562	A	2A5	
17	1	3	5	0.68469	C	2C5	
18	1	3	4	0.88493	C	2C4	*
19	2	3	6	0.09555	C	2C6	
20	2	2	2	0.10853	B	2B2	
21	2	2	1	0.17714	B	2B1	
22	2	3	1	0.25215	C	2C1	
23	2	2	6	0.32275	B	2B6	
24	2	3	2	0.33037	C	2C2	*
25	2	3	3	0.35151	C	2C3	
26	2	1	1	0.43424	A	2A1	
27	2	2	3	0.47595	B	2B3	
28	2	1	6	0.47886	A	2A6	
29	2	1	2	0.54611	A	2A2	
30	2	3	4	0.66566	C	2C4	*
31	2	2	5	0.73720	B	2B5	
32	2	1	4	0.79288	A	2A4	
33	2	1	5	0.82948	A	2A5	
34	2	3	5	0.85988	C	2C5	
35	2	2	4	0.91676	B	2B4	
36	2	1	3	0.97926	A	2A3	*
37	3	3	3	0.00275	C	2C3	
38	3	1	5	0.02144	A	2A5	
39	3	1	6	0.08661	A	2A6	
40	3	1	4	0.11286	A	2A4	
41	3	1	1	0.19395	A	2A1	
42	3	2	5	0.19567	B	2B5	*
43	3	3	4	0.36903	C	2C4	
44	3	1	2	0.37635	A	2A2	
45	3	2	3	0.39921	B	2B3	
46	3	3	1	0.41669	C	2C1	
47	3	3	6	0.44630	C	2C6	
48	3	2	2	0.44748	B	2B2	*

Analysis performed by M. Gaikowski SAS version 9.2 07:41 12MAY11

*Sample for Distribution 5-12-11 KLW

Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel species 6
AEH-11-PSUEDO-01
Random assignment of glochidia to test tank
Mussel species: Higgins eye

AEH-11-PSEUDO-01

Obs	round	row	tank	x	_row_	tankn
49	3	1	3	0.50721	A	2A3
50	3	3	5	0.60580	C	2C5
51	3	2	6	0.69776	B	2B6
52	3	2	4	0.72794	B	2B4
53	3	2	1	0.88609	B	2B1
54	3	3	2	0.93325	C	2C2 *
55	4	3	5	0.11301	C	2C5
56	4	2	1	0.21527	B	2B1
57	4	2	5	0.27124	B	2B5
58	4	1	1	0.38224	A	2A1
59	4	3	1	0.41408	C	2C1
60	4	1	5	0.44653	A	2A5 *
61	4	3	2	0.46886	C	2C2
62	4	2	3	0.51095	B	2B3
63	4	3	3	0.54719	C	2C3
64	4	2	2	0.60320	B	2B2
65	4	1	4	0.69545	A	2A4
66	4	2	4	0.69847	B	2B4 *
67	4	2	6	0.73361	B	2B6
68	4	3	4	0.73394	C	2C4
69	4	3	6	0.77816	C	2C6
70	4	1	3	0.80287	A	2A3
71	4	1	2	0.82110	A	2A2
72	4	1	6	0.86940	A	2A6 *
73	5	2	5	0.04537	B	2B5
74	5	1	1	0.09335	A	2A1
75	5	1	4	0.12238	A	2A4
76	5	3	6	0.22538	C	2C6
77	5	3	3	0.27257	C	2C3
78	5	1	5	0.37465	A	2A5 *
79	5	2	1	0.40649	B	2B1
80	5	3	4	0.46054	C	2C4
81	5	1	2	0.47441	A	2A2
82	5	1	3	0.55586	A	2A3
83	5	2	4	0.57846	B	2B4
84	5	3	5	0.58119	C	2C5 *
85	5	2	2	0.61383	B	2B2
86	5	2	3	0.62711	B	2B3
87	5	1	6	0.76919	A	2A6
88	5	2	6	0.77658	B	2B6
89	5	3	2	0.78874	C	2C2
90	5	3	1	0.98232	C	2C1 *
91	6	3	3	0.01723	C	2C3
92	6	3	1	0.06903	C	2C1
93	6	2	6	0.19358	B	2B6
94	6	2	5	0.26894	B	2B5
95	6	1	3	0.27596	A	2A3
96	6	3	6	0.29174	C	2C6

End: 11:49AM

PLM 12MAY11

Five rounds of glochidia were added to exposure chambers. Rounds 6-10 of the random assignment were not used. Statement added for clarification. TMS 5/14/12

Analysis performed by M. Gaikowski SAS version 9.2 07:41 12MAY11

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* sample aliquot taken for distribution number estimation 5-12-11 JRC

AEH-11-PSUEDO-01
Random assignment of glochidia to test tank
Mussel species: Higgins eye

AEH-11-PSEUDO-01

Obs	round	row	tank	x	_row_	tankn
97	6	1	4	0.33462	A	2A4
98	6	2	1	0.34254	B	2B1
99	6	2	3	0.42686	B	2B3
100	6	1	1	0.43456	A	2A1
101	6	2	2	0.59493	B	2B2
102	6	3	2	0.59626	C	2C2
103	6	1	6	0.59777	A	2A6
104	6	1	2	0.63322	A	2A2
105	6	3	4	0.76409	C	2C4
106	6	2	4	0.77924	B	2B4
107	6	1	5	0.82305	A	2A5
108	6	3	5	0.91307	C	2C5
109	7	3	3	0.01123	C	2C3
110	7	1	2	0.13433	A	2A2
111	7	2	4	0.15831	B	2B4
112	7	2	6	0.28654	B	2B6
113	7	3	2	0.29506	C	2C2
114	7	2	3	0.33579	B	2B3
115	7	1	6	0.43376	A	2A6
116	7	3	6	0.48583	C	2C6
117	7	3	1	0.52022	C	2C1
118	7	1	5	0.57020	A	2A5
119	7	1	1	0.57770	A	2A1
120	7	2	2	0.62832	B	2B2
121	7	2	5	0.73838	B	2B5
122	7	2	1	0.79867	B	2B1
123	7	3	4	0.81943	C	2C4
124	7	1	4	0.84167	A	2A4
125	7	3	5	0.92596	C	2C5
126	7	1	3	0.99065	A	2A3
127	8	3	3	0.06761	C	2C3
128	8	2	3	0.16831	B	2B3
129	8	2	2	0.23416	B	2B2
130	8	2	5	0.29976	B	2B5
131	8	1	1	0.30323	A	2A1
132	8	3	4	0.31483	C	2C4
133	8	1	4	0.34192	A	2A4
134	8	3	1	0.35878	C	2C1
135	8	3	5	0.38611	C	2C5
136	8	1	2	0.39165	A	2A2
137	8	2	1	0.42688	B	2B1
138	8	3	2	0.43867	C	2C2
139	8	2	4	0.47883	B	2B4
140	8	1	3	0.58091	A	2A3
141	8	1	6	0.67692	A	2A6
142	8	2	6	0.74573	B	2B6
143	8	3	6	0.89282	C	2C6
144	8	1	5	0.91038	A	2A5

Analysis performed by M. Gaikowski SAS version 9.2 07:41 12MAY11

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AEH-11-PSUEDO-01
Random assignment of glochidia to test tank
Mussel species: Higgins eye

AEH-11-PSEUDO-01

Obs	round	row	tank	x	_row_	tankn
145	9	1	1	0.06975	A	2A1
146	9	2	6	0.08304	B	2B6
147	9	3	6	0.14826	C	2C6
148	9	2	4	0.17937	B	2B4
149	9	2	5	0.19933	B	2B5
150	9	1	3	0.36557	A	2A3
151	9	3	2	0.38197	C	2C2
152	9	1	4	0.44056	A	2A4
153	9	2	3	0.53384	B	2B3
154	9	2	1	0.55441	B	2B1
155	9	3	1	0.60235	C	2C1
156	9	2	2	0.60503	B	2B2
157	9	1	6	0.62606	A	2A6
158	9	3	4	0.85074	C	2C4
159	9	3	5	0.85759	C	2C5
160	9	3	3	0.86157	C	2C3
161	9	1	2	0.93100	A	2A2
162	9	1	5	0.96584	A	2A5
163	10	1	4	0.01688	A	2A4
164	10	3	5	0.05395	C	2C5
165	10	2	1	0.06399	B	2B1
166	10	3	1	0.08176	C	2C1
167	10	3	6	0.08998	C	2C6
168	10	2	2	0.19534	B	2B2
169	10	2	4	0.32319	B	2B4
170	10	3	4	0.37064	C	2C4
171	10	1	5	0.47450	A	2A5
172	10	2	6	0.52635	B	2B6
173	10	1	2	0.59619	A	2A2
174	10	1	3	0.61180	A	2A3
175	10	1	6	0.63640	A	2A6
176	10	1	1	0.70002	A	2A1
177	10	2	3	0.76863	B	2B3
178	10	3	3	0.89173	C	2C3
179	10	2	5	0.94232	B	2B5
180	10	3	2	0.97204	C	2C2

Effects of Psuedomonas fluoroscens (Pf-CL145A) to glochidia from seven unionid mussel species 9
AEH-11-PSUEDO-01
Random assignment of glochidia to test tank
Mussel species: Fatmucket

AEH-11-PSEUDO-01

Obs	round	row	tank	x	_row_	tankn	
1	1	1	6	0.06425	A	3A6	
2	1	2	4	0.23487	B	3B4	
3	1	3	3	0.28423	C	3C3	
4	1	2	3	0.29083	B	3B3	
5	1	1	3	0.31575	A	3A3	
6	1	2	1	0.34859	B	3B1	*
7	1	2	2	0.44260	B	3B2	
8	1	3	2	0.46605	C	3C2	
9	1	2	6	0.50123	B	3B6	
10	1	3	5	0.58107	C	3C5	
11	1	1	1	0.61879	A	3A1	
12	1	2	5	0.63059	B	3B5	*
13	1	3	1	0.68919	C	3C1	
14	1	1	5	0.69536	A	3A5	
15	1	1	4	0.71002	A	3A4	
16	1	3	6	0.79854	C	3C6	
17	1	1	2	0.84196	A	3A2	
18	1	3	4	0.92360	C	3C4	*
19	2	3	3	0.21575	C	3C3	
20	2	2	3	0.21897	B	3B3	
21	2	3	5	0.27131	C	3C5	
22	2	1	5	0.36197	A	3A5	
23	2	3	2	0.45861	C	3C2	
24	2	1	6	0.46067	A	3A6	*
25	2	2	6	0.56608	B	3B6	
26	2	1	1	0.56645	A	3A1	
27	2	2	1	0.58251	B	3B1	
28	2	1	2	0.74533	A	3A2	
29	2	2	5	0.81915	B	3B5	
30	2	3	6	0.82026	C	3C6	*
31	2	3	4	0.83029	C	3C4	
32	2	2	4	0.83100	B	3B4	
33	2	1	4	0.83244	A	3A4	
34	2	3	1	0.87722	C	3C1	
35	2	2	2	0.89739	B	3B2	
36	2	1	3	0.97844	A	3A3	*
37	3	2	2	0.13149	B	3B2	
38	3	1	1	0.14025	A	3A1	
39	3	2	3	0.18648	B	3B3	
40	3	1	2	0.28969	A	3A2	
41	3	2	1	0.31270	B	3B1	
42	3	1	4	0.32951	A	3A4	*
43	3	1	3	0.40179	A	3A3	
44	3	2	6	0.40607	B	3B6	
45	3	3	6	0.48170	C	3C6	
46	3	2	4	0.59046	B	3B4	
47	3	3	4	0.71623	C	3C4	
48	3	3	5	0.74142	C	3C5	*

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* Sample aliquot taken for distribution number estimation
5-12-11 JW

AEH-11-PSUEDO-01
Random assignment of glochidia to test tank
Mussel species: Fatmucket

AEH-11-PSEUDO-01

Obs	round	row	tank	x	_row_	tankn
49	3	3	3	0.84026	C	3C3
50	3	1	6	0.84108	A	3A6
51	3	2	5	0.94185	B	3B5
52	3	1	5	0.94902	A	3A5
53	3	3	2	0.95766	C	3C2
54	3	3	1	0.97980	C	3C1 *
55	4	1	6	0.00485	A	3A6
56	4	3	2	0.04653	C	3C2
57	4	2	6	0.08927	B	3B6
58	4	1	2	0.26321	A	3A2
59	4	2	4	0.27095	B	3B4
60	4	2	5	0.27927	B	3B5 *
61	4	3	5	0.35826	C	3C5
62	4	3	6	0.43440	C	3C6
63	4	3	1	0.57582	C	3C1
64	4	2	2	0.57707	B	3B2
65	4	1	1	0.65035	A	3A1
66	4	1	3	0.66001	A	3A3 *
67	4	3	4	0.73456	C	3C4
68	4	2	3	0.75676	B	3B3
69	4	2	1	0.75680	B	3B1
70	4	1	4	0.77378	A	3A4
71	4	3	3	0.91226	C	3C3
72	4	1	5	0.98150	A	3A5 *
73	5	1	2	0.02882	A	3A2
74	5	3	2	0.08059	C	3C2
75	5	2	1	0.11022	B	3B1
76	5	2	3	0.14474	B	3B3
77	5	3	3	0.15604	C	3C3
78	5	2	4	0.16623	B	3B4 *
79	5	3	4	0.25175	C	3C4
80	5	2	5	0.32841	B	3B5
81	5	2	6	0.36155	B	3B6
82	5	1	5	0.41550	A	3A5
83	5	3	1	0.45032	C	3C1
84	5	1	3	0.48246	A	3A3 *
85	5	1	4	0.56627	A	3A4
86	5	1	6	0.63987	A	3A6
87	5	2	2	0.72266	B	3B2
88	5	1	1	0.89897	A	3A1
89	5	3	6	0.92983	C	3C6
90	5	3	5	0.93030	C	3C5 *
91	6	2	4	0.03396	B	3B4
92	6	3	2	0.05586	C	3C2
93	6	3	6	0.22820	C	3C6
94	6	1	4	0.25609	A	3A4
95	6	3	4	0.25905	C	3C4
96	6	1	5	0.27999	A	3A5

Five rounds of glochidia were added to exposure chambers. Rounds 6-10 of the random assignment were not used. Statement added for clarification. MS 5/14/12

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* Sample aliquot taken for distribution number estimation 5-12-11 JRW

Random assignment of glochidia to test tank
Mussel species: Fatmucket

AEH-11-PSEUDO-01

Obs	round	row	tank	x	_row_	tankn
97	6	1	6	0.30692	A	3A6
98	6	3	3	0.30840	C	3C3
99	6	2	6	0.33386	B	3B6
100	6	2	3	0.41121	B	3B3
101	6	2	2	0.52134	B	3B2
102	6	1	3	0.64758	A	3A3
103	6	3	1	0.70774	C	3C1
104	6	3	5	0.73370	C	3C5
105	6	1	1	0.74906	A	3A1
106	6	2	5	0.85655	B	3B5
107	6	1	2	0.89278	A	3A2
108	6	2	1	0.98807	B	3B1
109	7	3	6	0.01515	C	3C6
110	7	2	1	0.27756	B	3B1
111	7	3	5	0.28546	C	3C5
112	7	1	4	0.31072	A	3A4
113	7	3	4	0.33916	C	3C4
114	7	1	5	0.35827	A	3A5
115	7	1	2	0.46221	A	3A2
116	7	2	3	0.51697	B	3B3
117	7	1	6	0.59115	A	3A6
118	7	2	2	0.60333	B	3B2
119	7	2	5	0.66243	B	3B5
120	7	3	1	0.68544	C	3C1
121	7	3	2	0.69659	C	3C2
122	7	2	4	0.87014	B	3B4
123	7	1	3	0.89719	A	3A3
124	7	1	1	0.91395	A	3A1
125	7	2	6	0.92644	B	3B6
126	7	3	3	0.96712	C	3C3
127	8	3	3	0.03720	C	3C3
128	8	2	3	0.07049	B	3B3
129	8	3	4	0.17372	C	3C4
130	8	2	6	0.23243	B	3B6
131	8	3	6	0.26338	C	3C6
132	8	1	4	0.27158	A	3A4
133	8	1	3	0.29892	A	3A3
134	8	2	2	0.42426	B	3B2
135	8	2	1	0.53568	B	3B1
136	8	3	2	0.53767	C	3C2
137	8	3	1	0.59074	C	3C1
138	8	1	1	0.65401	A	3A1
139	8	2	4	0.77251	B	3B4
140	8	1	5	0.81738	A	3A5
141	8	1	6	0.87037	A	3A6
142	8	1	2	0.88416	A	3A2
143	8	2	5	0.94410	B	3B5
144	8	3	5	0.98817	C	3C5

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AEH-11-PSUEDO-01
Random assignment of glochidia to test tank
Mussel species: Fatmucket

AEH-11-PSEUDO-01

Obs	round	row	tank	x	_row_	tankn
145	9	1	1	0.10991	A	3A1
146	9	2	4	0.13007	B	3B4
147	9	2	3	0.18093	B	3B3
148	9	2	6	0.18945	B	3B6
149	9	1	3	0.19692	A	3A3
150	9	1	2	0.21057	A	3A2
151	9	3	3	0.27487	C	3C3
152	9	3	5	0.32850	C	3C5
153	9	3	1	0.39289	C	3C1
154	9	3	2	0.40678	C	3C2
155	9	3	4	0.45710	C	3C4
156	9	3	6	0.54291	C	3C6
157	9	1	6	0.61225	A	3A6
158	9	1	5	0.73560	A	3A5
159	9	2	5	0.75981	B	3B5
160	9	2	1	0.90249	B	3B1
161	9	2	2	0.91701	B	3B2
162	9	1	4	0.93682	A	3A4
163	10	3	6	0.03932	C	3C6
164	10	1	1	0.05556	A	3A1
165	10	2	5	0.09212	B	3B5
166	10	2	4	0.16615	B	3B4
167	10	1	2	0.43469	A	3A2
168	10	1	4	0.45582	A	3A4
169	10	1	3	0.46757	A	3A3
170	10	2	6	0.56078	B	3B6
171	10	1	5	0.61491	A	3A5
172	10	1	6	0.61818	A	3A6
173	10	3	5	0.67004	C	3C5
174	10	2	2	0.69138	B	3B2
175	10	2	1	0.76353	B	3B1
176	10	3	3	0.78170	C	3C3
177	10	3	1	0.90674	C	3C1
178	10	3	2	0.93409	C	3C2
179	10	3	4	0.94792	C	3C4
180	10	2	3	0.99146	B	3B3

MM

Analysis performed by M. Gaikowski SAS version 9.2 07:41 12MAY11

```
/*********************************************************************
*  Study Number  : AEH-11-PSUEDO-01
*  Study Director: Jim Luoma
*  date created  : 22Apr11 - MPG
*  date revised  : 12May11 - MPG mm
*  Verified by:  _____ (Date:_____)                page ____ of ____
*  Random allocation of glochidia to tank.sas
*********************************************************************/
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

FOOTNOTE1 'Analysis performed by M. Gaikowski SAS version ' &SYSVER &SYSTIME &SYSDATE;

options /*ls=85 ps=40 formdlim='-' */ pageno = 1 nocenter nodate nosource2;

/*Random distribution of glochidia to experimental tanks*/
/* tank 1 to 18 = tank A1 to A6 (1-6), tank B1 to B6 (7-12), tank C1 to C6 (13-18)
   round = distribution rounds 1 to 2, place one aliquot of glochidia per tank per round */

/**********************************************************************************************

/*Mussel species: Plain pocketbook*/
data glochidia;
 do round = 1 to 10 by 1;
 do row = 1 to 3 by 1;
  do tank = 1 to 6 by 1;
   x = ranuni(-1);
   output;
  end;
  end;
 end;
run;
data glochidiadist; set glochidia;
if row = 1 then _row_ = 'A';
if row = 2 then _row_ = 'B';
if row = 3 then _row_ = 'C';
 if row = 1 and tank = 1 then tankn = '1A1';
  if row = 1 and tank = 2 then tankn = '1A2';
   if row = 1 and tank = 3 then tankn = '1A3';
    if row = 1 and tank = 4 then tankn = '1A4';
     if row = 1 and tank = 5 then tankn = '1A5';
      if row = 1 and tank = 6 then tankn = '1A6';
 if row = 2 and tank = 1 then tankn = '1B1';
  if row = 2 and tank = 2 then tankn = '1B2';
   if row = 2 and tank = 3 then tankn = '1B3';
    if row = 2 and tank = 4 then tankn = '1B4';
     if row = 2 and tank = 5 then tankn = '1B5';
      if row = 2 and tank = 6 then tankn = '1B6';
 if row = 3 and tank = 1 then tankn = '1C1';
  if row = 3 and tank = 2 then tankn = '1C2';
   if row = 3 and tank = 3 then tankn = '1C3';
    if row = 3 and tank = 4 then tankn = '1C4';
     if row = 3 and tank = 5 then tankn = '1C5';
      if row = 3 and tank = 6 then tankn = '1C6'; run;
proc sort data= glochidiadist;
 by round x;
 run;
proc print data = glochidiadist;
```

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```
title1 h=2 'Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel
title2 h=1.5 'AEH-11-PSUEDO-01';
title3 h=1 'Random assignment of glochidia to test tanks';
title4 h=1 'Mussel species: Plain pocketbook';
run;

/*********************************************************************************************

/*Mussel species: Higgins eye */
data glochidia;
 do round = 1 to 10 by 1;
 do row = 1 to 3 by 1;
  do tank = 1 to 6 by 1;
   x = ranuni(-1);
   output;
  end;
  end;
 end;
run;
data glochidiadist; set glochidia;
if row = 1 then _row_ = 'A';
if row = 2 then _row_ = 'B';
if row = 3 then _row_ = 'C';
 if row = 1 and tank = 1 then tankn = '2A1';
  if row = 1 and tank = 2 then tankn = '2A2';
   if row = 1 and tank = 3 then tankn = '2A3';
    if row = 1 and tank = 4 then tankn = '2A4';
     if row = 1 and tank = 5 then tankn = '2A5';
      if row = 1 and tank = 6 then tankn = '2A6';
 if row = 2 and tank = 1 then tankn = '2B1';
  if row = 2 and tank = 2 then tankn = '2B2';
   if row = 2 and tank = 3 then tankn = '2B3';
    if row = 2 and tank = 4 then tankn = '2B4';
     if row = 2 and tank = 5 then tankn = '2B5';
      if row = 2 and tank = 6 then tankn = '2B6';
 if row = 3 and tank = 1 then tankn = '2C1';
  if row = 3 and tank = 2 then tankn = '2C2';
   if row = 3 and tank = 3 then tankn = '2C3';
    if row = 3 and tank = 4 then.tankn = '2C4';
     if row = 3 and tank = 5 then tankn = '2C5';
      if row = 3 and tank = 6 then tankn = '2C6'; run;
proc sort data= glochidiadist;
 by round x;
run;
proc print data = glochidiadist;
title1 h=2 'Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel
title2 h=1.5 'AEH-11-PSUEDO-01';
title3 h=1 'Random assignment of glochidia to test tank';
title4 h=1 'Mussel species: Higgins eye';
run;

/*********************************************************************************************

/*Mussel species: Fatmucket */
data glochidia;
 do round = 1 to 10 by 1;
 do row = 1 to 3 by 1;
```

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```
  do tank = 1 to 6 by 1;
   x = ranuni(-1);
   output;
  end;
  end;
 end;
run;
data glochidiadist; set glochidia;
if row = 1 then _row_ = 'A';
if row = 2 then _row_ = 'B';
if row = 3 then _row_ = 'C';
 if row = 1 and tank = 1 then tankn = '3A1';
  if row = 1 and tank = 2 then tankn = '3A2';
   if row = 1 and tank = 3 then tankn = '3A3';
    if row = 1 and tank = 4 then tankn = '3A4';
     if row = 1 and tank = 5 then tankn = '3A5';
      if row = 1 and tank = 6 then tankn = '3A6';
 if row = 2 and tank = 1 then tankn = '3B1';
  if row = 2 and tank = 2 then tankn = '3B2';
   if row = 2 and tank = 3 then tankn = '3B3';
    if row = 2 and tank = 4 then tankn = '3B4';
     if row = 2 and tank = 5 then tankn = '3B5';
      if row = 2 and tank = 6 then tankn = '3B6';
 if row = 3 and tank = 1 then tankn = '3C1';
  if row = 3 and tank = 2 then tankn = '3C2';
   if row = 3 and tank = 3 then tankn = '3C3';
    if row = 3 and tank = 4 then tankn = '3C4';
     if row = 3 and tank = 5 then tankn = '3C5';
      if row = 3 and tank = 6 then tankn = '3C6'; run;
proc sort data= glochidiadist;
 by round x;
run;
proc print data = glochidiadist;
title1 h=2 'Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel
title2 h=1.5 'AEH-11-PSUEDO-01';
title3 h=1 'Random assignment of glochidia to test tank';
title4 h=1 'Mussel species: Fatmucket';
run;
```

```
* date revised  : 12May11 - MPG
* Verified by:  ______ (Date:______)
* Random allocation of glochidia to tank.sas
*********************************************************************/
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

FOOTNOTE1 'Analysis performed by M. Gaikowski SAS version ' &SYSVER &SYSTIME &SYSDATE;
WARNING: The FOOTNOTE statement is ambiguous due to invalid options or unquoted text.

options /*ls=85 ps=40 formdlim='-' */ pageno = 1 nocenter nodate nosource2;

/*Random distribution of glochidia to experimental tanks*/
/* tank 1 to 18 = tank A1 to A6 (1-6), tank B1 to B6 (7-12), tank C1 to C6 (13-18)
   round = distribution rounds 1 to 2, place one aliquot of glochidia per tank per round */

/*******************************************************************************************
! **********/

/*Mussel species: Plain pocketbook*/
data glochidia;
 do round = 1 to 10 by 1;
 do row = 1 to 3 by 1;
  do tank = 1 to 6 by 1;
   x = ranuni(-1);
   output;
  end;
  end;
 end;
run;

NOTE: The data set WORK.GLOCHIDIA has 180 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.10 seconds
      cpu time            0.07 seconds


data glochidiadist; set glochidia;
if row = 1 then _row_ = 'A';
if row = 2 then _row_ = 'B';
if row = 3 then _row_ = 'C';
 if row = 1 and tank = 1 then tankn = '1A1';
  if row = 1 and tank = 2 then tankn = '1A2';
   if row = 1 and tank = 3 then tankn = '1A3';
    if row = 1 and tank = 4 then tankn = '1A4';
     if row = 1 and tank = 5 then tankn = '1A5';
      if row = 1 and tank = 6 then tankn = '1A6';
 if row = 2 and tank = 1 then tankn = '1B1';
  if row = 2 and tank = 2 then tankn = '1B2';
   if row = 2 and tank = 3 then tankn = '1B3';
    if row = 2 and tank = 4 then tankn = '1B4';
     if row = 2 and tank = 5 then tankn = '1B5';
      if row = 2 and tank = 6 then tankn = '1B6';
 if row = 3 and tank = 1 then tankn = '1C1';
  if row = 3 and tank = 2 then tankn = '1C2';
   if row = 3 and tank = 3 then tankn = '1C3';
    if row = 3 and tank = 4 then tankn = '1C4';
     if row = 3 and tank = 5 then tankn = '1C5';
```

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```
        if row = 3 and tank = 6 then tankn = '1C6'; run;

NOTE: There were 180 observations read from the data set WORK.GLOCHIDIA.
NOTE: The data set WORK.GLOCHIDIADIST has 180 observations and 6 variables.
NOTE: DATA statement used (Total process time):
      real time           0.03 seconds
      cpu time            0.01 seconds


  proc sort data= glochidiadist;
   by round x;
   run;

NOTE: There were 180 observations read from the data set WORK.GLOCHIDIADIST.
NOTE: The data set WORK.GLOCHIDIADIST has 180 observations and 6 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.04 seconds
      cpu time            0.03 seconds


  proc print data = glochidiadist;
  title1 h=2 'Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid
! mussel species';
  title2 h=1.5 'AEH-11-PSUEDO-01';
  title3 h=1 'Random assignment of glochidia to test tanks';
  title4 h=1 'Mussel species: Plain pocketbook';
  run;

NOTE: There were 180 observations read from the data set WORK.GLOCHIDIADIST.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.06 seconds
      cpu time            0.04 seconds



  /*********************************************************************************************
! **********/

  /*Mussel species: Higgins eye */
  data glochidia;
   do round = 1 to 10 by 1;
   do row = 1 to 3 by 1;
    do tank = 1 to 6 by 1;
     x = ranuni(-1);
     output;
    end;
    end;
   end;
  run;

NOTE: The data set WORK.GLOCHIDIA has 180 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds

  data glochidiadist; set glochidia;
```

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```
if row = 1 then _row_ = 'A';
if row = 2 then _row_ = 'B';
if row = 3 then _row_ = 'C';
 if row = 1 and tank = 1 then tankn = '2A1';
  if row = 1 and tank = 2 then tankn = '2A2';
   if row = 1 and tank = 3 then tankn = '2A3';
    if row = 1 and tank = 4 then tankn = '2A4';
     if row = 1 and tank = 5 then tankn = '2A5';
      if row = 1 and tank = 6 then tankn = '2A6';
 if row = 2 and tank = 1 then tankn = '2B1';
  if row = 2 and tank = 2 then tankn = '2B2';
   if row = 2 and tank = 3 then tankn = '2B3';
    if row = 2 and tank = 4 then tankn = '2B4';
     if row = 2 and tank = 5 then tankn = '2B5';
      if row = 2 and tank = 6 then tankn = '2B6';
 if row = 3 and tank = 1 then tankn = '2C1';
  if row = 3 and tank = 2 then tankn = '2C2';
   if row = 3 and tank = 3 then tankn = '2C3';
    if row = 3 and tank = 4 then tankn = '2C4';
     if row = 3 and tank = 5 then tankn = '2C5';
      if row = 3 and tank = 6 then tankn = '2C6'; run;

NOTE: There were 180 observations read from the data set WORK.GLOCHIDIA.
NOTE: The data set WORK.GLOCHIDIADIST has 180 observations and 6 variables.
NOTE: DATA statement used (Total process time):
      real time           0.03 seconds
      cpu time            0.03 seconds


proc sort data= glochidiadist;
 by round x;
run;

NOTE: There were 180 observations read from the data set WORK.GLOCHIDIADIST.
NOTE: The data set WORK.GLOCHIDIADIST has 180 observations and 6 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc print data = glochidiadist;
title1 h=2 'Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid
mussel species';
title2 h=1.5 'AEH-11-PSUEDO-01';
title3 h=1 'Random assignment of glochidia to test tank';
title4 h=1 'Mussel species: Higgins eye';
run;

NOTE: There were 180 observations read from the data set WORK.GLOCHIDIADIST.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


/*****************************************************************************************************
**********/
```

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```

/*Mussel species: Fatmucket */
data glochidia;
 do round = 1 to 10 by 1;
 do row = 1 to 3 by 1;
  do tank = 1 to 6 by 1;
   x = ranuni(-1);
   output;
  end;
  end;
 end;
run;

NOTE: The data set WORK.GLOCHIDIA has 180 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


data glochidiadist; set glochidia;
if row = 1 then _row_ = 'A';
if row = 2 then _row_ = 'B';
if row = 3 then _row_ = 'C';
 if row = 1 and tank = 1 then tankn = '3A1';
  if row = 1 and tank = 2 then tankn = '3A2';
   if row = 1 and tank = 3 then tankn = '3A3';
    if row = 1 and tank = 4 then tankn = '3A4';
     if row = 1 and tank = 5 then tankn = '3A5';
      if row = 1 and tank = 6 then tankn = '3A6';
 if row = 2 and tank = 1 then tankn = '3B1';
  if row = 2 and tank = 2 then tankn = '3B2';
   if row = 2 and tank = 3 then tankn = '3B3';
    if row = 2 and tank = 4 then tankn = '3B4';
     if row = 2 and tank = 5 then tankn = '3B5';
      if row = 2 and tank = 6 then tankn = '3B6';
 if row = 3 and tank = 1 then tankn = '3C1';
  if row = 3 and tank = 2 then tankn = '3C2';
   if row = 3 and tank = 3 then tankn = '3C3';
    if row = 3 and tank = 4 then tankn = '3C4';
     if row = 3 and tank = 5 then tankn = '3C5';
      if row = 3 and tank = 6 then tankn = '3C6'; run;

NOTE: There were 180 observations read from the data set WORK.GLOCHIDIA.
NOTE: The data set WORK.GLOCHIDIADIST has 180 observations and 6 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc sort data= glochidiadist;
 by round x;
run;

NOTE: There were 180 observations read from the data set WORK.GLOCHIDIADIST.
NOTE: The data set WORK.GLOCHIDIADIST has 180 observations and 6 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.00 seconds
```

```
     cpu time            0.00 seconds

proc print data = glochidiadist;
title1 h=2 'Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid
mussel species';
title2 h=1.5 'AEH-11-PSUEDO-01';
title3 h=1 'Random assignment of glochidia to test tank';
title4 h=1 'Mussel species: Fatmucket';
run;

NOTE: There were 180 observations read from the data set WORK.GLOCHIDIADIST.
NOTE: PROCEDURE PRINT used (Total process time):
     real time           0.01 seconds
     cpu time            0.01 seconds
```

MM 12 May 11

File Folder 14B

PROOFED BY
Initials: TMS Date: 5/14/12
REVIEWED BY
Initials: ____ Date: ____

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```
/*********************************************************************
*  Study Number  : AEH-11-PSUEDO-01
*  Study Director: Jim Luoma
*  date created  : 14Oct11 - MPG mm
*  Verified by:  ______ (Date:_______)                    page ____ of ____
*  Random allocation of glochidia to tank.sas
*********************************************************************/
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

FOOTNOTE1 'Analysis performed by M. Gaikowski SAS version ' &SYSVER &SYSTIME &SYSDATE;

options /*ls=85 ps=40 formdlim='-' */ pageno = 1 nocenter nodate nosource2;

/*Random distribution of glochidia to experimental tanks*/
/* tank 1 to 18 = tank A1 to A6 (1-6), tank B1 to B6 (7-12), tank C1 to C6 (13-18)
   round = distribution rounds 1 to 2, place one aliquot of glochidia per tank per round */

/*******************************************************************************************

/*Mussel species: Washboard*/
data glochidia;
 do round = 1 to 10 by 1;
 do row = 1 to 3 by 1;
  do tank = 1 to 6 by 1;
   x = ranuni(-1);
   output;
  end;
  end;
 end;
run;
data glochidiadist; set glochidia;
if row = 1 then _row_ = 'A';
if row = 2 then _row_ = 'B';
if row = 3 then _row_ = 'C';
 if row = 1 and tank = 1 then tankn = '1A1';
  if row = 1 and tank = 2 then tankn = '1A2';
   if row = 1 and tank = 3 then tankn = '1A3';
    if row = 1 and tank = 4 then tankn = '1A4';
     if row = 1 and tank = 5 then tankn = '1A5';
      if row = 1 and tank = 6 then tankn = '1A6';
 if row = 2 and tank = 1 then tankn = '1B1';
  if row = 2 and tank = 2 then tankn = '1B2';
   if row = 2 and tank = 3 then tankn = '1B3';
    if row = 2 and tank = 4 then tankn = '1B4';
     if row = 2 and tank = 5 then tankn = '1B5';
      if row = 2 and tank = 6 then tankn = '1B6';
 if row = 3 and tank = 1 then tankn = '1C1';
  if row = 3 and tank = 2 then tankn = '1C2';
   if row = 3 and tank = 3 then tankn = '1C3';
    if row = 3 and tank = 4 then tankn = '1C4';
     if row = 3 and tank = 5 then tankn = '1C5';
      if row = 3 and tank = 6 then tankn = '1C6'; run;
proc sort data= glochidiadist;
 by round x;
 run;
proc print data = glochidiadist;
title1 h=2 'Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel
title2 h=1.5 'AEH-11-PSUEDO-01';
title3 h=1 'Random assignment of glochidia to test tanks';
title4 h=1 'Mussel species: Washboard';
run;
```

AEH-11-PSEUDO-01

File Folder 14B
Book 1 p. 21-22

Item No. 2

PROOFED BY
Initials: JMS Date: 5/14/12
REVIEWED BY
Initials ______ Date ______

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```
/***************************************************************************************************

/*Mussel species: Fatmucket */
data glochidia;
 do round = 1 to 10 by 1;
 do row = 1 to 3 by 1;
  do tank = 1 to 6 by 1;
   x = ranuni(-1);
   output;
  end;
  end;
 end;
run;
data glochidiadist; set glochidia;
if row = 1 then _row_ = 'A';
if row = 2 then _row_ = 'B';
if row = 3 then _row_ = 'C';
 if row = 1 and tank = 1 then tankn = '3A1';
  if row = 1 and tank = 2 then tankn = '3A2';
   if row = 1 and tank = 3 then tankn = '3A3';
    if row = 1 and tank = 4 then tankn = '3A4';
     if row = 1 and tank = 5 then tankn = '3A5';
      if row = 1 and tank = 6 then tankn = '3A6';
 if row = 2 and tank = 1 then tankn = '3B1';
  if row = 2 and tank = 2 then tankn = '3B2';
   if row = 2 and tank = 3 then tankn = '3B3';
    if row = 2 and tank = 4 then tankn = '3B4';
     if row = 2 and tank = 5 then tankn = '3B5';
      if row = 2 and tank = 6 then tankn = '3B6';
 if row = 3 and tank = 1 then tankn = '3C1';
  if row = 3 and tank = 2 then tankn = '3C2';
   if row = 3 and tank = 3 then tankn = '3C3';
    if row = 3 and tank = 4 then tankn = '3C4';
     if row = 3 and tank = 5 then tankn = '3C5';
      if row = 3 and tank = 6 then tankn = '3C6'; run;
proc sort data= glochidiadist;
 by round x;
run;
proc print data = glochidiadist;
title1 h=2 'Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel
title2 h=1.5 'AEH-11-PSUEDO-01';
title3 h=1 'Random assignment of glochidia to test tank';
title4 h=1 'Mussel species: Fatmucket';
run;
```

```
*  date created  : 14Oct11 - MPG
*  Verified by:  ______ (Date:______)                     page ____ of ____
*  Random allocation of glochidia to tank.sas
********************************************************************/
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;
```

AEH-11-PSEUDO-01

```
FOOTNOTE1 'Analysis performed by M. Gaikowski SAS version ' &SYSVER &SYSTIME &SYSDATE;
WARNING: The FOOTNOTE statement is ambiguous due to invalid options or unquoted text.

options /*ls=85 ps=40 formdlim='-' */ pageno = 1 nocenter nodate nosource2;

/*Random distribution of glochidia to experimental tanks*/
/* tank 1 to 18 = tank A1 to A6 (1-6), tank B1 to B6 (7-12), tank C1 to C6 (13-18)
   round = distribution rounds 1 to 2, place one aliquot of glochidia per tank per round */

/*****************************************************************************************
**********/

/*Mussel species: Washboard*/
data glochidia;
 do round = 1 to 10 by 1;
 do row = 1 to 3 by 1;
  do tank = 1 to 6 by 1;
   x = ranuni(-1);
   output;
  end;
  end;
 end;
run;

NOTE: The data set WORK.GLOCHIDIA has 180 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.00 seconds
      cpu time            0.01 seconds


data glochidiadist; set glochidia;
if row = 1 then _row_ = 'A';
if row = 2 then _row_ = 'B';
if row = 3 then _row_ = 'C';
 if row = 1 and tank = 1 then tankn = '1A1';
  if row = 1 and tank = 2 then tankn = '1A2';
   if row = 1 and tank = 3 then tankn = '1A3';
    if row = 1 and tank = 4 then tankn = '1A4';
     if row = 1 and tank = 5 then tankn = '1A5';
      if row = 1 and tank = 6 then tankn = '1A6';
 if row = 2 and tank = 1 then tankn = '1B1';
  if row = 2 and tank = 2 then tankn = '1B2';
   if row = 2 and tank = 3 then tankn = '1B3';
    if row = 2 and tank = 4 then tankn = '1B4';
     if row = 2 and tank = 5 then tankn = '1B5';
      if row = 2 and tank = 6 then tankn = '1B6';
 if row = 3 and tank = 1 then tankn = '1C1';
  if row = 3 and tank = 2 then tankn = '1C2';
   if row = 3 and tank = 3 then tankn = '1C3';
    if row = 3 and tank = 4 then tankn = '1C4';
     if row = 3 and tank = 5 then tankn = '1C5';
      if row = 3 and tank = 6 then tankn = '1C6'; run;

NOTE: There were 180 observations read from the data set WORK.GLOCHIDIA.
NOTE: The data set WORK.GLOCHIDIADIST has 180 observations and 6 variables.
```

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```
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.03 seconds


proc sort data= glochidiadist;
 by round x;
 run;

NOTE: There were 180 observations read from the data set WORK.GLOCHIDIADIST.
NOTE: The data set WORK.GLOCHIDIADIST has 180 observations and 6 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.00 seconds
      cpu time            0.01 seconds


proc print data = glochidiadist;
title1 h=2 'Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid
mussel species';
title2 h=1.5 'AEH-11-PSUEDO-01';
title3 h=1 'Random assignment of glochidia to test tanks';
title4 h=1 'Mussel species: Washboard';
run;

NOTE: There were 180 observations read from the data set WORK.GLOCHIDIADIST.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.00 seconds
      cpu time            0.01 seconds



/*********************************************************************************************
**********/

/*Mussel species: Fatmucket */
data glochidia;
 do round = 1 to 10 by 1;
 do row = 1 to 3 by 1;
  do tank = 1 to 6 by 1;
   x = ranuni(-1);
   output;
  end;
  end;
 end;
run;

NOTE: The data set WORK.GLOCHIDIA has 180 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.00 seconds


data glochidiadist; set glochidia;
if row = 1 then _row_ = 'A';
if row = 2 then _row_ = 'B';
if row = 3 then _row_ = 'C';
 if row = 1 and tank = 1 then tankn = '3A1';
  if row = 1 and tank = 2 then tankn = '3A2';
   if row = 1 and tank = 3 then tankn = '3A3';
    if row = 1 and tank = 4 then tankn = '3A4';
     if row = 1 and tank = 5 then tankn = '3A5';
```

```
      if row = 1 and tank = 6 then tankn = '3A6';
 if row = 2 and tank = 1 then tankn = '3B1';
  if row = 2 and tank = 2 then tankn = '3B2';
   if row = 2 and tank = 3 then tankn = '3B3';
    if row = 2 and tank = 4 then tankn = '3B4';
     if row = 2 and tank = 5 then tankn = '3B5';
      if row = 2 and tank = 6 then tankn = '3B6';
 if row = 3 and tank = 1 then tankn = '3C1';
  if row = 3 and tank = 2 then tankn = '3C2';
   if row = 3 and tank = 3 then tankn = '3C3';
    if row = 3 and tank = 4 then tankn = '3C4';
     if row = 3 and tank = 5 then tankn = '3C5';
      if row = 3 and tank = 6 then tankn = '3C6'; run;

NOTE: There were 180 observations read from the data set WORK.GLOCHIDIA.
NOTE: The data set WORK.GLOCHIDIADIST has 180 observations and 6 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc sort data= glochidiadist;
 by round x;
run;

NOTE: There were 180 observations read from the data set WORK.GLOCHIDIADIST.
NOTE: The data set WORK.GLOCHIDIADIST has 180 observations and 6 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.00 seconds
      cpu time            0.01 seconds


proc print data = glochidiadist;
title1 h=2 'Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid
mussel species';
title2 h=1.5 'AEH-11-PSUEDO-01';
title3 h=1 'Random assignment of glochidia to test tank';
title4 h=1 'Mussel species: Fatmucket';
run;

NOTE: There were 180 observations read from the data set WORK.GLOCHIDIADIST.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds
```

Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel species 1
AEH-11-PSUEDO-01
Random assignment of glochidia to test tanks
Mussel species: Washboard

AEH-11-PSEUDO-01

Obs	round	row	tank	x	_row_	tankn
1	1	2	3	0.04838	B	1B3 ✓
2	1	2	1	0.15057	B	1B1 ✓
3	1	2	5	0.16278	B	1B5 ✓
4	1	3	6	0.18466	C	1C6 ✓
5	1	2	4	0.36690	B	1B4 ✓
6	1	3	5	0.37006	C	1C5 ✓ * ✓
7	1	1	1	0.44366	A	1A1 ✓
8	1	3	2	0.44674	C	1C2 ✓
9	1	1	5	0.45064	A	1A5 ✓
10	1	1	2	0.49027	A	1A2 ✓
11	1	1	6	0.56970	A	1A6 ✓
12	1	2	6	0.63330	B	1B6 ✓ * ✓
13	1	3	4	0.70052	C	1C4 ✓
14	1	3	1	0.77579	C	1C1 ✓
15	1	3	3	0.77615	C	1C3 ✓
16	1	1	3	0.77930	A	1A3 ✓
17	1	1	4	0.82716	A	1A4 ✓
18	1	2	2	0.86307	B	1B2 ✓ * ✓
19	2	2	6	0.05096	B	1B6 ✓
20	2	1	3	0.13832	A	1A3 ✓
21	2	3	3	0.21191	C	1C3 ✓
22	2	3	2	0.36940	C	1C2 ✓
23	2	1	2	0.38742	A	1A2 ✓
24	2	2	3	0.41658	B	1B3 ✓ * ✓
25	2	1	4	0.54504	A	1A4 ✓
26	2	3	5	0.58445	C	1C5 ✓
27	2	2	1	0.61804	B	1B1 ✓
28	2	1	6	0.66695	A	1A6 ✓
29	2	2	2	0.77401	B	1B2 ✓
30	2	3	1	0.84946	C	1C1 ✓ * ✓
31	2	2	4	0.85021	B	1B4 ✓
32	2	3	6	0.88869	C	1C6 ✓
33	2	1	1	0.90716	A	1A1 ✓
34	2	2	5	0.96065	B	1B5 ✓
35	2	1	5	0.98905	A	1A5 ✓
36	2	3	4	0.99356	C	1C4 ✓ *
37	3	3	3	0.00319	C	1C3 ✓
38	3	2	1	0.00885	B	1B1 ✓
39	3	3	6	0.06275	C	1C6 ✓
40	3	1	4	0.15052	A	1A4 ✓
41	3	2	3	0.20804	B	1B3 ✓
42	3	3	4	0.46083	C	1C4 ✓ *
43	3	2	2	0.47385	B	1B2 ✓
44	3	2	6	0.56072	B	1B6 ✓
45	3	2	4	0.56497	B	1B4 ✓
46	3	3	2	0.57337	C	1C2 ✓
47	3	3	1	0.63977	C	1C1 ✓
48	3	1	2	0.65438	A	1A2 ✓ *
49	3	2	5	0.73127	B	1B5 ✓
50	3	3	5	0.82134	C	1C5 ✓
51	3	1	6	0.82425	A	1A6 ✓
52	3	1	5	0.92219	A	1A5 ✓

Analysis performed by M. Gaikowski SAS version 9.2 11:03 14OCT11 MTL

Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel species 2
AEH-11-PSUEDO-01
Random assignment of glochidia to test tanks
Mussel species: Washboard

AEH-11-PSEUDO-01

Obs	round	row	tank	x	_row_	tankn
53	3	1	1	0.96150	A	1A1
54	3	1	3	0.99602	A	1A3 ①
55	4	1	2	0.01675	A	1A2
56	4	2	3	0.03225	B	1B3
57	4	1	6	0.07077	A	1A6
58	4	3	3	0.09805	C	1C3
59	4	3	2	0.10623	C	1C2
60	4	2	6	0.27354	B	1B6
61	4	2	5	0.29696	B	1B5
62	4	2	1	0.29785	B	1B1
63	4	3	6	0.31372	C	1C6
64	4	1	1	0.43548	A	1A1
65	4	1	4	0.49463	A	1A4
66	4	1	5	0.57987	A	1A5
67	4	3	1	0.72352	C	1C1
68	4	2	4	0.91329	B	1B4
69	4	3	4	0.91675	C	1C4
70	4	2	2	0.92533	B	1B2
71	4	1	3	0.96086	A	1A3
72	4	3	5	0.97561	C	1C5
73	5	1	1	0.01106	A	1A1
74	5	2	1	0.18516	B	1B1
75	5	2	6	0.21654	B	1B6
76	5	3	1	0.26751	C	1C1
77	5	1	2	0.30069	A	1A2
78	5	2	2	0.43225	B	1B2
79	5	1	6	0.48596	A	1A6
80	5	2	5	0.48743	B	1B5
81	5	3	4	0.54357	C	1C4
82	5	3	3	0.63776	C	1C3
83	5	2	4	0.73840	B	1B4
84	5	3	5	0.75303	C	1C5
85	5	3	2	0.77589	C	1C2
86	5	2	3	0.80186	B	1B3
87	5	3	6	0.80385	C	1C6
88	5	1	3	0.88062	A	1A3
89	5	1	5	0.91017	A	1A5
90	5	1	4	0.93914	A	1A4
91	6	1	3	0.10293	A	1A3
92	6	1	2	0.12505	A	1A2
93	6	2	2	0.13401	B	1B2
94	6	3	1	0.17936	C	1C1
95	6	2	5	0.27550	B	1B5
96	6	1	4	0.31435	A	1A4
97	6	1	6	0.35475	A	1A6
98	6	3	4	0.37608	C	1C4
99	6	1	1	0.38250	A	1A1
100	6	2	4	0.45321	B	1B4
101	6	3	6	0.48700	C	1C6
102	6	2	3	0.49334	B	1B3
103	6	2	6	0.57207	B	1B6
104	6	1	5	0.64539	A	1A5

Five rounds of glochidia were added to exposure chambers. Rounds 6-10 of the random assignment were not used. Statement added for clarification TMS 5/14/12

Analysis performed by W. Gaikowski SAS version 9.2 11:03 14OCT11

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① Distribution aliquot after 1A3 not 1A1 JKW 18OCT11

Random assignment of glochidia to test tanks
Mussel species: Washboard

AEH-11-PSEUDO-01

Obs	round	row	tank	x	_row_	tankn
105	6	2	1	0.65028	B	1B1
106	6	3	3	0.69455	C	1C3
107	6	3	2	0.74112	C	1C2
108	6	3	5	0.91209	C	1C5
109	7	2	2	0.07653	B	1B2
110	7	3	4	0.12108	C	1C4
111	7	1	4	0.12753	A	1A4
112	7	2	3	0.14355	B	1B3
113	7	1	3	0.21451	A	1A3
114	7	3	1	0.21495	C	1C1
115	7	3	6	0.24565	C	1C6
116	7	3	2	0.35784	C	1C2
117	7	2	6	0.37076	B	1B6
118	7	1	5	0.48846	A	1A5
119	7	1	6	0.49531	A	1A6
120	7	3	5	0.62835	C	1C5
121	7	1	1	0.66040	A	1A1
122	7	2	1	0.78412	B	1B1
123	7	3	3	0.84819	C	1C3
124	7	2	5	0.84879	B	1B5
125	7	2	4	0.91208	B	1B4
126	7	1	2	0.93405	A	1A2
127	8	1	4	0.07285	A	1A4
128	8	2	2	0.13071	B	1B2
129	8	3	3	0.17105	C	1C3
130	8	1	6	0.17484	A	1A6
131	8	1	1	0.17628	A	1A1
132	8	3	4	0.21731	C	1C4
133	8	3	1	0.29783	C	1C1
134	8	2	3	0.39927	B	1B3
135	8	3	2	0.44313	C	1C2
136	8	3	5	0.46488	C	1C5
137	8	2	5	0.48661	B	1B5
138	8	2	1	0.65784	B	1B1
139	8	3	6	0.74051	C	1C6
140	8	1	5	0.74383	A	1A5
141	8	1	2	0.75499	A	1A2
142	8	2	4	0.77273	B	1B4
143	8	2	6	0.85171	B	1B6
144	8	1	3	0.98418	A	1A3
145	9	2	5	0.00420	B	1B5
146	9	1	5	0.07321	A	1A5
147	9	2	4	0.10943	B	1B4
148	9	1	1	0.12746	A	1A1
149	9	1	3	0.12940	A	1A3
150	9	2	3	0.21011	B	1B3
151	9	3	2	0.22581	C	1C2
152	9	3	4	0.23737	C	1C4
153	9	3	3	0.24321	C	1C3
154	9	3	6	0.56414	C	1C6
155	9	1	4	0.63829	A	1A4
156	9	2	6	0.63940	B	1B6

AEH-11-PSUEDO-01
Random assignment of glochidia to test tanks
Mussel species: Washboard

AEH-11-PSEUDO-01

Obs	round	row	tank	x	_row_	tankn
157	9	3	1	0.78984	C	1C1
158	9	1	2	0.83409	A	1A2
159	9	2	2	0.83827	B	1B2
160	9	2	1	0.86606	B	1B1
161	9	1	6	0.93247	A	1A6
162	9	3	5	0.97047	C	1C5
163	10	1	5	0.13868	A	1A5
164	10	2	3	0.19793	B	1B3
165	10	2	1	0.22524	B	1B1
166	10	2	2	0.26319	B	1B2
167	10	1	1	0.33809	A	1A1
168	10	1	2	0.35208	A	1A2
169	10	2	6	0.40875	B	1B6
170	10	2	5	0.49523	B	1B5
171	10	3	2	0.54407	C	1C2
172	10	3	6	0.62890	C	1C6
173	10	3	4	0.66996	C	1C4
174	10	1	3	0.69539	A	1A3
175	10	1	6	0.73423	A	1A6
176	10	3	1	0.77376	C	1C1
177	10	2	4	0.81274	B	1B4
178	10	3	5	0.83455	C	1C5
179	10	3	3	0.98332	C	1C3
180	10	1	4	0.99509	A	1A4

Analysis performed by M. Gaikowski SAS version 9.2 11:03 14OCT11

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Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel species 5
AEH-11-PSUEDO-01
Random assignment of glochidia to test tank
Mussel species: ~~Fatmucket~~ ① Plain Pocketbook

① Species changed to Plain pocket book due to availability 10-18-11 JTL

AEH-11-PSEUDO-01

Obs	round	row	tank	x	_row_	tankn
1	1	2	5	0.01984	B	3B5 *
2	1	2	4	0.02206	B	3B4
3	1	2	2	0.03535	B	3B2
4	1	2	1	0.06789	B	3B1
5	1	3	4	0.09694	C	3C4
6	1	3	6	0.14775	C	3C6 *
7	1	3	3	0.16191	C	3C3
8	1	3	1	0.20688	C	3C1
9	1	1	3	0.20991	A	3A3
10	1	1	6	0.30468	A	3A6
11	1	1	2	0.36183	A	3A2
12	1	2	3	0.45100	B	3B3 *
13	1	3	5	0.54897	C	3C5
14	1	2	6	0.68545	B	3B6
15	1	1	4	0.69170	A	3A4
16	1	1	1	0.70064	A	3A1
17	1	1	5	0.70843	A	3A5
18	1	3	2	0.90442	C	3C2 *
19	2	3	1	0.00545	C	3C1
20	2	2	5	0.11329	B	3B5
21	2	1	2	0.13927	A	3A2
22	2	3	6	0.17332	C	3C6
23	2	1	6	0.22802	A	3A6
24	2	2	4	0.31714	B	3B4 *
25	2	2	6	0.38788	B	3B6
26	2	1	1	0.39340	A	3A1
27	2	1	5	0.40032	A	3A5
28	2	2	2	0.47171	B	3B2
29	2	2	1	0.54855	B	3B1
30	2	3	3	0.57131	C	3C3 *
31	2	3	5	0.61824	C	3C5
32	2	3	2	0.72234	C	3C2
33	2	1	3	0.79335	A	3A3
34	2	2	3	0.82843	B	3B3
35	2	1	4	0.93168	A	3A4
36	2	3	4	0.99853	C	3C4 *
37	3	3	2	0.02972	C	3C2
38	3	3	4	0.05996	C	3C4
39	3	1	1	0.20484	A	3A1
40	3	2	3	0.29113	B	3B3
41	3	2	5	0.29706	B	3B5
42	3	1	6	0.31291	A	3A6 *
43	3	2	6	0.38686	B	3B6
44	3	3	1	0.42806	C	3C1
45	3	1	5	0.53249	A	3A5
46	3	1	3	0.57085	A	3A3
47	3	1	2	0.57194	A	3A2
48	3	3	3	0.58116	C	3C3 *
49	3	2	2	0.65021	B	3B2
50	3	3	5	0.68760	C	3C5
51	3	2	4	0.91176	B	3B4
52	3	3	6	0.93952	C	3C6

Analysis performed by M. Gaikowski SAS version 9.2 11:03 14OCT11

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* sample for initial distribution count. 10-18-11 JTL

Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel species 6
AEH-11-PSUEDO-01
Random assignment of glochidia to test tank
Mussel species: ~~Fatmucket~~ Plain Pocketbook

D species changed to Plain pocketbook due to availability 10-18-11 JAL

AEH-11-PSEUDO-01

Obs	round	row	tank	x	_row_	tankn
53	3	2	1	0.96219	B	3B1 ✓
54	3	1	4	0.98562	A	3A4 ✓ * ✓
55	4	1	1	0.11087	A	3A1 ✓
56	4	3	4	0.12171	C	3C4 ✓
57	4	2	3	0.26431	B	3B3 ✓
58	4	1	2	0.33085	A	3A2 ✓
59	4	3	3	0.37231	C	3C3 ✓
60	4	3	1	0.44582	C	3C1 ✓ * ✓
61	4	2	2	0.45637	B	3B2 ✓
62	4	2	1	0.51454	B	3B1 ✓
63	4	3	5	0.56189	C	3C5 ✓
64	4	2	6	0.58070	B	3B6 ✓
65	4	1	3	0.67822	A	3A3 ✓
66	4	2	4	0.70090	B	3B4 ✓ * ✓
67	4	2	5	0.75720	B	3B5 ✓
68	4	1	6	0.77556	A	3A6 ✓
69	4	1	5	0.80028	A	3A5 ✓
70	4	1	4	0.89069	A	3A4 ✓
71	4	3	6	0.96558	C	3C6 ✓
72	4	3	2	0.97468	C	3C2 ✓ * ✓
73	5	1	2	0.01511	A	3A2 ✓
74	5	3	6	0.10971	C	3C6 ✓
75	5	1	6	0.12110	A	3A6 ✓
76	5	3	5	0.12841	C	3C5 ✓
77	5	3	3	0.23289	C	3C3 ✓
78	5	1	3	0.26407	A	3A3 ✓ * ✓
79	5	2	6	0.27565	B	3B6 ✓
80	5	2	2	0.30196	B	3B2 ✓
81	5	1	4	0.42720	A	3A4 ✓
82	5	2	4	0.57471	B	3B4 ✓
83	5	2	3	0.71329	B	3B3 ✓
84	5	2	1	0.73755	B	3B1 ✓ * ✓
85	5	1	1	0.76979	A	3A1 ✓
86	5	3	1	0.78149	C	3C1 ✓
87	5	3	4	0.89971	C	3C4 ✓
88	5	1	5	0.90033	A	3A5 ✓
89	5	2	5	0.93155	B	3B5 ✓
90	5	3	2	0.96067	C	3C2 ✓ * ✓
91	6	3	5	0.09556	C	3C5
92	6	2	3	0.12167	B	3B3
93	6	3	3	0.15818	C	3C3
94	6	3	1	0.18872	C	3C1
95	6	3	4	0.20937	C	3C4
96	6	1	3	0.41650	A	3A3
97	6	1	2	0.50092	A	3A2
98	6	1	6	0.54739	A	3A6
99	6	1	5	0.57758	A	3A5
100	6	2	5	0.58475	B	3B5
101	6	3	2	0.74069	C	3C2
102	6	2	4	0.84537	B	3B4
103	6	2	6	0.88420	B	3B6
104	6	1	4	0.89658	A	3A4

Five rounds of glochidia were added to exposure chambers. Rounds 6-10 of the random assignment were not used. statement added for clarification TMS 5/14/12

Analysis performed by M. Gaikowski SAS version 9.2 11:03 14OCT11

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AEH-11-PSUEDO-01
Random assignment of glochidia to test tank
Mussel species: ~~Fatmucket~~ ① Plain pocket book

① species changed to Plain pocket book due to availability 10-18-11 JAL

AEH-11-PSEUDO-01

Obs	round	row	tank	x	_row_	tankn
105	6	2	1	0.90354	B	3B1
106	6	2	2	0.90435	B	3B2
107	6	3	6	0.96706	C	3C6
108	6	1	1	0.97874	A	3A1
109	7	2	4	0.02158	B	3B4
110	7	2	6	0.06957	B	3B6
111	7	3	3	0.11259	C	3C3
112	7	1	6	0.18067	A	3A6
113	7	1	2	0.27586	A	3A2
114	7	1	5	0.28208	A	3A5
115	7	3	2	0.29497	C	3C2
116	7	2	2	0.35074	B	3B2
117	7	3	4	0.38960	C	3C4
118	7	2	1	0.50335	B	3B1
119	7	1	3	0.51187	A	3A3
120	7	2	5	0.77789	B	3B5
121	7	1	1	0.80690	A	3A1
122	7	3	1	0.89798	C	3C1
123	7	1	4	0.95446	A	3A4
124	7	3	5	0.96114	C	3C5
125	7	2	3	0.99428	B	3B3
126	7	3	6	0.99761	C	3C6
127	8	1	3	0.01429	A	3A3
128	8	3	1	0.07414	C	3C1
129	8	1	5	0.14921	A	3A5
130	8	3	4	0.21611	C	3C4
131	8	2	5	0.26547	B	3B5
132	8	1	1	0.31691	A	3A1
133	8	3	5	0.34881	C	3C5
134	8	3	6	0.37552	C	3C6
135	8	2	2	0.37813	B	3B2
136	8	1	6	0.39360	A	3A6
137	8	2	1	0.49526	B	3B1
138	8	2	6	0.50407	B	3B6
139	8	2	4	0.65842	B	3B4
140	8	3	2	0.71088	C	3C2
141	8	1	4	0.76065	A	3A4
142	8	1	2	0.83296	A	3A2
143	8	3	3	0.94481	C	3C3
144	8	2	3	0.97151	B	3B3
145	9	3	3	0.09622	C	3C3
146	9	1	2	0.13471	A	3A2
147	9	1	4	0.18266	A	3A4
148	9	3	4	0.37323	C	3C4
149	9	2	2	0.41030	B	3B2
150	9	3	2	0.41378	C	3C2
151	9	1	1	0.42871	A	3A1
152	9	3	1	0.46783	C	3C1
153	9	2	6	0.59253	B	3B6
154	9	1	5	0.59983	A	3A5
155	9	2	5	0.61762	B	3B5
156	9	1	3	0.66848	A	3A3

Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel species 8
AEH-11-PSUEDO-01
Random assignment of glochidia to test tank
Mussel species: ~~Fatmucket~~ plain pocketbook ①

① Species changed to plain pocketbook due to availability 12-18-11 JAL

AEH-11-PSEUDO-01

File Folder 14B

Obs	round	row	tank	x	_row_	tankn
157	9	2	3	0.83063	B	3B3
158	9	1	6	0.84236	A	3A6
159	9	2	4	0.88438	B	3B4
160	9	3	5	0.92844	C	3C5
161	9	3	6	0.93473	C	3C6
162	9	2	1	0.95001	B	3B1
163	10	1	3	0.01342	A	3A3
164	10	2	5	0.09717	B	3B5
165	10	3	3	0.16608	C	3C3
166	10	1	1	0.23652	A	3A1
167	10	1	5	0.32192	A	3A5
168	10	3	6	0.38088	C	3C6
169	10	3	2	0.43876	C	3C2
170	10	2	6	0.51029	B	3B6
171	10	2	1	0.54732	B	3B1
172	10	3	4	0.57027	C	3C4
173	10	1	4	0.72514	A	3A4
174	10	2	3	0.74935	B	3B3
175	10	1	6	0.80066	A	3A6
176	10	2	2	0.83958	B	3B2
177	10	3	1	0.87588	C	3C1
178	10	1	2	0.88810	A	3A2
179	10	3	5	0.93787	C	3C5
180	10	2	4	0.98796	B	3B4

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Initials: ____ Date: ____

Analysis performed by M. Gaikowski SAS version 9.2 11:03 14OCT11 MPG

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ffects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel species 1
EH-11-PSJEDO-01
andom assignment of glochidia to test tanks Jn✓
ussel species: Black sandshell

Logbook 1, pg 30-31
AEH-11-PSEUDO-01
File folder 14B

Initiated at 1026 h JKW 17JAN11

Item No. 3

bs	round	row	tank	x	_row_	tankn
1	1	1	1	0.03540	A	1A1 ✓ *✓
2	1	2	5	0.15720	B	1B5 ✓
3	1	1	2	0.16134	A	1A2 ✓
4	1	2	3	0.36077	B	1B3 ✓
5	1	1	5	0.39275	A	1A5 ✓
6	1	3	6	0.39514	C	1C6 ✓
7	1	2	2	0.42251	B	1B2 ✓ *✓
8	1	1	6	0.43934	A	1A6 ✓
9	1	3	4	0.45629	C	1C4 ✓
10	1	3	1	0.46298	C	1C1 ✓
11	1	1	4	0.46826	A	1A4 ✓
12	1	3	3	0.49509	C	1C3 ✓
13	1	3	2	0.53590	C	1C2 ✓ *✓
14	1	2	4	0.63459	B	1B4 ✓
15	1	1	3	0.72859	A	1A3 ✓
16	1	3	5	0.73196	C	1C5 ✓
17	1	2	6	0.85674	B	1B6 ✓
18	1	2	1	0.86215	B	1B1 ✓
19	2	1	1	0.00152	A	1A1 ✓ *✓ 1032
20	2	2	6	0.01044	B	1B6 ✓
21	2	2	1	0.01183	B	1B1 ✓
22	2	1	6	0.09963	A	1A6 ✓
23	2	3	6	0.16381	C	1C6 ✓
24	2	3	1	0.37238	C	1C1 ✓
25	2	3	2	0.39250	C	1C2 ✓ *✓
26	2	1	5	0.39924	A	1A5 ✓
27	2	1	3	0.48046	A	1A3 ✓
28	2	2	3	0.57825	B	1B3 ✓
29	2	3	4	0.65481	C	1C4 ✓
30	2	2	2	0.71580	B	1B2 ✓
31	2	3	3	0.73748	C	1C3 ✓ *✓
32	2	3	5	0.76035	C	1C5 ✓
33	2	1	4	0.79744	A	1A4 ✓
34	2	1	2	0.87184	A	1A2 ✓
35	2	2	4	0.93146	B	1B4 ✓
36	2	2	5	0.94375	B	1B5 ✓
37	3	1	3	0.08401	A	1A3 ✓ *✓ 1037
38	3	3	5	0.11492	C	1C5 ✓
39	3	1	4	0.12051	A	1A4 ✓
40	3	1	1	0.17095	A	1A1 ✓
41	3	2	6	0.25392	B	1B6 ✓
42	3	2	3	0.26144	B	1B3 ✓
43	3	3	4	0.40117	C	1C4 ✓ *✓
44	3	1	6	0.41257	A	1A6 ✓
45	3	1	2	0.45539	A	1A2 ✓
46	3	2	5	0.51031	B	1B5 ✓
47	3	1	5	0.55130	A	1A5 ✓

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Initials: Jn Date: 10/2/12

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* Sample for distribution aliquot KLW 17JAN12

nalysis performed by J. Luoma SAS version 9.2 12:27 09JAN12

ffects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel species
EH-11-PSUEDO-01
andom assignment of glochidia to test tanks
ussel species: Black sandshell

AEH-11-PSEUDO-01

bs	round	row	tank	x	_row_	tankn	
48	3	3	6	0.63349	C	1C6	*
49	3	2	4	0.66683	B	1B4	
50	3	2	1	0.67735	B	1B1	
51	3	2	2	0.74551	B	1B2	
52	3	3	1	0.79592	C	1C1	
53	3	3	2	0.85372	C	1C2	
54	3	3	3	0.97376	C	1C3	* 1041
55	4	2	6	0.04165	B	1B6	
56	4	3	3	0.12059	C	1C3	
57	4	3	5	0.19203	C	1C5	
58	4	2	2	0.23442	B	1B2	
59	4	2	3	0.31512	B	1B3	
60	4	1	3	0.42558	A	1A3	*
61	4	2	1	0.46156	B	1B1	
62	4	3	6	0.49274	C	1C6	
63	4	2	5	0.53001	B	1B5	
64	4	1	4	0.64966	A	1A4	
65	4	1	5	0.66283	A	1A5	
66	4	1	6	0.71016	A	1A6	*
67	4	3	2	0.75088	C	1C2	
68	4	1	1	0.82426	A	1A1	
69	4	1	2	0.86272	A	1A2	
70	4	3	1	0.88343	C	1C1	
71	4	2	4	0.90920	B	1B4	
72	4	3	4	0.94484	C	1C4	* 1048
73	5	2	6	0.05331	B	1B6	
74	5	1	5	0.07180	A	1A5	
75	5	2	2	0.07704	B	1B2	
76	5	3	3	0.12926	C	1C3	
77	5	3	4	0.21732	C	1C4	
78	5	2	3	0.29334	B	1B3	*
79	5	1	1	0.29504	A	1A1	
80	5	1	4	0.32955	A	1A4	
81	5	2	1	0.34201	B	1B1	
82	5	3	2	0.35173	C	1C2	
83	5	1	2	0.46220	A	1A2	
84	5	3	6	0.64764	C	1C6	*
85	5	1	3	0.66290	A	1A3	
86	5	2	4	0.82630	B	1B4	
87	5	3	1	0.92558	C	1C1	
88	5	2	5	0.92810	B	1B5	
89	5	3	5	0.95883	C	1C5	
90	5	1	6	0.98341	A	1A6	*
91	6	1	6	0.00717	A	1A6	
92	6	2	1	0.03283	B	1B1	
93	6	2	3	0.05644	B	1B3	
94	6	1	4	0.09740	A	1A4	

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Completed at 1051 h JKW 17JAN12

* Sample for distribution aliquot KLW 12JAN12

nalysis performed by J. Luoma SAS version 9.2 12:27 09JAN12

andom assignment of glochidia to test tanks
ussel species: Black sandshell

AEH-11-PSEUDO-01

bs	round	row	tank	x	_row_	tankn
95	6	3	4	0.16739	C	1C4
96	6	2	6	0.21512	B	1B6
97	6	2	4	0.35347	B	1B4
98	6	2	2	0.36195	B	1B2
99	6	3	2	0.52497	C	1C2
00	6	1	3	0.60721	A	1A3
01	6	3	3	0.61745	C	1C3
02	6	2	5	0.63064	B	1B5
03	6	3	1	0.64129	C	1C1
04	6	3	6	0.66559	C	1C6
05	6	3	5	0.67314	C	1C5
06	6	1	2	0.78109	A	1A2
07	6	1	5	0.79067	A	1A5
08	6	1	1	0.93271	A	1A1
09	7	2	6	0.05320	B	1B6
10	7	3	3	0.09042	C	1C3
11	7	1	5	0.16011	A	1A5
12	7	1	6	0.20313	A	1A6
13	7	3	6	0.25107	C	1C6
14	7	2	5	0.25202	B	1B5
15	7	2	3	0.35410	B	1B3
16	7	1	2	0.36411	A	1A2
17	7	2	2	0.38468	B	1B2
18	7	2	1	0.40012	B	1B1
19	7	1	3	0.54025	A	1A3
20	7	3	4	0.62582	C	1C4
21	7	1	4	0.64380	A	1A4
22	7	1	1	0.77253	A	1A1
23	7	3	1	0.77724	C	1C1
24	7	3	2	0.94665	C	1C2
25	7	2	4	0.96571	B	1B4
26	7	3	5	0.96636	C	1C5
27	8	3	2	0.03099	C	1C2
28	8	2	2	0.03762	B	1B2
29	8	1	2	0.04247	A	1A2
30	8	1	5	0.09006	A	1A5
31	8	3	5	0.09815	C	1C5
32	8	2	4	0.13921	B	1B4
33	8	3	6	0.16122	C	1C6
34	8	1	4	0.26608	A	1A4
35	8	1	3	0.29674	A	1A3
36	8	2	3	0.32798	B	1B3
37	8	2	1	0.35775	B	1B1
38	8	2	5	0.45371	B	1B5
39	8	1	6	0.50449	A	1A6
40	8	3	1	0.56444	C	1C1
41	8	3	3	0.72173	C	1C3

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* Sample for distribution alignment
11JAN12 TAW12

nalysis performed by J. Luoma SAS version 9.2 12:27 09JAN12

EH-11-PSUEDO-01
andom assignment of glochidia to test tanks
ussel species: Black sandshell

AEH-11-PSEUDO-01

bs	round	row	tank	x	_row_	tankn	
42	8	2	6	0.74113	B	1B6	
43	8	1	1	0.94768	A	1A1	
44	8	3	4	0.94903	C	1C4	*
45	9	2	6	0.03961	B	1B6	
46	9	3	5	0.05729	C	1C5	
47	9	3	1	0.13174	C	1C1	
48	9	1	5	0.14749	A	1A5	
49	9	3	6	0.22306	C	1C6	
50	9	1	6	0.22381	A	1A6	*
51	9	2	4	0.24007	B	1B4	
52	9	1	4	0.35483	A	1A4	
53	9	1	1	0.35749	A	1A1	
54	9	1	3	0.41038	A	1A3	
55	9	2	1	0.43514	B	1B1	
56	9	3	2	0.55984	C	1C2	*
57	9	2	5	0.77391	B	1B5	
58	9	2	3	0.77465	B	1B3	
59	9	2	2	0.80819	B	1B2	
60	9	3	3	0.85688	C	1C3	
61	9	1	2	0.90994	A	1A2	
62	9	3	4	0.93858	C	1C4	*
63	10	2	4	0.02628	B	1B4	
64	10	3	2	0.02875	C	1C2	
65	10	1	3	0.05560	A	1A3	
66	10	1	1	0.11087	A	1A1	
67	10	2	3	0.20975	B	1B3	
68	10	1	5	0.24947	A	1A5	*
69	10	3	5	0.26831	C	1C5	
70	10	3	4	0.36314	C	1C4	
71	10	3	6	0.36828	C	1C6	
72	10	1	2	0.41707	A	1A2	
73	10	3	1	0.52680	C	1C1	
74	10	1	4	0.53721	A	1A4	*
75	10	2	5	0.66343	B	1B5	
76	10	2	2	0.75209	B	1B2	
77	10	3	3	0.85367	C	1C3	
78	10	1	6	0.85835	A	1A6	
79	10	2	1	0.94702	B	1B1	
80	10	2	6	0.96842	B	1B6	*

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* sample for distribution aliquot
KLW 12JAN12

ıalysis performed by J. Luoma SAS version 9.2 12:27 09JAN12

EH-11-PSUEDO-01
andom assignment of glochidia to test tank
ussel species: Mucket

AEH-11-PSEUDO-01
Initiated @ 1200

bs	round	row	tank	x	_row_	tankn	
1	1	1	2	0.00098	A	2A2	*
2	1	2	4	0.01664	B	2B4	
3	1	3	5	0.17106	C	2C5	
4	1	1	1	0.19629	A	2A1	
5	1	3	3	0.21688	C	2C3	
6	1	2	3	0.22886	B	2B3	
7	1	1	3	0.32034	A	2A3	*
8	1	3	2	0.32988	C	2C2	
9	1	2	2	0.33127	B	2B2	
10	1	1	5	0.39490	A	2A5	
11	1	3	6	0.41756	C	2C6	
12	1	2	1	0.61317	B	2B1	
13	1	1	4	0.65694	A	2A4	*
14	1	2	6	0.65809	B	2B6	
15	1	1	6	0.70590	A	2A6	
16	1	3	4	0.71470	C	2C4	
17	1	2	5	0.85421	B	2B5	
18	1	3	1	0.97547	C	2C1	
19	2	1	2	0.04473	A	2A2	* 1209
20	2	3	3	0.05814	C	2C3	
21	2	2	6	0.09603	B	2B6	
22	2	2	1	0.14342	B	2B1	
23	2	1	6	0.29490	A	2A6	
24	2	1	1	0.32005	A	2A1	
25	2	2	3	0.34073	B	2B3	*
26	2	1	3	0.38231	A	2A3	
27	2	3	4	0.38258	C	2C4	
28	2	3	2	0.54309	C	2C2	
29	2	2	5	0.66187	B	2B5	
30	2	2	2	0.68421	B	2B2	
31	2	3	5	0.77307	C	2C5	*
32	2	3	1	0.77572	C	2C1	
33	2	2	4	0.81987	B	2B4	
34	2	1	5	0.84082	A	2A5	
35	2	3	6	0.92784	C	2C6	
36	2	1	4	0.96058	A	2A4	
37	3	3	4	0.01582	C	2C4	* 1214
38	3	2	6	0.02269	B	2B6	
39	3	1	3	0.10073	A	2A3	
40	3	3	6	0.24126	C	2C6	
41	3	3	1	0.38566	C	2C1	
42	3	1	2	0.40678	A	2A2	
43	3	3	3	0.47539	C	2C3	*
44	3	1	5	0.62337	A	2A5	
45	3	2	5	0.63294	B	2B5	
46	3	2	3	0.71107	B	2B3	
47	3	3	2	0.76563	C	2C2	

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* Sample for distribution aliquot KLW 12JAN12

nalysis performed by J. Luoma SAS version 9.2 12:27 09JAN12

EH-11-PSUEDO-01
andom assignment of glochidia to test tanks
ussel species: Mucket

AEH-11-PSEUDO-01

bs	round	row	tank	x	_row_	tankn
48	3	1	6	0.77490	A	2A6 ✓ ——— * ✓
49	3	1	1	0.79056	A	2A1 ✓
50	3	3	5	0.82629	C	2C5 ✓
51	3	2	1	0.84457	B	2B1 ✓
52	3	2	2	0.86062	B	2B2 ✓
53	3	2	4	0.88782	B	2B4 ✓
54	3	1	4	0.90326	A	2A4 ✓ ——— * ✓ 1120
55	4	1	3	0.02466	A	2A3 ✓
56	4	2	1	0.07961	B	2B1 ✓
57	4	3	1	0.09021	C	2C1 ✓
58	4	3	6	0.10964	C	2C6 ✓
59	4	1	2	0.15271	A	2A2 ✓
60	4	2	2	0.22284	B	2B2 ✓ ——— * ✓
61	4	1	5	0.36666	A	2A5 ✓
62	4	2	4	0.41787	B	2B4 ✓
63	4	2	3	0.43712	B	2B3 ✓
64	4	3	5	0.50663	C	2C5 ✓
65	4	1	4	0.58751	A	2A4 ✓
66	4	2	5	0.60800	B	2B5 ✓ ——— * ✓
67	4	3	3	0.66845	C	2C3 ✓
68	4	1	1	0.75786	A	2A1 ✓
69	4	3	4	0.78458	C	2C4 ✓
70	4	3	2	0.94567	C	2C2 ✓
71	4	2	6	0.96083	B	2B6 ✓
72	4	1	6	0.96962	A	2A6 ✓ ——— * ✓ 1226
73	5	2	2	0.03379	B	2B2 ✓
74	5	3	4	0.09290	C	2C4 ✓
75	5	1	4	0.17092	A	2A4 ✓
76	5	2	3	0.35518	B	2B3 ✓
77	5	3	3	0.41766	C	2C3 ✓
78	5	1	2	0.45670	A	2A2 ✓ ——— * ✓
79	5	3	5	0.54443	C	2C5 ✓
30	5	2	4	0.65095	B	2B4 ✓
31	5	1	5	0.65645	A	2A5 ✓
32	5	2	5	0.66093	B	2B5 ✓
33	5	1	1	0.67867	A	2A1 ✓
34	5	3	2	0.67998	C	2C2 ✓ ——— * ✓
35	5	2	1	0.68374	B	2B1 ✓
36	5	1	3	0.70107	A	2A3 ✓
37	5	1	6	0.73423	A	2A6 ✓
38	5	3	6	0.76479	C	2C6 ✓
39	5	3	1	0.80740	C	2C1 ✓
90	5	2	6	0.90553	B	2B6 ✓ ——— * ✓ completed at 1229 JKW 17JAN12
91	6	1	6	0.06769	A	2A6
92	6	1	2	0.08744	A	2A2
93	6	2	1	0.14791	B	2B1
94	6	1	3	0.45638	A	2A3

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* Sample for distribution aliquot KW 12JAN12

nalysis performed by J. Luoma SAS version 9.2 12:27 09JAN12

ffects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel species
EH-11-PSUEDO-01
andom assignment of glochidia to test tank
ussel species: Mucket

AEH-11-PSEUDO-01

os	round	row	tank	x	_row_	tankn
95	6	3	3	0.46071	C	2C3
96	6	3	6	0.49449	C	2C6 *
97	6	1	5	0.52489	A	2A5
98	6	3	1	0.61590	C	2C1
99	6	3	4	0.70732	C	2C4
00	6	2	5	0.71307	B	2B5
01	6	3	5	0.75936	C	2C5
02	6	1	1	0.78284	A	2A1 *
03	6	2	3	0.83665	B	2B3
04	6	3	2	0.84431	C	2C2
05	6	2	4	0.84525	B	2B4
06	6	2	2	0.88368	B	2B2
07	6	2	6	0.90205	B	2B6
08	6	1	4	0.96568	A	2A4 *
09	7	2	1	0.04213	B	2B1
10	7	2	2	0.05244	B	2B2
11	7	3	5	0.08552	C	2C5
12	7	1	6	0.11230	A	2A6
13	7	3	2	0.16857	C	2C2
14	7	2	5	0.22141	B	2B5 *
15	7	2	6	0.22335	B	2B6
16	7	2	4	0.25653	B	2B4
17	7	1	4	0.33381	A	2A4
18	7	1	1	0.40108	A	2A1
19	7	2	3	0.56124	B	2B3
20	7	3	1	0.60276	C	2C1 *
21	7	3	6	0.78412	C	2C6
22	7	1	2	0.81734	A	2A2
23	7	3	3	0.82288	C	2C3
24	7	3	4	0.88472	C	2C4
25	7	1	3	0.96413	A	2A3
26	7	1	5	0.99816	A	2A5 *
27	8	1	4	0.01906	A	2A4
28	8	2	1	0.02411	B	2B1
29	8	3	4	0.06716	C	2C4
30	8	3	5	0.23709	C	2C5
31	8	2	3	0.26067	B	2B3
32	8	2	5	0.30873	B	2B5 *
33	8	1	1	0.40705	A	2A1
34	8	1	5	0.48246	A	2A5
35	8	3	2	0.64868	C	2C2
36	8	3	1	0.69547	C	2C1
37	8	2	6	0.71882	B	2B6
38	8	2	2	0.74359	B	2B2
39	8	2	4	0.76087	B	2B4 *
40	8	1	2	0.80410	A	2A2
41	8	1	3	0.82372	A	2A3

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* Sample for distribution aliquot KLW 12JAN12

nalysis performed by J. Luoma SAS version 9.2 12:27 09JAN12

EH-11-PSUEDO-01
andom assignment of glochidia to test tank
ussel species: Mucket

AEH-11-PSEUDO-01

bs	round	row	tank	x	_row_	tankn	
42	8	3	6	0.91183	C	2C6	
43	8	1	6	0.92576	A	2A6	
44	8	3	3	0.92879	C	2C3	*
45	9	3	2	0.05308	C	2C2	
46	9	2	3	0.18267	B	2B3	
47	9	3	3	0.22758	C	2C3	
48	9	1	4	0.28404	A	2A4	
49	9	3	4	0.31054	C	2C4	
50	9	1	2	0.37890	A	2A2	*
51	9	3	6	0.40119	C	2C6	
52	9	2	1	0.40611	B	2B1	
53	9	2	2	0.46055	B	2B2	
54	9	1	6	0.52324	A	2A6	
55	9	2	4	0.60900	B	2B4	
56	9	1	3	0.61136	A	2A3	*
57	9	1	5	0.72902	A	2A5	
58	9	1	1	0.74745	A	2A1	
59	9	2	6	0.76650	B	2B6	
60	9	2	5	0.81084	B	2B5	
61	9	3	1	0.90941	C	2C1	
62	9	3	5	0.91173	C	2C5	*
63	10	1	3	0.07174	A	2A3	
64	10	2	1	0.24946	B	2B1	
65	10	3	5	0.26949	C	2C5	
66	10	1	1	0.35243	A	2A1	
67	10	3	3	0.37878	C	2C3	
68	10	3	2	0.38445	C	2C2	*
69	10	2	3	0.38997	B	2B3	
70	10	1	2	0.53325	A	2A2	
71	10	3	6	0.54136	C	2C6	
72	10	1	4	0.59608	A	2A4	
73	10	1	6	0.60197	A	2A6	
74	10	2	6	0.60681	B	2B6	*
75	10	1	5	0.77531	A	2A5	
76	10	3	1	0.84612	C	2C1	
77	10	3	4	0.84761	C	2C4	
78	10	2	4	0.85964	B	2B4	
79	10	2	5	0.88245	B	2B5	
30	10	2	2	0.97126	B	2B2	*

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* Sample for distribution aliquot
KW 12JAN12

ıalysis performed by J. Luoma SAS version 9.2 12:27 09JAN12

EH-11-PSUEDO-01
andom assignment of glochidia to test tank Jn
ussel species: Hickorynut

AEH-11-PSEUDO-01

bs	round	row	tank	x	_row_	tankn	
1	1	2	3	0.09694	B	3B3 ✓	* ✓ Began distribution at 0855 JKW 12JAN12
2	1	3	2	0.12386	C	3C2 ✓	
3	1	2	2	0.24003	B	3B2 ✓	
4	1	1	1	0.35718	A	3A1 ✓	
5	1	2	1	0.36418	B	3B1 ✓	
6	1	1	5	0.39705	A	3A5 ✓	* ✓
7	1	2	4	0.39869	B	3B4 ✓	
8	1	3	6	0.43999	C	3C6 ✓	
9	1	2	6	0.45944	B	3B6 ✓	
10	1	3	1	0.48797	C	3C1 ✓	
11	1	2	5	0.55122	B	3B5 ✓	
12	1	3	5	0.61015	C	3C5 ✓	* ✓
13	1	1	2	0.84062	A	3A2 ✓	
14	1	1	4	0.84562	A	3A4 ✓	
15	1	1	3	0.91209	A	3A3 ✓	
16	1	3	3	0.93867	C	3C3 ✓	
17	1	1	6	0.97246	A	3A6 ✓	
18	1	3	4	0.97311	C	3C4 ✓	* ✓ 0900
19	2	2	3	0.10321	B	3B3 ✓	
20	2	3	4	0.11034	C	3C4 ✓	
21	2	1	4	0.12373	A	3A4 ✓	
22	2	3	1	0.17907	C	3C1 ✓	
23	2	2	6	0.18162	B	3B6 ✓	
24	2	1	2	0.21762	A	3A2 ✓	* ✓
25	2	2	1	0.27501	B	3B1 ✓	
26	2	1	5	0.28241	A	3A5 ✓	
27	2	1	3	0.28415	A	3A3 ✓	
28	2	3	3	0.37881	C	3C3 ✓	
29	2	2	5	0.37999	B	3B5 ✓	
30	2	1	6	0.49017	A	3A6 ✓	* ✓
31	2	1	1	0.63859	A	3A1 ✓	
32	2	2	2	0.65262	B	3B2 ✓	
33	2	3	6	0.69186	C	3C6 ✓	
34	2	3	2	0.69369	C	3C2 ✓	
35	2	2	4	0.70827	B	3B4 ✓	
36	2	3	5	0.78579	C	3C5 ✓	* ✓ 0904
37	3	2	1	0.01179	B	3B1 ✓	
38	3	2	4	0.10882	B	3B4 ✓	
39	3	1	3	0.11746	A	3A3 ✓	
40	3	1	6	0.13722	A	3A6 ✓	
41	3	2	3	0.16069	B	3B3 ✓	
42	3	1	2	0.35818	A	3A2 ✓	* ✓
43	3	1	1	0.41017	A	3A1 ✓	
44	3	3	5	0.53766	C	3C5 ✓	
45	3	3	4	0.63046	C	3C4 ✓	
46	3	3	2	0.67755	C	3C2 ✓	
47	3	1	4	0.68602	A	3A4 ✓	

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* Sample for distribution aliquot KLW 12JAN12

nalysis performed by J. Luoma SAS version 9.2 12:27 09JAN12

EH-11-PSUEDO-01
andom assignment of glochidia to test tank JM✓
ussel species: Hickorynut

AEH-11-PSEUDO-01

bs	round	row	tank	x	_row_	tankn	
48	3	1	5	0.69543	A	3A5 ✓	*✓
49	3	2	5	0.75556	B	3B5 ✓	
50	3	2	6	0.79147	B	3B6 ✓	
51	3	3	6	0.84903	C	3C6 ✓	
52	3	3	1	0.97540	C	3C1 ✓	
53	3	2	2	0.97573	B	3B2 ✓	
54	3	3	3	0.97702	C	3C3 ✓	*✓0910
55	4	1	5	0.07050	A	3A5 ✓	
56	4	3	2	0.14045	C	3C2 ✓	
57	4	2	2	0.21126	B	3B2 ✓	
58	4	3	3	0.21630	C	3C3 ✓	
59	4	1	6	0.22909	A	3A6 ✓	
60	4	3	1	0.29698	C	3C1 ✓	*✓
61	4	1	2	0.44271	A	3A2 ✓	
62	4	1	4	0.52535	A	3A4 ✓	
63	4	2	3	0.55913	B	3B3 ✓	
64	4	1	3	0.62375	A	3A3 ✓	
65	4	3	4	0.62433	C	3C4 ✓	
66	4	2	5	0.68598	B	3B5 ✓	*✓
67	4	1	1	0.69146	A	3A1 ✓	
68	4	2	1	0.69765	B	3B1 ✓	
69	4	3	6	0.72655	C	3C6 ✓	
70	4	3	5	0.82123	C	3C5 ✓	
71	4	2	6	0.88000	B	3B6 ✓	
72	4	2	4	0.92653	B	3B4 ✓	*✓ 0916
73	5	3	2	0.00824	C	3C2 ✓	
74	5	1	4	0.01230	A	3A4 ✓	
75	5	2	1	0.02548	B	3B1 ✓	
76	5	1	1	0.06230	A	3A1 ✓	
77	5	3	5	0.13003	C	3C5 ✓	
78	5	1	5	0.24109	A	3A5 ✓	*✓
79	5	2	5	0.39376	B	3B5 ✓	
30	5	2	3	0.41701	B	3B3 ✓	
31	5	1	2	0.45119	A	3A2 ✓	
32	5	2	4	0.47559	B	3B4 ✓	
33	5	1	6	0.48506	A	3A6 ✓	
34	5	3	1	0.68921	C	3C1 ✓	*✓
35	5	3	3	0.70606	C	3C3 ✓	
36	5	1	3	0.73924	A	3A3 ✓	
37	5	2	6	0.75774	B	3B6 ✓	
38	5	3	6	0.88193	C	3C6 ✓	
39	5	3	4	0.96848	C	3C4 ✓	
90	5	2	2	0.99055	B	3B2 ✓	*✓ 0918 aliquot distribution complete
91	6	1	6	0.12821	A	3A6	
92	6	3	2	0.16364	C	3C2	
93	6	3	4	0.24726	C	3C4	
94	6	1	5	0.25910	A	3A5	

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* Sample for distribution aliquot KLW 12JAN12

nalysis performed by J. Luoma SAS version 9.2 12:27 09JAN12

EH-11-PSUEDO-01
andom assignment of glochidia to test tank
ussel species: Hickorynut

AEH-11-PSEUDO-01

bs	round	row	tank	x	_row_	tankn
95	6	2	1	0.26299	B	3B1
96	6	3	3	0.30397	C	3C3
97	6	1	3	0.30555	A	3A3
98	6	3	6	0.32338	C	3C6
99	6	2	4	0.33703	B	3B4
00	6	1	2	0.39379	A	3A2
01	6	2	2	0.40541	B	3B2
02	6	2	6	0.58897	B	3B6
03	6	3	1	0.59051	C	3C1
04	6	2	3	0.61685	B	3B3
05	6	1	1	0.63296	A	3A1
06	6	2	5	0.79550	B	3B5
07	6	1	4	0.83396	A	3A4
08	6	3	5	0.84557	C	3C5
09	7	1	6	0.02148	A	3A6
10	7	1	4	0.02382	A	3A4
11	7	1	1	0.02963	A	3A1
12	7	3	4	0.14361	C	3C4
13	7	2	5	0.19079	B	3B5
14	7	3	5	0.20467	C	3C5
15	7	1	2	0.27057	A	3A2
16	7	3	6	0.31717	C	3C6
17	7	1	3	0.38801	A	3A3
18	7	3	2	0.39049	C	3C2
19	7	2	6	0.39349	B	3B6
20	7	3	1	0.42828	C	3C1
21	7	2	4	0.54735	B	3B4
22	7	3	3	0.55535	C	3C3
23	7	2	1	0.68413	B	3B1
24	7	2	2	0.72020	B	3B2
25	7	2	3	0.76838	B	3B3
26	7	1	5	0.83621	A	3A5
27	8	3	3	0.13585	C	3C3
28	8	2	5	0.16375	B	3B5
29	8	3	4	0.18530	C	3C4
30	8	2	3	0.19336	B	3B3
31	8	3	2	0.26637	C	3C2
32	8	1	6	0.34703	A	3A6
33	8	1	3	0.35070	A	3A3
34	8	3	5	0.37603	C	3C5
35	8	2	4	0.38254	B	3B4
36	8	2	2	0.44956	B	3B2
37	8	1	2	0.56573	A	3A2
38	8	3	6	0.66244	C	3C6
39	8	2	1	0.69048	B	3B1
40	8	2	6	0.69651	B	3B6
41	8	1	4	0.77204	A	3A4

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* Sample for distribution aliquot KLW 12JAN12

nalysis performed by J. Luoma SAS version 9.2 12:27 09JAN12

EH-11-PSUEDO-01
andom assignment of glochidia to test tank
ussel species: Hickorynut

AEH-11-PSEUDO-01

bs	round	row	tank	x	_row_	tankn
42	8	3	1	0.82651	C	3C1
43	8	1	1	0.86643	A	3A1
44	8	1	5	0.87486	A	3A5 *
45	9	2	2	0.11291	B	3B2
46	9	1	1	0.13740	A	3A1
47	9	1	4	0.21325	A	3A4
48	9	1	5	0.31054	A	3A5
49	9	2	1	0.37708	B	3B1
50	9	2	6	0.49338	B	3B6 *
51	9	3	2	0.54631	C	3C2
52	9	3	6	0.57931	C	3C6
53	9	2	3	0.59069	B	3B3
54	9	3	3	0.59828	C	3C3
55	9	3	4	0.68249	C	3C4
56	9	3	1	0.68708	C	3C1 *
57	9	3	5	0.76617	C	3C5
58	9	1	3	0.81233	A	3A3
59	9	1	6	0.82266	A	3A6
60	9	2	5	0.84778	B	3B5
61	9	1	2	0.84787	A	3A2
62	9	2	4	0.97792	B	3B4 *
63	10	1	3	0.08787	A	3A3
64	10	2	5	0.10147	B	3B5
65	10	3	1	0.16544	C	3C1
66	10	2	2	0.19042	B	3B2
67	10	2	6	0.24519	B	3B6
68	10	3	5	0.27066	C	3C5 *
69	10	1	5	0.30115	A	3A5
70	10	3	4	0.33208	C	3C4
71	10	1	6	0.34559	A	3A6
72	10	2	1	0.55190	B	3B1
73	10	2	3	0.57019	B	3B3
74	10	1	1	0.59400	A	3A1 *
75	10	1	2	0.64943	A	3A2
76	10	1	4	0.65495	A	3A4
77	10	2	4	0.69299	B	3B4
78	10	3	6	0.71444	C	3C6
79	10	3	2	0.74016	C	3C2
80	10	3	3	0.90388	C	3C3 *

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* Sample for distribution aliquot KLW 12JAN12

nalysis performed by J. Luoma SAS version 9.2 12:27 09JAN12

```
*  date revised  : 09Jan12 - JAL
*  Verified by:  ________ (Date:_______)                    page ____ of ____
*  Random allocation of glochidia to tank.sas
*****************************************************************/
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;
```

AEH-11-PSEUDO-01

```
FOOTNOTE1 'Analysis performed by J. Luoma SAS version ' &SYSVER &SYSTIME &SYSDATE;
ARNING: The FOOTNOTE statement is ambiguous due to invalid options or unquoted text.

options /*ls=85 ps=40 formdlim='-' */ pageno = 1 nocenter nodate nosource2;

/*Random distribution of glochidia to experimental tanks*/
/* tank 1 to 18 = tank A1 to A6 (1-6), tank B1 to B6 (7-12), tank C1 to C6 (13-18)
   round = distribution rounds 1 to 2, place one aliquot of glochidia per tank per round */

/***************************************************************************************
*******/

/*Mussel species: Black Sandshell*/
data glochidia;
 do round = 1 to 10 by 1;
 do row = 1 to 3 by 1;
  do tank = 1 to 6 by 1;
   x = ranuni(-1);
   output;
  end;
  end;
 end;
run;

OTE: The data set WORK.GLOCHIDIA has 180 observations and 4 variables.
OTE: DATA statement used (Total process time):
     real time           0.01 seconds
     cpu time            0.01 seconds


data glochidiadist; set glochidia;
if row = 1 then _row_ = 'A';
if row = 2 then _row_ = 'B';
if row = 3 then _row_ = 'C';
 if row = 1 and tank = 1 then tankn = '1A1';
  if row = 1 and tank = 2 then tankn = '1A2';
   if row = 1 and tank = 3 then tankn = '1A3';
    if row = 1 and tank = 4 then tankn = '1A4';
     if row = 1 and tank = 5 then tankn = '1A5';
      if row = 1 and tank = 6 then tankn = '1A6';
 if row = 2 and tank = 1 then tankn = '1B1';
  if row = 2 and tank = 2 then tankn = '1B2';
   if row = 2 and tank = 3 then tankn = '1B3';
    if row = 2 and tank = 4 then tankn = '1B4';
     if row = 2 and tank = 5 then tankn = '1B5';
      if row = 2 and tank = 6 then tankn = '1B6';
 if row = 3 and tank = 1 then tankn = '1C1';
  if row = 3 and tank = 2 then tankn = '1C2';
   if row = 3 and tank = 3 then tankn = '1C3';
    if row = 3 and tank = 4 then tankn = '1C4';
```

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```
      if row = 3 and tank = 5 then tankn = '1C5';
       if row = 3 and tank = 6 then tankn = '1C6'; run;

OTE: There were 180 observations read from the data set WORK.GLOCHIDIA.
OTE: The data set WORK.GLOCHIDIADIST has 180 observations and 6 variables.
OTE: DATA statement used (Total process time):
     real time           0.04 seconds
     cpu time            0.04 seconds
```

AEH-11-PSEUDO-01

```
proc sort data= glochidiadist;
 by round x;
 run;

OTE: There were 180 observations read from the data set WORK.GLOCHIDIADIST.
OTE: The data set WORK.GLOCHIDIADIST has 180 observations and 6 variables.
OTE: PROCEDURE SORT used (Total process time):
     real time           0.01 seconds
     cpu time            0.01 seconds


proc print data = glochidiadist;
title1 h=2 'Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid
mussel species';
title2 h=1.5 'AEH-11-PSUEDO-01';
title3 h=1 'Random assignment of glochidia to test tanks';
title4 h=1 'Mussel species: Black sandshell';
run;

OTE: There were 180 observations read from the data set WORK.GLOCHIDIADIST.
OTE: PROCEDURE PRINT used (Total process time):
     real time           0.00 seconds
     cpu time            0.00 seconds


/**********************************************************************************************
*******/

/*Mussel species: Mucket */
data glochidia;
 do round = 1 to 10 by 1;
 do row = 1 to 3 by 1;
  do tank = 1 to 6 by 1;
   x = ranuni(-1);
   output;
  end;
  end;
 end;
run;

OTE: The data set WORK.GLOCHIDIA has 180 observations and 4 variables.
OTE: DATA statement used (Total process time):
     real time           0.01 seconds
     cpu time            0.01 seconds
```

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AEH-11-PSEUDO-01

```
data glochidiadist; set glochidia;
if row = 1 then _row_ = 'A';
if row = 2 then _row_ = 'B';
if row = 3 then _row_ = 'C';
 if row = 1 and tank = 1 then tankn = '2A1';
  if row = 1 and tank = 2 then tankn = '2A2';
   if row = 1 and tank = 3 then tankn = '2A3';
    if row = 1 and tank = 4 then tankn = '2A4';
     if row = 1 and tank = 5 then tankn = '2A5';
      if row = 1 and tank = 6 then tankn = '2A6';
 if row = 2 and tank = 1 then tankn = '2B1';
  if row = 2 and tank = 2 then tankn = '2B2';
   if row = 2 and tank = 3 then tankn = '2B3';
    if row = 2 and tank = 4 then tankn = '2B4';
     if row = 2 and tank = 5 then tankn = '2B5';
      if row = 2 and tank = 6 then tankn = '2B6';
 if row = 3 and tank = 1 then tankn = '2C1';
  if row = 3 and tank = 2 then tankn = '2C2';
   if row = 3 and tank = 3 then tankn = '2C3';
    if row = 3 and tank = 4 then tankn = '2C4';
     if row = 3 and tank = 5 then tankn = '2C5';
      if row = 3 and tank = 6 then tankn = '2C6'; run;

OTE: There were 180 observations read from the data set WORK.GLOCHIDIA.
OTE: The data set WORK.GLOCHIDIADIST has 180 observations and 6 variables.
OTE: DATA statement used (Total process time):
     real time           0.03 seconds
     cpu time            0.03 seconds


proc sort data= glochidiadist;
 by round x;
run;

OTE: There were 180 observations read from the data set WORK.GLOCHIDIADIST.
OTE: The data set WORK.GLOCHIDIADIST has 180 observations and 6 variables.
OTE: PROCEDURE SORT used (Total process time):
     real time           0.01 seconds
     cpu time            0.01 seconds


proc print data = glochidiadist;
title1 h=2 'Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid
mussel species';
title2 h=1.5 'AEH-11-PSUEDO-01';
title3 h=1 'Random assignment of glochidia to test tank';
title4 h=1 'Mussel species: Mucket';
run;

OTE: There were 180 observations read from the data set WORK.GLOCHIDIADIST.
OTE: PROCEDURE PRINT used (Total process time):
     real time           0.01 seconds
     cpu time            0.01 seconds
```

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AEH-11-PSEUDO-01

```
/***********************************************************************************************
*******/

/*Mussel species: Hickorynut */
data glochidia;
 do round = 1 to 10 by 1;
 do row = 1 to 3 by 1;
  do tank = 1 to 6 by 1;
   x = ranuni(-1);
   output;
  end;
  end;
 end;
run;

OTE: The data set WORK.GLOCHIDIA has 180 observations and 4 variables.
OTE: DATA statement used (Total process time):
     real time           0.01 seconds
     cpu time            0.01 seconds


data glochidiadist; set glochidia;
if row = 1 then _row_ = 'A';
if row = 2 then _row_ = 'B';
if row = 3 then _row_ = 'C';
 if row = 1 and tank = 1 then tankn = '3A1';
  if row = 1 and tank = 2 then tankn = '3A2';
   if row = 1 and tank = 3 then tankn = '3A3';
    if row = 1 and tank = 4 then tankn = '3A4';
     if row = 1 and tank = 5 then tankn = '3A5';
      if row = 1 and tank = 6 then tankn = '3A6';
 if row = 2 and tank = 1 then tankn = '3B1';
  if row = 2 and tank = 2 then tankn = '3B2';
   if row = 2 and tank = 3 then tankn = '3B3';
    if row = 2 and tank = 4 then tankn = '3B4';
     if row = 2 and tank = 5 then tankn = '3B5';
      if row = 2 and tank = 6 then tankn = '3B6';
 if row = 3 and tank = 1 then tankn = '3C1';
  if row = 3 and tank = 2 then tankn = '3C2';
   if row = 3 and tank = 3 then tankn = '3C3';
    if row = 3 and tank = 4 then tankn = '3C4';
     if row = 3 and tank = 5 then tankn = '3C5';
      if row = 3 and tank = 6 then tankn = '3C6'; run;

OTE: There were 180 observations read from the data set WORK.GLOCHIDIA.
OTE: The data set WORK.GLOCHIDIADIST has 180 observations and 6 variables.
OTE: DATA statement used (Total process time):
     real time           0.01 seconds
     cpu time            0.01 seconds
```

Page 10 of 20

```
proc sort data= glochidiadist;
 by round x;
run;
```

```
)TE: There were 180 observations read from the data set WORK.GLOCHIDIADIST.
)TE: The data set WORK.GLOCHIDIADIST has 180 observations and 6 variables.
)TE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds
```

AEH-11-PSEUDO-01

```
proc print data = glochidiadist;
title1 h=2 'Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid
mussel species';
title2 h=1.5 'AEH-11-PSUEDO-01';
title3 h=1 'Random assignment of glochidia to test tank';
title4 h=1 'Mussel species: Hickorynut';
run;

)TE: There were 180 observations read from the data set WORK.GLOCHIDIADIST.
)TE: PROCEDURE PRINT used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds
```

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AEH-11-PSEUDO-01

```
*****************************************************************
  Study Number  : AEH-11-PSUEDO-01
  Study Director: Jim Luoma
  date created  : 22Apr11 - MPG
  date revised  : 12May11 - MPG
  date revised  : 09Jan12 - JAL
  Verified by:  _______ (Date:_______)                page ____ of ____
  Random allocation of glochidia to tank.sas
*****************************************************************/
M 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

OOTNOTE1 'Analysis performed by J. Luoma SAS version ' &SYSVER &SYSTIME &SYSDATE;

ptions /*ls=85 ps=40 formdlim='-' */ pageno = 1 nocenter nodate nosource2;

*Random distribution of glochidia to experimental tanks*/
* tank 1 to 18 = tank A1 to A6 (1-6), tank B1 to B6 (7-12), tank C1 to C6 (13-18)
  round = distribution rounds 1 to 2, place one aliquot of glochidia per tank per round */

*********************************************************************************************

*Mussel species: Black Sandshell*/
ata glochidia;
do round = 1 to 10 by 1;
do row = 1 to 3 by 1;
 do tank = 1 to 6 by 1;
  x = ranuni(-1);
  output;
 end;
 end;
end;
un;
ata glochidiadist; set glochidia;
f row = 1 then _row_ = 'A';
f row = 2 then _row_ = 'B';
f row = 3 then _row_ = 'C';
if row = 1 and tank = 1 then tankn = '1A1';
 if row = 1 and tank = 2 then tankn = '1A2';
  if row = 1 and tank = 3 then tankn = '1A3';
   if row = 1 and tank = 4 then tankn = '1A4';
    if row = 1 and tank = 5 then tankn = '1A5';
     if row = 1 and tank = 6 then tankn = '1A6';
if row = 2 and tank = 1 then tankn = '1B1';
 if row = 2 and tank = 2 then tankn = '1B2';
  if row = 2 and tank = 3 then tankn = '1B3';
   if row = 2 and tank = 4 then tankn = '1B4';
    if row = 2 and tank = 5 then tankn = '1B5';
     if row = 2 and tank = 6 then tankn = '1B6';
if row = 3 and tank = 1 then tankn = '1C1';
 if row = 3 and tank = 2 then tankn = '1C2';
  if row = 3 and tank = 3 then tankn = '1C3';
   if row = 3 and tank = 4 then tankn = '1C4';
    if row = 3 and tank = 5 then tankn = '1C5';
     if row = 3 and tank = 6 then tankn = '1C6'; run;
roc sort data= glochidiadist;
by round x;
```

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```
run;
roc print data = glochidiadist;
itle1 h=2 'Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel spe(
itle2 h=1.5 'AEH-11-PSUEDO-01';
itle3 h=1 'Random assignment of glochidia to test tanks';
itle4 h=1 'Mussel species: Black sandshell';
un;
```

AEH-11-PSEUDO-01

```
****************************************************************************************************

*Mussel species: Mucket */
ata glochidia;
do round = 1 to 10 by 1;
do row = 1 to 3 by 1;
 do tank = 1 to 6 by 1;
  x = ranuni(-1);
  output;
 end;
 end;
end;
un;
ata glochidiadist; set glochidia;
f row = 1 then _row_ = 'A';
f row = 2 then _row_ = 'B';
f row = 3 then _row_ = 'C';
if row = 1 and tank = 1 then tankn = '2A1';
 if row = 1 and tank = 2 then tankn = '2A2';
  if row = 1 and tank = 3 then tankn = '2A3';
   if row = 1 and tank = 4 then tankn = '2A4';
    if row = 1 and tank = 5 then tankn = '2A5';
     if row = 1 and tank = 6 then tankn = '2A6';
if row = 2 and tank = 1 then tankn = '2B1';
 if row = 2 and tank = 2 then tankn = '2B2';
  if row = 2 and tank = 3 then tankn = '2B3';
   if row = 2 and tank = 4 then tankn = '2B4';
    if row = 2 and tank = 5 then tankn = '2B5';
     if row = 2 and tank = 6 then tankn = '2B6';
if row = 3 and tank = 1 then tankn = '2C1';
 if row = 3 and tank = 2 then tankn = '2C2';
  if row = 3 and tank = 3 then tankn = '2C3';
   if row = 3 and tank = 4 then tankn = '2C4';
    if row = 3 and tank = 5 then tankn = '2C5';
     if row = 3 and tank = 6 then tankn = '2C6'; run;
roc sort data= glochidiadist;
by round x;
un;
roc print data = glochidiadist;
itle1 h=2 'Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel spe(
itle2 h=1.5 'AEH-11-PSUEDO-01';
itle3 h=1 'Random assignment of glochidia to test tank';
itle4 h=1 'Mussel species: Mucket';
un;
```

Page 19 of 20

```
****************************************************************************************************

*Mussel species: Hickorynut */
```

```
ata glochidia;
do round = 1 to 10 by 1;
do row = 1 to 3 by 1;
 do tank = 1 to 6 by 1;
  x = ranuni(-1);
  output;
 end;
 end;
end;
un;
ata glochidiadist; set glochidia;
f row = 1 then _row_ = 'A';
f row = 2 then _row_ = 'B';
f row = 3 then _row_ = 'C';
if row = 1 and tank = 1 then tankn = '3A1';
 if row = 1 and tank = 2 then tankn = '3A2';
  if row = 1 and tank = 3 then tankn = '3A3';
   if row = 1 and tank = 4 then tankn = '3A4';
    if row = 1 and tank = 5 then tankn = '3A5';
     if row = 1 and tank = 6 then tankn = '3A6';
if row = 2 and tank = 1 then tankn = '3B1';
 if row = 2 and tank = 2 then tankn = '3B2';
  if row = 2 and tank = 3 then tankn = '3B3';
   if row = 2 and tank = 4 then tankn = '3B4';
    if row = 2 and tank = 5 then tankn = '3B5';
     if row = 2 and tank = 6 then tankn = '3B6';
if row = 3 and tank = 1 then tankn = '3C1';
 if row = 3 and tank = 2 then tankn = '3C2';
  if row = 3 and tank = 3 then tankn = '3C3';
   if row = 3 and tank = 4 then tankn = '3C4';
    if row = 3 and tank = 5 then tankn = '3C5';
     if row = 3 and tank = 6 then tankn = '3C6'; run;
roc sort data= glochidiadist;
by round x;
un;
roc print data = glochidiadist;
itle1 h=2 'Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel spe
itle2 h=1.5 'AEH-11-PSUEDO-01';
itle3 h=1 'Random assignment of glochidia to test tank';
itle4 h=1 'Mussel species: Hickorynut';
un;
```

AEH-11-PSEUDO-01
File Folder 14B

PROOFED BY
Initials: TMS Date 5/14/12
REVIEWED BY
Initials: SA Date 6/1/12

Page 20 of 20

Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel species 1
AEH-11-PSUEDO-01
Random assignment of treatment to experimental tanks
Mussel speces: Plain pocketbook

AEH-11-PSEUDO-01

File Folder 14C
Book 1 p.4

Item No. 1

Obs	block	tank	x	tankn	trt
1	1	3	0.01295	1A3	control
2	3	3	0.02351	1C3	control
3	2	1	0.04483	1B1	control
4	1	1	0.08181	1A1	50
5	2	5	0.10591	1B5	50
6	3	1	0.16200	1C1	50
7	1	2	0.16274	1A2	100
8	2	4	0.19080	1B4	100
9	1	4	0.31663	1A4	100
10	2	2	0.37323	1B2	200
11	3	6	0.57147	1C6	200
12	3	4	0.60073	1C4	200
13	3	2	0.61414	1C2	300
14	3	5	0.73888	1C5	300
15	1	5	0.83704	1A5	300
16	2	6	0.88651	1B6	300-HD
17	1	6	0.88955	1A6	300-HD
18	2	3	0.92670	1B3	300-HD

PROOFED BY
Initials: TMS Date: 5/15/12
REVIEWED BY
Initials: ____ Date: ____

MG
Analysis performed by M. Gaikowski SAS version 9.2 08:56 10MAY11

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AEH-11-PSUEDO-01
Random assignment of treatment to experimental tanks
Mussel speces: Higgins eye

AEH-11-PSEUDO-01

Obs	block	tank	x	tankn	trt
1	3	6	0.02715	2C6	control
2	2	1	0.10793	2B1	control
3	3	3	0.24227	2C3	control
4	1	4	0.39065	2A4	50
5	1	2	0.41329	2A2	50
6	1	1	0.60081	2A1	50
7	3	5	0.63527	2C5	100
8	3	4	0.63939	2C4	100
9	1	3	0.67341	2A3	100
10	1	6	0.68308	2A6	200
11	2	2	0.70345	2B2	200
12	2	5	0.73297	2B5	200
13	2	3	0.77113	2B3	300
14	3	2	0.77978	2C2	300
15	3	1	0.83290	2C1	300
16	2	6	0.84501	2B6	300-HD
17	1	5	0.87678	2A5	300-HD
18	2	4	0.88010	2B4	300-HD

Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel species 3
AEH-11-PSUEDO-01
Random assignment of treatment to experimental tanks
Mussel speces: Fatmucket

AEH-11-PSEUDO-01

Obs	block	tank	x	tankn	trt
1	1	2	0.08330	3A2	control
2	2	2	0.10124	3B2	control
3	2	3	0.31999	3B3	control
4	3	5	0.32027	3C5	50
5	3	6	0.35692	3C6	50
6	2	5	0.45716	3B5	50
7	1	1	0.52092	3A1	100
8	1	3	0.52278	3A3	100
9	1	5	0.55777	3A5	100
10	2	4	0.63422	3B4	200
11	2	6	0.73185	3B6	200
12	3	4	0.78148	3C4	200
13	3	1	0.81279	3C1	300
14	2	1	0.83781	3B1	300
15	3	3	0.84275	3C3	300
16	1	4	0.84300	3A4	300-HD
17	3	2	0.89865	3C2	300-HD
18	1	6	0.91420	3A6	300-HD

MM

Analysis performed by M. Gaikowski SAS version 9.2 08:56 10MAY11

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```
*  date created  : 22Apr11 - MPG
*  Verified by:  _______ (Date:_______)                    page ____ of ____
*  Random allocation of treatment to tank.sas
*******************************************************************/
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

FOOTNOTE1 'Analysis performed by M. Gaikowski SAS version ' &SYSVER &SYSTIME &SYSDATE;
WARNING: The FOOTNOTE statement is ambiguous due to invalid options or unquoted text.

options /*ls=85 ps=40 formdlim='-' */ pageno = 1 nocenter nodate nosource2;

/*Random assignment of treatment to experimental tanks*/
/*Mussel speces: Pocketbook*/
data PPB;
 do block = 1 to 3 by 1;
  do tank = 1 to 6 by 1;
   x = ranuni(-1);
   output;
  end;
 end;
run;

NOTE: The data set WORK.PPB has 18 observations and 3 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


data PPB2; set PPB;
 if block = 1 and tank = 1 then tankn = '1A1';
  if block = 1 and tank = 2 then tankn = '1A2';
   if block = 1 and tank = 3 then tankn = '1A3';
    if block = 1 and tank = 4 then tankn = '1A4';
     if block = 1 and tank = 5 then tankn = '1A5';
      if block = 1 and tank = 6 then tankn = '1A6';
 if block = 2 and tank = 1 then tankn = '1B1';
  if block = 2 and tank = 2 then tankn = '1B2';
   if block = 2 and tank = 3 then tankn = '1B3';
    if block = 2 and tank = 4 then tankn = '1B4';
     if block = 2 and tank = 5 then tankn = '1B5';
      if block = 2 and tank = 6 then tankn = '1B6';
 if block = 3 and tank = 1 then tankn = '1C1';
  if block = 3 and tank = 2 then tankn = '1C2';
   if block = 3 and tank = 3 then tankn = '1C3';
    if block = 3 and tank = 4 then tankn = '1C4';
     if block = 3 and tank = 5 then tankn = '1C5';
      if block = 3 and tank = 6 then tankn = '1C6';
    run;

NOTE: There were 18 observations read from the data set WORK.PPB.
NOTE: The data set WORK.PPB2 has 18 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds

proc sort data=PPB2;
```

```
 by x;
run;

NOTE: There were 18 observations read from the data set WORK.PPB2.
NOTE: The data set WORK.PPB2 has 18 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


data assign_trt_PPB; set PPB2;
 if _n_ = 1 then trt = 'control';
  if _n_ = 2 then trt = 'control';
   if _n_ = 3 then trt = 'control';
    if _n_ = 4 then trt = '50';
 if _n_ = 5 then trt = '50';
  if _n_ = 6 then trt = '50';
   if _n_ = 7 then trt = '100';
    if _n_ = 8 then trt = '100';
 if _n_ = 9 then trt = '100';
  if _n_ = 10 then trt = '200';
   if _n_ = 11 then trt = '200';
    if _n_ = 12 then trt = '200';
  if _n_ = 13 then trt = '300';
   if _n_ = 14 then trt = '300';
    if _n_ = 15 then trt = '300';
  if _n_ = 16 then trt = '300-HD';
   if _n_ = 17 then trt = '300-HD';
    if _n_ = 18 then trt = '300-HD';
    run;

NOTE: There were 18 observations read from the data set WORK.PPB2.
NOTE: The data set WORK.ASSIGN_TRT_PPB has 18 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc print data= assign_trt_PPB;
title1 h=2 'Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid
mussel species';
title2 h=1.5 'AEH-11-PSUEDO-01';
title3 h=1 'Random assignment of treatment to experimental tanks';
title4 h=1 'Mussel speces: Plain pocketbook';
run;

NOTE: There were 18 observations read from the data set WORK.ASSIGN_TRT_PPB.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


/***************************************************************************************************
*******************/

```

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```
/*Mussel speces: Higgins eye*/
data HGE;
 do block = 1 to 3 by 1;
  do tank = 1 to 6 by 1;
   x = ranuni(-1);
   output;
  end;
 end;
run;

NOTE: The data set WORK.HGE has 18 observations and 3 variables.
NOTE: DATA statement used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds


data HGE2; set HGE;
 if block = 1 and tank = 1 then tankn = '2A1';
  if block = 1 and tank = 2 then tankn = '2A2';
   if block = 1 and tank = 3 then tankn = '2A3';
    if block = 1 and tank = 4 then tankn = '2A4';
     if block = 1 and tank = 5 then tankn = '2A5';
      if block = 1 and tank = 6 then tankn = '2A6';
 if block = 2 and tank = 1 then tankn = '2B1';
  if block = 2 and tank = 2 then tankn = '2B2';
   if block = 2 and tank = 3 then tankn = '2B3';
    if block = 2 and tank = 4 then tankn = '2B4';
     if block = 2 and tank = 5 then tankn = '2B5';
      if block = 2 and tank = 6 then tankn = '2B6';
 if block = 3 and tank = 1 then tankn = '2C1';
  if block = 3 and tank = 2 then tankn = '2C2';
   if block = 3 and tank = 3 then tankn = '2C3';
    if block = 3 and tank = 4 then tankn = '2C4';
     if block = 3 and tank = 5 then tankn = '2C5';
      if block = 3 and tank = 6 then tankn = '2C6';
    run;

NOTE: There were 18 observations read from the data set WORK.HGE.
NOTE: The data set WORK.HGE2 has 18 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc sort data=HGE2;
 by x;
run;

NOTE: There were 18 observations read from the data set WORK.HGE2.
NOTE: The data set WORK.HGE2 has 18 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds


data assign_treat_HGE; set HGE2;
```

```
 if _n_ = 1 then trt = 'control';
  if _n_ = 2 then trt = 'control';
   if _n_ = 3 then trt = 'control';
    if _n_ = 4 then trt = '50';
 if _n_ = 5 then trt = '50';
  if _n_ = 6 then trt = '50';
   if _n_ = 7 then trt = '100';
    if _n_ = 8 then trt = '100';
 if _n_ = 9 then trt = '100';
  if _n_ = 10 then trt = '200';
   if _n_ = 11 then trt = '200';
    if _n_ = 12 then trt = '200';
  if _n_ = 13 then trt = '300';
   if _n_ = 14 then trt = '300';
    if _n_ = 15 then trt = '300';
  if _n_ = 16 then trt = '300-HD';
   if _n_ = 17 then trt = '300-HD';
    if _n_ = 18 then trt = '300-HD';
    run;

NOTE: There were 18 observations read from the data set WORK.HGE2.
NOTE: The data set WORK.ASSIGN_TREAT_HGE has 18 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc print data= assign_treat_HGE;
title1 h=2 'Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid
mussel species';
title2 h=1.5 'AEH-11-PSUEDO-01';
title3 h=1 'Random assignment of treatment to experimental tanks';
title4 h=1 'Mussel speces: Higgins eye';
run;

NOTE: There were 18 observations read from the data set WORK.ASSIGN_TREAT_HGE.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds


/****************************************************************************************
*******************/

/*Mussel speces: Fatmucket*/
data FAM;
 do block = 1 to 3 by 1;
  do tank = 1 to 6 by 1;
   x = ranuni(-1);
   output;
  end;
 end;
run;

NOTE: The data set WORK.FAM has 18 observations and 3 variables.
NOTE: DATA statement used (Total process time):
```

```
     real time           0.01 seconds
     cpu time            0.01 seconds
```



```
data FAM2; set FAM;
 if block = 1 and tank = 1 then tankn = '3A1';
  if block = 1 and tank = 2 then tankn = '3A2';
   if block = 1 and tank = 3 then tankn = '3A3';
    if block = 1 and tank = 4 then tankn = '3A4';
     if block = 1 and tank = 5 then tankn = '3A5';
      if block = 1 and tank = 6 then tankn = '3A6';
 if block = 2 and tank = 1 then tankn = '3B1';
  if block = 2 and tank = 2 then tankn = '3B2';
   if block = 2 and tank = 3 then tankn = '3B3';
    if block = 2 and tank = 4 then tankn = '3B4';
     if block = 2 and tank = 5 then tankn = '3B5';
      if block = 2 and tank = 6 then tankn = '3B6';
 if block = 3 and tank = 1 then tankn = '3C1';
  if block = 3 and tank = 2 then tankn = '3C2';
   if block = 3 and tank = 3 then tankn = '3C3';
    if block = 3 and tank = 4 then tankn = '3C4';
     if block = 3 and tank = 5 then tankn = '3C5';
      if block = 3 and tank = 6 then tankn = '3C6';
    run;

NOTE: There were 18 observations read from the data set WORK.FAM.
NOTE: The data set WORK.FAM2 has 18 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc sort data=FAM2;
 by x;
run;

NOTE: There were 18 observations read from the data set WORK.FAM2.
NOTE: The data set WORK.FAM2 has 18 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


data assign_treat_FAM; set FAM2;
 if _n_ = 1 then trt = 'control';
  if _n_ = 2 then trt = 'control';
   if _n_ = 3 then trt = 'control';
    if _n_ = 4 then trt = '50';
 if _n_ = 5 then trt = '50';
  if _n_ = 6 then trt = '50';
   if _n_ = 7 then trt = '100';
    if _n_ = 8 then trt = '100';
 if _n_ = 9 then trt = '100';
  if _n_ = 10 then trt = '200';
   if _n_ = 11 then trt = '200';
    if _n_ = 12 then trt = '200';
```

```
  if _n_ = 13 then trt = '300';
   if _n_ = 14 then trt = '300';
    if _n_ = 15 then trt = '300';
  if _n_ = 16 then trt = '300-HD';
   if _n_ = 17 then trt = '300-HD';
    if _n_ = 18 then trt = '300-HD';
    run;

NOTE: There were 18 observations read from the data set WORK.FAM2.
NOTE: The data set WORK.ASSIGN_TREAT_FAM has 18 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc print data= assign_treat_FAM;
title1 h=2 'Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid
mussel species';
title2 h=1.5 'AEH-11-PSUEDO-01';
title3 h=1 'Random assignment of treatment to experimental tanks';
title4 h=1 'Mussel speces: Fatmucket';
run;

NOTE: There were 18 observations read from the data set WORK.ASSIGN_TREAT_FAM.
NOTE: PROCEDURE PRINT used (Total process time):
      real timo           0.01 seconds
      cpu time            0.01 seconds
```

```
/***********************************************************************
*  Study Number  : AEH-11-PSUEDO-01
*  Study Director: Jim Luoma
*  date created  : 22Apr11 - MPG MM
*  Verified by:  _____ (Date:_______)                    page ____ of ____
*  Random allocation of treatment to tank.sas
***********************************************************************/
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

FOOTNOTE1 'Analysis performed by M. Gaikowski SAS version ' &SYSVER &SYSTIME &SYSDATE;

options /*ls=85 ps=40 formdlim='-' */ pageno = 1 nocenter nodate nosource2;

/*Random assignment of treatment to experimental tanks*/
/*Mussel speces: Pocketbook*/
data PPB;
 do block = 1 to 3 by 1;
  do tank = 1 to 6 by 1;
   x = ranuni(-1);
   output;
  end;
 end;
run;
data PPB2; set PPB;
 if block = 1 and tank = 1 then tankn = '1A1';
  if block = 1 and tank = 2 then tankn = '1A2';
   if block = 1 and tank = 3 then tankn = '1A3';
    if block = 1 and tank = 4 then tankn = '1A4';
     if block = 1 and tank = 5 then tankn = '1A5';
      if block = 1 and tank = 6 then tankn = '1A6';
 if block = 2 and tank = 1 then tankn = '1B1';
  if block = 2 and tank = 2 then tankn = '1B2';
   if block = 2 and tank = 3 then tankn = '1B3';
    if block = 2 and tank = 4 then tankn = '1B4';
     if block = 2 and tank = 5 then tankn = '1B5';
      if block = 2 and tank = 6 then tankn = '1B6';
 if block = 3 and tank = 1 then tankn = '1C1';
  if block = 3 and tank = 2 then tankn = '1C2';
   if block = 3 and tank = 3 then tankn = '1C3';
    if block = 3 and tank = 4 then tankn = '1C4';
     if block = 3 and tank = 5 then tankn = '1C5';
      if block = 3 and tank = 6 then tankn = '1C6';
    run;
proc sort data=PPB2;
 by x;
run;

data assign_trt_PPB; set PPB2;
 if _n_ = 1 then trt = 'control';
  if _n_ = 2 then trt = 'control';
   if _n_ = 3 then trt = 'control';
    if _n_ = 4 then trt = '50';
 if _n_ = 5 then trt = '50';
  if _n_ = 6 then trt = '50';
   if _n_ = 7 then trt = '100';
    if _n_ = 8 then trt = '100';
 if _n_ = 9 then trt = '100';
```

```
  if _n_ = 10 then trt = '200';
   if _n_ = 11 then trt = '200';
    if _n_ = 12 then trt = '200';
  if _n_ = 13 then trt = '300';
   if _n_ = 14 then trt = '300';
    if _n_ = 15 then trt = '300';
  if _n_ = 16 then trt = '300-HD';
   if _n_ = 17 then trt = '300-HD';
    if _n_ = 18 then trt = '300-HD';
    run;
proc print data= assign_trt_PPB;
title1 h=2 'Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel
title2 h=1.5 'AEH-11-PSUEDO-01';
title3 h=1 'Random assignment of treatment to experimental tanks';
title4 h=1 'Mussel speces: Plain pocketbook';
run;

/*****************************************************************************************

/*Mussel speces: Higgins eye*/
data HGE;
 do block = 1 to 3 by 1;
  do tank = 1 to 6 by 1;
   x = ranuni(-1);
   output;
  end;
 end;
run;
data HGE2; set HGE;
 if block = 1 and tank = 1 then tankn = '2A1';
  if block = 1 and tank = 2 then tankn = '2A2';
   if block = 1 and tank = 3 then tankn = '2A3';
    if block = 1 and tank = 4 then tankn = '2A4';
     if block = 1 and tank = 5 then tankn = '2A5';
      if block = 1 and tank = 6 then tankn = '2A6';
 if block = 2 and tank = 1 then tankn = '2B1';
  if block = 2 and tank = 2 then tankn = '2B2';
   if block = 2 and tank = 3 then tankn = '2B3';
    if block = 2 and tank = 4 then tankn = '2B4';
     if block = 2 and tank = 5 then tankn = '2B5';
      if block = 2 and tank = 6 then tankn = '2B6';
 if block = 3 and tank = 1 then tankn = '2C1';
  if block = 3 and tank = 2 then tankn = '2C2';
   if block = 3 and tank = 3 then tankn = '2C3';
    if block = 3 and tank = 4 then tankn = '2C4';
     if block = 3 and tank = 5 then tankn = '2C5';
      if block = 3 and tank = 6 then tankn = '2C6';
    run;
proc sort data=HGE2;
 by x;
run;

data assign_treat_HGE; set HGE2;
 if _n_ = 1 then trt = 'control';
  if _n_ = 2 then trt = 'control';
   if _n_ = 3 then trt = 'control';
    if _n_ = 4 then trt = '50';
```

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```
 if _n_ = 5 then trt = '50';
  if _n_ = 6 then trt = '50';
   if _n_ = 7 then trt = '100 ;
    if _n_ = 8 then trt = '100';
 if _n_ = 9 then trt = '100';
  if _n_ = 10 then trt = '200';
   if _n_ = 11 then trt = '200';
    if _n_ = 12 then trt = '200';
  if _n_ = 13 then trt = '300';
   if _n_ = 14 then trt = '300';
    if _n_ = 15 then trt = '300';
  if _n_ = 16 then trt = '300-HD';
   if _n_ = 17 then trt = '300-HD';
    if _n_ = 18 then trt = '300-HD';
    run;
proc print data= assign_treat_HGE;
title1 h=2 'Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel
title2 h=1.5 'AEH-11-PSUEDO-01';
title3 h=1 'Random assignment of treatment to experimental tanks';
title4 h=1 'Mussel species: Higgins eye';
run;

/*********************************************************************************************

/*Mussel speces: Fatmucket*/
data FAM;
 do block = 1 to 3 by 1;
  do tank = 1 to 6 by 1;
   x = ranuni(-1);
   output;
  end;
 end;
run;
data FAM2; set FAM;
 if block = 1 and tank = 1 then tankn = '3A1';
  if block = 1 and tank = 2 then tankn = '3A2';
   if block = 1 and tank = 3 then tankn = '3A3';
    if block = 1 and tank = 4 then tankn = '3A4';
     if block = 1 and tank = 5 then tankn = '3A5';
      if block = 1 and tank = 6 then tankn = '3A6';
 if block = 2 and tank = 1 then tankn = '3B1';
  if block = 2 and tank = 2 then tankn = '3B2';
   if block = 2 and tank = 3 then tankn = '3B3';
    if block = 2 and tank = 4 then tankn = '3B4';
     if block = 2 and tank = 5 then tankn = '3B5';
      if block = 2 and tank = 6 then tankn = '3B6';
 if block = 3 and tank = 1 then tankn = '3C1';
  if block = 3 and tank = 2 then tankn = '3C2';
   if block = 3 and tank = 3 then tankn = '3C3';
    if block = 3 and tank = 4 then tankn = '3C4';
     if block = 3 and tank = 5 then tankn = '3C5';
      if block = 3 and tank = 6 then tankn = '3C6';
    run;
proc sort data=FAM2;
 by x;
run;
```

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```
data assign_treat_FAM; set FAM2;
 if _n_ = 1 then trt = 'control';
  if _n_ = 2 then trt = 'control';
   if _n_ = 3 then trt = 'control';
    if _n_ = 4 then trt = '50';
 if _n_ = 5 then trt = '50';
  if _n_ = 6 then trt = '50';
   if _n_ = 7 then trt = '100';
    if _n_ = 8 then trt = '100';
 if _n_ = 9 then trt = '100';
  if _n_ = 10 then trt = '200';
   if _n_ = 11 then trt = '200';
    if _n_ = 12 then trt = '200';
  if _n_ = 13 then trt = '300';
   if _n_ = 14 then trt = '300';
    if _n_ = 15 then trt = '300';
  if _n_ = 16 then trt = '300-HD';
   if _n_ = 17 then trt = '300-HD';
    if _n_ = 18 then trt = '300-HD';
    run;
proc print data= assign_treat_FAM;
title1 h=2 'Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel
title2 h=1.5 'AEH-11-PSUEDO-01';
title3 h=1 'Random assignment of treatment to experimental tanks';
title4 h=1 'Mussel speces: Fatmucket';
run;
```

AEH-11-PSEUDO-01

File Folder 14C

mm
10 May 11

PROOFED BY
Initials: TMS Date: 5/15/12
REVIEWED BY
Initials: ____ Date: ____

```
/*********************************************************************
*  Study Number  : AEH-11-PSUEDO-01
*  Study Director: Jim Luoma
*  date created  : 14Oct11 - MPG
*  Verified by:  _______ (Date:_______)                    page ____ of ____
*  Random allocation of treatment to tank.sas
*********************************************************************/
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

FOOTNOTE1 'Analysis performed by M. Gaikowski SAS version ' &SYSVER &SYSTIME &SYSDATE;

options /*ls=85 ps=40 formdlim='-' */ pageno = 1 nocenter nodate nosource2;

/*Random assignment of treatment to experimental tanks*/
/*Mussel speces: Washboard*/
data WASH;
 do block = 1 to 3 by 1;
  do tank = 1 to 6 by 1;
   x = ranuni(-1);
   output;
  end;
 end;
run;
data WASH2; set WASH;
 if block = 1 and tank = 1 then tankn = '1A1';
  if block = 1 and tank = 2 then tankn = '1A2';
   if block = 1 and tank = 3 then tankn = '1A3';
    if block = 1 and tank = 4 then tankn = '1A4';
     if block = 1 and tank = 5 then tankn = '1A5';
      if block = 1 and tank = 6 then tankn = '1A6';
 if block = 2 and tank = 1 then tankn = '1B1';
  if block = 2 and tank = 2 then tankn = '1B2';
   if block = 2 and tank = 3 then tankn = '1B3';
    if block = 2 and tank = 4 then tankn = '1B4';
     if block = 2 and tank = 5 then tankn = '1B5';
      if block = 2 and tank = 6 then tankn = '1B6';
 if block = 3 and tank = 1 then tankn = '1C1';
  if block = 3 and tank = 2 then tankn = '1C2';
   if block = 3 and tank = 3 then tankn = '1C3';
    if block = 3 and tank = 4 then tankn = '1C4';
     if block = 3 and tank = 5 then tankn = '1C5';
      if block = 3 and tank = 6 then tankn = '1C6';
    run;
proc sort data=WASH2;
 by x;
run;

data assign_trt_WASH; set WASH2;
 if _n_ = 1 then trt = 'control';
  if _n_ = 2 then trt = 'control';
   if _n_ = 3 then trt = 'control';
    if _n_ = 4 then trt = '50';
 if _n_ = 5 then trt = '50';
  if _n_ = 6 then trt = '50';
   if _n_ = 7 then trt = '100';
    if _n_ = 8 then trt = '100';
 if _n_ = 9 then trt = '100';
  if _n_ = 10 then trt = '200';
   if _n_ = 11 then trt = '200';
    if _n_ = 12 then trt = '200';
  if _n_ = 13 then trt = '300';
```

MPG

AEH-11-PSEUDO-01

File Folder 14C
Book 1 p. 21-22

Item No. 2

PROOFED BY
Initials: TMS Date: 5/15/12
REVIEWED BY
Initials: ____ Date: ____

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```
   if _n_ = 14 then trt = '300';
    if _n_ = 15 then trt = '300';
  if _n_ = 16 then trt = '300-HD';
   if _n_ = 17 then trt = '300-HD';
    if _n_ = 18 then trt = '300-HD';
    run;
proc print data= assign_trt_WASH;
title1 h=2 'Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel
title2 h=1.5 'AEH-11-PSUEDO-01';
title3 h=1 'Random assignment of treatment to experimental tanks';
title4 h=1 'Mussel speces: Washboard';
run;

/*****************************************************************************************************

/*Mussel speces: Fatmucket*/
data FAM;
 do block = 1 to 3 by 1;
  do tank = 1 to 6 by 1;
   x = ranuni(-1);
   output;
  end;
 end;
run;
data FAM2; set FAM;
 if block = 1 and tank = 1 then tankn = '3A1';
  if block = 1 and tank = 2 then tankn = '3A2';
   if block = 1 and tank = 3 then tankn = '3A3';
    if block = 1 and tank = 4 then tankn = '3A4';
     if block = 1 and tank = 5 then tankn = '3A5';
      if block = 1 and tank = 6 then tankn = '3A6';
 if block = 2 and tank = 1 then tankn = '3B1';
  if block = 2 and tank = 2 then tankn = '3B2';
   if block = 2 and tank = 3 then tankn = '3B3';
    if block = 2 and tank = 4 then tankn = '3B4';
     if block = 2 and tank = 5 then tankn = '3B5';
      if block = 2 and tank = 6 then tankn = '3B6';
 if block = 3 and tank = 1 then tankn = '3C1';
  if block = 3 and tank = 2 then tankn = '3C2';
   if block = 3 and tank = 3 then tankn = '3C3';
    if block = 3 and tank = 4 then tankn = '3C4';
     if block = 3 and tank = 5 then tankn = '3C5';
      if block = 3 and tank = 6 then tankn = '3C6';
    run;
proc sort data=FAM2;
 by x;
run;

data assign_treat_FAM; set FAM2;
 if _n_ = 1 then trt = 'control';
  if _n_ = 2 then trt = 'control';
   if _n_ = 3 then trt = 'control';
    if _n_ = 4 then trt = '50';
 if _n_ = 5 then trt = '50';
  if _n_ = 6 then trt = '50';
   if _n_ = 7 then trt = '100';
    if _n_ = 8 then trt = '100';
 if _n_ = 9 then trt = '100';
  if _n_ = 10 then trt = '200';
   if _n_ = 11 then trt = '200';
    if _n_ = 12 then trt = '200';
```

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```
  if _n_ = 13 then trt = '300';
   if _n_ = 14 then trt = '300';
    if _n_ = 15 then trt = '300';
  if _n_ = 16 then trt = '300-HD';
   if _n_ = 17 then trt = '300-HD';
    if _n_ = 18 then trt = '300-HD';
    run;
proc print data= assign_treat_FAM;
title1 h=2 'Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel
title2 h=1.5 'AEH-11-PSUEDO-01';
title3 h=1 'Random assignment of treatment to experimental tanks';
title4 h=1 'Mussel specos: Fatmucket';
run;
```

```
*  date created  : 14Oct11 - MPG
*  Verified by:  _______ (Date:_______)                    page ____ of ____ AEH-11-PSEUDO-01
*  Random allocation of treatment to tank.sas
*********************************************************************/
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

FOOTNOTE1 'Analysis performed by M. Gaikowski SAS version ' &SYSVER &SYSTIME &SYSDATE;
WARNING: The FOOTNOTE statement is ambiguous due to invalid options or unquoted text.

options /*ls=85 ps=40 formdlim='-' */ pageno = 1 nocenter nodate nosource2;

/*Random assignment of treatment to experimental tanks*/
/*Mussel speces: Washboard*/
data WASH;
 do block = 1 to 3 by 1;
  do tank = 1 to 6 by 1;
   x = ranuni(-1);
   output;
  end;
 end;
run;

NOTE: The data set WORK.WASH has 18 observations and 3 variables.
NOTE: DATA statement used (Total process time):
      real time           0.00 seconds
      cpu time            0.01 seconds


data WASH2; set WASH;
 if block = 1 and tank = 1 then tankn = '1A1';
  if block = 1 and tank = 2 then tankn = '1A2';
   if block = 1 and tank = 3 then tankn = '1A3';
    if block = 1 and tank = 4 then tankn = '1A4';
     if block = 1 and tank = 5 then tankn = '1A5';
      if block = 1 and tank = 6 then tankn = '1A6';
 if block = 2 and tank = 1 then tankn = '1B1';
  if block = 2 and tank = 2 then tankn = '1B2';
   if block = 2 and tank = 3 then tankn = '1B3';
    if block = 2 and tank = 4 then tankn = '1B4';
     if block = 2 and tank = 5 then tankn = '1B5';
      if block = 2 and tank = 6 then tankn = '1B6';
 if block = 3 and tank = 1 then tankn = '1C1';
  if block = 3 and tank = 2 then tankn = '1C2';
   if block = 3 and tank = 3 then tankn = '1C3';
    if block = 3 and tank = 4 then tankn = '1C4';
     if block = 3 and tank = 5 then tankn = '1C5';
      if block = 3 and tank = 6 then tankn = '1C6';
    run;

NOTE: There were 18 observations read from the data set WORK.WASH.
NOTE: The data set WORK.WASH2 has 18 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc sort data=WASH2;
 by x;
run;

NOTE: There were 18 observations read from the data set WORK.WASH2.
```

```
NOTE: The data set WORK.WASH2 has 18 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.00 seconds
      cpu time            0.01 seconds


data assign_trt_WASH; set WASH2;
 if _n_ = 1 then trt = 'control';
  if _n_ = 2 then trt = 'control';
   if _n_ = 3 then trt = 'control';
    if _n_ = 4 then trt = '50';
 if _n_ = 5 then trt = '50';
  if _n_ = 6 then trt = '50';
   if _n_ = 7 then trt = '100';
    if _n_ = 8 then trt = '100';
 if _n_ = 9 then trt = '100';
  if _n_ = 10 then trt = '200';
   if _n_ = 11 then trt = '200';
    if _n_ = 12 then trt = '200';
  if _n_ = 13 then trt = '300';
   if _n_ = 14 then trt = '300';
    if _n_ = 15 then trt = '300';
  if _n_ = 16 then trt = '300-HD';
   if _n_ = 17 then trt = '300-HD';
    if _n_ = 18 then trt = '300-HD';
    run;

NOTE: There were 18 observations read from the data set WORK.WASH2.
NOTE: The data set WORK.ASSIGN_TRT_WASH has 18 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc print data= assign_trt_WASH;
title1 h=2 'Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid
! mussel species';
title2 h=1.5 'AEH-11-PSUEDO-01';
title3 h=1 'Random assignment of treatment to experimental tanks';
title4 h=1 'Mussel speces: Washboard';
run;

NOTE: There were 18 observations read from the data set WORK.ASSIGN_TRT_WASH.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds


/*****************************************************************************************
! ******************/
                  ① Plain pocketbook
/*Mussel speces: ~~Eatmucket~~*/     ① species name was changed due to availability / lab entry TMS 5/15/12
data ① ~~FAM~~; PPB
 do block = 1 to 3 by 1;
  do tank = 1 to 6 by 1;
   x = ranuni(-1);
   output;
  end;
 end;
```

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run;

NOTE: The data set WORK.~~FAM~~ PPB ① has 18 observations and 3 variables.
NOTE: DATA statement used (Total process time):
real time 0.01 seconds
cpu time 0.01 seconds

data ~~FAM~~2 PPB ①; set ~~FAM~~ PPB ①;
if block = 1 and tank = 1 then tankn = '3A1';
if block = 1 and tank = 2 then tankn = '3A2';
if block = 1 and tank = 3 then tankn = '3A3';
if block = 1 and tank = 4 then tankn = '3A4';
if block = 1 and tank = 5 then tankn = '3A5';
if block = 1 and tank = 6 then tankn = '3A6';
if block = 2 and tank = 1 then tankn = '3B1';
if block = 2 and tank = 2 then tankn = '3B2';
if block = 2 and tank = 3 then tankn = '3B3';
if block = 2 and tank = 4 then tankn = '3B4';
if block = 2 and tank = 5 then tankn = '3B5';
if block = 2 and tank = 6 then tankn = '3B6';
if block = 3 and tank = 1 then tankn = '3C1';
if block = 3 and tank = 2 then tankn = '3C2';
if block = 3 and tank = 3 then tankn = '3C3';
if block = 3 and tank = 4 then tankn = '3C4';
if block = 3 and tank = 5 then tankn = '3C5';
if block = 3 and tank = 6 then tankn = '3C6';
run;

NOTE: There were 18 observations read from the data set WORK.~~FAM~~ PPB ①.
NOTE: The data set WORK.~~FAM~~2 PPB ① has 18 observations and 4 variables.
NOTE: DATA statement used (Total process time):
real time 0.01 seconds
cpu time 0.01 seconds

proc sort data=~~FAM~~2 PPB;
by x;
run;

NOTE: There were 18 observations read from the data set WORK.~~FAM~~2 PPB ①.
NOTE: The data set WORK.FAM2 has 18 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
real time 0.00 seconds
cpu time 0.00 seconds

data assign_treat_~~FAM~~ PPB ①; set ~~FAM~~2 PPB ①;
if _n_ = 1 then trt = 'control';
if _n_ = 2 then trt = 'control';
if _n_ = 3 then trt = 'control';
if _n_ = 4 then trt = '50';
if _n_ = 5 then trt = '50';
if _n_ = 6 then trt = '50';
if _n_ = 7 then trt = '100';
if _n_ = 8 then trt = '100';
if _n_ = 9 then trt = '100';
if _n_ = 10 then trt = '200';
if _n_ = 11 then trt = '200';
if _n_ = 12 then trt = '200';

① species name was changed due to availability late entry TMS 5/15/12

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AEH-11-PSEUDO-01

```
    if _n_ = 13 then trt = '300';
     if _n_ = 14 then trt = '300';
      if _n_ = 15 then trt = '300';
    if _n_ = 16 then trt = '300-HD';
     if _n_ = 17 then trt = '300-HD';
      if _n_ = 18 then trt = '300-HD';
      run;
                                                            PPB ①
NOTE: There were 18 observations read from the data set WORK.FAM2.
                                      PPB ①
NOTE: The data set WORK.ASSIGN_TREAT_FAM has 18 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.00 seconds

                                 PPB ①
proc print data= assign_treat_FAM;
title1 h=2 'Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid
mussel species';
title2 h=1.5 'AEH-11-PSUEDO-01';
title3 h=1 'Random assignment of treatment to experimental tanks';
title4 h=1 'Mussel speces: Fatmucket';
                      ① Plain Pocketbook
run;
                                                                        ① PPB
NOTE: There were 18 observations read from the data set WORK.ASSIGN_TREAT_FAM.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds
```

① species name changed due to availability late entry TMS 5/15/12

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Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel species 2
AEH-11-PSUEDO-01
Random assignment of treatment to experimental tanks
Mussel speces: ~~Fatmucket~~ (1) plain pocketbook (PPB)

(1) species changed due to availability 10-18-11 10 wrong notth 10-18-11 JR

Obs	block	tank	x	tankn	trt
1	2	5	0.01598	3B5	control
2	2	6	0.19581	3B6	control
3	1	6	0.22694	3A6	control
4	3	1	0.24156	3C1	50
5	1	4	0.24732	3A4	50
6	3	2	0.29907	3C2	50
7	2	3	0.35524	3B3	100
8	2	1	0.41973	3B1	100
9	3	6	0.46543	3C6	100
10	3	3	0.50889	3C3	200
11	3	5	0.53796	3C5	200
12	1	2	0.63093	3A2	200
13	2	4	0.81196	3B4	300
14	3	4	0.86321	3C4	300
15	1	3	0.88466	3A3	300
16	1	5	0.88937	3A5	300-HD
17	2	2	0.90543	3B2	300-HD
18	1	1	0.99497	3A1	300-HD

10/18/11 JR

AEH-11-PSEUDO-01

Analysis performed by M. Gaikowski SAS version 9.2 11:03 14OCT11 MM Page 8 of 9

Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel species 1
AEH-11-PSUEDO-01
Random assignment of treatment to experimental tanks
Mussel speces: Washboard

AEH-11-PSEUDO-01
File Folder 14c

Obs	block	tank	x	tankn	trt
1	2	2	0.04902	1B2	control
2	1	5	0.19550	1A5	control
3	2	4	0.22508	1B4	control
4	3	2	0.25731	1C2	50
5	1	1	0.26201	1A1	50
6	3	1	0.29013	1C1	50
7	3	6	0.48750	1C6	100
8	1	2	0.51734	1A2	100
9	1	6	0.60907	1A6	100
10	2	5	0.69893	1B5	200
11	3	4	0.75130	1C4	200
12	3	3	0.78912	1C3	200
13	2	1	0.79399	1B1	300
14	3	5	0.79401	1C5	300
15	1	4	0.80175	1A4	300
16	2	3	0.83625	1B3	300-HD
17	1	3	0.91376	1A3	300-HD
18	2	6	0.95637	1B6	300-HD

PROOFED BY
Initials: TMS Date: 5/15/12
REVIEWED BY
Initials: ____ Date: ____

Analysis performed by M. Gaikowski SAS version 9.2 11:03 14OCT11 mrl

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ffects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel species 1
EH-11-PSUEDO-01
andom assignment of treatment to experimental tanks Sm
ussel speces: Black Sandshell

AEH-11-PSEUDO-01

File Folder 14C
Logbook 1, pg 30-31

Item No. 3

bs	block	tank	x	tankn	trt
1	3	6	0.04349	1C6	control
2	1	5	0.05493	1A5	control
3	2	1	0.06777	1B1	control
4	1	1	0.06965	1A1	50
5	3	1	0.07510	1C1	50
6	1	3	0.07952	1A3	50
7	2	2	0.09559	1B2	100
8	2	3	0.21685	1B3	100
9	3	5	0.29355	1C5	100
10	1	6	0.32497	1A6	200
11	2	6	0.36986	1B6	200
12	2	5	0.41768	1B5	200
13	3	2	0.43417	1C2	300
14	2	4	0.48474	1B4	300
15	1	2	0.70423	1A2	300
16	3	4	0.72592	1C4	300-HD
17	3	3	0.74208	1C3	300-HD
18	1	4	0.80908	1A4	300-HD

PROOFED BY
Initials: MS Date: 5/15/12
REVIEWED BY
Initials: [illegible] Date: [illegible]

Page 1 of 13

nalysis performed by J. Luoma SAS version 9.2 11:32 09JAN12

EH-11-PSUEDO-01
andom assignment of treatment to experimental tanks 5~✓
ussel speces: Mucket

AEH-11-PSEUDO-01

bs	block	tank	x	tankn	trt
1	3	1	0.05499	2C1	control
2	2	5	0.14187	2B5	control
3	2	4	0.23886	2B4	control
4	2	6	0.25670	2B6	50
5	1	4	0.26143	2A4	50
6	3	3	0.34256	2C3	50
7	3	6	0.37326	2C6	100
8	1	2	0.37424	2A2	100
9	2	2	0.49338	2B2	100
10	3	2	0.55304	2C2	200
11	1	6	0.55610	2A6	200
12	1	5	0.73592	2A5	200
13	2	3	0.76571	2B3	300
14	2	1	0.79764	2B1	300
15	3	4	0.86801	2C4	300
16	1	3	0.92888	2A3	300-HD
17	3	5	0.97855	2C5	300-HD
18	1	1	0.98976	2A1	300-HD

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nalysis performed by J. Luoma SAS version 9.2 11:32 09JAN12

EH-11-PSUEDO-01
andom assignment of treatment to experimental tanks
ussel speces: Hickory Nut

AEH-11-PSEUDO-01

bs	block	tank	x	tankn	trt
1	3	4	0.01010	3C4	control
2	3	1	0.03487	3C1	control
3	1	2	0.04425	3A2	control
4	2	6	0.14354	3B6	50
5	2	3	0.31457	3B3	50
6	1	5	0.41691	3A5	50
7	2	1	0.52751	3B1	100
8	3	5	0.66356	3C5	100
9	3	2	0.67191	3C2	100
10	3	6	0.70303	3C6	200
11	1	4	0.71377	3A4	200
12	1	3	0.77824	3A3	200
13	1	6	0.78722	3A6	300
14	2	5	0.86606	3B5	300
15	2	2	0.89116	3B2	300
16	1	1	0.90987	3A1	300-HD
17	3	3	0.94304	3C3	300-HD
18	2	4	0.99299	3B4	300-HD

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nalysis performed by J. Luoma SAS version 9.2 11:32 09JAN12

```
**********************************************************************
  Study Number  : AEH-11-PSUEDO-01
  Study Director: Jim Luoma
  date created  : 09Jan12 - JAL
  Verified by:  _______ (Date:_______)                 page ____ of ____
  Random allocation of treatment to tank.sas
*********************************************************************/
M 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

OOTNOTE1 'Analysis performed by J. Luoma SAS version ' &SYSVER &SYSTIME &SYSDATE;

ptions /*ls=85 ps=40 formdlim='-' */ pageno = 1 nocenter nodate nosource2;

*Random assignment of treatment to experimental tanks*/
*Mussel speces: Black Sandshell*/
ata BLS;
do block = 1 to 3 by 1;
 do tank = 1 to 6 by 1;
  x = ranuni(-1);
  output;
 end;
end;
un;
ata BLS2; set BLS;
if block = 1 and tank = 1 then tankn = '1A1';
 if block = 1 and tank = 2 then tankn = '1A2';
  if block = 1 and tank = 3 then tankn = '1A3';
   if block = 1 and tank = 4 then tankn = '1A4';
    if block = 1 and tank = 5 then tankn = '1A5';
     if block = 1 and tank = 6 then tankn = '1A6';
if block = 2 and tank = 1 then tankn = '1B1';
 if block = 2 and tank = 2 then tankn = '1B2';
  if block = 2 and tank = 3 then tankn = '1B3';
   if block = 2 and tank = 4 then tankn = '1B4';
    if block = 2 and tank = 5 then tankn = '1B5';
     if block = 2 and tank = 6 then tankn = '1B6';
if block = 3 and tank = 1 then tankn = '1C1';
 if block = 3 and tank = 2 then tankn = '1C2';
  if block = 3 and tank = 3 then tankn = '1C3';
   if block = 3 and tank = 4 then tankn = '1C4';
    if block = 3 and tank = 5 then tankn = '1C5';
     if block = 3 and tank = 6 then tankn = '1C6';
   run;
roc sort data=BLS2;
by x;
un;

ata assign_trt_BLS; set BLS2;
if _n_ = 1 then trt = 'control';
 if _n_ = 2 then trt = 'control';
  if _n_ = 3 then trt = 'control';
   if _n_ = 4 then trt = '50';
if _n_ = 5 then trt = '50';
 if _n_ = 6 then trt = '50';
  if _n_ = 7 then trt = '100';
   if _n_ = 8 then trt = '100';
```

```
if _n_ = 9 then trt = '100';
 if _n_ = 10 then trt = '200';
  if _n_ = 11 then trt = '200';
   if _n_ = 12 then trt = '200';
 if _n_ = 13 then trt = '300';
  if _n_ = 14 then trt = '300';
   if _n_ = 15 then trt = '300';
 if _n_ = 16 then trt = '300-HD';
  if _n_ = 17 then trt = '300-HD';
   if _n_ = 18 then trt = '300-HD';
   run;
roc print data= assign_trt_BLS;
itle1 h=2 'Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel spe(
itle2 h=1.5 'AEH-11-PSUEDO-01';
itle3 h=1 'Random assignment of treatment to experimental tanks';
itle4 h=1 'Mussel speces: Black Sandshell';
un;

*******************************************************************************************************

*Mussel speces: Mucket*/
ata MUC;
do block = 1 to 3 by 1;
 do tank = 1 to 6 by 1;
  x = ranuni(-1);
  output;
 end;
end;
un;
ata MUC2; set MUC;
if block = 1 and tank = 1 then tankn = '2A1';
 if block = 1 and tank = 2 then tankn = '2A2';
  if block = 1 and tank = 3 then tankn = '2A3';
   if block = 1 and tank = 4 then tankn = '2A4';
    if block = 1 and tank = 5 then tankn = '2A5';
     if block = 1 and tank = 6 then tankn = '2A6';
if block = 2 and tank = 1 then tankn = '2B1';
 if block = 2 and tank = 2 then tankn = '2B2';
  if block = 2 and tank = 3 then tankn = '2B3';
   if block = 2 and tank = 4 then tankn = '2B4';
    if block = 2 and tank = 5 then tankn = '2B5';
     if block = 2 and tank = 6 then tankn = '2B6';
if block = 3 and tank = 1 then tankn = '2C1';
 if block = 3 and tank = 2 then tankn = '2C2';
  if block = 3 and tank = 3 then tankn = '2C3';
   if block = 3 and tank = 4 then tankn = '2C4';
    if block = 3 and tank = 5 then tankn = '2C5';
     if block = 3 and tank = 6 then tankn = '2C6';
   run;
roc sort data=MUC2;
by x;
un;

ata assign_trt_MUC; set MUC2;
if _n_ = 1 then trt = 'control';
 if _n_ = 2 then trt = 'control';
```

```
if _n_ = 3 then trt = 'control';
  if _n_ = 4 then trt = '50';
if _n_ = 5 then trt = '50';
 if _n_ = 6 then trt = '50';
  if _n_ = 7 then trt = '100';
   if _n_ = 8 then trt = '100';
if _n_ = 9 then trt = '100';
 if _n_ = 10 then trt = '200';
  if _n_ = 11 then trt = '200';
   if _n_ = 12 then trt = '200';
 if _n_ = 13 then trt = '300';
  if _n_ = 14 then trt = '300';
   if _n_ = 15 then trt = '300';
 if _n_ = 16 then trt = '300-HD';
  if _n_ = 17 then trt = '300-HD';
   if _n_ = 18 then trt = '300-HD';
   run;
roc print data= assign_trt_MUC;
itle1 h=2 'Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel spe
itle2 h=1.5 'AEH-11-PSUEDO-01';
itle3 h=1 'Random assignment of treatment to experimental tanks';
itle4 h=1 'Mussel species: Mucket';
un;

********************************************************************************************

*Mussel speces: Hickory Nut*/
ata HIC;
do block = 1 to 3 by 1;
 do tank = 1 to 6 by 1;
  x = ranuni(-1);
  output;
 end;
end;
un;
ata HIC2; set HIC;
if block = 1 and tank = 1 then tankn = '3A1';
 if block = 1 and tank = 2 then tankn = '3A2';
  if block = 1 and tank = 3 then tankn = '3A3';
   if block = 1 and tank = 4 then tankn = '3A4';
    if block = 1 and tank = 5 then tankn = '3A5';
     if block = 1 and tank = 6 then tankn = '3A6';
if block = 2 and tank = 1 then tankn = '3B1';
 if block = 2 and tank = 2 then tankn = '3B2';
  if block = 2 and tank = 3 then tankn = '3B3';
   if block = 2 and tank = 4 then tankn = '3B4';
    if block = 2 and tank = 5 then tankn = '3B5';
     if block = 2 and tank = 6 then tankn = '3B6';
if block = 3 and tank = 1 then tankn = '3C1';
 if block = 3 and tank = 2 then tankn = '3C2';
  if block = 3 and tank = 3 then tankn = '3C3';
   if block = 3 and tank = 4 then tankn = '3C4';
    if block = 3 and tank = 5 then tankn = '3C5';
     if block = 3 and tank = 6 then tankn = '3C6';
   run;
roc sort data=HIC2;
```

```
by x;
un;

ata assign_trt_HIC; set HIC2;
if _n_ = 1 then trt = 'control';
 if _n_ = 2 then trt = 'control';
  if _n_ = 3 then trt = 'control';
   if _n_ = 4 then trt = '50';
if _n_ = 5 then trt = '50';
 if _n_ = 6 then trt = '50';
  if _n_ = 7 then trt = '100';
   if _n_ = 8 then trt = '100';
if _n_ = 9 then trt = '100';
 if _n_ = 10 then trt = '200';
  if _n_ = 11 then trt = '200';
   if _n_ = 12 then trt = '200';
 if _n_ = 13 then trt = '300';
  if _n_ = 14 then trt = '300';
   if _n_ = 15 then trt = '300';
 if _n_ = 16 then trt = '300-HD';
  if _n_ = 17 then trt = '300-HD';
   if _n_ = 18 then trt = '300-HD';
   run;
roc print data= assign_trt_HIC;
itle1 h=2 'Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel spe
itle2 h=1.5 'AEH-11-PSUEDO-01';
itle3 h=1 'Random assignment of treatment to experimental tanks';
itle4 h=1 'Mussel speces: Hickory Nut';
un;
```

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```
*  date created   : 09Jan12 - JAL
*  Verified by:  _______  (Date:_______)                     page ____ of ____
*  Random allocation of treatment to tank.sas
*****************************************************************/
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

FOOTNOTE1 'Analysis performed by J. Luoma SAS version ' &SYSVER &SYSTIME &SYSDATE;
ARNING: The FOOTNOTE statement is ambiguous due to invalid options or unquoted text.

options /*ls=85 ps=40 formdlim='-' */ pageno = 1 nocenter nodate nosource2;

/*Random assignment of treatment to experimental tanks*/
/*Mussel speces: Black Sandshell*/
data BLS;
 do block = 1 to 3 by 1;
  do tank = 1 to 6 by 1;
   x = ranuni(-1);
   output;
  end;
 end;
run;

OTE: The data set WORK.BLS has 18 observations and 3 variables.
OTE: DATA statement used (Total process time):
     real time           0.01 seconds
     cpu time            0.01 seconds


data BLS2; set BLS;
 if block = 1 and tank = 1 then tankn = '1A1';
  if block = 1 and tank = 2 then tankn = '1A2';
   if block = 1 and tank = 3 then tankn = '1A3';
    if block = 1 and tank = 4 then tankn = '1A4';
     if block = 1 and tank = 5 then tankn = '1A5';
      if block = 1 and tank = 6 then tankn = '1A6';
 if block = 2 and tank = 1 then tankn = '1B1';
  if block = 2 and tank = 2 then tankn = '1B2';
   if block = 2 and tank = 3 then tankn = '1B3';
    if block = 2 and tank = 4 then tankn = '1B4';
     if block = 2 and tank = 5 then tankn = '1B5';
      if block = 2 and tank = 6 then tankn = '1B6';
 if block = 3 and tank = 1 then tankn = '1C1';
  if block = 3 and tank = 2 then tankn = '1C2';
   if block = 3 and tank = 3 then tankn = '1C3';
    if block = 3 and tank = 4 then tankn = '1C4';
     if block = 3 and tank = 5 then tankn = '1C5';
      if block = 3 and tank = 6 then tankn = '1C6';
    run;

OTE: There were 18 observations read from the data set WORK.BLS.
OTE: The data set WORK.BLS2 has 18 observations and 4 variables.
OTE: DATA statement used (Total process time):
     real time           0.01 seconds
     cpu time            0.01 seconds
```

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```
proc sort data=BLS2;
 by x;
run;

OTE: There were 18 observations read from the data set WORK.BLS2.
OTE: The data set WORK.BLS2 has 18 observations and 4 variables.
OTE: PROCEDURE SORT used (Total process time):
     real time           0.01 seconds
     cpu time            0.01 seconds


data assign_trt_BLS; set BLS2;
 if _n_ = 1 then trt = 'control';
  if _n_ = 2 then trt = 'control';
   if _n_ = 3 then trt = 'control';
    if _n_ = 4 then trt = '50';
 if _n_ = 5 then trt = '50';
  if _n_ = 6 then trt = '50';
   if _n_ = 7 then trt = '100';
    if _n_ = 8 then trt = '100';
 if _n_ = 9 then trt = '100';
  if _n_ = 10 then trt = '200';
   if _n_ = 11 then trt = '200';
    if _n_ = 12 then trt = '200';
  if _n_ = 13 then trt = '300';
   if _n_ = 14 then trt = '300';
    if _n_ = 15 then trt = '300';
  if _n_ = 16 then trt = '300-HD';
   if _n_ = 17 then trt = '300-HD';
    if _n_ = 18 then trt = '300-HD';
    run;

OTE: There were 18 observations read from the data set WORK.BLS2.
OTE: The data set WORK.ASSIGN_TRT_BLS has 18 observations and 5 variables.
OTE: DATA statement used (Total process time):
     real time           0.01 seconds
     cpu time            0.01 seconds


proc print data= assign_trt_BLS;
title1 h=2 'Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid
mussel species';
title2 h=1.5 'AEH-11-PSUEDO-01';
title3 h=1 'Random assignment of treatment to experimental tanks';
title4 h=1 'Mussel speces: Black Sandshell';
run;

OTE: There were 18 observations read from the data set WORK.ASSIGN_TRT_BLS.
OTE: PROCEDURE PRINT used (Total process time):
     real time           0.00 seconds
     cpu time            0.00 seconds


/*********************************************************************************************
```

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```
^^^^^^^^^^^^^^^^/

/*Mussel speces: Mucket*/
data MUC;
 do block = 1 to 3 by 1;
  do tank = 1 to 6 by 1;
   x = ranuni(-1);
   output;
  end;
 end;
run;

OTE: The data set WORK.MUC has 18 observations and 3 variables.
OTE: DATA statement used (Total process time):
     real time           0.00 seconds
     cpu time            0.00 seconds


data MUC2; set MUC;
 if block = 1 and tank = 1 then tankn = '2A1';
  if block = 1 and tank = 2 then tankn = '2A2';
   if block = 1 and tank = 3 then tankn = '2A3';
    if block = 1 and tank = 4 then tankn = '2A4';
     if block = 1 and tank = 5 then tankn = '2A5';
      if block = 1 and tank = 6 then tankn = '2A6';
 if block = 2 and tank = 1 then tankn = '2B1';
  if block = 2 and tank = 2 then tankn = '2B2';
   if block = 2 and tank = 3 then tankn = '2B3';
    if block = 2 and tank = 4 then tankn = '2B4';
     if block = 2 and tank = 5 then tankn = '2B5';
      if block = 2 and tank = 6 then tankn = '2B6';
 if block = 3 and tank = 1 then tankn = '2C1';
  if block = 3 and tank = 2 then tankn = '2C2';
   if block = 3 and tank = 3 then tankn = '2C3';
    if block = 3 and tank = 4 then tankn = '2C4';
     if block = 3 and tank = 5 then tankn = '2C5';
      if block = 3 and tank = 6 then tankn = '2C6';
    run;

OTE: There were 18 observations read from the data set WORK.MUC.
OTE: The data set WORK.MUC2 has 18 observations and 4 variables.
OTE: DATA statement used (Total process time):
     real time           0.00 seconds
     cpu time            0.00 seconds


proc sort data=MUC2;
 by x;
run;

OTE: There were 18 observations read from the data set WORK.MUC2.
OTE: The data set WORK.MUC2 has 18 observations and 4 variables.
OTE: PROCEDURE SORT used (Total process time):
     real time           0.01 seconds
     cpu time            0.01 seconds
```

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```
data assign_trt_MUC; set MUC2;
 if _n_ = 1 then trt = 'control';
  if _n_ = 2 then trt = 'control';
   if _n_ = 3 then trt = 'control';
    if _n_ = 4 then trt = '50';
 if _n_ = 5 then trt = '50';
  if _n_ = 6 then trt = '50';
   if _n_ = 7 then trt = '100';
    if _n_ = 8 then trt = '100';
 if _n_ = 9 then trt = '100';
  if _n_ = 10 then trt = '200';
   if _n_ = 11 then trt = '200';
    if _n_ = 12 then trt = '200';
  if _n_ = 13 then trt = '300';
   if _n_ = 14 then trt = '300';
    if _n_ = 15 then trt = '300';
  if _n_ = 16 then trt = '300-HD';
   if _n_ = 17 then trt = '300-HD';
    if _n_ = 18 then trt = '300-HD';
    run;

OTE: There were 18 observations read from the data set WORK.MUC2.
OTE: The data set WORK.ASSIGN_TRT_MUC has 18 observations and 5 variables.
OTE: DATA statement used (Total process time):
     real time           0.01 seconds
     cpu time            0.01 seconds


proc print data= assign_trt_MUC;
title1 h=2 'Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid
mussel species';
title2 h=1.5 'AEH-11-PSUEDO-01';
title3 h=1 'Random assignment of treatment to experimental tanks';
title4 h=1 'Mussel speces: Mucket';
run;

OTE: There were 18 observations read from the data set WORK.ASSIGN_TRT_MUC.
OTE: PROCEDURE PRINT used (Total process time):
     real time           0.00 seconds
     cpu time            0.00 seconds


/*****************************************************************************************
***************/

/*Mussel speces: Hickory Nut*/
data HIC;
 do block = 1 to 3 by 1;
  do tank = 1 to 6 by 1;
   x = ranuni(-1);
   output;
  end;
 end;
```

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```
run;

OTE: The data set WORK.HIC has 18 observations and 3 variables.
OTE: DATA statement used (Total process time):
     real time           0.00 seconds
     cpu time            0.00 seconds
```

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```
data HIC2; set HIC;
 if block = 1 and tank = 1 then tankn = '3A1';
  if block = 1 and tank = 2 then tankn = '3A2';
   if block = 1 and tank = 3 then tankn = '3A3';
    if block = 1 and tank = 4 then tankn = '3A4';
     if block = 1 and tank = 5 then tankn = '3A5';
      if block = 1 and tank = 6 then tankn = '3A6';
 if block = 2 and tank = 1 then tankn = '3B1';
  if block = 2 and tank = 2 then tankn = '3B2';
   if block = 2 and tank = 3 then tankn = '3B3';
    if block = 2 and tank = 4 then tankn = '3B4';
     if block = 2 and tank = 5 then tankn = '3B5';
      if block = 2 and tank = 6 then tankn = '3B6';
 if block = 3 and tank = 1 then tankn = '3C1';
  if block = 3 and tank = 2 then tankn = '3C2';
   if block = 3 and tank = 3 then tankn = '3C3';
    if block = 3 and tank = 4 then tankn = '3C4';
     if block = 3 and tank = 5 then tankn = '3C5';
      if block = 3 and tank = 6 then tankn = '3C6';
    run;

OTE: There were 18 observations read from the data set WORK.HIC.
OTE: The data set WORK.HIC2 has 18 observations and 4 variables.
OTE: DATA statement used (Total process time):
     real time           0.01 seconds
     cpu time            0.01 seconds


proc sort data=HIC2;
 by x;
run;

OTE: There were 18 observations read from the data set WORK.HIC2.
OTE: The data set WORK.HIC2 has 18 observations and 4 variables.
OTE: PROCEDURE SORT used (Total process time):
     real time           0.01 seconds
     cpu time            0.01 seconds



data assign_trt_HIC; set HIC2;
 if _n_ = 1 then trt = 'control';
  if _n_ = 2 then trt = 'control';
   if _n_ = 3 then trt = 'control';
    if _n_ = 4 then trt = '50';
 if _n_ = 5 then trt = '50';
  if _n_ = 6 then trt = '50';
   if _n_ = 7 then trt = '100';
```

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```
    if _n_ = 8 then trt = '100';
 if _n_ = 9 then trt = '100';
  if _n_ = 10 then trt = '200';
   if _n_ = 11 then trt = '200';
    if _n_ = 12 then trt = '200';
  if _n_ = 13 then trt = '300';
   if _n_ = 14 then trt = '300';
    if _n_ = 15 then trt = '300';
  if _n_ = 16 then trt = '300-HD';
   if _n_ = 17 then trt = '300-HD';
    if _n_ = 18 then trt = '300-HD';
    run;

OTE: There were 18 observations read from the data set WORK.HIC2.
OTE: The data set WORK.ASSIGN_TRT_HIC has 18 observations and 5 variables.
OTE: DATA statement used (Total process time):
     real time           0.01 seconds
     cpu time            0.01 seconds


proc print data= assign_trt_HIC;
title1 h=2 'Effects of Psuedomonas fluorescens (Pf-CL145A) to glochidia from seven unionid
mussel species';
title2 h=1.5 'AEH-11-PSUEDO-01';
title3 h=1 'Random assignment of treatment to experimental tanks';
title4 h=1 'Mussel speces: Hickory Nut';
run;

OTE: There were 18 observations read from the data set WORK.ASSIGN_TRT_HIC.
OTE: PROCEDURE PRINT used (Total process time):
     real time           0.00 seconds
     cpu time            0.00 seconds
```

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File Folder 14c

PROOFED BY
Initials: TMS Date: 5/15/12
REVIEWED BY
Initials: JR Date: [illegible]

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Appendix 4. Test Article Information

Item number	Item description	Number of pages	Report page number
1	Material Safety Data Sheet: MBI-401 Spray Dried Powder	2	238
2	Material Safety Data Sheet: MBI-401 Freeze Dried Powder	2	240
3	MBI-401 FDP (lot # 110607WB-FD-E) Test Article: Certificate of Analysis	1	242
4	Copy of test article information from test article log book for MBI-401 SDP; lot number MBI-401-110308AI-BD-3	5	243
5	Copy of test article information from test article log book for MBI-401 FDP; lot number 110607WB-FD-E	4	248
6	Copy of test article information from test article log book for MBI-401 SDP; lot number MBI-401 SDP 4655-12-Mix	4	252
7	NYSM Post-Treatment Product Validation Assay (lot number MBI-401 SDP 110308AI-BD-3)	2	256
8	NYSM Post-Treatment Product Validation Assay (lot number MBI-401 FDP 110607WB-FD-E)	2	258
9	NYSM Post-Treatment Product Validation Assay (lot number MBI-401 SDP 4655-12-Mix)	2	260
10	Test Chemical Weights (12-May-11)	1	262
11	Glochidia Exposure Dosing Form and Chemical Stock Solution Determination (PPB SDP)	2	263
12	Glochidia Exposure Dosing Form and Chemical Stock Solution Determination (HGE SDP)	2	265
13	Glochidia Exposure Dosing Form and Chemical Stock Solution Determination (FAM SDP)	2	267
14	Test Chemical Stock Preparation Data Form (18-Oct-11)	1	269
15	Glochidia Exposure Dosing Form and Chemical Stock Solution Determination (PPB FDP)	2	270
16	Glochidia Exposure Dosing Form and Chemical Stock Solution Determination (WAS FDP)	2	272
17	Test Chemical Stock Preparation Data Form (17-Jan-12)	1	274
18	Glochidia Exposure Dosing Form and Chemical Stock Solution Determination (BLS SDP)	2	275
19	Glochidia Exposure Dosing Form and Chemical Stock Solution Determination (MUC SDP)	2	277
20	Test Chemical Stock Preparation Data Form (19-Jan-12)	1	279
21	Glochidia Exposure Dosing Form and Chemical Stock Solution Determination (HIC SDP)	2	280

MATERIAL SAFETY DATA SHEET

Product Name: MBI-401 SDP

Trade names/ Synonyms:	MBI-401 SDP
EPA Registration Number:	None, Experimental
PMRA Research Authorization #:	0030-RP-10
Primary Hazards:	Inhalation

Contact: Marrone Bio Innovations, 2121 Second Street,
Suite B-107, Davis, CA 95618
Phone (Business hours): 530-750-2800
www.marronebioinnovations.com
For emergencies such as leaks or spills call CHEMTREC 24-hour toll-free hotline at 1.800.424.9300

SECTION 1: MATERIAL IDENTIFICATION

INGREDIENT 1

Common Name:	CL145A strain of *Pseudomonas fluorescens*
Chemical Name:	Not applicable
Molecular Formula:	Not applicable
CAS Number:	Not applicable
Percent:	50%

OTHER INGREDIENTS: inert, non-reactive

SECTION 2: PHYSICAL DATA

Boiling Point:	Not applicable
Melting Point:	Not applicable
Bulk Density:	0.78 g/ml
Solubility in Water:	Dispersible in water
Appearance:	Powder
Color:	Tan
Odor:	Sweet, musty

SECTION 3: FIRE AND EXPLOSION DATA

Flash Point:	Not flammable
Method:	Not applicable
Extinguishing Media:	Use extinguishing media appropriate for the surrounding fire
Special Fire Fighting Procedures:	None
Unusual Fire and Explosion Hazards:	None

SECTION 4: REACTIVITY

Stability:	Material is non-reactive
Hazardous Polymerization:	Does not occur
Incompatibility:	None known
Hazardous Decomposition Products:	None known
Conditions to avoid:	None known

SECTION 5: HEALTH HAZARDS

Primary Route of Entry:	Skin contact, Eye, Inhalation
Exposure Limit:	Not established
Corrosive:	Not corrosive
Inhalation:	May be irritating to respiratory tract for some individuals. Avoid breathing dust.
Skin/ Eye Irritation:	May be irritating to skin and eyes for some individuals.
Effects of Overexposure:	If product comes in contact with eyes or skin, irritation may occur.
Toxicity:	None of the components of this product are listed as carcinogenic by NTP, IARC, OSHA
Acute studies:.	
Acute Oral LD_{50} (Rat):	>5,000 mg/kg (very low toxicity)
Acute Dermal LD_{50} (Rabbit)	>2,000 mg/kg (non-irritating, mild or slight irritation)
Primary Dermal Irritation	Slight Irritation, Class 4
Eye Irritation	Minimal Irritation, Class 4
Inhalation:	>2.25 g/ml, Class 4

SECTION 6: FIRST AID

Emergency First Aid Procedures:

If in eyes:	Hold eye open and rinse slowly and gently with water for 15-20 minutes. Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye. Call a poison control center of doctor for treatment advice.
If inhaled:	Move person to fresh air. If person is not breathing, call 911 or an ambulance, then give artificial respiration, preferably mouth-to-mouth if possible.
If on skin:	Take off contaminated clothing. Rinse skin immediately with plenty of water for 15-20 minutes. Call a poison control center or doctor for further treatment advice.
If swallowed:	Call a poison control center or doctor immediately for treatment advice. Have person sip a glass of water if able to swallow. Do not induce vomiting unless told to by a poison control center or doctor. Do not give anything by mouth to an unconscious person.

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Initials: DS Date: 08FEB12

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Initials: TJS Date: 08FEB12

MATERIAL SAFETY DATA SHEET

MBI-401 Spray Dried Powder, August 2010

SECTION 7: SPILL, LEAK AND DISPOSAL PROCEDURES

Steps to be taken in case material is released or spilled:

Wear suitable protective clothing such as long-sleeved shirt, pants, waterproof gloves and shoes with socks. Carefully mop or sweep up spill and place in a closed container for disposal.

Waste disposal method: Dispose of in accordance with all applicable federal, state, and local environmental regulations.

For emergencies such as leaks or spills, call CHEMTREC 24-hour toll-free hotline at 1.800.424.9300.

SECTION 8: SPECIAL HANDLING

Respiratory:	Use a NIOSH approved respirator with any N-95, P-95, R-95 or HE filter for biological products when mixing/loading the product.
Protective gloves:	Wear gloves made of Latex or other impervious material.
Eye protection:	Safety goggles or safety glasses with side shields recommended.
Other protective clothes:	Clothing to prevent prolonged skin contact as needed such as long-sleeved shirt, long pants and shoes with socks.

SECTION 9: SPECIAL PRECAUTIONS

Precautions to be taken in handling and storing:

Use a NIOSH approved respirator with any N-95, P-95, R-95 or HE filter for biological products when mixing/loading the product.

Store in a dry area inaccessible to children. Store in original containers only. Keep container closed when not in use.

Empty container completely and dispose of in accordance with all applicable federal, state, and local environmental regulations.

Wash any contamination from skin or eyes immediately. Wash hands and exposed skin before eating, drinking, smoking after work or using the toilet.

SECTION 10: SHIPPING REGULATIONS

Proper shipping name:	None
DOT Label (s) Required:	None
Freight Classification:	Insecticides, Fungicides N.O.I., Other Than Poisons. NMFC 102120 Class 60

SARA Title III Hazard Classification:

Immediate (acute) Health:	None
Delayed (chronic) Health:	None
Fire:	None
Sudden Release of Pressure:	None
Reactivity:	None

National Fire Protection Association Rating:

Health:	None
Flammability:	None
Reactivity:	None

Marrone™ Bio Innovations

CERTIFIED COPY

Initials: TS Date: 08FEB12

AEH-11-PSEUDO-01

Product Name: MBI-401 FDP

Trade names/ Synonyms:	Zequanox FDP, MBI-401 FDP
EPA Registration Number:	None, Experimental
PMRA Research Authorization #.	0053-RA-11
Primary Hazards:	Inhalation

Contact: Marrone Bio Innovations, 2121 Second Street, Suite B-107, Davis, CA 95618
Phone (Business hours) 530-750-2800
www.marronebioinnovations.com
For emergencies such as leaks or spills call CHEMTREC 24-hour toll-free hotline at 1.800.424.9300

SECTION 1: MATERIAL IDENTIFICATION

INGREDIENT 1

Common Name:	CL145A strain of *Psuedomonas fluorescens* cells, powder formulation
Chemical Name:	Not applicable
Molecular Formula:	Not applicable
CAS Number.	Not applicable
Percent	100%

OTHER INGREDIENTS. inert, non-reactive

SECTION 2 PHYSICAL DATA

Boiling Point:	Not applicable
Melting Point	Not applicable
Bulk Density:	0.61 g/mL
Solubility in Water:	Dispersible in water
Appearance	Powder
Color:	Tan
Odor:	Musty

SECTION 3 FIRE AND EXPLOSION DATA

Flash Point.	Not flammable
Method	Not applicable
Extinguishing Media:	Use extinguishing media appropriate for the surrounding fire
Special Fire Fighting Procedures.	None
Unusual Fire and Explosion Hazards:	None

SECTION 4: REACTIVITY

Stability:	Material is non-reactive
Hazardous Polymerization.	Does not occur
Incompatibility.	None known
Hazardous Decomposition Products.	None known
Conditions to avoid.	None known

SECTION 5: HEALTH HAZARDS: POTENTIAL SENSITIZER

Primary Route of Entry:	Skin contact, Eye, Inhalation
Exposure Limit:	Not established
Corrosive:	Not corrosive
Inhalation:	May be irritating to respiratory tract for some individuals. Avoid breathing dust.
Skin/ Eye Irritation.	May be irritating to skin and eyes for some individuals
Effects of Overexposure:	If product comes in contact with eyes or skin, irritation may occur.
Toxicity:	None of the components of this product are listed as carcinogenic by NTP, IARC, or OSHA
Acute studies:	
Acute Oral LD_{50} (Rat):	>5,000 mg/kg (very low toxicity)
Acute Dermal LD_{50} (Rabbit)	>2,000 mg/kg (non-irritating, mild or slight irritation)
Primary Dermal Irritation	Non- Irritating, Class 4
Eye Irritation	Minimal Irritation, Class 4
Inhalation	>2.25 g/mL Class 4

SECTION 6: FIRST AID

Emergency First Aid Procedures:

If in eyes:	Hold eye open and rinse slowly and gently with water for 15-20 minutes. Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye. Call a poison control center of doctor for treatment advice
If inhaled:	Move person to fresh air. If person in not breathing, call 911 or an ambulance, then give artificial respiration, preferably mouth-to-mouth if possible
If on skin:	Take off contaminated clothing. Rinse skin immediately with plenty of water for 15-20 minutes. Call a poison control center or doctor for further treatment advice.
If swallowed:	Call a poison control center or doctor immediately for treatment advice. Have person sip a glass of water if able to swallow. Do not induce vomiting unless told to by a poison control center or doctor. Do not give anything by mouth to an unconscious person.

Updated: June 23, 2011

AEH-11-PSEUDO-01 **CERTIFIED COPY**

Initials: TDS Date: 08FEB12

SECTION 7 SPILL, LEAK AND DISPOSAL PROCEDURES

Steps to be taken in case material is released or spilled

Wear suitable protective clothing such as long-sleeved shirt, pants, waterproof gloves and shoes with socks. Carefully mop or sweep up spill and place in a closed container for disposal.

Waste disposal method: Dispose of in accordance with all applicable federal, state, and local environmental regulations.

For emergencies such as leaks or spills, call CHEMTREC 24-hour toll-free hotline at 1 800 424 9300

SECTION 8 SPECIAL HANDLING

Respiratory	Use a NIOSH approved respirator with any N-95, R-95, P-95 or HE filter for biological products when mixing/loading the product
Protective gloves:	Latex gloves or other impervious, waterproof material recommended when mixing/loading the product
Eye protection	Safety goggles or safety glasses with side shields recommended when mixing/loading the product
Other protective clothes:	Clothing to prevent prolonged skin contact as needed such as long-sleeved shirt, long pants and shoes with socks.

SECTION 9 SPECIAL PRECAUTIONS

Precautions to be taken in handling and storing

Use a NIOSH approved respirator with any N-95, R-95, P-95 or HE filter for biological products when mixing/loading the product.

Store in a dry area inaccessible to children. Store in original containers only. Keep container closed when not in use.

Empty container completely and dispose of in accordance with all applicable federal, state, and local environmental regulations

Wash from exposed skin or from eyes immediately. Wash hands and exposed skin before eating, drinking, smoking after work or using the toilet.

SECTION 10 SHIPPING REGULATIONS

Proper shipping name	None
DOT Label (s) Required:	None
Freight Classification:	Insecticides, Fungicides N.O.I., Other Than Poisons. NMFC 102120 Class 60

SARA Title III Hazard Classification

Immediate (acute) Health	No
Delayed (chronic) Health	No
Fire	No
Sudden Release of Pressure:	No
Reactivity:	No

National Fire Protection Association Rating.

Health:	0
Flammability	0
Reactivity.	0

This document set forth is based on information that Marrone Bio Innovations, Inc (MBI) believes to be accurate. No warranty, expressed or implied, is intended. The information is provided solely for your information and consideration and MBI assumes no legal responsibility for use or reliance thereon.

Marrone Bio Innovations

Updated June 23, 2011

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CERTIFICATE OF ANALYSIS

Name of Product:	MBI-401 FDP
Active Ingredient:	100% *Pseudomonas fluorescens* strain CL145A cells and spent fermentation media
Cfu/g:	0 cfu/g *Pseudomonas fluorescens* strain CL145A
Lot Number:	110607WB-FD-E
Mussel Bioassay:	Pass
Appearance:	Tan powder
Storage Conditions:	4°C, in the dark
Date of Manufacture:	5/31/11
Expiration Date:	This product is stable for 6 months from date of manufacture provided the sample is stored under the recommended storage conditions.

I hereby certify that the above information is true and correct.

Analyst: [redacted] Date: 7/11/11
James Waller, Applications Engineer

Approved by: [redacted] Date: 7/11/2011
Ray Lam, Vice President Process Development

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Initials: TJS Date: 08FEB12

CHEMICAL LOG BOOK

MBI-401 SDP

(CL145A strain of *Pseudomonas fluorescens*)

[redacted]

011 by [redacted] Pro

Manufacturer:

Marrone Bio Innovations (MBI)
Davis, California

Lot Number:

MBI-401-110308AI-BD-3

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AEH-11-PSEUDO-01

SIGNATURE PAGE

All personnel making an entry in this log must fill out the form below in accordance with SOP GEN 009.

PRINTED NAME	SIGNATURE	INITIALS	DATE
Kerry L. Weber	[redacted]	KLW	28 APR 11
Todd J. Severson	[redacted]	TJS	29APR11

"This Use and Maintenance Log Book has been inspected and found to be in compliance with SOP GEN 009.

Inspected and sealed on May 11, 2011 by [redacted]."

Date Quality Assurance Unit

CERTIFIED COPY
Initials: DS Date: 08FEB12

Zequanox Spray Dried Powder - Material Specification Sheet

Prepared By:
Denise Mayer
New York State Museum
Field Research Laboratory
51 Fish Hatchery Road
Cambridge, NY 12816
(518) 677-8245

Shipped to:
Attn: Jim Luoma
Upper Midwest Environmental Sciences Center
2630 Fanta Reed Rd.
LaCrosse, WI 54603
(608)783-6451

Shipment Date:
4/27/11

Storage Information:
Store below 4C at all times.

Material Specs:
Material Lot#: **MBI-401-110308AI-BD-3**
Formulation: SDP
Dry Cell Weight: 50% (by weight of powder)
Volume per Aliquot: 96g (= 48 g active ingredient)
Number of Aliquots: 1

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SOP No. GEN 012.2
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Form GEN 012.2a
Page 1 of 1

TEST CHEMICAL DATA FORM

Test Material (Chemical Name) CL145A strain of Pseudomonas flucrescens

Trade Name of Chemical (Synonyms) MBI-401 SDP

Source of Chemical (Manufacturer) Marrone Bio Innovations (MBI)

Storage Location Room 122 Hotpoint Refrigerator in lockbox

Date Received 28 APR 2011 Date Opened 28 APR 2011 Expiration Date N/A
(5 years unless otherwise stated)

Test Chemical Lot Number MBI-401-110308A1-BD-3 Purity of Chemical 50%

Amount of Test Chemical Available or Received (if known) 96g

Initial Weight (with cover on) of Test Chemical and Container 130.678g

Characterization of Test Chemical: Color Tan
Physical State: liquid ☐ solid ☒
Solid Form: powder ☒ crystal ☐ pellet ☐

Chemical Abstract Service Number N/A

Manufacturer Certificate of Analysis Yes ☐ No ☒

Additional Comments About Test Chemical:

This lot of Pf-CL145A was used for L. Cardium, L. siliquoidea, and L. higginsii glochidia (dosing date: 12 MAY 11). TDS 08FEB12

Sample Placed in Archives: Yes ☒ No ☐ (Entries should also be made on Form GEN 012.2b) by Jane Rivera, QAO on 5/11/2011

Archive # RF-III-F3 Material Safety Data Sheet Available Yes ☒ No ☐

Signature of person (Study Director or designated representative) initiating Test Chemical Use Log and date:

Signature [redacted]

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Date 26 APR 2011

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SOP No. GEN 012.2
Page 6 of 6

Form GEN 012.2b
Page 1 of 1

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REVIEWED BY
Initials: ____ Date: ____

TEST CHEMICAL USE LOG

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ITEM № → 1

Test Chemical: PF-CL145A/MBI-401 SDP Manufacturer: Marrone Bio Innovations (MBI) Lot #: MBI-401-110308A1-BD-3

A Initial weight of chemical & container (with cap/lid on)(g)	B Amount removed (g)	C Weight of chemical & container (g) after removal (with cap/lid on)	D Study # and Purpose	E Comments	F Date/Initials
*1 130.678	1.00147	129.62	*Archived Sample AEH-11-PSEUDO-01		KLW 28APR11
129.62	0.03054	129.18 ①	AEH-11-PSEUDO-01	Preliminary Microscope Observations	29APR11 DS
129.22	8.00117	121.14	AEH-11-PSEUDO-01	Glochidia Exposure Experiment (~~LAS~~, HGE + PPB) ②FAM KLW 5/12/11	12MAY11 KLW
				Of the 8.00117 g of test chemical removed	
				4 aliquots were made. There weights were	
				1.99910g, 2.00062g, 2.00008g and	
				2.00062g. Used to make stock solutions.	12MAY11 KLW
121.17	8.43266	112.59	AEH-11-PSEUDO-01	Sample to be sent to New York State Museum (Denise Mayer) for analysis	17MAY11 KLW

① Weight of chemical is low by approximately 0.3g due to a piece of parafilm that was sealing container and thrown away. From now on container will be weighed prior to re-sealing with parafilm. DS 29APR11

② Wrong species FAM is correct KLW 5/12/2011

Page 7 of 7

* The first entry should be the chemical placed in the Chemical Archives. Follow GEN 011.
* The initial weight is also entered on Form GEN 012.2a.

A The weight of the chemical and its container shall be measured by weighing it on a balance that has been verified to be accurate (SOP GEN 013)

B The chemical removed from the test chemical container will be weighed into a tared vessel.

C After the chemical has been removed from the test chemical container, reweigh the container and its contents (including the cap or

AEH-11-PSEUDO-01

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CHEMICAL LOG BOOK

[redacted] **MBI-401 FDP**

d on 10/27/11

[redacted]

Marrone Bio Innovations
Davis, California

LOT NUMBER: 110607WB-FD-E

FILE FOLDER → 8

ITEM # → 2

Page 1 of 7

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Initials: TJS Date: 03FEB12

SIGNATURE PAGE

All personnel making an entry in this log must fill out the form below in accordance with SOP GEN 009.

PRINTED NAME	SIGNATURE	INITIALS	DATE
Kerry L. Weber	[redacted]	KLW	14 Oct 11

"This Use and Maintenance Log Book has been inspected and found to be in compliance with SOP GEN 009.

Inspected and sealed on 10/07/2011 by [redacted] QAO."

Date — Quality Assurance Unit

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SOP No. GEN 012.2
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Form GEN 012.2a
Page 1 of 1

TEST CHEMICAL DATA FORM

Test Material (Chemical Name) MBI-401 FDP

Trade Name of Chemical (Synonyms) PF-CL145A; Zequanox

Source of Chemical (Manufacturer) Marrone Bio Innovations

Storage Location Refrigerator in Rm 122 in lockbox (4°C)

Date Received 14OCT11 Date Opened 14OCT11 Expiration Date 31 Nov 2011
(5 years unless otherwise stated)

Test Chemical Lot Number 110607WB-FD-E Purity of Chemical 100%

Amount of Test Chemical Available or Received (if known) 100g

Initial Weight (with cover on) of Test Chemical and Container 161.68g

Characterization of Test Chemical: Color Tan
Physical State: liquid ☐ solid ☒
Solid Form: powder ☒ crystal ☐ pellet ☐

Chemical Abstract Service Number N/A

Manufacturer Certificate of Analysis Yes ☒ No ☐

Additional Comments About Test Chemical:
Temperature in cooler was 4.4°C upon receipt.
This lot of PF-CL145A was used for L. cardium and M. nervosa glochidia (dosing date: 18OCT11). TJS 08FEB12

Sample Placed in Archives: Yes ☒ No ☐ (Entries should also be made on Form GEN 012.2b)

Archive # R/III-f-9 Material Safety Data Sheet Available: Yes ☐ No ☐
OKO 19OCT11

Signature of person (Study Director or designated representative) initiating Test Chemical Use Log and date:

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Signature [redacted] Date 14OCT 2011

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Initials: TJS Date: 08FEB12

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Form GEN 012.2b
Page 1 of 1

ORIGINAL

TEST CHEMICAL USE LOG

Test Chemical: PFCL145A/MBI-401 FDP Manufacturer: Marrone Bio Innovations (MBI) Lot #: 110607WB-FD-E

A Initial weight of chemical & container (with cap/lid on)(g)	B Amount removed (g)	C Weight of chemical & container (g) after removal (with cap/lid on)	D Study # and Purpose	E Comments	F Date/Initials
*1 161.68	* ② ~~0.9982~~	* 160.66	*Archived Sample	AEH-11-PSEUDO-01 ① Archiving Sample Washboard ~~Model species~~	KLW 14OCT11
161.082	1.00005	160.047		AEH-11-PSEUDO-01 Deactive Stock	KLW 18OCT11
160.047	1.00014	159.046		AEH-11-PSEUDO-01 Washboard Active Stock	KLW 18OCT11
159.046	1.00072	158.044		AEH-11-PSEUDO-01 PPB Active Stock	KLW 18OCT11
158.078	6.1789	151.881		AEH-11-PSEUDO-01 Sample to NYSM for activity verification	KLW 25OCT11

* = The first entry should be the chemical placed in the Chemical Archives. Follow GEN 011.
1 = The initial weight is also entered on Form GEN 012.2a.

① Plain Pocketbook was used instead of model. KLW 18OCT11
② NL Should be 0.9962g KLW18OCT11

A The weight of the chemical and its container shall be measured by weighing it on a balance that has been verified to be accurate (SOP GEN 013)
B The chemical removed from the test chemical container will be weighed into a tared vessel.
C After the chemical has been removed from the test chemical container, reweigh the container and its contents (including the cap or

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CHEMICAL LOG BOOK

MBI-401 SDP

Marrone Bio Innovations
Davis, California

LOT NUMBER: MBI-401 SDP 4655-12-Mix

[redacted]

[redacted] 000
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PRINTED NAME	SIGNATURE	INITIALS	DATE
Kerry L. Weber	[redacted]	Klw	10JAN12

"This Use and Maintenance Log Book has been inspected and found to be in compliance with SOP GEN 009.

Inspected and sealed on 10 January 2012 by [redacted]."

Date — Quality Assurance Unit

Page 2 of 6

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Initials: TJS Date: 08FEB12

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Form GEN 012.3a
Page 1 of 1

TEST CHEMICAL DATA FORM

Test Chemical (Chemical Name) MBI -401 SDP

Circle one: Test Article Control Article

Trade Name of Test Chemical (Synonyms) Pf-CL145A; Zequanox

Source of Test Chemical (Manufacturer) Marrone Bio Innovations

Storage Location Refrigerator in Rm 122 in lockbox (0-5°C)

Date Received 10 JAN 2012 Date Opened 10 JAN 2012 Expiration Date 10 JAN 2017
(5 years unless otherwise stated) (None provided)

Test Chemical Lot Number MBI-401 SDP 4655-12-Mix Purity of Chemical 50%

Amount of Test Chemical Available or Received (if known) 100g

Initial Mass (with cover on) of Test Chemical and Container 162.057 g

Characterization of Test Chemical: Color Tan
Physical State: liquid __ solid X
Solid Form: powder X crystal __ pellet __

Chemical Abstract Service Number N/A

Manufacturer Certificate of Analysis Yes __ No X

Additional Comments about the Test Chemical:
Temperature in cooler upon receipt was 3.1°C.
This lot of Pf-CL145A was used for L. recta, A. ligamentina, and O. olivaria glochidia (dosing dates: 17JAN12 and 19JAN12). TJS 08FEB12.

Sample Placed in Archives: Yes X No __ (Entries should also be made on Form GEN 012.b)

Archive Location R1 III-F11 Material Safety Data Sheet Available: Yes X No __

Signature of Study Director or designee initiating Test Chemical Use Log and date:

Signature [redacted] Date 10 JAN 2012

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Form GEN 012.3b
Page 1 of 1

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TEST CHEMICAL USE LOG

Test Chemical: PF-CL145A/MBI-401 SDF/Zequanox | Manufacturer: Marrone Bio Innovations (MBI) | Lot or Batch #: MBI-401 SDP 4655-12-Mix

A Initial mass of test chemical & container with cap/ lid on (g)	B Amount removed (g)	C Mass of test chemical & container after removal, with cap/lid on (g)	D Study Number	E Purpose and Other Comments	F Date	G Initials
*1 162.057	* 4.5436	* 157.404	AEH-11-PSEUDO-01	*Archived Sample	10JAN12	KLW
157.413	2.0013	155.359	AEH-11-PSEUDO-01	Heat deactive test chemical for BLS and MLC	17JAN12	KLW
155.359	2.0037	153.339	AEH-11-PSEUDO-01	Test chemical for BLS	17JAN12	KLW
153.339	2.0020	151.300	AEH-11-PSEUDO-01	Test chemical for MLC	17JAN12	KLW
151.375	2.0002	149.338	AEH-11-PSEUDO-01	Heat deactive test chemical for HIC	19JAN12	KLW
149.338	2.0014	147.386	AEH-11-PSEUDO-01	Test chemical for HIC	19JAN12	KLW
147.287	10.0553	137.150	AEH-11-PSEUDO-02	Heat deactive test chemical for FAM	27JAN12	KLW
137.150	10.0406	126.969	AEH-11-PSEUDO-02	Active Test Chemical for FAM	27JAN12	KLW
126.979	5.1433	121.801	AEH-11-PSEUDO-01 AEH-11-PSEUDO-02	Test chemical to WFSM for analysis	31JAN12	KLW

*1 The first entry should be the test chemical sample placed in the Chemical Archives. Follow GEN 011.
The initial mass is also entered on Form GEN 012.a.
A. The mass of the test chemical and its container will be determined using a balance that has been verified to be accurate (SOP GEN 013).
B. The test chemical removed from the container will be placed into a tared vessel.
C. After the test chemical has been removed from the container, determine the mass of the container and its contents (with cap/lid on).

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NYSM Post-Treatment Product Validation Assay

MBI-401 SDP 110308AI-BD-3 (USGS Study #AEH-11-PSEUDO-01)

Date product received from USGS: 2011/05/18
Date of start of test: 09/13/2011

BACKGROUND: As standard protocol for the USEPA project, each time a batch of Zequanox product is used in a test a UMESC, a portion of the product is bioassayed by the NYSM to validate toxicity post-treatment.

- MBI-401 SDP 110308AI-BD-3
  - USGS Study #AEH-11-PSEUDO-01: Glochidia (FAM, HGE, PPB)

PURPOSE: Post-test product validation of MBI-401 SDP 110308AI-BD-3 from USGS-UMESC.

MATERIALS AND METHODS:

Preparation of product for testing:
Product was shipped under cold conditions and held in the laboratory refrigerator at 4°C until use. Within 30 min of treatment application, prepare each at treatment stock of each MBI-401 formulated product:

MBI 401-110308AI-BD-3 (SDP – 50% active ingredient): Weigh out 1.0 g of the powder into a labeled 50 ml centrifuge tube and bring volume up to 20 ml with dilution water. Vortex until evenly dispersed = 50 mg product/ml or 25 mg ai/ml. For 200 ppm ai treatments in testing jars, add 4 ml to each jar (500 ml).

Cambridge CF (Standard for Positive Controls):
As an efficacy standard, use *Pf*-CL145A killed CF that was maintained at -80°C (Cambridge CF). Since its production in 2005, this material has been valuable as a reference standard. The Cambridge CF was produced in 2005 (2005-0027) in 100-L batches 10, 11 and 12 and E-beamed to kill the cells. The solution, at 110 mg/ml dry weight, is stored in 1 cm thick sheets in the Cambridge ultrafreezer at -80°C. A section of the sheet was broken off and weighed to determine volume (ca. 1 g = 1 ml).

For this bioassay, the positive control suspension was produced on 5-2-2011 by weighing out 33.84 g of frozen Batch 10-12 block (killed cell suspension from test 2005-0027) and diluting with ca. 35 ml dilution water. 7 ml of this suspension were dispersed into 50-ml centrifuge tubes and placed in the ultrafreezer (11 tubes). The dry weight of the material was 43.75 mg/ml; therefore 2.3 ml were added to each testing jar to treat at 200 ppm.

Mussel collections:
Mussels attached to small stones were collected from Hedges Lake (Washington County, NY) and brought back to the Cambridge Lab in coolers. Mussels were scraped from substrates at the lab and placed in pint-sized canning jars, covered with mesh, and placed in aquaria containing unchlorinated tap water with circulation (1 Whisper filter) and aeration at ambient laboratory temperature (20-23°C).

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Mussel collection and handling:

Species	Collection site	Collection date	Date in lab (20°C)	Picked for test
Zebra mussels	Hedges Lake (Washington County)	09/09/2011	09/09/2011	09/13/2011 KP

Experimental design:
For validation of efficacy the following treatments will be set up:

Zebra mussels (25 mussels/jar):

- 3 – Untreated Control
- 3 – 200 ppm (ai) Positive Control - Cambridge CF (A, B, C)
- 3 – 200 ppm (ai) SDP MBI 401-110308AI-BD-3 (A, B, C)

Testing jar bioassay protocol:
On the day prior to treatment (09/13/2011) mussels were carefully examined and 25 mussels placed into each testing jar containing ca. 100 ml aerated hard water and allowed to attach overnight. The next morning (09/14/2011), unattached mussels were removed and replaced with attached mussels from an extra glass Petri dish. Water was replaced with 500 ml fresh aerated hard water.

After at least one hour, the treatment was applied. The optical density of each jar was measured in duplicate ($A_{660\ nm}$ Genesys Spectrophotometer).

After 24 hr of treatment, mussel mortality was checked and mussels were transferred to square plastic dishes with fresh aerated hard water. Mortality was checked and recorded each day with water replacements, for an additional 22 days. On the final day of mortality checks, 20 mussels were measured from the untreated controls using a caliper.

Results:

Mussel length: Zebra mussels 12.35 ± 2.33 mm.

Optical density of treatments:

Treatment	Mean (±SD) OD ($A_{660\ nm}$)
Untreated Control	-0.006 ± 0.001
Positive Control - Cambridge CF	0.175 ± 0.009
SDP MBI-401 110308AI-BD-3	0.246 ± 0.002

Zebra mussel mortality: Mussels were treated in triplicate testing jars (500 ml) at 20°C for 24 hr and mortality was recorded for a total of 23 days.

Treatment	Mean % mortality (±SD)
Untreated Control	1.3 ± 2.3%
Positive Control - Cambridge CF	80.0 ± 6.9%
SDP MBI-401 110308AI-BD-3	86.7 ± 4.6% Pass

Activity of MBI-401 SDP 110308AI-BD-3 was acceptable for this study.

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NYSM Post-Treatment Product Validation Assay

MBI-401 FDP 110607~~10~~B-FD-E (USGS Study #AEH-11-PSEUDO-01)

*-W 1/23/12 JAM

Date product arrived from USGS: 2011/10/22

Date of start of test: 11/15/2011

BACKGROUND: As standard protocol for the USEPA project, each time a batch of Zequanox product is used in a test a UMESC, a portion of the product is bioassayed by the NYSM to validate toxicity post-treatment. W 1/23/12 JAM

- MBI-401 FDP 110607~~10~~B-FD-E
  - USGS Study #AEH-11-PSEUDO-01: Glochidia (WAS, PPB)

PURPOSE: Post-test product validation of MBI-401 FDP 110607~~10~~B-FD-E from USGS-UMESC. W 1/23/12 JAM

MATERIALS AND METHODS:

Preparation of product for testing:

Product was shipped under cold conditions and held in the laboratory refrigerator at 4°C until use. Within 30 min of treatment application, prepare treatment stock of MBI 401 formulated product:

W wrong lot number TMS 2/8/12

MBI 110607~~10~~B-FD-E (FDP – 100% active ingredient): Weigh out 1.0 g of the powder into a labeled 50 ml centrifuge tube and bring volume up to 20 ml with dilution water. Vortex until evenly dispersed = 50 mg product/ml or 50 mg ai/ml. For 200 ppm ai treatments in testing jars, add 2 ml to each jar (500 ml).

Cambridge CF (Standard):

As an efficacy standard, use *Pf*-CL145A killed CF that was maintained at -80°C (Cambridge CF). Since its production in 2005, this material has been valuable as a reference standard. The Cambridge CF was produced in 2005 (2005-0027) in 100-L batches 10, 11 and 12 and E-beamed to kill the cells. The solution, at 110 mg/ml dry weight, is stored in 1 cm thick sheets in the Cambridge ultrafreezer at -80°C. A section of the sheet was broken off and weighed to determine volume (ca. 1 g = 1 ml).

For this bioassay, the positive control suspension was produced on 5-2-2011 by weighing out 33.84 g of frozen Batch 10-12 block (killed cell suspension from test 2005-0027) and diluting with ca. 35 ml dilution water. 7 ml of this suspension were dispersed into 50-ml centrifuge tubes and placed in the ultrafreezer (11 tubes). The dry weight of the material was 43.75 mg/ml; therefore 2.3 ml were added to each testing jar to treat at 200 ppm.

In this test verified the activity of a new batch of positive control suspension produced on 11-14-2011; produced as described above and having a dry weight of 65.65 mg/ml. 1.52 ml were added to each testing jar (500 ml) to treat at 200 ppm.

Mussel collections:

Mussels were scraped from substrates (rocks) in the field and placed in plastic bins. Bins containing mussels were set in a cooler with towels and frozen ice packs to maintain temperature to be transported back to the laboratory in Cambridge. In the laboratory, mussels were placed in pint-sized canning jars, covered with mesh, and placed in aquaria containing unchlorinated tap water with circulation (1 Whisper filter) and aeration at ambient laboratory temperature (20°C).

*Entry error within the product lot #

FILE FOLDER → 8a
ITEM # → 2

PROOFED BY
Initials: TJS Date: 10FEB12
REVIEWED BY
Initials: ____ Date: ____

Page 1 of 2

W wrong lot number TMS 2/8/12

Mussel collection and handling:

Species	Collection site	Collection date	Date in lab (20°C)	Picked for test
Zebra mussels	Hedges Lake (Washington County)	10/18/2011	10/18/2011	11/14/2011 PS

Experimental design:
For validation of efficacy the following treatments will be set up:

Zebra mussels (25 mussels/jar):

3 – Untreated Control
3 – 200 ppm (ai) Cambridge CF Positive Control A (A, B, C) (produced on 5-2-2011)
3 – 200 ppm (ai) Cambridge CF Positive Control B (A, B, C) (produced on 11-14-2011)
3 – 200 ppm (ai) MBI 110607~~10~~B-FD-E (A, B, C
W 1/23/12 DAM

Testing jar bioassay protocol:
On the day prior to treatment (11/14/2011) mussels were carefully examined and 25 mussels placed into each testing jar containing ca. 100 ml aerated hard water and allowed to attach overnight. The next morning (11/15/2011), unattached mussels were removed and replaced with attached mussels from an extra glass Petri dish. Water was replaced with 500 ml fresh aerated hard water.

After at least one hour, the treatment was applied. The optical density of each jar was measured in duplicate ($A_{660\,nm}$ Genesys Spectrophotometer).

After 24 hr of treatment, mussel mortality was checked and mussels were transferred to square plastic dishes with fresh aerated hard water. Mortality was checked and recorded each day with water replacements, for an additional 22 days. On the final day of mortality checks, 20 mussels were measured from the untreated controls using a caliper.

Results:

Mussel length: Zebra mussels 12.46 ± 2.29 mm.

Optical density of treatments:

Treatment	Mean (±SD) OD ($A_{660\,nm}$)
Untreated Control	-0.005 ± 0.001
Cambridge CF A (Positive Control)	0.171 ± 0.013
Cambridge CF B (Positive Control)	0.176 ± 0.002
MBI-401 FDP-110607~~10~~B-FD-E	0.184 ± 0.003

W 1/23/12 DAM

Zebra mussel mortality: Mussels were treated in triplicate testing jars (500 ml) at 20°C for 24 hr and mortality was recorded for a total of 10 days.

Treatment	Mean % mortality (±SD)
Untreated Control	0.0 ± 0.0%
Cambridge CF A (Positive Control)	88.0 ± 4.0%
Cambridge CF B (Positive Control)	79.6 ± 11.0%
MBI-401 FDP-110607~~10~~B-FD-E	94.7 ± 6.1% **Pass**

W 1/23/12 DAM

Activity of MBI-401 FDP-110607~~10~~B-FD-E was acceptable for this study.

W 1/23/12 DAM

FILE FOLDER → 8a
ITEM # → 2

PROOFED BY
Initials: TJS Date: 10FEB12
REVIEWED BY
Initials: ____ Date: ____

Page 2 of 2

NYSM Post-Treatment Product Validation Assay

MBI-401 SDP 4655-12-Mix (USGS Study #AEH-11-PSEUDO-01)

FILE FOLDER → 8a

ITEM # → 4

Date product received from USGS: 2012/02/01

Date of start of test: 02/09/2012

BACKGROUND: As standard protocol for the USEPA project, each time a batch of Zequanox product is used in a test a UMESC, a portion of the product is bioassayed by the NYSM to validate toxicity post-treatment.

- MBI-401 SDP 4655-12-Mix
  - USGS Study #AEH-11-PSEUDO-01: Glochidia (BLS, MUC, HIC)
  - USGS Study #AEH-11-PSEUDO-02: Juvenile (FAM)

PURPOSE: Post-test product validation of MBI-401 SDP 4655-12-Mix from USGS-UMESC.

MATERIALS AND METHODS:

Preparation of product for testing:

Product was shipped under cold conditions and held in the laboratory refrigerator at 4°C until use. Within 30 min of treatment application, prepare each at treatment stock of each MBI-401 formulated product:

MBI-401 SDP 4655-12-Mix (SDP – 50% active ingredient): Weigh out 2 g of the powder and add slowly to a beaker with water stirring for even suspension. Total volume should be 20 ml in dilution water. Transfer to a 50 ml centrifuge and store in refrigerator until ready to use. Mix until evenly dispersed = 100 mg product/ml or 50 mg a.i./ml. For 200 ppm ai treatments in testing jars, add 2 ml to each jar (500 ml).

Cambridge CF (Standard for Positive Controls):

As an efficacy standard, use *Pf*-CL145A killed CF that was maintained at -80°C (Cambridge CF). Since its production in 2005, this material has been valuable as a reference standard. The Cambridge CF was produced in 2005 (2005-0027) in 100-L batches 10, 11 and 12 and E-beamed to kill the cells. The solution, at 110 mg/ml dry weight, is stored in 1 cm thick sheets in the Cambridge ultrafreezer at -80°C. A section of the sheet was broken off and weighed to determine volume (ca. 1 g = 1 ml).

For this bioassay, the positive control suspension was produced on 11-14-2011 and then stored at -80°C in 50-ml centrifuge tubes. The dry weight of the material is 65.65 mg/ml; therefore 1.5 ml was added to each testing jar to treat at 200 ppm.

Mussel collections:

Mussels were scraped from substrates (rocks) in the field and placed in plastic bins. Bins containing mussels were set in a cooler with towels and frozen ice packs to maintain temperature to be transported back to the laboratory in Cambridge. In the laboratory, mussels were placed in pint-sized canning jars, covered with mesh, and placed in aquaria containing unchlorinated tap water with circulation (1 Whisper filter) and aeration at ambient laboratory temperature (20°C).

PROOFED BY
Initials: TMS Date: 4/26/12
REVIEWED BY
Initials ____ Date: ____

Page 1 of 2

Mussel collection and handling:

Species	Collection site	Collection date	Date in lab (20°C)	Picked for test
Zebra mussels	Hedges Lake (Washington County)	11/30/2011	01/31/2012	02/08/2012

Experimental design:
For validation of efficacy the following treatments will be set up: **AEH-11-PSEUDO-01**

Zebra mussels (25 mussels/jar):
3 – Untreated Control
3 – 200 ppm (a.i.) Cambridge CF Positive Control B (A, B, C) (produced on 11-14-2011)
3 – 200 ppm (a.i.) MBI-401 SDP 4655-12-Mix (A, B, C)

Testing jar bioassay protocol:
On the day prior to treatment (02/08/2012) mussels were carefully examined and 25 mussels placed into each testing jar containing ca. 100 ml aerated hard water and allowed to attach overnight. The next morning (02/09/2012), unattached mussels were removed and replaced with attached mussels from an extra glass Petri dish. Water was replaced with 500 ml fresh aerated hard water.

After at least one hour, the treatment was applied. The optical density of each jar was measured in duplicate ($A_{660\ nm}$ Genesys Spectrophotometer).

After 24 hr of treatment, mussel mortality was checked and mussels were transferred to square plastic dishes with fresh aerated hard water. Mortality was checked and recorded each day with water replacements, for an additional 13 days. On the final day of mortality checks, 20 mussels were measured from the untreated controls using a caliper.

Results:

Mussel length: Zebra mussels 12.28 ± 2.89 mm.

Optical density of treatments:

Treatment	Mean (±SD) OD ($A_{660\ nm}$)
Cambridge CF (Positive Control)	0.191 ± 0.008
MBI-401 SDP 4655-12-Mix	0.283 ± 0.007

Zebra mussel mortality: Mussels were treated in triplicate testing jars (500 ml) at 20°C for 24 hr and mortality was recorded for a total of 14 days.

Treatment	Mean % mortality (±SD)
Untreated Control	0.0 ± 0.0%
Cambridge CF (Positive Control)	78.4 ± 6.0%
MBI-401 SDP 4655-12-Mix	85.3 ± 11.5%

MBI-401 SDP 4655-12-Mix PASSED the post-test bioassay validation (85% mortality). Untreated control mortality was 0%.

Page 2 of 2

FILE FOLDER → 8a
ITEM № → 4

PROOFED BY
Initials: TMS Date: 4/26/12
REVIEWED BY
Initials: ____ Date: ____

Study Number: AEH-11-PSEUDO-01 | MK Folder 10

Pf-CL 14A Test chemical - Pseudomonas fluorescens (lot # MBI-401-1103080AI-BD-3 | Logbook 1, pg 8

AEH-11-PSEUDO-01

Test Chemical Weights (12 MAY 2011 KLW) Proofed: TDS 07FEB12 Reviewed: ______

1.99990g PF-CL145A KLW — used to make 20,000 mg/L of deactivated stock solution. Brought up to 100 mL of acclimated well water. Deactivated by placing in water bath at 70°C for 45 minutes.

[redacted]

Item No. 1

2.00062g PF-CL145A KLW — used to make 20,000 mg/L of active stock solution for L. cardium. Placed in 100mL volumetric flask and brought to volume.

AEH-11-PSEUDO-01 Prep: 12MAY11
2.00062g PF-CL145A @ 1055
in 100mL Acclimated wellwater Exp: 17MAY11
20,000mg/L TDS

2.00008g PF-CL145A TDS — used to make 20,000 mg/L of active stock solution for L. higginsii. Placed in 100mL volumetric flask and brought to volume. KLW 5/12/2011

AEH-11-PSEUDO-01 Prep: 12MAY11
2.00008g PF-CL145A @ 1124
in acclimated wellwater Exp: 17MAY11
20,000 mg/L TDS

PROOFED BY
Initials: ____ Date: ____
REVIEWED BY
Initials: ____ Date: ____
not needed see above mrs 5/14/12

AEH-11-PSEUDO-01 Prep: 12MAY11
2.00062g PF-CL145A @ 1255
in 100mL acclimated well water TDS
20,000mg/L Exp: 17MAY11

2.00062g PF-CL145A TDS — used to make 20,000 mg/L of active stock solution for L. siliquoidea. Placed in 100mL volumetric flask and brought to volume. KLW 5/12/2011

Page 1 of 2

12 MAY 2011 KLW

Study Title: **"Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"**

Study number: AEH-11-PSEUDO-01

Approved by [redacted] Date 5-5-11

Book #1
File Folder: 16 [redacted] Lab book/pgs: 9/41 Proofed: TJS 03FEB12 Reviewed:

Glochidia Exposure Dosing Form

Item No. 2

Species: L. Cardium **UMESC lot number:** 111400

Test Block Assignment (circle one): 1 2 3

Estimated # glochidia/Chamber >1,000

Date/time (military) of dosing initiation: 1036

Date/time (military) of dosing completion: 1040

Additional information: chambers contained 96.5mL of well water
added 5-100mL aliquots with stockwells
Controls = 3.0 mL of well water
50 mg/L = 2.5 mL of well water + 0.5mL of 10,000 mg/L A.I. stock
100 mg/L = 2.0 mL of well water + 1.0mL of 10,000 mg/L A.I. stock
200 mg/L = 1.0 mL of well water + 2.0 mL of 10,000 mg/L A.I. stock
300 mg/L = 3.0 mL of 10,000 mg/L A.I. stock
300 mg/L Heat Deactivated = 3.0 mL of heat deactivated stock

Witness and form recorded by:

James A. Luoma	[redacted]	UMESC	5/12/11
Printed Name	**Signature**	**Affiliation**	**Date**

Page 1 of 2

Study Number AEH-11-PSEUDO-01

Approved by: [redacted] Date 5/12/11

File Folder 16
Book 1 p. 9

Proofed: TDS 03FEB12 Reviewed:

Pocketbook 5/12/11 Jm

Chemical Stock Solution Determination

Stock A = Control (20 °C well water); **Stock B** = 10,000 mg/L active material (2.0g/100 mL product = 10.0mg/mL active ingredient);
Stock C = 10,000 mg/L detoxified active material (2.0g/100 mL - Heat deactivated for 45 minutes at 70°C/cooled to 20 °C prior to use

Dosage Level (mg/L)	Dose concentration	Exposure volume (mL)	Time added	Deactivated mg required	Active mg required	(well) Aliquot Stock A (mL)	(active) Aliquot Stock B (mL)	(deactivated) Aliquot Stock C (mL)	Color Code Assignment
0	control (well water blank)	100	1029	0	0	3.0	0.0	0.0	White
300	mg/L (Deactivated)	100		30	0	0.0	0.0	(5) 3.0	White/black stripes
50	mg/L	100		0	5	2.5	(1) 0.5	0.0	Yellow
100	mg/L	100		0	10	2.0	(2) 1.0	0.0	Yellow/black stripes
200	mg/L	100		0	20	1.0	(3) 2.0	0.0	Blue
300	mg/L	100		0	30	0.0	(4) 3.0	0.0	Blue/black stripes
Total						8.5	6.5	3.0	
Total per species						25.5	19.5	9.0	
Total per 3 species						76.5	58.5	27.0	

(1) Dose @ 1036
(2) Dose @ 1037
(3) Dose @ 1038
(4) Dose @ 1039
(5) Dose @ 1040
5/12/11 Jm

Page 2 of 2

Exposure chambers w/ mussels to test system at 10:45
Exposure chambers placed and air adjusted to 5-10 bubbles/sec at 1058 h 5-12-11 Jm

Study Title: "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"

Study number: AEH-11-PSEUDO-01

Approved by [redacted] Date 5-5-11

File Folder: 16 [redacted] Lab book/pgs: Book #1 10-11 Proofed: TS 07FEB12 Reviewed:

Glochidia Exposure Dosing Form

Item No. 3

Species: Higgins Eye **UMESC lot number:** 11500

Test Block Assignment (circle one): 1 2 3

Estimated # glochidia/Chamber >1000

Date/time (military) of dosing initiation: 1155

Date/time (military) of dosing completion: 1204

Additional information:

Chambers contained 96.5 mls H_2O

added 5 100 ul aliquots with glochidia (0.5 mL)

Each chamber received 3 mL of a stock or combination of stocks.

Control = 3 mL of well water

50 mg/L = 2.5 mL of well water and 0.5 mL of 10,000 mg/L A.I. stock.

100 mg/L = 2.0 mL of well water and 1.0 mL of 10,000 mg/L A.I. stock

200 mg/L = 1.0 mL of well water and 2.0 mL of 10,000 mg/L A.I. stock

Witness and form recorded by:

James A. Luoma	[redacted]	UMESC	5/12/11
Printed Name	**Signature**	**Affiliation**	**Date**

300 mg/L = 3.0 mL of 10,000 mg/L A.I. stock.

300 mg/L Heat deactivate = 3.0 mL of Heat deactivated stock.

Page 1 of 2

Proofed: BS 03FEB12 Reviewed: ________

Study Number AEH-11-PSEUDO-01

Approved by: [redacted] **Date** 5/12/11

File Folder 10
Book 1 p. 10

Higgins Eye
5-12-11
Jow

Chemical Stock Solution Determination

Stock A = Control (20 °C well water); **Stock B** = 10,000 mg/L active material (2.0g/100 mL product = 10.0mg/mL active ingredient);
Stock C = 10,000 mg/L detoxified active material (2.0g/100 mL - Heat deactivated for 45 minutes at 70°C/cooled to 20 °C prior to use

Dosage Level (mg/L)	Dose concentration	Exposure volume (mL)	Deactivated mg required	Active mg required	well Aliquot Stock A (mL)	active Aliquot Stock B (mL)	deactive Aliquot Stock C (mL)	Color Code Assignment
0	control (well water blank)	100	0	0	(1) 3.0	0.0	0.0	White
300	mg/L (Deactivated)	100	30	0	0.0	0.0	(6) 3.0	White/black stripes
50	mg/L	100	0	5	2.5	(2) 0.5	0.0	Yellow
100	mg/L	100	0	10	2.0	(3) 1.0	0.0	Yellow/black stripes
200	mg/L	100	0	20	1.0	(4) 2.0	0.0	Blue
300	mg/L	100	0	30	0.0	(5) 3.0	0.0	Blue/black stripes
Total					8.5	6.5	3.0	
Total per species					25.5	19.5	9.0	
Total per 3 species					76.5	58.5	27.0	

(1) Dose complete 1155
(2) Dose complete 1200
(3) Dose complete 1202
(4) Dose complete 1203
(5) Dose complete 1204
(6) Dose complete 1204

May 12, 2011
Jow

Page 2 of 2

Study Title: "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"

Study number: AEH-11-PSEUDO-01

Approved by [redacted] Date 5-5-11

File Folder: 16 Lab book/pgs: Book #2 10+11 Proofed: JS 03FEB12 Reviewed: ______

Glochidia Exposure Dosing Form

Item No. 4

Species: Fat Mucket **UMESC lot number:** 111600

Test Block Assignment (circle one): 1 2 3

Estimated # glochidia/Chamber >1000

Date/time (military) of dosing initiation: 1317

Date/time (military) of dosing completion: 1325

Additional information: chambers contained 96.5 mL of well water added 5 100 uL aliquots with glochidia (0.5 mL)

Controls = 3.0 mL of well water

50 mg/L = 2.5 mL of well water + 0.5 mL of 10,000 mg/L A.I. stock

100 mg/L = 2.0 mL of well water + 1.0 mL of 10,000 mg/L A.I. stock

200 mg/L = 1.0 mL of well water + 2.0 mL of 10,000 mg/L A.I. stock

300 mg/L = 3.0 mL of 10,000 mg/L A.I. stock

300 mg/L heat deactivated = 3.0 mL of heat deactivated stock

Witness and form recorded by:

James A. Luoma	[redacted]	UMESC	5/12/11
Printed Name	**Signature**	**Affiliation**	**Date**

Page 1 of 2

Study Number AEH-11-PSEUDO-01

Proofed: DS 03FEB12 Reviewed: ______

Approved by: [redacted] Date 5-12-11

Fat mudlat 5-12-11 JTV

File Folder 16
Book 1 p. 10

Chemical Stock Solution Determination

Stock A = Control (20 °C well water); **Stock B** = 10,000 mg/L active material (2.0g/100 mL product = 10.0mg/mL active ingredient);
Stock C = 10,000 mg/L detoxified active material (2.0g/100 mL - Heat deactivated for 45 minutes at 70°C/cooled to 20 °C prior to use

Dosage Level (mg/L)	Dose concentration	Exposure volume (mL)	Deactivated mg required	Active mg required	(well) Aliquot Stock A (mL)	(active) Aliquot Stock B (mL)	(deactivated) Aliquot Stock C (mL)	Color Code Assignment
0	control (well water blank)	100	0	0	(1) 3.0	0.0	0.0	White
300	mg/L (Deactivated)	100	30	0	0.0	0.0	(6) 3.0	White/black stripes
50	mg/L	100	0	5	(2) 2.5	0.5	0.0	Yellow
100	mg/L	100	0	10	(3) 2.0	1.0	0.0	Yellow/black stripes
200	mg/L	100	0	20	(4) 1.0	2.0	0.0	Blue
300	mg/L	100	0	30	0.0	(5) 3.0	0.0	Blue/black stripes
Total					8.5	6.5	3.0	
Total per species					25.5	19.5	9.0	
Total per 3 species					76.5	58.5	27.0	

Page 2 of 2

May 12, 2011
JTV

(1) Dosing complete at 1317
(2) Dosing complete at 13~~18~~22 wrote incorrect time 5-12-11 JTV
(3) Dosing complete at 1322
(4) Dosing complete at 1323
(5) Dosing complete at 1324
(6) Dosing complete at 1325

Study Title: "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven different unionid species"

Study number: AEH-11-PSEUDO-01

Item No. 5

Approved by [redacted] Date 10-18-11

File Folder: 18 Lab book/pgs: 1/23 Proofed: TJS 03FEB12 Reviewed:

Test Chemical Stock Preparation Data Form

Test Chemical: *Pseudomonas fluorescens* strain 145A

Test Chemical Lot # 110607WB-FD-E Date Rec'd 14OCT 2011 Exp. Date 11/31/11

Chemical Weighing:

Sample I.D.	Sample wt. (g)	Date/Time	Initials
Deactive	1.00005	18 OCT 11/0725	KLW
WAS Active	1.00014	18 OCT 11/0735	KLW
PPB Active	1.00072	18 OCT 11/0740	KLW
		②	

*Chemical samples to be stored refrigerated until used for stock preparation.

Stock Preparation:

Sample I.D.	Dilution Vol. (ml)	Dilution time	Use (ie: Active stock for IIGE)	Date/ Time	Initials
Deactive	100 mL	0855	Deactive into bath 0750 out bath 0835	18 OCT 11/ 0900	KLW
WAS Active	100 mL	1250	WAS Active 1.00014g	~~18 Oct 11~~ ① 1250	JA
PPB Active	100 mL	1120	PPB Active 1.00072g	18 Oct 11/ 1120	JA
			②		

*Stocks to be prepared immediately before use, except for heat deactivated stock which will be prepared at least 1 hr prior to use to allow for deactivation and cooling.

② No data recorded. TJS 03FEB12.

① Incorrect date date should read 18 Oct 11 JA

Page 1 of 1

Study Title: "**Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species**"

Study number: AEH-11-PSEUDO-01 Item No. 6

Approved by [redacted] Date 5-5-11

File Folder: 16 Lab book/pgs: 1/23 Proofed: TS 03FEB12 Reviewed: ______

Glochidia Exposure Dosing Form

Species: L. Cardium **UMESC lot number:** 115400

Test Block Assignment (circle one): 1 2 3

Estimated # glochidia/Chamber 1000

Date/time (military) of dosing initiation: 1137

Date/time (military) of dosing completion: 1146

Additional information:

Witness and form recorded by:

Susan M Schleis	[redacted]	UMESC	18OCT11
Printed Name	**Signature**	**Affiliation**	**Date**

Page 1 of 2

Study Number AEH-11-PSEUDO-01
Approved by: [redacted] Date: 10/17/11

Species Dosed: PPB
Date of Dosing: 18OCT11

File Folder 16
Book 1 p.25-26

Chemical Stock Solution Determination

Stock A = Control (20 °C well water); **Stock B** = 10,000 mg/L active material (1.0g/100 mL product = 10.0mg/mL active ingredient);
Stock C = 10,000 mg/L detoxified active material (1.0g/100 mL - Heat deactivated for 45 minutes at 70°C/cooled to 20 °C prior to use

Dosage Level (mg/L)	Dose concentration	Exposure Volume (mL)	Deactivated mg Required	Active mg required	*Well Water* Aliquot Stock A (mL)	*Active Stock* Aliqot Stock B (mL)	*Deactive Stock* Aliquot Stock C (mL)	Color Code Assignment
0	control (well water blank)	100	0	0	3.0	0.0	0.0	White
300	mg/L (Deactivated)	100	30	0	0.0	0.0	3.0	White/black stripes
50	mg/L	100	0	5	2.5	0.5	0.0	Yellow
100	mg/L	100	0	10	2.0	1.0	0.0	Yellow/black stripes
200	mg/L	100	0	20	1.0	2.0	0.0	Blue
300	mg/L	100	0	30	0.0	3.0	0.0	Blue/black stripes
Total					8.5	6.5	3.0	
Total per species					25.5	19.5	9.0	
Total per 3 species					76.5	58.5	27.0	

Proofed: TDS 03FEB12 Reviewed: ____________

Concentration	Time Dosing Started	Time Dosing Completed	Date	Initials
0 mg/L	1137	1138	18OCT11	JKW
300 mg/L Deactive	1146	1146	18OCT11	JKW
50 mg/L	1139	1140	18OCT11	JKW
100 mg/L	1141	1143	18OCT11	JKW
200 mg/L	1144	1145	18OCT11	JKW
300 mg/L Active	1145	1146	18OCT11	JKW

Page 2 of 2

17OCT2011/vs1.1

Study Title: "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"

Study number: AEH-11-PSEUDO-01 Item No. 7

Approved by [redacted] Date 5-5-11

File Folder: 16 [redacted] Lab book/pgs: 1/23 Proofed: TJS 03FEB12 Reviewed: ______

Glochidia Exposure Dosing Form

Species: Washboard **UMESC lot number:** 115500

Test Block Assignment (circle one): 1 2 3

Estimated # glochidia/Chamber 500

Date/time (military) of dosing initiation: 1305

Date/time (military) of dosing completion: 1313

Additional information:

Witness and form recorded by:

Susan M Schleis	[redacted]	UMESC	18OCT11
Printed Name	**Signature**	**Affiliation**	**Date**

Page 1 of 2

Study Number AEH-11-PSEUDO-01
Approved by: [redacted] Date: 10/17/11

Species Dosed: WAS
Date of Dosing: 18OCT11

File Folder 16
Book 1 p.25-26

Chemical Stock Solution Determination

Stock A = Control (20 °C well water); **Stock B** = 10,000 mg/L active material (1.0g/100 mL product = 10.0mg/mL active ingredient);
Stock C = 10,000 mg/L detoxified active material (1.0g/100 mL - Heat deactivated for 45 minutes at 70°C/cooled to 20 °C prior to use

Dosage Level (mg/L)	Dose concentration	Exposure Volume (mL)	Deactivated mg Required	Active mg required	*Well Water* Aliquot Stock A (mL)	*Active Stock* Aliqot Stock B (mL)	*Deactive Stock* Aliquot Stock C (mL)	Color Code Assignment
0	control (well water blank)	100	0	0	3.0	0.0	0.0	White
300	mg/L (Deactivated)	100	30	0	0.0	0.0	3.0	White/black stripes
50	mg/L	100	0	5	2.5	0.5	0.0	Yellow
100	mg/L	100	0	10	2.0	1.0	0.0	Yellow/black stripes
200	mg/L	100	0	20	1.0	2.0	0.0	Blue
300	mg/L	100	0	30	0.0	3.0	0.0	Blue/black stripes
Total					8.5	6.5	3.0	
Total per species					25.5	19.5	9.0	
Total per 3 species					76.5	58.5	27.0	

Proofed: JJS 03FEB12 Reviewed: ____________

Concentration	Time Dosing Started	Time Dosing Completed	Date	Initials
0 mg/L	1305	1307	18OCT11	JKW
300 mg/L Deactive	1308	1308	18OCT11	JKW
50 mg/L	1308	1310	18OCT11	JKW
100 mg/L	1310	1311	18OCT11	JKW
200 mg/L	1311	1312	18OCT11	JKW
300 mg/L Active	1312	1313	18OCT11	JKW

Page 2 of 2

17OCT2011/vs1.1

Study Title: "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven different unionid species"

Study number: AEH-11-PSEUDO-01 Item No. 8

File Folder: 16 Lab book/pgs: 1|31 Reviewed: TJS 07FEB12 Verified: ______

Test Chemical Stock Preparation Data Form

Test Chemical: *Pseudomonas fluorescens* strain 145A

Test Chemical Lot # ~~4655-12-Mix~~ MBI-401 SDP Date Rec'd 10 JAN 2012 Exp. Date 10 JAN 2017 (none provided)

Mussel Species BLS + MUC Block ID BLS = 1 ; MUC = 2

Instruments: BAL 1, BAL 4 and WTS 2

Chemical Weighing:

Sample I.D.	Sample wt. (g)	Date/Time	Initials
Deactive	2.0013	17 JAN 12/0800	KLW
Active BLS	2.0037	17 JAN 12/0807	KLW
Active MUC	2.0020	17 JAN 12/0815	KLW
		① KLW 17 JAN 2012	

*Chemical samples to be stored refrigerated until used for stock preparation.

Stock Preparation:

Sample I.D.	Dilution Vol. (ml)	Dilution time	Use (ie: Active stock for HGE)	Date/ Time	Initials
Deactive	100 mL	Into heat at 0545 out @ 0935 finished @ 1000	Deactive stock for BLS + MUC	17 JAN 12 0845	KLW
Active BLS	100 mL	1035	Active stock for BLS	17 JAN 12 1035	KLW
Active MUC	100 mL	1205	Active stock for MUC	17 JAN 12 1205	TJS
			① KLW 17 JAN 2012		

*Stocks to be prepared immediately before use, except for heat deactivated stock which will be prepared prior to use to allow for deactivation and cooling.

Page 1 of 1

This datasheet was approved by [redacted] on 12 JAN 2012
11JAN2012/version 1.1

① Only 2 species were dosed. KLW 17 JAN 2012

Study Title: "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid species"

Study number: AEH-11-PSEUDO-01 Item No. 9

File Folder: 16 Lab book/pgs: 1|32 Reviewed: TRS 07FEB12 Verified: ________

Glochidia Exposure Dosing Form

Species: BLS **UMESC lot number:** 120800

Test System Assignment (circle one): 1 2 3

Estimated # glochidia/chamber ≤ 500

Date/time (military) of dosing initiation: 1100

Date/time (military) of dosing completion: ~~1150~~ ① 1111

Additional information:

Chambers contained 96.5mL H_2O. Added 5 100µL aliquots with glochidia (0.5mL). Each chamber received 3mL of a stock or combination of stocks. Control = 3mL of well water; 50mg/L = 25mL of well water and 0.5mL of 10,000mg/L A.I. stock; 100mg/L = 2.0mL of well water and 1.0mL of 10,000mg/L A.I. stock; 200mg/L = 1.0mL of well water and 2.0mL of 10,000mg/L A.I. stock; 300mg/L = 3mL of A.I. stock; 300mg/L Deactivated = 3mL of 10,000mg/L deactivated stock. KMO 17JAN2012

Witness and form recorded by:

Jeremy K. Wise	[redacted]	UMESC	17JAN12
Printed Name	**Signature**	**Affiliation**	**Date**

Page 1 of 2

This datasheet was approved by [redacted] on 17JAN2012.
13JAN2012/version1.1

① Misentry. JKW 17JAN12

Study Nun EH-11-PSEUDO-01
Reviewed: TRS 07FEB12
Verified: ______

File Folder: 16
Lab book/pgs: 1/31
Species: Black Sandshell
Date of Dosing: 17 JAN 2012

Chemical Stock Solution Determination and Preparation

Formulation and lot #: MBI-401 SDP 4655-12-Mix

AEH-11-PSEUDO-01

Stock A = Control (20 °C well water)

Stock B = 10,000 mg/L active material (2.0 g/100 mL product = 1.0 g/100 mL active ingredient)

Stock C = 10,000 mg/L detoxified active material (2.0 g/100 mL product = 1.0 g/100 mL active ingredient - Heat deactivated for 45 minutes at 70°C/cooled to 20 °C prior to use)

Dosage Level (mg/L)	Exposure Volume (mL)	Deactivated mg Required	Active mg required	*Well Water* Aliquot Stock A (mL)	*Active Stock* Aliqot Stock B (mL)	*Deactive Stock* Aliquot Stock C (mL)	Color Code Assignment
0	100	0	0	3.0	0.0	0.0	White
300 HD	100	30	0	0.0	0.0	3.0	White/black stripes
50	100	0	5	2.5	0.5	0.0	Yellow
100	100	0	10	2.0	1.0	0.0	Yellow/black stripes
200	100	0	20	1.0	2.0	0.0	Blue
300 Active	100	0	30	0.0	3.0	0.0	Blue/black stripes
Total per rep					8.5	6.5	3.0
Total per species					25.5	19.5	9.0
Total per 3 species					76.5	58.5	27.0

Concentration	Stock Preperation Time	Time Dosing Started	Time Dosing Completed	Date	Initials
0 mg/L	1100	1100	1100	17JAN12	SMS
300 mg/L Deactive	1000	1107	1107	17JAN12	SMS
50 mg/L	1035	1102 ~~1108~~ (2)	1108	17JAN12	SMS
100 mg/L	1035	1102	1109	17JAN12	SMS
200 mg/L	1036	~~1103~~ 1106 (1)	1110	17JAN12	SMS
300 mg/L Active	1035	1111	1111	17JAN12	SMS

(1) wrong time recorded SMS 17JAN12 (2) wrote in wrong slot SMS 17JAN12

Page 2 of 2

13JAN2012/vs1.2 This datasheet was approved by [redacted] on 17 JAN2012.

Study Title: "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid species"

Study number: AEH-11-PSEUDO-01 Item No. 10

File Folder: 16 Lab book/pgs: 1|32 Reviewed: DS 17FEB12 Verified: ________

Glochidia Exposure Dosing Form

Species: MLLC **UMESC lot number:** 120700

Test System Assignment (circle one): 1 2 3

Estimated # glochidia/chamber ≤ 500

Date/time (military) of dosing initiation: 1232 / 17JAN12

Date/time (military) of dosing completion: 1238 / 17JAN12

Additional information:

Chambers contained 96.5mL H_2O. Added 5 100µL aliquots with glochidia (0.5mL). Each chamber received 3mL of a stock or combination of stocks. Control = 3mL of well water; 50mg/L = 2.5mL of well water and 0.5mL of ①~~100mg/L~~ A.I. Stock; 100mg/L = 2.0mL of well water and 1.0mL of 10,000mg/L A.I. stock; 200mg/L = 1.0mL of well water and 2.0mL of 10,000mg/L A.I stock; 300mg/L Active = 3mL of 10,000mg/L A.I stock; 300mg/L Deactivated = 3mL of 10,000mg/L deactivated Stock. KLW 17JAN2012

Witness and form recorded by:

Jeremy K. Wise	[redacted]	UMESC	17JAN12
Printed Name	**Signature**	**Affiliation**	**Date**

① DEC. Should be 10,000mg/L KLW 17JAN2012

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This datasheet was approved by [redacted] on 17JAN2012.
13JAN2012/version1.1

Study Nun EH-11-PSEUDO-01
Reviewed: TJS 07FEB12
Verified: ______

File Folder: 16
Lab book/pgs: 1/31
Species: MUCKET
Date of Dosing: 17 JAN 2012

Chemical Stock Solution Determination and Preparation

Formulation and lot #: MBI-401 SDF 4655-12-Mix

AEH-11-PSEUDO-01

Stock A = Control (20 °C well water)

Stock B = 10,000 mg/L active material (2.0 g/100 mL product = 1.0 g/100 mL active ingredient)

Stock C = 10,000 mg/L detoxified active material (2.0 g/100 mL product = 1.0 g/100 mL active ingredient - Heat deactivated for 45 minutes at 70°C/cooled to 20 °C prior to use)

Dosage Level (mg/L)	Exposure Volume (mL)	Deactivated mg Required	Active mg required	*Well Water* Aliquot Stock A (mL)	*Active Stock* Aliqot Stock B (mL)	*Deactive Stock* Aliquot Stock C (mL)	Color Code Assignment
0	100	0	0	3.0	0.0	0.0	White
300 HD	100	30	0	0.0	0.0	3.0	White/black stripes
50	100	0	5	2.5	0.5	0.0	Yellow
100	100	0	10	2.0	1.0	0.0	Yellow/black stripes
200	100	0	20	1.0	2.0	0.0	Blue
300 Active	100	0	30	0.0	3.0	0.0	Blue/black stripes
Total per rep					8.5	6.5	3.0
Total per species					25.5	19.5	9.0
Total per 3 species					76.5	58.5	27.0

Concentration	Stock Preperation Time	Time Dosing Started	Time Dosing Completed	Date	Initials
0 mg/L	1232	1232	1232	17JAN12	SMS
300 mg/L Deactive	1000	1235	1235	17JAN12	SMS
50 mg/L	1205	1233	1236	17JAN12	SMS
100 mg/L	1205	1233	1237	17JAN12	SMS
200 mg/L	1205	1234	1238	17JAN12	SMS
300 mg/L Active	1205	1238	1238	17JAN12	SMS

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13JAN2012/vs1.2

This datasheet was approved by [redacted] on 17 JAN 2012.

Study Title: "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven different unionid species"

Study number: AEH-11-PSEUDO-01

Item No. 11

File Folder: 16 Lab book/pgs: 1/33,34 Reviewed: TJS 07FEB12 Verified: ________

Test Chemical Stock Preparation Data Form

Test Chemical: *Pseudomonas fluorescens* strain 145A

Test Chemical Lot # MBI-401 SDP 4655-12-Mix Date Rec'd 10JAN12 Exp. Date 10 JAN 2017 (none provided)

Mussel Species HIC Block ID 3

Instruments: BAL 1, BAL 4, WTS

Chemical Weighing:

Sample I.D.	Sample wt. (g)	Date/Time	Initials
Deactive Stock	2.0002	19 JAN 12/0756	KW
Active Stock HIC	2.0014	19 JAN 12/0804	KW
		Only one stock needed for active ad deactive as only one species is being dosed. KW 19JAN12	

*Chemical samples to be stored refrigerated until used for stock preparation.

Stock Preparation:

Sample I.D.	Dilution Vol. (ml)	Dilution time	Use (ie: Active stock for HGE)	Date/ Time	Initials
Deactive Stock	100	Into heat @ 0808	~~RE~~ KW 19JAN12 Deactive stock for HIC	19JAN12/0808	TJS
Active Stock	100	0900	Active Stock for HIC	19JAN12/0900	TJS
			Only one species	dosed. KW	19 JAN 12

*Stocks to be prepared immediately before use, except for heat deactivated stock which will be prepared prior to use to allow for deactivation and cooling.

Page 1 of 1

This datasheet was approved by [redacted] on 12 JAN 2012.
11JAN2012/version 1.1

Study Title: **"Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid species"**

Study number: AEH-11-PSEUDO-01 Item No. 12

File Folder: 16 Lab book/pgs: 1/34 Reviewed: PS 07FEB12 Verified: ________

Glochidia Exposure Dosing Form

Species: HIC **UMESC lot number:** 120900

Test System Assignment (circle one): 1 2 3

Estimated # glochidia/chamber ≤1000

Date/time (military) of dosing initiation: 0925

Date/time (military) of dosing completion: 0929

Additional information:

Chambers contained 96.5mL H_2O. Added 5 100uL aliquots with glochidia (0.5mL). Each chamber received 3mL of a stock or concentration of stocks. Control = 3mL of well water; 50mg/L = 2.5mL of well water and 0.5mL of 10,000mg/L active stock; 100mg/L = ① E KW 19JAN12 ~~2.0~~ mL of well water and 1.0mL of 10,000mg/L active stock; 200mg/L = 1.0mL of well water and 2.0mL of 10,000mg/L active stock; 300mg/L active = 3mL of 10,000mg/L active stock; 300mg/L Deactivated = 3mL of 10,000mg/L deactivated stock. KW 19JAN12

Witness and form recorded by:

Jeremy K. Wise	[redacted]	UMESC	19JAN12
Printed Name	**Signature**	**Affiliation**	**Date**

① Should be 2.0mg/L KW 19JAN12

Page 1 of 2

This datasheet was approved by [redacted] on 17JAN2012.
13JAN2012/version1.1

Study Nun :H-11-PSEUDO-01
Reviewed: TRS 07FEB12
Verified: ____________

File Folder: 16
Lab book/pgs: 1/34
Species: HIC
Date of Dosing: 19JAN12

Chemical Stock Solution Determination and Preparation

Formulation and lot #: MBI-401 SDP 4655-12-Mix

AEH-11-PSEUDO-01

Stock A = Control (20 °C well water)
Stock B = 10,000 mg/L active material (2.0 g/100 mL product = 1.0 g/100 mL active ingredient)
Stock C = 10,000 mg/L detoxified active material (2.0 g/100 mL product = 1.0 g/100 mL active ingredient - Heat deactivated for 45 minutes at 70°C/cooled to 20 °C prior to use)

Dosage Level (mg/L)	Exposure Volume (mL)	Deactivated mg Required	Active mg required	*Well Water* Aliquot Stock A (mL)	*Active Stock* Aliqot Stock B (mL)	*Deactive Stock* Aliquot Stock C (mL)	Color Code Assignment
0	100	0	0	3.0	0.0	0.0	White
300 HD	100	30	0	0.0	0.0	3.0	White/black stripes
50	100	0	5	2.5	0.5	0.0	Yellow
100	100	0	10	2.0	1.0	0.0	Yellow/black stripes
200	100	0	20	1.0	2.0	0.0	Blue
300 Active	100	0	30	0.0	3.0	0.0	Blue/black stripes
Total per rep					8.5	6.5	3.0
Total per species					25.5	19.5	9.0
Total per 3 species					76.5	58.5	27.0

Concentration	Stock Preperation Time	Time Dosing Started	Time Dosing Completed	Date	Initials
0 mg/L	~~0900~~ ① 0925	0925	0926	19JAN12	JKW
300 mg/L Deactive	0808	0928	0928	↑	JKW
50 mg/L	0900	0926	0926		JKW
100 mg/L	0900	0927	0927		JKW
200 mg/L	0900	0927	0928	↓	JKW
300 mg/L Active	0900	0928	0929	19JAN12	JKW

Page 2 of 2

① Stock prepared when control treatment initiated, 0925 JKW 19JAN12

13JAN2012/vs1.2 This datasheet was approved by [redacted] on 17 Jan/2012.

Appendix 5. Test Animal Information

Study Title: "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"

Study number: AEH-11-PSEUDO-01

Approved by [redacted] Date 29 APR 2011

Test Organism Species List, Collection and Inclusion Criteria

Common name	Scientific name
Black sandshell	*Ligumia recta*
Fatmucket	*Lampsilis siliquoidea*
Hickorynut	*Obovaria olivaria*
Higgins eye	*Lampsilis higginsii*
Mucket	*Actinonaias ligamentina*
Plain pocketbook	*Lampsilis cardium*
Washboard	*Megalonaias nervosa*

Collection and inclusion criteria (see protocol sections 4.1- 4.2):

* Gravid donor mussels will be collected by biologists from the Genoa National Fish Hatchery from the Upper Iowa, Upper Mississippi or St. Croix Rivers. Mussels will be identified to species as described in Cummings and Mayer (1992) and Watters et al. (2009) and transported to the Genoa National Fish Hatchery. Glochidia from at least three gravid female mussels of each species will be extracted and evaluated for viability and if acceptable (≥80%) glochidia groups will pooled for use by the Genoa NFH according to the procedures outlined in ASTM E2455-06 and immediately transferred to the UMESC in a cooler at ~20°C. Glochidia will be acclimated to test water by the addition of 50% UMESC well water at the Genoa NFH and upon arrival at UMESC. Prior to exposure initiation, viability will be assessed in a subsample of glochidia by the addition of a saturated sodium chloride solution (12 g NaCl/ 50 mL of D.I. H_2O [ASTM E2455-06]). Viability will be assessed ~1 minute after addition of sodium chloride and must exhibit > 80% viability for use in exposures.

* This section was updated due to Amendment #1.
SEE Amended Test organism Species List, Collection and Inclusion criteria dated 5-11-11
May 11, 2011
JaL

Study Title: "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"

Study number: AEH-11-PSEUDO-01

Approved by [redacted] Date 5-11-2011

Amended Test Organism Species List, Collection and Inclusion Criteria

Common name	Scientific name
Black sandshell	*Ligumia recta*
Fatmucket	*Lampsilis siliquoidea*
Hickorynut	*Obovaria olivaria*
Higgins eye	*Lampsilis higginsii*
Mucket	*Actinonaias ligamentina*
Plain pocketbook	*Lampsilis cardium*
Washboard	*Megalonaias nervosa*

Collection and inclusion criteria (see protocol sections 4.1- 4.2):

Gravid donor mussels will be collected by biologists from the Genoa National Fish Hatchery from the Upper Iowa, Upper Mississippi or St. Croix Rivers. Mussels will be identified to species as described in Cummings and Mayer (1992) and Watters et al. (2009) and transported to the Genoa National Fish Hatchery. Approximately 72 h prior to test initiation the donor mussels will be transferred from the Genoa NFH to the UMESC and acclimated to test temperature. Glochidia from at least three gravid female mussels of each species will be extracted by personnel from the Genoa NFH. The viability of glochidia from each mussel will be assessed by drawing aliquots (e.g. 100 µL) of settled glochidia from the stock container with an adjustable manual pipet fitted with a wide bore tip and placing it on a pre-labeled 35mm petri dish. The sample will be diluted with a known and consistent volume (e.g. 200 µL) of acclimated well water to enhance dispersion of glochidia for more accurate enumeration. The sample will be viewed under a dissecting microscope, adjusted for maximum clarity and magnification, and a photomicrograph will be recorded. Approximately one drop of a saturated sodium chloride solution (12 g NaCl/ 50 mL of D.I. H_2O [ASTM E2455-06]) will be added to the sample and after one minute a second photomicrograph will be recorded. Enumerations will be conducted for the total number of glochidia within the sample and the number of glochidia closed or open before and after sodium chloride addition. The percentage of viable glochidia (glochidia that respond to sodium chloride exposure by valve closure) will be calculated by the following method:

Percent viable = ([Total number of glochidia in sample – {total number of glochidia closed before addition of sodium chloride + number of glochidia open after sodium chloride addition}]/Total number of glochidia in sample) * 100

If viability is acceptable (≥80%) for a mussel, the glochidia from that mussel will be pooled with glochidia from other mussels of that species. An appropriate amount of settled glochidia (e.g.: 500 µL) will be randomly drawn from the pooled glochidia stock and aliquoted to an exposure chamber (80 mm x 40 mm crystallizing dish) containing exposure water to achieve a final volume of 100 mL. Between 500-1,000 glochidia will be transferred to each exposure chamber in a minimum of two aliquots (dependent on number of glochidia per aliquot and the estimated number of glochidia available) per chamber (e.g. two 100 µL aliquots that contain ~250 glochidia each). Once all of the exposure chambers receive test animals the exposures will be initiated by the addition of the appropriate amount of MBI-401 SDP, deactivated MBI-401 SDP (positive control) or plain water (control) in a completely randomized design to achieve the desired test concentrations of 0 (control), 300 mg/L deactivated (positive control), or 50, 100, 200, and 300 mg/L of active MBI-401 SDP. The exposure chambers will be gently mixed with a glass rod or stainless steel spatula to ensure uniform distribution of the test chemical. All concentrations will be tested in triplicate for a total of 18 exposure chambers per species. Exposure chambers will be held at 20°C in a water bath or controlled environment room and maintained on an 18:6 h light/dark cycle. Viability of a subsample of approximately 100 glochidia from each chamber will be assessed at 6, 12 and 24 h post exposure by enumeration from photomicrograph records pre- and post-sodium chloride addition. The study will be terminated after 24 h post exposure evaluations are completed.

Study Number AEH-11-PSEUDO-01

Book #1

File Folder: 7a Lab book/pgs: 48 Proofed: TJS 02FEB12 Reviewed: ____

Daily Care Worksheet

Item No. 1

Tank #: 1A **Room #:** 11 **Month/Year:** 05/2011
Species: L. Cardium
Lot Number: 11100

Day	Feed Time (military)	Flow Rate (mL/min) Inflow	Pump	Temperature (°C)	Dissolved Oxygen (mg/L)	pH	Initials
1							
2							
3				①			
4							
5							
6							
7							
8							
9	1330	1100	9.8	13.0	10.18	8.19	KLW
10	1335	1100	9.7	19.5	7.97	8.07	TJS
11	1240	1100	9.5	19.4	7.81	7.90	TJS
12	0810	1100	9.3	21.0	8.15	7.98	KLW
13							
14				①			
15							
16	Note: Mussel holding tanks 1A, 1B, and 1C are three sections						
17	of one tank divided by mesh screens. These water						
18	quality parameters were measured only in Section						
19	1C, yet recorded on sheets for 1A and 1B as well.						
20	TJS 12MAY11						
21							
22							
23							
24				①			
25						PROOFED BY Initials: TMS Date: 2/7/12	
26						REVIEWED BY	
27						Initials: Date:	
28							
29						Disregard stamped on to! TMS 2/7/12	
30							
31							

Daily Food Prep: Mix 44.0g Shellfish diet, 35.3g *Tetraselmis*, and 92.4g *Thalassiosira weissflogii* with 14,400 mL well water each day, to be delivered by peristaltic pump at 10 mL per minute. Target water inflow rate is 1,100 mL/min. Prepare fresh diet daily.

Page 1 of 1

① No data recorded. TJS 12MAY11

Approved by: [redacted] Date: 5-9-11

Study Number AEH-11-PSEUDO-01

Book #1

File Folder: 7a Lab book/pgs: 4-8 Proofed: TJS 02FEB12 Reviewed: ____

Daily Care Worksheet

Item No. 2

Tank #: 1B Room #: 11 Month/Year: 05/2011

Species: L. siliquoidea

Lot Number: 111200

Day	Feed Time (military)	Flow Rate (mL/min) Inflow	Pump	Temperature (°C)	Dissolved Oxygen (mg/L)	pH	Initials
1							
2							
3				②			
4							
5							
6							
7							
8							
9	1330	1100	9.8	13.0	10.18	8.19	KLW
10	1335	1100	9.7	19.5	7.97	8.07	TJS
11	1240	1100	9.5	19.4	7.81	7.90	TJS
12	0810	1100	9.3	21.0	8.15	7.98	KLW
13							
14				②			
15							
16							
17							
18							
19							
20							
21							
22							
23							
24							
25				②			
26							
27							
28							
29							
30							
31							

Note: Mussel holding tanks 1A, 1B, and 1C are ①three ~~theee~~ sections of one tank divided by mesh screens. These water quality parameters were measured only in section 1C, yet were recorded on sheets for 1A and 1B as well. TJS 12MAY11

Daily Food Prep: Mix 44.0g Shellfish diet, 35.3g *Tetraselmis*, and 92.4g *Thalassiosira weissflogii* with 14,400 mL well water each day, to be delivered by peristaltic pump at 10 mL per minute. Target water inflow rate is 1,100 mL/min. Prepare fresh diet daily.

page 1 of 1

Approved by: [redacted] Date: 5-9-11

① Spelling error. TJS 12MAY11

② No data recorded. TJS 12MAY11

Study Number AEH-11-PSEUDO-01 Book #1

File Folder: 7a Lab book/pgs: 4-8 Proofed: TJS 02FEB12 Reviewed: ________

Daily Care Worksheet

Item No. 3

Tank #: 1C Room #: 11 Month/Year: 05/2011
Species: L. Higginsii
Lot Number: 111300

Day	Feed Time (military)	Flow Rate (mL/min) Inflow	Flow Rate (mL/min) Pump	Temperature (°C)	Dissolved Oxygen (mg/L)	pH	Initials
1							
2							
3				(1)			
4							
5							
6							
7							
8							
9	1330	1100	9.8	13.0	10.18	8.19	KLW
10	1335	1100	9.7	19.5	7.97	8.07	TJS
11	1240	1100	9.5	19.4	7.81	7.90	TJS
12	0810	1100	9.3	21.0	8.15	7.98	KLW
13							
14				(1)			
15							
16							
17							
18							
19							
20							
21							
22							
23							
24							
25				(1)			
26							
27							
28							
29							
30							
31							

Note: Mussel holding tanks 1A, 1B, and 1C are three sections of one tank divided by mesh screens. These water quality parameters were measured only in section 1C, yet were recorded on sheets for 1A and 1B as well. TJS 12MAY11

Daily Food Prep: Mix 44.0g Shellfish diet, 35.3g *Tetraselmis*, and 92.4g *Thalassiosira weissflogii* with 14,400 mL well water each day, to be delivered by peristaltic pump at 10 mL per minute. Target water inflow rate is 1,100 mL/min. Prepare fresh diet daily.

page 1 of 1 (1) No data recorded. 12MAY11 TJS

Approved by: [redacted] Date: 5-9-11

Study Number AEH-11-PSEUDO-01

File Folder: 7a Lab book/pgs: 1/31,33,34 Reviewed: TJS 02FEB12 Verified: ____

Daily Care Worksheet

Donor Mussel Holding

Item No. 5

Tank #: 1 Section #: C Room #: 16 Month/Year: JAN 2012

Species: HIC Lot Number: 120600

Instruments: DO4, pH3, THERM-8

Day	Feed Time (military)	Flow Rate (mL/min) Inflow	Pump	Temperature (°C)	Dissolved Oxygen (mg/L)	pH	Initials
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11				No Data Entry prior to test			
12				Initiation. TJS 17JAN12			
13							
14							
15							
16							
17	0915	1100	10.0	20.1	8.50	7.97	JKW
18	0825	1100	9.9	19.9	7.98	7.93	TJS
19	0724	1100	10.0	20.0	7.88	7.89	TJS
20							
21							
22							
23							
24							
25							
26					HIC mussels were only held		
27					until 19JAN12. TJS 19JAN12		
28							
29							
30							
31							

page 1 of 1

This datasheet was approved by [redacted] on 12JAN2012.
11JAN2012/version1.1

Study Number AEH-11-PSEUDO-01

File Folder: 7a Lab book/pgs: 1/31 Reviewed: TJS 02FEB12 Verified: ________

Daily Care Worksheet

Donor Mussel Holding **Item No.** 6

Tank #: 1 **Section #:** A **Room #:** 16 **Month/Year:** JAN 2012

Species: BLS **Lot Number:** 120400

Instruments: DO4, pH3, THERM8

Day	Feed Time (military)	Flow Rate (mL/min) Inflow	Flow Rate (mL/min) Pump	Temperature (°C)	Dissolved Oxygen (mg/L)	pH	Initials
1							
2							
3							
4							
5							
6							
7							
8							
9							
10					No Data Entry Prior to test		
11					Initiation. TJS 17JAN12		
12							
13							
14							
15							
16							
17	0915	1100	10.0	20.1	8.50	7.97	JKW
18							
19							
20							
21							
22							
23							
24							
25					BLS Mussels were only held		
26					for one day. TJS 17JAN12		
27							
28							
29							
30							
31							

page 1 of 1

This datasheet was approved by [redacted] on 12JAN2012.
11JAN2012/version1.1

Study Number AEH-11-PSEUDO-01

File Folder: 7a Lab book/pgs: 1 / 31 Reviewed: TJS 02FEB12 Verified: ________

Daily Care Worksheet

Donor Mussel Holding

Item No. 7

Tank #: 1 **Section #:** B **Room #:** 16 **Month/Year:** JAN 2012

Species: MUC **Lot Number:** 120500

Instruments: DO4, pH3, THERM-8

Day	Feed Time (military)	Flow Rate (mL/min) Inflow	Pump	Temperature (°C)	Dissolved Oxygen (mg/L)	pH	Initials
1							
2							
3							
4							
5							
6							
7							
8							
9					No Data Entry	Prior to	test
10					Initiation.	TJS 17JAN12	
11							
12							
13							
14							
15							
16							
17	0915	1100	10.0	20.1	8.50	7.97	JKW
18							
19							
20							
21							
22							
23							
24							
25					MUC mussels	were only	held
26					for one day.	TJS 17JAN12	
27							
28							
29							
30							
31							

page 1 of 1

This datasheet was approved by [redacted] on 12JAN2012.
11JAN2012/version1.1

AEH-11-PSEUDO-01

File Folder: 7A

Adult Mussel Holding Daily Algal Diet
1/13/2012

Item No. 8

Tank Dimensions and volume
48"x 14" x 6" (121.92 cm x 35.56 cm x 15.24 cm) = 66,073 cm^3
6" (15.24 cm) standpipe: 66.073 L

Flow rate (1 turnover per hour): 1.1 L/m)

Daily water volume: 1.1 L/m X 60 m/h X 24 h/d = 1584 L/d

Diet weights (50% *Tetraselmis* and 50% *Nannochloropsis*)
to achieve 10 mg/L by dry weight:

Tetraselmis: Target 5.0 mg/L (17.93% Dry Wt); *Nannochloropsis*: Target 5.0 mg/L (20.48% Dry Wt)

Tetraselmis: 27.89 mg/L; *Nannochloropsis*: 24.41mg/L

X 1584 L/d

Tetraselmis: 44.2 g/d; *Nannochloropsis*: 38.7g/d

Diet Inflow Rate: 10 mL/min X 60 m/h X 24 h/d = 14400 mL/d

Daily Care:
Mix 44.2g *Tetraselmis* and 38.7g *Nannochloropsis* with 14,400 mL well water each day, to be delivered by peristaltic pump at 10 mL per minute. Prepare fresh diet daily.

Approved by: [redacted] Date: 13 Jan 2012

Page 1 of 1

PROOFED BY
Initials: mS Date: 2/7/12
REVIEWED BY
Initials: ____ Date: ____

Study Title: "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"

Study number: AEH-11-PSEUDO-01 **Item No.** 1

Approved by [redacted] Date 5-5-11

File Folder: 7B Lab book/pgs: Book 1 p. 3 Proofed: TJS 02FEB12 Reviewed: ______

Donor Mussel UMESC Lot Number Assignment Form

DONOR MUSSEL INFORMATION:

Species: Plain Pocketbook (Lampsilis cardium)

Number of Donor Mussels: 4 **Collection Date:** 4/29/11

Collection Location: St. Croix River @ Hudson Narrows

Species Identification performed by: Nathan Eckert

Title/affiliation of identifier: Mussel Biologist / Genoa NFH

UMESC Arrival Date: 5-9-11 1130 h

Receiving UMESC tank/room # 1A Room 11

Additional information:

Recieved @ 53°F (11.7°C) ramp started from 12°C to 20°C @ 1230 set to run for 60 h

UMESC LOT NUMBER DESIGNATION: 11100

PROOFED BY
Initials: Date:
REVIEWED BY
Initials: Date:
not needed TJS 02FEB12

Witness and form recorded by:

James A. Luoma	[redacted]	UMESC	5/9/11
Printed Name	**Signature**	**Affiliation**	**Date**

page 1 of 1

Study Title: "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"

Study number: AEH-11-PSEUDO-01 **Item No.** 2

Approved by [redacted] Date 5-5-11

File Folder: 7B Lab book/pgs: Book 1 pg. 3 Proofed: TJS 02FEB12 Reviewed: ___

Donor Mussel UMESC Lot Number Assignment Form

DONOR MUSSEL INFORMATION:

Species: Fat Mucket (L. siliquoidea)

Number of Donor Mussels: 4 **Collection Date:** 4/29/11

Collection Location: St. Croix River @ Hudson Narrows

Species Identification performed by: Nathan Eckert

Title/affiliation of identifier: Mussel Biologist / Genoa NFH

UMESC Arrival Date: 5-9-11 1130 h

Receiving UMESC tank/room # 1 B Room 11

Additional information:

Received @ 53°F (11.7°C) Ramp started from 12°C to 20°C @ 1230h set to run for 60 h

UMESC LOT NUMBER DESIGNATION: 111200

Witness and form recorded by:

James A. Luoma	[redacted]	UMESC	5/9/11
Printed Name	**Signature**	**Affiliation**	**Date**

page 1 of 1

Study Title: "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"

Study number: AEH-11-PSEUDO-01 **Item No.** 3

Approved by [redacted] Date 5-5-11

File Folder: 7B Lab book/pgs: Book 1 p.3 Proofed: TPS 02FEB12 Reviewed: _____

Donor Mussel UMESC Lot Number Assignment Form

DONOR MUSSEL INFORMATION:

Species: Higgins Eye (L. higginsii)

Number of Donor Mussels: 4 **Collection Date:** 4/29/11

Collection Location: St. Croix River @ Hudson Narrows

Species Identification performed by: Nathan Eckert

Title/affiliation of identifier: Mussel Biologist / Genoa NFH

UMESC Arrival Date: 5-9-11 @ 1130 hr

Receiving UMESC tank/room # 1C Room 11

Additional information:

Received @ 53°F (11.7°C) Ramp started @ 1230 h to ramp from 12°C to 20°C over 60 h.

UMESC LOT NUMBER DESIGNATION: 111300

Witness and form recorded by:

James A. Luoma	[redacted]	UMESC	5-9-11
Printed Name	**Signature**	**Affiliation**	**Date**

page 1 of 1

Study Title: "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"

Study number: AEH-11-PSEUDO-01 **Item No.** 4

Approved by [redacted] Date 5-5-11

File Folder: 7B Lab book/pgs: Book-1 p. 22-23 Proofed: TS 02 FEB 12 Reviewed: ____

Donor Mussel UMESC Lot Number Assignment Form

DONOR MUSSEL INFORMATION:

Species: Lampsilis cardium (Plain Pocketbook)

Number of Donor Mussels: 3 **Collection Date:** 10/8/11

Collection Location: UMR Pool 9

Species Identification performed by: Genoa NFH Nathan Eckert

Title/affiliation of identifier: Mussel Biologist

UMESC Arrival Date: 10-18-11

Receiving UMESC tank/room # 15

Additional information:

UMESC LOT NUMBER DESIGNATION: 115200

Witness and form recorded by:

James R. Luoma	[redacted]	UMESC	10/18/11
Printed Name	**Signature**	**Affiliation**	**Date**

page 1 of 1

Study Title: "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"

Study number: AEH-11-PSEUDO-01 **Item No.** 5

Approved by [redacted] Date 5-5-11

File Folder: 7B Lab book/pgs: Book 1 p. 33-37 Proofed: TTS 02 FEB 12 Reviewed:______

Donor Mussel UMESC Lot Number Assignment Form

DONOR MUSSEL INFORMATION:

Species: Washboard (Megalonaias nervosa)

Number of Donor Mussels: 4 **Collection Date:** 10-17-11

Collection Location: UMR Pool 9

Species Identification performed by: Nathan Eckert

Title/affiliation of identifier: Mussel Biologist Genoa NFH

UMESC Arrival Date: 10-18-11

Receiving UMESC tank/room # RM 15

Additional information:

____

____

UMESC LOT NUMBER DESIGNATION: 115300

Witness and form recorded by:

James Luoma	[redacted]	UMESC	10/18/11
Printed Name	**Signature**	**Affiliation**	**Date**

page 1 of 1

Study Title: "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"

Study number: AEH-11-PSEUDO-01 **Item No.** 6

File Folder: 7b Lab book/pgs: 1/31 Reviewed: ms 02 FEB 12 Verified: ______

Donor Mussel UMESC Lot Number Assignment Form

DONOR MUSSEL INFORMATION:

Species: Black Sandshell

Number of Donor Mussels: 4 **Collection Date:** 25 OCT 2011

Collection Location: Chippewa River

Species Identification performed by: Nathan Eckert

Title/affiliation of identifier: Mussel Biologist / GNFH

UMESC Arrival Date: 17 JAN 2012

Receiving UMESC tank/room # UMESC Rm 15

Additional information:

Arrived at 0915 @ 18°C. Placed in 50% UMESC water followed by 100% UMESC water @ 20°C.

UMESC LOT NUMBER DESIGNATION: 120400

Witness and form recorded by:

Kerry L. Weber	[redacted]	UMESC	17 JAN 12
Printed Name	**Signature**	**Affiliation**	**Date**

Page 1 of 1

This datasheet was approved by [redacted] on 12 JAN 2012.

11JAN2012/Version 1.1

Study Title: **"Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"**

Study number: AEH-11-PSEUDO-01 **Item No.** 7

File Folder: 7b Lab book/pgs: 1/31 Reviewed: DS 02FEB12 Verified: ______

Donor Mussel UMESC Lot Number Assignment Form

DONOR MUSSEL INFORMATION:

Species: Mucket

Number of Donor Mussels: 4 **Collection Date:** 20 SEP 2011

Collection Location: St. Croix River

Species Identification performed by: Nathan Eckert

Title/affiliation of identifier: Mussel Biologist / GNFH

UMESC Arrival Date: 17 JAN 2012

Receiving UMESC tank/room # UMESC Rm 15

Additional information:

Arrived at 0915 @ 18°C. Placed in 50% UMESC water followed by 100% UMESC water @ 20°C.

UMESC LOT NUMBER DESIGNATION: 120500

Witness and form recorded by:

Kerry L. Weber	[redacted]	UMESC	17 JAN 12
Printed Name	**Signature**	**Affiliation**	**Date**

page 1 of 1

This datasheet was approved by [redacted] on 12 JAN 2012.

11JAN2012/Version 1.1

Study Title: "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"

Study number: AEH-11-PSEUDO-01 **Item No.** 8

File Folder: 7b Lab book/pgs: 1/31 Reviewed: TJS 02 FEB 12 Verified: ______

Donor Mussel UMESC Lot Number Assignment Form

DONOR MUSSEL INFORMATION:

Species: Hickorynut

Number of Donor Mussels: 4 **Collection Date:** 25 OCT 2011

Collection Location: Chippewa River

Species Identification performed by: Nathan Eckert

Title/affiliation of identifier: Mussel Biologist / GNFH

UMESC Arrival Date: 17 JAN 2012

Receiving UMESC tank/room # UMESC Rm 15

Additional information:

Arrived at 0915 @ 18°C. Placed in 50% UMESC water followed by 100% UMESC water @ 20°C.

UMESC LOT NUMBER DESIGNATION: 120600

Witness and form recorded by:

Kerry L. Weber	[redacted]	UMESC	17 JAN 12
Printed Name	**Signature**	**Affiliation**	**Date**

Page 1 of 1

This datasheet was approved by [redacted] on 17 JAN 2012.

11JAN2012/Version 1.1

AEH-11-PSEUDO-01

USGS Upper Midwest Environmental Sciences Center

CERTIFIED COPY
Initials: TJS Date: 09FEB12

Fish/Animal History Form

NA = not applicable IN = inside OUT = outside Rm = room T = tank

RW = raceway Est = estimate

Log Book / Pages 1/31
File Folder 7b
Initials KW Date 9 FEB 2012

UMESC Lot Number: 120600

Source Facility Lot #:

Origin/Source: Gen NFH

Species/Strain: Hickory Nut

Species Verified: ✓

Number Received: 4

Date received/lot # assigned: 1/17/12

Life stage at receipt/lot #: egg larval juvenile adult

Health certificate: Yes No

If egg, disinfected:

Date spawn: Actual/Estimate

Date hatch: Actual/Estimate

First UMESC rearing unit: Rm 15
(e.g. room, tank, raceway, pond and rearing unit number)

Comments: Collected from Chippewa River 10/25/11
by Genoa NFH

Date completed: 1/17/12

Completed by: [redacted]

FILE FOLDER → 7b

ITEM # → 9

PROOFED BY
Initials: TJS Date 09FEB12
REVIEWED BY
Initials: ____ Date: ____

AEH-11-PSEUDO-01

USGS Upper Midwest Environmental Sciences Center

Fish/Animal History Form

CERTIFIED COPY
Initials: TJS Date: 09FEB12

NA = not applicable IN = inside OUT = outside Rm = room T = tank

RW = raceway Est = estimate

Log Book / Pages 1/34
File Folder 7b
Initials KLW Date 09FEB 2012

UMESC Lot Number: 111200

Source Facility Lot #:

Origin/Source: St. Croix River (Collected by Genoa NFH)

Species/Strain: Fat mucket (L. siliquoidea) mussel

Species Verified: ✓

Number Received: 4

Date received/lot # assigned: 5/9/11

Life stage at receipt/lot #: egg larval juvenile adult

Health certificate: Yes No

If egg, disinfected:

Date spawn: Actual/Estimate

Date hatch: Actual/Estimate

First UMESC rearing unit: Room 11 tank 1B
(e.g. room, tank, raceway, pond and rearing unit number)

Comments: Collected 4/29/11 by Genoa NFH
transferred to UMESC 5/9/11

Date completed: 5/9/11

Completed by: [redacted]

FILE FOLDER → 7b

ITEM # → ~~7~~ 10 wrote incorrect item number. TJS 09FEB12

PROOFED BY
Initials: TJS Date: 09FEB12
REVIEWED BY
Initials: _____ Date: _____

USGS Upper Midwest Environmental Sciences Center

CERTIFIED COPY
Initials: TJS Date: 09FEB12

Fish/Animal History Form

NA = not applicable IN = inside OUT = outside Rm = room T = tank

RW = raceway Est = estimate

Log Book / Pages 1 | 3 4
File Folder 7B
Initials kw Date 09FEB12

UMESC Lot Number: 11/100

Source Facility Lot #:

Origin/Source: St. Croix River (from Genoa NFH)

Species/Strain: plain pocketbook (L. cardium)

Species Verified: ✓

Number Received: 4

Date received/lot # assigned: 5/9/11

Life stage at receipt/lot #: egg larval juvenile adult

Health certificate: Yes No

If egg, disinfected:

Date spawn: Actual/Estimate

Date hatch: Actual/Estimate

First UMESC rearing unit: Rm 11 tank 1A
(e.g. room, tank, raceway, pond and rearing unit number)

Comments: Collected on 4/29/11
from Genoa transferred to UMESC

Date completed: [redacted] 5/9/11

Completed by: [redacted]

FILE FOLDER → 7b
ITEM # → ~~8~~ 11 Wrote incorrect item number. TJS 09FEB12

PROOFED BY
Initials: TJS Date: 09FEB12
REVIEWED BY
Initials: ____ Date: ____

AEH-11-PSEUDO-01

USGS Upper Midwest Environmental Sciences Center

CERTIFIED COPY

Initials: TS Date: 09FEB12

Fish/Animal History Form

NA = not applicable IN = inside OUT = outside Rm = room T = tank

RW = raceway Est = estimate

Log Book / Pages 1/31

File Folder 7B

Initials YW Date 04FEB2012

UMESC Lot Number: 120500

Source Facility Lot #:

Origin/Source: Gen NCH

Species/Strain: Muckett Mussel

Species Verified: ✓

Number Received: 4

Date received/lot # assigned: 1/17/12

Life stage at receipt/lot #: egg larval juvenile adult

Health certificate: Yes No

If egg, disinfected:

Date spawn: Actual/Estimate

Date hatch: Actual/Estimate

First UMESC rearing unit: Rm 15

(e.g. room, tank, raceway, pond and rearing unit number)

Comments: Collected from ST. Croix River 9/20/11 by Genoa NFH

Date completed: [redacted]1/17/12

Completed by: [redacted]

FILE FOLDER → 7b

ITEM # → 12

PROOFED BY

Initials: TS Date: 09FEB12

REVIEWED BY

Initials: Date:

USGS Upper Midwest Environmental Sciences Center

CERTIFIED COPY
Initials: TDS Date: 09FEB12

Fish/Animal History Form

NA = not applicable IN = inside OUT = outside Rm = room T = tank

RW = raceway Est = estimate

Log Book / Pages 1/31
File Folder 7B
Initials KLW Date 09FEB2012

UMESC Lot Number: 120400

Source Facility Lot #:

Origin/Source: Gen NFH

Species/Strain: Black Sandshell mussel

Species Verified: ✓

Number Received: 4

Date received/lot # assigned: 1/17/12

Life stage at receipt/lot #: egg larval juvenile adult

Health certificate: Yes No

If egg, disinfected:

Date spawn: Actual/Estimate

Date hatch: Actual/Estimate

First UMESC rearing unit: Rm 15
(e.g. room, tank, raceway, pond and rearing unit number)

Comments:

Collected on 10/25/11

Date completed: 1/17/12

FILE FOLDER → 7b Completed by: [redacted]

ITEM # → 13

PROOFED BY
Initials: TDS Date: 09FEB12
REVIEWED BY
Initials: ____ Date: ____

USGS Upper Midwest Environmental Sciences Center

CERTIFIED COPY
Initials: TJS Date: 09FEB12

Fish/Animal History Form

NA = not applicable IN = inside OUT = outside Rm = room T = tank

RW = raceway Est = estimate

Log Book / Pages 1/23
File Folder 76
Initials KW Date 09FEB2012

UMESC Lot Number: 115300

Source Facility Lot #:

Origin/Source: Pool 9 Upper Missi. River

Species/Strain: Washboard (Megalonaias Nervosa)

Species Verified: ✓ (Gerald & Nathan Eckert)

Number Received: 4

Date received/lot # assigned: 10/18/11

Life stage at receipt/lot #: egg larval juvenile adult

Health certificate: Yes No

If egg, disinfected:

Date spawn: Actual/Estimate

Date hatch: Actual/Estimate

First UMESC rearing unit: Rm 15
(e.g. room, tank, raceway, pond and rearing unit number)

Comments: Collected on 10/17/11

Date completed: 10/20/11

FILE FOLDER → 76

Completed by: [redacted]

ITEM # → 14

PROOFED BY
Initials: TJS Date: 09FEB12
REVIEWED BY
Initials: ____ Date: ____

AEH-11-PSEUDO-01

USGS Upper Midwest Environmental Sciences Center

Fish/Animal History Form

NA = not applicable IN = inside OUT = outside Rm = room T = tank

RW = raceway Est = estimate

UMESC Lot Number: 115200

Source Facility Lot #:

Origin/Source: Pool 9 UMR

Species/Strain: Plain Pocketbook

Species Verified: ✓ (Genoa Biologist Nathan Eckert)

Number Received: 3

Date received/lot # assigned: 10/18/11

Life stage at receipt/lot #: egg larval juvenile adult

Health certificate: Yes No

If egg, disinfected:

Date spawn: Actual/Estimate

Date hatch: Actual/Estimate

First UMESC rearing unit: Room 15
(e.g. room, tank, raceway, pond and rearing unit number)

Comments: Collected on 10/8/11

Date completed: 10/20/11

FILE FOLDER → 76
Completed by: [redacted] [redacted]

ITEM № → 15

AEH-11-PSEUDO-01

USGS Upper Midwest Environmental Sciences Center

CERTIFIED COPY
Initials: TS Date: 09FEB12

Fish/Animal History Form

NA = not applicable IN = inside OUT = outside Rm = room T = tank
RW = raceway Est = estimate

Log Book / Pages 1/314
File Folder 7B
Initials KW Date 09FEB2012

UMESC Lot Number: 111300

Source Facility Lot #:

Origin/Source: St Croix River (collected by Genoa NFH)

Species/Strain: Higgins eye (L. higginsii)

Species Verified: ✓

Number Received: 4

Date received/lot # assigned: 5/9/11

Life stage at receipt/lot #: egg larval juvenile adult

Health certificate: Yes No

If egg, disinfected:

Date spawn: Actual/Estimate

Date hatch: Actual/Estimate

First UMESC rearing unit: Rm 11 tank 1C
(e.g. room, tank, raceway, pond and rearing unit number)

Comments: collected on 4/28/11 by Genoa NFH
transferred to UMESC on 5/9/11

Date completed: [redacted] 5/9/11

Completed by: [redacted]

FILE FOLDER → 7b
ITEM № → 16

PROOFED BY
Initials: TS Date: 09FEB12
REVIEWED BY
Initials: ____ Date: ____

Study Title: "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"

Study number: AEH-11-PSEUDO-01

Approved by [redacted] Date 5-5-11

File Folder: 7C Lab book/pgs: Book 1 1-11 p.8 - extraction p.11 - Lot number Assignment Proofed: TJS 02FEB12 Reviewed:________

Glochidia Test Organism UMESC Lot Number Assignment Form

Species: Lampsilis cardium

Number of Donor Mussels: 3 **Donor Mussel Lot #:** 11100

Extraction Date: 05 / 12 /2011 **Extraction Time (military)** 0900 to 0922

Extraction Location: UMESC rm 15 **Container ID:** Pool Block 1

Approximate Number of glochidia: 40,000

Glochidia extracted by: Nathan Eckert **Affiliation:** Genoa NFH

Additional information:

UMESC GLOCHIDIA LOT NUMBER DESIGNATION: 11400

Witness and form recorded by:

Kerry L. Weber	[redacted]	UMESC	05/12/2011
Printed Name	**Signature**	**Affiliation**	**Date**

ITEM # → 9

Page 1 of 1

Study Title: "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"

Study number: AEH-11-PSEUDO-01

Approved by [redacted] Date 5-5-11

File Folder: 7c Lab book/pgs: Book 1 1-11 Pg. 9 - extraction Pg. 11 - Lot Number Assignment Proofed: TJS 02FEB12 Reviewed:

Glochidia Test Organism UMESC Lot Number Assignment Form

Species: L. higginsii

Number of Donor Mussels: 3 **Donor Mussel Lot #:** 111300

Extraction Date: 05/12/2011 **Extraction Time (military)** 1033 to 1045

Extraction Location: UMESC rm 15 **Container ID:** Pool Block 2

Approximate Number of glochidia: ~~40,000~~ ① RE, KLW 12 MAY 2011 25,000

Glochidia extracted by: Nathan Eckert **Affiliation:** Genoa NFH

Additional information:

UMESC GLOCHIDIA LOT NUMBER DESIGNATION: 111500

Witness and form recorded by:

Kerry L. Weber	[redacted]	UMESC	05/12/2011
Printed Name	**Signature**	**Affiliation**	**Date**

① Wrong Number Recorded 12 MAY 2011 KLW

ITEM # → 10

Page 1 of 1

Study Title: "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"

Study number: AEH-11-PSEUDO-01

Approved by [redacted] Date 5-5-11

File Folder: 7C Lab book/pgs: Book 1 1-11 Proofed: DS 02FEB12 Reviewed:

P.10 - extraction
P.11 - Lot Number Assignment

Glochidia Test Organism UMESC Lot Number Assignment Form

Species: L. siliquoidea

Number of Donor Mussels: 3 **Donor Mussel Lot #:** 111200

Extraction Date: 05 / 12 /2011 **Extraction Time (military)** 1215 to 1229

Extraction Location: UMESC rm 15 **Container ID:** Pool Block 3

Approximate Number of glochidia: 40,000

Glochidia extracted by: Nathan Eckert **Affiliation:** Genoa NFH

Additional information:

UMESC GLOCHIDIA LOT NUMBER DESIGNATION: 111600

Witness and form recorded by:

Kerry L. Weber	[redacted]	UMESC	05/12/2011
Printed Name	**Signature**	**Affiliation**	**Date**

ITEM № → 11

Page 1 of 1

Study Title: "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"

Study number: AEH-11-PSEUDO-01

Approved by [redacted] Date 5-5-11

Book 1

File Folder: 7C Lab book/pgs: 23-25 Proofed: TJS 02FEB12 Reviewed: ____

Glochidia Test Organism UMESC Lot Number Assignment Form

Species: L. Cardium

Number of Donor Mussels: 3 **Donor Mussel Lot #:** 115200

Extraction Date: 10 / 18 /2011 **Extraction Time (military)** 0945

Extraction Location: UMESC rm 15 **Container ID:** 1, 2 + 3

Approximate Number of glochidia: ± 5,000 from each

Glochidia extracted by: Jim Luoma **Affiliation:** UMESC

Additional information:

UMESC GLOCHIDIA LOT NUMBER DESIGNATION: 115400

Witness and form recorded by:

Printed Name	Signature	Affiliation	Date
James A. Luoma	[redacted]	UMESC	18OCT11

ITEM # → 12

Page 1 of 1

Study Title: "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"

Study number: AEH-11-PSEUDO-01

Approved by [redacted] Date 5-5-11

File Folder: 7C Lab book/pgs: Book 1 (extraction and Lot Number Assignment) pgs 23-25 Proofed: TJS 02FEB12 Reviewed: ______

Glochidia Test Organism UMESC Lot Number Assignment Form

Species: Washboard (Megalonaias nervosa) wrong lot number entered jms 2/8/12

Number of Donor Mussels: 4 **Donor Mussel Lot #:** ~~115500~~ 115300

Extraction Date: 10 / 18 /2011 **Extraction Time (military)** 1015

Extraction Location: UMESC rm 15 **Container ID:** 1, 2, 3 + 4

Approximate Number of glochidia: 3-4,000 Each

Glochidia extracted by: Jorge Buening **Affiliation:** Genoa NFH

Additional information:

UMESC GLOCHIDIA LOT NUMBER DESIGNATION: 115500

Witness and form recorded by:

James A. Luoma	[redacted]	UMESC	10/18/11
Printed Name	**Signature**	**Affiliation**	**Date**

ITEM # → 13

Page 1 of 1 .

Study Title: "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"

Study number: AEH-11-PSEUDO-01

File Folder: 7c Lab book/pgs: 1/31 Reviewed: DS 02FEB12 Verified: ________

Glochidia Test Organism UMESC Lot Number Assignment Form

Species: Black Sandshell

Number of Donor Mussels: 4 **Donor Mussel Lot #:** 120400

Extraction Date: 01 /17 /2012 **Extraction Time (military)** 0945

Extraction Location: UMESC Rm 15 **Container ID:** BLS 1,2+3

Approximate Number of glochidia: 20,000

Glochidia extracted by: [redacted] **Affiliation:** Genoa NFH

Additional information:

UMESC GLOCHIDIA LOT NUMBER DESIGNATION: 120800

Witness and form recorded by:

Kerry L. Weber	[redacted]	UMESC	17JAN12
Printed Name	**Signature**	**Affiliation**	**Date**

ITEM # → 14

This datasheet was approved by [redacted] on 12 JAN 2012.

11JAN2012/Version 1.1

Page 1 of 1

Study Title: "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"

Study number: AEH-11-PSEUDO-01

File Folder: 7C Lab book/pgs: 1/31 Reviewed: TJS 02FEB12 Verified: ________

Glochidia Test Organism UMESC Lot Number Assignment Form

Species: Mucket

Number of Donor Mussels: 4 **Donor Mussel Lot #:** 120500

Extraction Date: 01 / 17 / 2012 **Extraction Time (military)** 1100

Extraction Location: UMESC Rm 15 **Container ID:** MUC 1,2,3

Approximate Number of glochidia: 20,000 [redacted]

Glochidia extracted by: [redacted] **Affiliation:** Genoa NFH

Additional information:

UMESC GLOCHIDIA LOT NUMBER DESIGNATION: 120700

Witness and form recorded by:

Kerry L Weber	[redacted]	UMESC	17JAN12
Printed Name	**Signature**	**Affiliation**	**Date**

ITEM # → 15

This datasheet was approved by [redacted] on 12 JAN 2012.

11JAN2012/Version 1.1

Page 1 of 1

Study Title: "Effects of *Pseudomonas fluorescens* (*Pf*-CL145A) to glochidia from seven unionid mussel species"

Study number: AEH-11-PSEUDO-01

File Folder: 7c Lab book/pgs: 1/34 Reviewed: TJS 02 FEB 12 Verified: ________

Glochidia Test Organism UMESC Lot Number Assignment Form

Species: Hickorynut

Number of Donor Mussels: 4 **Donor Mussel Lot #:** 120600

Extraction Date: 01 / 19 / 2012 **Extraction Time (military)** 0832

Extraction Location: UMESC Rm. 15 **Container ID:** HIC 1, 2 + 3

Approximate Number of glochidia: 20,000

Glochidia extracted by: Jim Luoma **Affiliation:** UMESC

Additional information:

UMESC GLOCHIDIA LOT NUMBER DESIGNATION: 120900

Witness and form recorded by:

Kerry L. Weber	[redacted]	UMESC	19JAN12
Printed Name	**Signature**	**Affiliation**	**Date**

ITEM # → 16

This datasheet was approved by [redacted] on 12 JAN 2012.

11JAN2012/Version 1.1

Page 1 of 1

USGS Upper Midwest Environmental Sciences Center

Fish/Animal History Form

NA = not applicable IN = inside OUT = outside Rm = room T = tank RW = raceway Est = estimate

Log Book / Pages 1/3-4
File Folder 7c
Initials KW Date 09FEB2012

CERTIFIED COPY
Initials: TJS Date: 09FEB12

UMESC Lot Number: 120900

Source Facility Lot #:

Origin/Source: Umesc

Species/Strain: Hickory nut mussel

Species Verified: ✓

Number Received: ~ 20,000 glochidia

Date received/lot # assigned: 1/19/2012

Life stage at receipt/lot #: egg larval juvenile adult

Health certificate: Yes No

If egg, disinfected:

Date spawn: Actual/Estimate 1/19/2012

Date hatch: Actual/Estimate

First UMESC rearing unit: RM-15 Cont HTC 1,2,3
(e.g. room, tank, raceway, pond and rearing unit number)

Comments: extracted 1/19/2012 at Umesc

Date completed: 1/19/2012

Completed by: [redacted]

FILE FOLDER → 7c

ITEM № → 1

PROOFED BY
Initials: TJS Date: 09FEB12
REVIEWED BY
Initials: Date:

USGS Upper Midwest Environmental Sciences Center

Fish/Animal History Form

NA = not applicable IN = inside OUT = outside Rm = room T = tank

RW = raceway Est = estimate

Log Book / Pages 1/31
File Folder 7C
Initials [initials] 04FEB2012

CERTIFIED COPY
Initials: TJS Date: 09FEB12

UMESC Lot Number: 120800

Source Facility Lot #:

Origin/Source: Umesc

Species/Strain: Black sandshell glochidia mussel

Species Verified: ✓

Number Received: ~ 4,000 - 20,000

Date received/lot # assigned: 1/17/12

Life stage at receipt/lot #: egg larval juvenile adult

Health certificate: Yes No

If egg, disinfected:

Date spawn: ~~Actual/Estimate~~ ~~Adults spawned~~ wrong entry 1/17/12 SOR

Date hatch: Actual/Estimate

First UMESC rearing unit: Rm 15 Cont to BLS 1, 2, 3
(e.g. room, tank, raceway, pond and rearing unit number)

Comments:

glochidia extracted 1/17/12
at Umesc

Date completed: 1/17/12

Completed by: [redacted]

FILE FOLDER → 7c

ITEM № → 2

PROOFED BY
Initials: TJS Date: 09FEB12
REVIEWED BY
Initials: ____ Date: ____

USGS Upper Midwest Environmental Sciences Center

CERTIFIED COPY

Initials: TJS Date: 09FEB12

Fish/Animal History Form

NA = not applicable IN = inside OUT = outside Rm = room T = tank

RW = raceway Est = estimate

Log Book / Pages 1/32

File Folder 7C

Initials KW Date 09FEB2012

UMESC Lot Number: 120700

Source Facility Lot #:

Origin/Source: Umesc

Species/Strain: muckett glochidia mussels

Species Verified: ✓

Number Received: ~20,000

Date received/lot # assigned: 1/17/12

Life stage at receipt/lot #: egg larval juvenile adult

Health certificate: Yes No

If egg, disinfected:

Date spawn: ~~Actual/Estimate Spawn Adults 1/17/12~~ wrong entry 1/17/12 SOR

Date hatch: Actual/Estimate

First UMESC rearing unit: Rm 15 . tank muc 1, 2, 3

(e.g. room, tank, raceway, pond and rearing unit number)

Comments: Glochidia Extracted from Adults at Umesc

~~Adults spawned at Umesc~~ wrong Entry 1/17/12 SOR

Date completed: 1/17/12

Completed by: [redacted]

FILE FOLDER → 7C

ITEM # → 3

PROOFED BY
Initials: TJS Date: 09FEB12
REVIEWED BY
Initials: ____ Date: ____

AEH-11-PSEUDO-01

USGS Upper Midwest Environmental Sciences Center

Fish/Animal History Form

CERTIFIED COPY
Initials: 7JS Date: 09FEB12

NA = not applicable IN = inside OUT = outside Rm = room T = tank

RW = raceway Est = estimate

Log Book / Pages 1/85
File Folder 7c
Initials [illegible] Date 09 FEB 2012

UMESC Lot Number: 115506

Source Facility Lot #:

Origin/Source: Umesc

Species/Strain: Washboard mussel (M. nervosa)

Species Verified: ✓ (Jim Luoma)

Number Received: ±16,000 3,000 – 4,000 / gravid female

Date received/lot # assigned: 10/18/11

Life stage at receipt/lot #: egg larval juvenile adult

Health certificate: Yes No

If egg, disinfected:

Date spawn: Actual/Estimate

Date hatch: Actual/Estimate 10/18/11

First UMESC rearing unit: Rm 15 container 1, 2, 3, 4
(e.g. room, tank, raceway, pond and rearing unit number)

Comments: glochidia Extracted by (George B). (George NED)

Date completed: 10/20/11

Completed by: [redacted]

FILE FOLDER → 7c

ITEM № → 4

PROOFED BY
Initials: 7JS Date: 09FEB12
REVIEWED BY
Initials: ____ Date: ____

AEH-11-PSEUDO-01

USGS Upper Midwest Environmental Sciences Center

CERTIFIED COPY
Initials: TS Date: 09FEB12

Fish/Animal History Form

NA = not applicable IN = inside OUT = outside Rm = room T = tank

RW = raceway Est = estimate

Log Book/Pages 1/25
File Folder 7C
Initials [illegible] Date 09FEB2012

UMESC Lot Number: 115400

Source Facility Lot #:

Origin/Source:

Species/Strain: plain pocketbook (L. cardium)

Species Verified: ✓ (Jim Luoma)

Number Received: ± 15,000

Date received/lot # assigned: 10/18/11

Life stage at receipt/lot #: egg larval juvenile adult

Health certificate: Yes No

If egg, disinfected:

Date spawn: Actual/Estimate 10/18/11

Date hatch: Actual/Estimate

First UMESC rearing unit: Rm 15 tank 1,2,3
(e.g. room, tank, raceway, pond and rearing unit number)

Comments: Extracted from gravid female at UMESC
5,000/gravid ♀

Date completed: 10/20/11

Completed by: [redacted]

FILE FOLDER → 7c

ITEM № → 5

PROOFED BY
Initials: TS Date: 09FEB12
REVIEWED BY
Initials: ____ Date: ____

USGS Upper Midwest Environmental Sciences Center

CERTIFIED COPY
Initials: TKS Date: 09FEB12

Fish/Animal History Form

NA = not applicable IN = inside OUT = outside Rm = room T = tank

RW = raceway Est = estimate

Log Book / Pages 1/11
File Folder 7C
Initials [illegible] Date 09FEB2012

UMESC Lot Number: 111600

Source Facility Lot #:

Origin/Source: Umesc

Species/Strain: Fat muckett (L. siliquoidea) mussel glochidia

Species Verified: ✓

Number Received: ≈ 40,000

Date received/lot # assigned: 5/12/11

Life stage at receipt/lot #: egg larval juvenile adult

Health certificate: Yes No

If egg, disinfected:

Date spawn: Actual/Estimate 5/12/11

Date hatch: Actual/Estimate

First UMESC rearing unit: Rm 15 Cont. Jo # Block # 3
(e.g. room, tank, raceway, pond and rearing unit number)

Comments: Donor mussel lot # 111200
glochidia extracted by Glenn NFH

Date completed: 5/12/11

FILE FOLDER → 7C Completed by: [redacted]

ITEM # → 6

PROOFED BY
Initials: TKS Date: 09FEB12
REVIEWED BY
Initials: ____ Date: ____

AEH-11-PSEUDO-01

USGS Upper Midwest Environmental Sciences Center

CERTIFIED COPY
Initials: TJS Date: 09FEB12

Fish/Animal History Form

NA = not applicable IN = inside OUT = outside Rm = room T = tank

RW = raceway Est = estimate

Log Book / Pages 1/11
File Folder 7C
Initials lw Date 09FEB2012

UMESC Lot Number: 111500

Source Facility Lot #:

Origin/Source: Umesc

Species/Strain: higgins eye mussel glochidia

Species Verified: —

Number Received: ~ 25,000

Date received/lot # assigned: 5/12/11

Life stage at receipt/lot #: egg larval juvenile adult

Health certificate: Yes No

If egg, disinfected:

Date spawn: Actual/Estimate 5/12/11

Date hatch: Actual/Estimate

First UMESC rearing unit: Rm 15 Cont I.D. Block # 2
(e.g. room, tank, raceway, pond and rearing unit number)

Comments: Donor mussel Lot # 111300
glochidia extracted in Rm 15 Block # 2
by genus NC4

Date completed: 5/12/11

Completed by: [redacted]

FILE FOLDER → 7c
ITEM # → 7

PROOFED BY
Initials: TJS Date: 09FEB12
REVIEWED BY
Initials: ____ Date: ____

USGS Upper Midwest Environmental Sciences Center CERTIFIED COPY

Fish/Animal History Form

Initials: TDS Date: ~~8FEB12~~ wrong date. 09FEB12 TDS

NA = not applicable IN = inside OUT = outside Rm = room T = tank

RW = raceway Est = estimate

Log Book / Pages 1/11
File Folder 7C
Initials [illegible] Date 09FEB2012

UMESC Lot Number: 111400

Source Facility Lot #:

Origin/Source: Umesc

Species/Strain: Plain Pocket book (L. cardium)

Species Verified: ✓

Number Received: ≈ 40,000

Date received/lot # assigned: 5/12/11

Life stage at receipt/lot #: egg larval juvenile adult

Health certificate: Yes No

If egg, disinfected:

Date spawn: Actual/Estimate 5/12/11

Date hatch: Actual/Estimate

First UMESC rearing unit:
(e.g. room, tank, raceway, pond and rearing unit number)

Comments: Donor mussel lot # 111100
glochidia extracted in Rm 15 lot pbl Block #1
by Bence N[illegible]

Date completed: 5/12/11

Completed by: [redacted]

FILE FOLDER → 7C
ITEM # → 8

PROOFED BY
Initials: TDS Date: 09FEB12
REVIEWED BY
Initials: ___ Date: ___

Appendix 6. Water Quality

Item number	Item description	Number of pages	Report page number
1	Initial (Pre Exposure) Water Chemistry – Data Summary	2	326
2	Exposure Water Chemistry – Data Summary	9	328
3	Exposure Light Intensity – Data Summary	2	337
4	Termination Water Chemistry – Data Summary	2	339
5	Report of Analysis – Ammonia Report from Water Quality Laboratory at UMESC – Report Dated November 9, 2011	1	341
6	Report of Analysis – Ammonia Report from Water Quality Laboratory at UMESC – Report Dated February 3, 2012	1	342
7	Total Ammonia Nitrogen – Data Summary	10	343

		Action	Date	Initials
Study Number: AEH-11-PSEUDO-01		Created......	3-Mar-15	TJS TJS
Lab Notebook: 1		Revised......	4-Mar-15	TJS TJS
Data Source: File Folder: 10a		Reviewed...	4-MAR-15	TJS
Forms:	Water Quality - Form 1 Initial (Dilution Water Hardness, Alkalinity, and Temperature) [Conductivity]	Certified...	JT 3-13-15	JT
File Name:	I:\AEH-11-PSEUDO-01\Data\Water Chemistry\[Initial (Pre Exposure) Water Chemistry Summary.xlsx]Initial Coversheet		wrong column 3-13-15 JT	

Initial (Pre Exposure) Water Chemistry

Test Article: *Pseudomonas fluorescens* (*Pf*-CL145A) spray dried powder (SDP) and freeze dried powder (FDP)
Article Lot #s: SDP = MBI-401-110308AI-BD-3 and MBI-401 SDP 4655-12-Mix; FDP = 110607WB-FD-E
Exposure Dates: 12-May-11, 18-Oct-11, 17-Jan-12, and 19-Jan-12
Species Tested: PPB, FAM, HGE, WAS, BLS, MUC, and HIC

Data Explanation:

Species Abbreviations
- PPB - plain pocketbook (*Lampsilis cardium*)
- FAM - fatmucket (*Lampsilis siliquoidea*)
- HGE - Higgins eye (*Lampsilis higginsii*)
- WAS - washboard (Megalonaias nervosa)
- BLS - black sandshell (*Ligumia recta*)
- MUC - mucket (*Actinonaias ligamentina*)
- HIC - hickorynut (*Obovaria olivaria*)

Data anomalies and deviations:

1) Initial conductivity reading was not measured on 12-MAY-11.
2) Only one replicate sample of conductivity was measured on 18-Oct-11.
3) Initial water chemistry parameters were measured in triplicate for each exposure day (12-May-11, 18-Oct-11, 17-Jan-12, and 19-Jan-12) but not separately for each species, therefore, for species that were tested on the same day the data is transcribed for clarity.

Item Number 7
Page 1 of 2

Study Number: AEH-11-PSEUDO-01
Lab Notebook 1
Data Source: File Folder: 10a
Forms: Water Quality - Form 1
Initial (Dilution Water Hardness, Alkalinity, and Temperature) (Conductivity)

Test Article: MBI 401 (Pf-CL 145A [SDP] and [FDP])
Article Lot #: MBI-401-110308AI-BD-3, MBI-401 SDP 4655-12-Mix, 110607WB-FD-E
Exposure Dates: 12-May-11, 18-Oct-11, 17-Jan-12, and 19-Jan-12
Species Tested: PPB, FAM, HGE, WAS, BLS, MUC, and HIC

Initial (Pre Exposure) Water Chemistry

Species	Formulation	Test Date	Replicate	Hardness (mg/L $CaCO_3$)	Alkalinity (mg/L $CaCO_3$)	Conductivity (µS/cm)	Temperature (°C)
PPB	SDP	12-May-11	1	170	130	ND	19.4
			2	172	130	ND	19.3
			3	170	130	ND	19.3
			Mean	171	130	ND	19.3
			SD	1	0	ND	0.1
			Min	170	130	ND	19.3
			Max	172	130	ND	19.4
FAM	SDP	12-May-11	1	170	130	ND	19.4
			2	172	130	ND	19.3
			3	170	130	ND	19.3
			Mean	171	130	ND	19.3
			SD	1	0	ND	0.1
			Min	170	130	ND	19.3
			Max	172	130	ND	19.4
HGE	SDP	12-May-11	1	170	130	ND	19.4
			2	172	130	ND	19.3
			3	170	130	ND	19.3
			Mean	171	130	ND	19.3
			SD	1	0	ND	0.1
			Min	170	130	ND	19.3
			Max	172	130	ND	19.4
PPB	FDP	18-Oct-11	1	174	126	391	18.4
			2	174	126	ND	18.4
			3	174	127	ND	18.4
			Mean	174	126	391	18.4
			SD	0	1	ND	0.0
			Min	174	126	391	18.4
			Max	174	127	391	18.4
WAS	FDP	18-Oct-11	1	174	126	391	18.4
			2	174	126	ND	18.4
			3	174	127	ND	18.4
			Mean	174	126	391	18.4
			SD	0	1	ND	0.0
			Min	174	126	391	18.4
			Max	174	127	391	18.4
BLS	SDP	17-Jan-12	1	172	125	412	19.9
			2	171	124	425	19.9
			3	170	124	429	19.9
			Mean	171	124	422	19.9
			SD	1	1	9	0.0
			Min	170	124	412	19.9
			Max	172	125	429	19.9
MUC	SDP	17-Jan-12	1	172	125	412	19.9
			2	171	124	425	19.9
			3	170	124	429	19.9
			Mean	171	124	422	19.9
			SD	1	1	9	0.0
			Min	170	124	412	19.9
			Max	172	125	429	19.9
HIC	SDP	19-Jan-12	1	174	121	382	20.1
			2	174	122	381	19.9
			3	176	122	385	19.8
			Mean	175	122	383	19.9
			SD	1	1	2	0.2
			Min	174	121	381	19.8
			Max	176	122	385	20.1

ND = No data. Pre-exposure conductivity was not collected on 12-May-11 and only one sample was measured for conductivity on 18-Oct-11.

Item Number 7
Page 2 of 2

Study Number: AEH-11-PSEUDO-01		Action	Date	Initials
Lab Notebook: 1		Created......	3-Mar-15	TJS TJS
Data Source: File Folder: 10b		Revised......	9-Mar-15	TJS TJS
Forms:	Water Quality - Form 2	Reviewed...	9-MAR-15	TJS
	During Exposure (Dissolved Oxygen, pH, Temperature) [Light Intensity]	Certified...	3/12/15	JN
File Name:	I:\AEH-11-PSEUDO-01\Data\Water Chemistry\[Exposure Water Chemistry Summary.xlsx]Exposure Coversheet			

Exposure Water Chemistry

Test Article: *Pseudomonas fluorescens* (*Pf*-CL145A) spray dried powder (SDP) and freeze dried powder (FDP)
Article Lot #s: SDP = MBI-401-110308AI-BD-3 and MBI-401 SDP 4655-12-Mix; FDP = 110607WB-FD-E
Exposure Dates: 12-May-11, 18-Oct-11, 17-Jan-12, and 19-Jan-12
Species Tested: PPB, FAM, HGE, WAS, BLS, MUC, and HIC

Data Explanation:

300 HD = 300 mg/L Heat Deactivated

Species Abbreviations
- PPB - plain pocketbook (*Lampsilis cardium*)
- FAM - fatmucket (*Lampsilis siliquoidea*)
- HGE - Higgins eye (*Lampsilis higginsii*)
- WAS - washboard (Megalonaias nervosa)
- BLS - black sandshell (*Ligumia recta*)
- MUC - mucket (*Actinonaias ligamentina*)
- HIC - hickorynut (*Obovaria olivaria*)

Water chemistry was measured twice during exposure:
1) Upon exposure initiation
2) Prior to exposure termination

For PPB, FAM, and HGE (SDP), water chemistry parameters were measured in all replicates (3) for controls (0 mg/L) and 300 mg/L treatments, however, only one replicate was measured for 50, 100, 200 mg/L, and 300 mg/L heat deactivated (HD). All other species had all replicates measured for all treatment concentrations.

pH means calculated on hydrogen ion concentration; pH standard deviations calculated on pH values.

Data anomalies and deviations:

None

Item Number 17
Page 1 of 9

Study Number: AEH-11-PSEJDO-01
Lab Notebook 1
Data Source: File Folder: 10b
Forms: Water Quality - Form 2
During Exposure (Dissolved Oxygen, pH, Temperature)

Test Article: MBI 401 (PF-CL 145A [SDP])
Article Lot #: MBI-401-110308AI-BD-3
Exposure Date: 12-May-11
Species Tested: PPB
Glochidia Lot # 111400

Exposure Water Chemistry - Plain Pocketbook SDP

Test Chamber	Treatment (mg/L)	Sample Time	Dissolved Oxygen (mg/L)	pH	Temperature (°C)
1A3	0	1	8.57	8.42	19.3
		2	8.39	8.23	19.1
1B1		1	8.43	8.30	20.0
		2	8.55	8.35	19.5
1C3		1	8.66	8.39	20.0
		2	8.39	8.41	19.0
		Mean	8.50	8.35	19.5
		SD	0.11	0.07	0.4
		Min	8.39	8.23	19.0
		Max	8.66	8.42	20.0
1B5	50	1	8.23	8.39	20.2
1A1		2	8.07	8.25	19.2
		Mean	8.15	8.33	19.7
		SD	0.11	0.10	0.7
		Min	8.07	8.25	19.2
		Max	8.23	8.39	20.2
1B4	100	1	8.68	8.39	19.6
1A4		2	7.40	8.03	19.0
		Mean	8.04	8.25	19.3
		SD	0.91	0.25	0.4
		Min	7.40	8.03	19.0
		Max	8.68	8.39	19.6
1C6	200	1	8.27	8.36	19.5
1C4		2	7.04	8.09	19.0
		Mean	7.66	8.25	19.3
		SD	0.87	0.19	0.4
		Min	7.04	8.09	19.0
		Max	8.27	8.36	19.5
1A5	300	1	8.80	8.27	20.0
		2	6.20	7.85	19.0
1C2		1	8.54	8.29	20.0
		2	5.83	7.87	19.5
1C5		1	8.68	8.27	20.0
		2	5.22	7.74	19.1
		Mean	7.21	8.11	19.6
		SD	1.63	0.25	0.5
		Min	5.22	7.74	19.0
		Max	8.80	8.29	20.0
1B3	300 HD	1	8.29	8.37	20.0
1A6		2	6.63	8.07	19.1
		Mean	7.46	8.25	19.6
		SD	1.17	0.21	0.6
		Min	6.63	8.07	19.1
		Max	8.29	8.37	20.0

Sample Time 1 was measured upon exposure initiation and Sample Time 2 was measured just prior to exposure termination.

Item Number 17
Page 2 of 9

Study Number: AEH-11-PSEUDO-01
Lab Notebook 1
Data Source: File Folder: 10b
Forms: Water Quality - Form 2
During Exposure (Dissolved Oxygen, pH, Temperature)

Test Article: MBI 401 (PF-C_145A [SDP])
Article Lot #: MBI-401-110308AI-BD-3
Exposure Date: 12-May-11
Species Tested: FAM
Glochidia Lot # 111600

Exposure Water Chemistry - Fatmucket SDP

Test Chamber	Treatment (mg/L)	Sample Time	Dissolved Oxygen (mg/L)	pH	Temperature (°C)
3A2	0	1	8.65	8.39	19.7
		2	8.86	8.47	19.0
3B2		1	8.56	8.48	19.4
		2	8.05	8.43	19.0
3B3		1	8.67	8.44	20.0
		2	8.75	8.42	19.1
		Mean	8.59	8.44	19.4
		SD	0.28	0.03	0.4
		Min	8.05	8.39	19.0
		Max	8.86	8.48	20.0
3C5	50	1	8.11	8.36	19.5
3B5		2	7.31	8.05	19.0
		Mean	7.71	8.21	19.3
		SD	0.57	0.22	0.4
		Min	7.31	8.05	19.0
		Max	8.11	8.36	19.5
3A5	100	1	8.15	8.35	20.0
3A3		2	7.01	8.09	18.9
		Mean	7.58	8.24	19.5
		SD	0.81	0.18	0.8
		Min	7.01	8.09	18.9
		Max	8.15	8.35	20.0
3C4	200	1	8.60	8.26	19.9
3B4		2	6.02	7.73	19.1
		Mean	7.31	8.07	19.5
		SD	1.82	0.37	0.6
		Min	6.02	7.73	19.1
		Max	8.60	8.26	19.9
3B1	300	1	8.52	8.24	20.3
		2	4.97	7.57	19.0
3C1		1	8.50	8.37	20.3
		2	7.31	7.92	19.0
3C3		1	8.50	8.27	20.2
		2	3.71	7.52	19.1
		Mean	6.92	8.10	19.7
		SD	2.09	0.37	0.7
		Min	3.71	7.52	19.0
		Max	8.52	8.37	20.3
3C2	300 HD	1	8.36	8.31	19.2
3A4		2	5.19	7.68	19.0
		Mean	6.78	8.10	19.1
		SD	2.24	0.45	0.1
		Min	5.19	7.68	19.0
		Max	8.36	8.31	19.2

Sample Time 1 was measured upon exposure initiation and Sample Time 2 was measured just prior to exposure termination.

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Study Number: AEH-11-PSEUDO-01
Lab Notebook 1
Data Source: File Folder: 10b
Forms: Water Quality - Form 2
During Exposure (Dissolved Oxygen, pH, Temperature)

Test Article: MBI 401 (Pf-CL 145A [SDP])
Article Lot # MBI-401-110308AI-BD-3
Exposure Date: 12-May-11
Species Tested: HGE
Glochidia Lot # 111500

Exposure Water Chemistry - Higgins Eye SDP

Test Chamber	Treatment (mg/L)	Sample Time	Dissolved Oxygen (mg/L)	pH	Temperature (°C)
2B1	0	1	7.82	8.41	19.9
		2	8.28	8.39	19.0
2C3		1	7.85	8.38	19.0
		2	7.97	8.44	18.5
2C6		1	8.23	8.46	20.1
		2	8.15	8.44	19.0
		Mean	8.05	8.42	19.3
		SD	0.20	0.03	0.6
		Min	7.82	8.38	18.5
		Max	8.28	8.46	20.1
2A4	50	1	7.77	8.38	19.4
2A2		2	7.40	8.08	19.1
		Mean	7.59	8.26	19.3
		SD	0.26	0.21	0.2
		Min	7.40	8.08	19.1
		Max	7.77	8.38	19.4
2C5	100	1	8.55	8.29	19.7
2C4		2	7.48	8.00	18.2
		Mean	8.02	8.17	19.0
		SD	0.76	0.21	1.1
		Min	7.48	8.00	18.2
		Max	8.55	8.29	19.7
2B2	200	1	7.97	8.30	19.3
2B2		2	7.01	8.03	18.0
		Mean	7.49	8.19	18.7
		SD	0.68	0.19	0.9
		Min	7.01	8.03	18.0
		Max	7.97	8.30	19.3
2B3	300	1	7.70	8.26	20.2
		2	4.08	7.58	18.5
2C1		1	7.77	8.41	19.8
		2	7.78	7.99	18.5
2C2		1	7.81	8.39	20.0
		2	8.11	8.05	18.5
		Mean	7.21	8.19	19.3
		SD	1.54	0.31	0.8
		Min	4.08	7.58	18.5
		Max	8.11	8.41	20.2
2B4	300 HD	1	7.97	8.34	20.0
2B4		2	6.07	7.85	19.0
		Mean	7.02	8.16	19.5
		SD	1.34	0.35	0.7
		Min	6.07	7.85	19.0
		Max	7.97	8.34	20.0

Sample Time 1 was measured upon exposure initiation and Sample Time 2 was measured just prior to exposure termination.

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Study Number: AEH-11-PSEUDO-01
Lab Notebook 1
Data Source: File Folder: 10b
Forms: Water Quality - Form 2
During Exposure (Dissolved Oxygen, pH, Temperature)

Test Article: MBI 401 (PF-CL 145A [FDP])
Article Lot #: 110607WB-FD-E
Exposure Date: 18-Oct-11
Species Tested: WAS
Glochidia Lot # 115500

Exposure Water Chemistry - Washboard FDP

Test Chamber	Treatment (mg/L)	Sample Time	Dissolved Oxygen (mg/L)	pH	Temperature (°C)
1A5	0	1	7.58	8.41	18.6
		2	7.36	8.41	18.3
1B2		1	7.66	8.39	18.7
		2	7.37	8.39	18.6
1B4		1	7.45	8.40	18.7
		2	7.13	8.40	18.7
		Mean	7.43	8.40	18.6
		SD	0.19	0.01	0.2
		Min	7.13	8.39	18.3
		Max	7.66	8.41	18.7
1A1	50	1	7.75	8.40	18.1
		2	6.80	8.39	17.7
1C1		1	8.11	8.40	17.4
		2	7.11	8.40	17.3
1C2		1	8.55	8.39	18.3
		2	7.54	8.40	18.1
		Mean	7.64	8.40	17.8
		SD	0.64	0.01	0.4
		Min	6.80	8.39	17.3
		Max	8.55	8.40	18.3
1A2	100	1	9.25	8.41	18.8
		2	8.00	8.39	18.6
1A6		1	9.11	8.41	18.6
		2	7.68	8.39	18.1
1C6		1	8.90	8.41	18.2
		2	7.40	8.39	17.7
		Mean	8.39	8.40	18.3
		SD	0.79	0.01	0.4
		Min	7.40	8.39	17.7
		Max	9.25	8.41	18.8
1B5	200	1	9.09	8.40	18.7
		2	7.44	8.49	18.5
1C3		1	8.30	8.37	18.0
		2	7.60	8.37	18.2
1C4		1	8.72	8.35	18.8
		2	7.59	8.35	18.7
		Mean	8.12	8.39	18.5
		SD	0.69	0.05	0.3
		Min	7.44	8.35	18.0
		Max	9.09	8.49	18.8
1A4	300	1	8.64	8.38	18.5
		2	7.37	8.37	18.2
1B1		1	8.09	8.39	17.9
		2	7.36	8.36	17.6
1C5		1	8.23	8.37	18.3
		2	7.55	8.35	18.3
		Mean	7.87	8.37	18.1
		SD	0.53	0.01	0.3
		Min	7.36	8.35	17.6
		Max	8.64	8.39	18.5
1A3	300 HD	1	8.44	8.40	18.6
		2	7.42	8.37	18.3
1B3		1	8.09	8.39	18.3
		2	7.27	8.37	18.0
1B6		1	8.74	8.37	18.6
		2	7.07	8.37	18.3
		Mean	7.84	8.38	18.4
		SD	0.68	0.01	0.2
		Min	7.07	8.37	18.0
		Max	8.74	8.40	18.6

Sample Time 1 was measured upon exposure initiation and Sample Time 2 was measured just prior to exposure termination.

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Study Number: AEH-11-PSEUDO-01
Lab Notebook 1
Data Source: File Folder: 10b
Forms: Water Quality - Form 2
During Exposure (Dissolved Oxygen, pH, Temperature)

Test Article: MBI 401 (Pf CL 145A (FDP))
Article Lot #: 110607WB-FD-E
Exposure Date: 18-Oct-11
Species Tested: PPB
Glochidia Lot # 115400

Exposure Water Chemistry - Plain Pocketbook FDP

Test Chamber	Treatment (mg/L)	Sample Time	Dissolved Oxygen (mg/L)	pH	Temperature (°C)
3A6	0	1	8.43	8.32	18.3
		2	8.00	8.29	17.7
3B5		1	9.10	8.12	17.7
		2	7.74	8.22	17.2
3B6		1	8.72	8.19	18.1
		2	8.04	8.25	17.6
		Mean	8.34	8.24	17.8
		SD	0.51	0.07	0.4
		Min	7.74	8.12	17.2
		Max	9.10	8.32	18.3
3A4	50	1	8.70	8.26	18.1
		2	7.81	8.27	17.4
3C1		1	8.64	8.28	17.3
		2	7.54	8.31	16.8
3C2		1	8.50	8.26	17.2
		2	7.59	8.31	16.6
		Mean	8.13	8.28	17.2
		SD	0.54	0.02	0.5
		Min	7.54	8.26	16.6
		Max	8.70	8.31	18.1
3B1	100	1	8.90	8.34	17.9
		2	7.46	8.31	17.4
3B3		1	8.88	8.33	17.8
		2	7.25	8.31	17.3
3C6		1	8.65	8.34	17.7
		2	7.31	8.31	17.1
		Mean	8.08	8.32	17.5
		SD	0.81	0.02	0.3
		Min	7.25	8.31	17.1
		Max	8.90	8.34	17.9
3A2	200	1	8.50	8.31	17.7
		2	7.55	8.31	17.1
3C3		1	8.41	8.34	17.3
		2	7.21	8.31	16.8
3C5		1	8.55	8.31	18.1
		2	7.29	8.31	17.4
		Mean	7.92	8.32	17.4
		SD	0.63	0.01	0.5
		Min	7.21	8.31	16.8
		Max	8.55	8.34	18.1
3A3	300	1	8.47	8.29	18.2
		2	7.71	8.29	17.6
3B4		1	8.45	8.31	18.0
		2	7.54	8.30	17.3
3C4		1	7.88	8.33	17.5
		2	6.81	8.31	16.8
		Mean	7.81	8.31	17.6
		SD	0.62	0.02	0.5
		Min	6.81	8.29	16.8
		Max	8.47	8.33	18.2
3A1	300 HD	1	8.04	8.34	18.2
		2	6.04	8.31	17.7
3A5		1	7.85	8.36	18.0
		2	6.90	8.32	17.5
3B2		1	7.97	8.34	17.7
		2	6.32	8.34	17.2
		Mean	7.19	8.34	17.7
		SD	0.89	0.02	0.4
		Min	6.04	8.31	17.2
		Max	8.04	8.36	18.2

Sample Time 1 was measured upon exposure initiation and Sample Time 2 was measured just prior to exposure termination.

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Study Number: AEH-11-PSEUDO-01
Lab Notebook 1
Data Source: File Folder: 10b
Forms: Water Quality - Form 2
During Exposure (Dissolved Oxygen, pH, Temperature)

Test Article: MBI 401 (Pf-CL 145A (SDP))
Article Lot #: MBI-401 SDP 4655-12-Mix
Exposure Date: 17-Jan-12
Species Tested: BLS
Glochidia Lot # 120800

Exposure Water Chemistry - Black Sandshell SDP

Test Chamber	Treatment (mg/L)	Sample Time	Dissolved Oxygen (mg/L)	pH	Temperature (°C)
1A5	0	1	8.69	8.27	20.2
		2	8.90	8.55	20.0
1B1		1	9.39	8.40	21.2
		2	9.27	8.56	20.1
1C6		1	9.50	8.42	20.6
		2	9.24	8.55	20.4
		Mean	9.17	8.47	20.4
		SD	0.31	0.12	0.4
		Min	8.69	8.27	20.0
		Max	9.50	8.56	21.2
1A1	50	1	9.47	8.41	21.0
		2	9.67	8.42	20.4
1A3		1	9.68	8.41	20.6
		2	9.68	8.44	20.0
1C1		1	8.45	8.39	21.9
		2	9.18	8.34	20.4
		Mean	9.36	8.40	20.7
		SD	0.48	0.03	0.7
		Min	8.45	8.34	20.0
		Max	9.68	8.44	21.9
1B2	100	1	10.68	8.36	21.3
		2	8.97	8.33	20.5
1B3		1	10.49	8.37	20.1
		2	8.90	8.38	20.3
1C5		1	10.03	8.37	20.4
		2	8.44	8.32	20.3
		Mean	9.59	8.36	20.5
		SD	0.94	0.02	0.4
		Min	8.44	8.32	20.1
		Max	10.68	8.38	21.3
1A6	200	1	9.15	8.31	20.4
		2	8.46	8.30	20.2
1B5		1	9.10	8.36	19.8
		2	8.34	8.37	19.8
1B6		1	9.37	8.31	20.3
		2	7.90	8.21	20.5
		Mean	8.72	8.31	20.2
		SD	0.57	0.06	0.3
		Min	7.90	8.21	19.8
		Max	9.37	8.37	20.5
1A2	300	1	10.23	8.26	20.8
		2	7.86	8.15	20.5
1B4		1	8.11	8.31	20.2
		2	8.44	8.30	20.3
1C2		1	10.02	8.28	21.2
		2	7.85	8.15	20.5
		Mean	8.75	8.25	20.6
		SD	1.09	0.07	0.4
		Min	7.85	8.15	20.2
		Max	10.23	8.31	21.2
1A4	300 HD	1	8.34	8.33	18.3
		2	8.65	8.50	18.5
1C3		1	10.30	8.37	20.4
		2	9.11	8.39	20.0
1C4		1	10.45	8.37	20.3
		2	8.88	8.27	20.1
		Mean	9.29	8.38	19.6
		SD	0.88	0.08	0.9
		Min	8.34	8.27	18.3
		Max	10.45	8.50	20.4

Sample Time 1 was measured upon exposure initiation and Sample Time 2 was measured just prior to exposure termination.

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Study Number: AEH-11-PSEUDO-01
Lab Notebook 1
Data Source: File Folder: 10b
Forms: Water Quality - Form 2
During Exposure (Dissolved Oxygen, pH, Temperature)

Test Article: MBI 401 (Pf-CL 145A [SDP])
Article Lot #: MBI-401 SDP 4655-12-Mix
Exposure Date: 17-Jan-12
Species Tested: MUC
Glochidia Lot # 120700

Exposure Water Chemistry - Mucket SDP

Test Chamber	Treatment (mg/L)	Sample Time	Dissolved Oxygen (mg/L)	pH	Temperature (°C)
2B4	0	1	8.64	8.34	20.1
		2	7.44	8.52	20.1
2B5		1	8.02	8.37	20.0
		2	8.32	8.53	20.0
2C1		1	9.30	8.38	20.2
		2	8.66	8.52	20.3
		Mean	8.40	8.45	20.1
		SD	0.63	0.09	0.1
		Min	7.44	8.34	20.0
		Max	9.30	8.53	20.3
2A4	50	1	9.85	8.33	20.0
		2	8.89	8.38	20.3
2B6		1	9.55	8.36	20.1
		2	8.80	8.38	20.3
2C3		1	9.43	8.36	20.0
		2	8.70	8.43	20.0
		Mean	9.20	8.37	20.1
		SD	0.47	0.03	0.1
		Min	8.70	8.33	20.0
		Max	9.85	8.43	20.3
2A2	100	1	8.88	8.32	20.1
		2	8.72	8.37	20.3
2B2		1	8.72	8.34	19.7
		2	8.44	8.31	20.2
2C6		1	8.72	8.35	19.3
		2	8.43	8.43	19.2
		Mean	8.65	8.36	19.8
		SD	0.18	0.04	0.5
		Min	8.43	8.31	19.2
		Max	8.88	8.43	20.3
2A5	200	1	9.44	8.32	19.9
		2	8.46	8.35	19.8
2A6		1	9.14	8.30	19.9
		2	8.56	8.35	19.5
2C2		1	9.60	8.26	19.6
		2	8.57	8.40	19.7
		Mean	8.96	8.33	19.7
		SD	0.50	0.05	0.2
		Min	8.46	8.26	19.5
		Max	9.60	8.40	19.9
2B1	300	1	8.33	8.29	20.1
		2	8.78	8.34	20.3
2B3		1	8.85	8.29	19.6
		2	8.83	8.32	19.6
2C4		1	9.07	8.30	19.8
		2	8.60	8.38	19.6
		Mean	8.74	8.32	19.8
		SD	0.25	0.04	0.3
		Min	8.33	8.29	19.6
		Max	9.07	8.38	20.3
2A1	300 HD	1	8.93	8.30	20.0
		2	8.33	8.54	19.9
2A3		1	8.71	8.32	18.8
		2	8.26	8.23	20.5
2C5		1	9.00	8.33	19.7
		2	8.55	8.42	19.4
		Mean	8.63	8.33	19.7
		SD	0.31	0.06	0.6
		Min	8.26	8.23	18.8
		Max	9.00	8.42	20.5

Sample Time 1 was measured upon exposure initiation and Sample Time 2 was measured just prior to exposure termination.

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Study Number: AEH-11-PSEUDO-01
Lab Notebook 1
Data Source: File Folder: 10b
Forms: Water Quality - Form 2
During Exposure (Dissolved Oxygen, pH, Temperature)

Test Article: MBI 401 (Pf-CL 145A (SDP))
Article Lot #: MBI-401 SDP 4655-12-Mix
Exposure Date: 19-Jan-12
Species Tested: HIC
Glochidia Lot # 120900

Exposure Water Chemistry - Hickorynut SDP

Test Chamber	Treatment (mg/L)	Sample Time	Dissolved Oxygen (mg/L)	pH	Temperature (°C)
3A2	0	1	10.32	8.36	20.1
		2	10.73	8.51	19.9
3C1		1	8.46	8.36	20.1
		2	9.55	8.43	19.8
3C4		1	9.88	8.36	20.0
		2	9.95	8.50	19.5
		Mean	9.82	8.42	19.9
		SD	0.78	0.07	0.2
		Min	8.46	8.36	19.5
		Max	10.73	8.51	20.1
3A5	50	1	10.37	8.33	20.0
		2	10.06	8.42	19.5
3B3		1	11.00	8.37	19.7
		2	10.43	8.48	18.8
3B6		1	10.36	8.32	20.0
		2	10.23	8.34	19.9
		Mean	10.41	8.38	19.7
		SD	0.32	0.06	0.5
		Min	10.06	8.32	18.8
		Max	11.00	8.48	20.0
3B1	100	1	10.91	8.34	20.1
		2	10.03	8.38	19.7
3C2		1	10.00	8.36	19.6
		2	9.91	8.45	18.6
3C5		1	10.35	8.32	20.0
		2	9.74	8.30	19.6
		Mean	10.16	8.36	19.6
		SD	0.42	0.05	0.5
		Min	9.74	8.30	18.6
		Max	10.91	8.45	20.1
3A3	200	1	9.95	8.24	20.0
		2	9.08	8.10	19.9
3A4		1	9.87	8.27	19.9
		2	9.49	8.24	19.7
3C6		1	9.96	8.32	19.9
		2	9.66	8.35	18.6
		Mean	9.67	8.26	19.7
		SD	0.34	0.09	0.5
		Min	9.08	8.10	18.6
		Max	9.96	8.35	20.0
3A6	300	1	10.70	8.18	19.9
		2	8.58	8.10	19.8
3B2		1	10.45	8.21	19.8
		2	8.96	8.15	19.7
3B5		1	10.31	8.30	19.7
		2	8.95	8.26	19.4
		Mean	9.66	8.21	19.7
		SD	0.93	0.07	0.2
		Min	8.58	8.10	19.4
		Max	10.70	8.30	19.9
3A1	300 HD	1	10.75	8.11	20.1
		2	9.97	8.14	19.7
3B4		1	10.64	8.13	20.0
		2	10.68	8.36	17.4
3C3		1	10.76	8.15	19.8
		2	10.61	8.32	19.0
		Mean	10.57	8.21	19.3
		SD	0.30	0.11	1.0
		Min	9.97	8.11	17.4
		Max	10.76	8.36	20.1

Sample Time 1 was measured upon exposure initiation and Sample Time 2 was measured just prior to exposure termination.

Item Number 17
Page 9 of 9

Study Number: AEH-11-PSEUDO-01		
Lab Notebook: 1		
Data Source: File Folder: 10b		
Forms:	Water Quality - Form 2	
	During Exposure (Dissolved Oxygen, pH, Temperature) (Light Intensity)	
File Name:	I:\AEH-11-PSEUDO-01\Data\Water Chemistry\[Exposure Water Chemistry Summary.xlsx]Light Intensity Coversheet	

Action	Date	Initials
Created......	9-Mar-15	TJS TJS
Revised......	9-Mar-15	TJS TJS
Reviewed...	9-MAR-15	TJS
Certified...		

Exposure Light Intensity

Test Article: *Pseudomonas fluorescens* (*Pf*-CL145A) spray dried powder (SDP) and freeze dried powder (FDP)
Article Lot #s: SDP = MBI-401-110308AI-BD-3 and MBI-401 SDP 4655-12-Mix; FDP = 110607WB-FD-E
Exposure Dates: 12-May-11, 18-Oct-11, 17-Jan-12, and 19-Jan-12
Species Tested: PPB, FAM, HGE, WAS, BLS, MUC, and HIC

Data Explanation:

Light intensity was measured twice during exposure:
1) Upon exposure initiation
2) Prior to exposure termination

Species Abbreviations
PPB - plain pocketbook (*Lampsilis cardium*)
FAM - fatmucket (*Lampsilis siliquoidea*)
HGE - Higgins eye (*Lampsilis higginsii*)
WAS - washboard (Megalonaias nervosa)
BLS - black sandshell (*Ligumia recta*)
MUC - mucket (*Actinonaias ligamentina*)
HIC - hickorynut (*Obovaria olivaria*)

Data anomalies and deviations:

none

Item Number 18
Page 1 of 2

Exposure Light Intensity

Species	Formulation	Test Date	Sample Time	Light Intensity (lux)
PPB	SDP	12-May-11	1	204
			2	242
			Mean	223
			SD	27
FAM	SDP	12-May-11	1	239
			2	211
			Mean	225
			SD	20
HGE	SDP	12-May-11	1	218
			2	220
			Mean	219
			SD	1
PPB	FDP	18-Oct-11	1	120
			2	96
			Mean	108
			SD	17
WAS	FDP	18-Oct-11	1	500
			2	571
			Mean	536
			SD	50
BLS	SDP	17-Jan-12	1	283
			2	465
			Mean	374
			SD	129
MUC	SDP	17-Jan-12	1	176
			2	277
			Mean	227
			SD	71
HIC	SDP	19-Jan-12	1	228
			2	295
			Mean	262
			SD	47
			Grand Mean	272
			SD	132
			Min	96
			Max	571

Sample Time 1 was measured upon exposure initiation and Sample Time 2 was measured just prior to exposure termination.

Item Number 18
Page 2 of 2

Study Number: AEH-11-PSEUDO-01	Action	Date	Initials
Lab Notebook: 1	Created......	9-Mar-15	TJS TJS
Data Source: File Folder: 10c	Revised......	9-Mar-15	TJS TJS
Forms: Water Quality - Form 3	Reviewed...	9-MAR-15	TJS
Upon Termination (Hardness, Alkalinity, Conductivity, and Ammonia)	Certified...	3/13/15	JR
File Name: I:\AEH-11-PSEUDO-01\Data\Water Chemistry\[Termination Water Chemistry Summary.xlsx]Termination Coversheet			

Termination Water Chemistry

Test Article: *Pseudomonas fluorescens* (*Pf*-CL145A) spray dried powder (SDP) and freeze dried powder (FDP)
Article Lot #s: SDP = MBI-401-110308AI-BD-3 and MBI-401 SDP 4655-12-Mix; FDP = 110607WB-FD-E
Exposure Dates: 12-May-11, 18-Oct-11, 17-Jan-12, and 19-Jan-12
Species Tested: PPB, FAM, HGE, WAS, BLS, MUC, and HIC

Data Explanation:

300 mg/L HD = Heat Deactivated

Species Abbreviations
- PPB - plain pocketbook (*Lampsilis cardium*)
- FAM - fatmucket (*Lampsilis siliquoidea*)
- HGE - Higgins eye (*Lampsilis higginsii*)
- WAS - washboard (Megalonaias nervosa)
- BLS - black sandshell (*Ligumia recta*)
- MUC - mucket (*Actinonaias ligamentina*)
- HIC - hickorynut (*Obovaria olivaria*)

Upon termination, all replicate treatment chambers (3) were pooled and water chemistry was measured from pooled sample.

Data anomalies and deviations:

Upon termination of 12-May-11 SDP exposure, one control replicate from chamber 2B1 (HGE) was accidentally pooled with control replicates from block 1 (PPB); this resulted in controls for PPB having 4 pooled control replicates and controls for HGE having only 2 pooled replicates.

Item Number 9
Page 1 of 2

Study Number: AEH-11-PSEUDO-01
Lab Notebook 1
Data Source: File Folder: 10c
Forms: Water Quality - Form 3
Upon Termination (Hardness, Alkalinity, Conductivity, and Ammonia)

Test Article: MBI 401 (*Pf*-CL 145A [SDP] and [FDP])
Article Lot #: MBI-401-110308AI-BD-3, MBI-401 SDP 4655-12-Mix, 110607WB-FD-E
Exposure Dates: 12-May-11, 18-Oct-11, 17-Jan-12, and 19-Jan-12
Species Tested: PPB, FAM, HGE, WAS, BLS, MUC, and HIC

Termination Water Chemistry

Species	Formulation	Test Date	Pooled Concentration	Hardness (mg/L $CaCO_3$)	Alkalinity (mg/L $CaCO_3$)	Conductivity (µS/cm)
PPB	SDP	12-May-11	0 mg/L	182	134	380
			50 mg/L	180	134	383
			100 mg/L	180	136	374
			200 mg/L	179	136	394
			300 mg/L	178	138	403
			300 mg/L HD	181	142	411
			Mean	180	137	391
			SD	1	3	14
			Min	178	134	374
			Max	182	142	411
HGE	SDP	12-May-11	0 mg/L	180	138	375
			50 mg/L	182	138	363
			100 mg/L	186	141	384
			200 mg/L	185	144	389
			300 mg/L	190	145	409
			300 mg/L HD	186	147	403
			Mean	185	142	387
			SD	3	4	17
			Min	180	138	363
			Max	190	147	409
FAM	SDP	12-May-11	0 mg/L	180	135	354
			50 mg/L	180	135	350
			100 mg/L	182	135	367
			200 mg/L	178	139	382
			300 mg/L	183	139	377
			300 mg/L HD	181	140	370
			Mean	181	137	367
			SD	2	2	13
			Min	178	135	350
			Max	183	140	382
PPB	FDP	18-Oct-11	0 mg/L	190	138	410
			50 mg/L	197	144	424
			100 mg/L	194	145	432
			200 mg/L	200	146	437
			300 mg/L	200	147	443
			300 mg/L HD	195	143	439
			Mean	196	144	431
			SD	4	3	12
			Min	190	138	410
			Max	200	147	443
WAS	FDP	18-Oct-11	0 mg/L	198	139	413
			50 mg/L	198	148	431
			100 mg/L	200	147	434
			200 mg/L	202	150	447
			300 mg/L	203	152	454
			300 mg/L HD	203	153	463
			Mean	201	148	440
			SD	2	5	18
			Min	198	139	413
			Max	203	153	463
BLS	SDP	17-Jan-12	0 mg/L	196	143	414
			50 mg/L	195	147	412
			100 mg/L	192	148	423
			200 mg/L	200	156	434
			300 mg/L	198	161	445
			300 mg/L HD	210	164	468
			Mean	199	153	433
			SD	6	8	21
			Min	192	143	412
			Max	210	164	468
MUC	SDP	17-Jan-12	0 mg/L	196	146	403
			50 mg/L	196	148	420
			100 mg/L	199	153	437
			200 mg/L	197	156	448
			300 mg/L	198	159	465
			300 mg/L HD	202	156	467
			Mean	198	153	440
			SD	2	5	25
			Min	196	146	403
			Max	202	159	467
HIC	SDP	19-Jan-12	0 mg/L	208	135	414
			50 mg/L	208	145	456
			100 mg/L	210	146	460
			200 mg/L	209	148	460
			300 mg/L	208	150	456
			300 mg/L HD	218	156	498
			Mean	210	147	457
			SD	4	7	27
			Min	208	135	414
			Max	218	156	498

Item Number 9
Page 2 of 2

File Folder : 10 d

Sample Description: 3 mL acidified exposure water (0.45 μm filtrated)
Study #: AEH-11-PSEUDO-01

Upper Midwest Environmental Sciences Center
attn: James Luoma
2630 Fanta Reed Road
La Crosse, WI54603

Water Quality Laboratory
Upper Midwest Environmental Sciences Center
USGS
2630 Fanta Reed Road
La Crosse, WI 54603

Analyzing Date: 11/07 /2011
Report Date: 11/09/2011

REPORT OF ANALYSIS

Sample Code	Results (Total Ammonia Nitrogen, mg NH_3-N/L)
PPB3A1	0.193
PPB3A2	0.226
PPB3A3	0.299
PPB3A4	0.104
PPB3A5	0.204
PPB3A6	0.059
PPB3B1	0.148
PPB3B2	0.181
PPB3B3	0.136
PPB3B4	0.306
PPB3B5	0.074
PPB3B6	0.042
PPB3C1	0.122
PPB3C2	0.096
PPB3C3	0.238
PPB3C4	0.283
PPB3C5	0.210
PPB3C6	0.126
WAS1A1	0.054
WAS1A2	0.035
WAS1A3	0.096
WAS1A4	0.111
WAS1A5	0.027
WAS1A6	0.059
WAS1B1	0.185
WAS1B2	0.022
WAS1B3	0.100
WAS1B4	0.030
WAS1B5	0.053
WAS1B6	0.063
WAS1C1	0.058
WAS1C2	0.039
WAS1C3	0.056
WAS1C4	0.033
WAS1C5	0.074
WAS1C6	0.073

USGS
Water Quality Laboratory
Laboratory Director

Pg 1 of 1

[redacted]
Xiaoli Yuan

File Folder: 10d

Sample Description: 3 mL acidified exposure water (0.45 µm filtrated)
Study #: AEH-11-PSEUDO-01

Upper Midwest Environmental Sciences Center
attn: James Luoma
2630 Fanta Reed Road
La Crosse, WI54603

Water Quality Laboratory
Upper Midwest Environmental Sciences Center
USGS
2630 Fanta Reed Road
La Crosse, WI54603

Analyzing Date: 01/25/2012
Report Date: 02/03/2012

REPORT OF ANALYSIS

Sample Code	Results (Total Ammonia Nitrogen, mg NH_3-N/L)
BLS1A1T24	0.215
BLS1A2T24	1.661
BLS1A3T24	0.241
BLS1A4T24	0.247
BLS1A5T24	0.082
BLS1A6T24	1.078
BLS1B1T24	0.079
BLS1B2T24	0.698
BLS1B3T24	0.719
BLS1B4T24	1.187
BLS1B5T24	0.916
BLS1B6T24	1.167
BLS1C1T24	0.436
BLS1C2T24	1.509
BLS1C3T24	0.245
BLS1C4T24	0.197
BLS1C5T24	0.724
BLS1C6T24	0.08
HIC3A1T24	0.885
HIC3A2T24	0.082
HIC3A3T24	0.254
HIC3A4T24	0.263
HIC3A5T24	0.265
HIC3A6T24	0.482
HIC3B1T24	0.533
HIC3B2T24	0.231
HIC3B3T24	0.098
HIC3B4T24	0.107
HIC3B5T24	0.192
HIC3B6T24	0.266
HIC3C1T24	0.084
HIC3C2T24	0.094
HIC3C3T24	0.474
HIC3C4T24	0.032
HIC3C5T24	0.473
HIC3C6T24	0.213
MUC2A1T24	0.537
MUC2A2T24	0.694
MUC2A3T24	0.796
MUC2A4T24	0.088
MUC2A5T24	0.861
MUC2A6T24	0.777
MUC2B1T24	1.136
MUC2B2T24	0.43
MUC2B3T24	0.916
MUC2B4T24	0.037
MUC2B5T24	0.052
MUC2B6T24	0.533
MUC2C1T24	0.11
MUC2C2T24	0.921
MUC2C3T24	0.183
MUC2C4T24	1.088
MUC2C5T24	0.409
MUC2C6T24	0.212

USGS
Water Quality Laboratory
Laboratory Director
[redacted]
Xiaoli Yuan

Pg *1* of *1*

Study Number: AEH-11-PSEUDO-01	
Lab Notebook: 1	
Data Source: File Folder: 10b, 10c, and 10d	
Forms: Water Quality - Form 2 and 3; LTRM Water Quality Laboratory Report	
File Name: I:\AEH-11-PSEUDO-01\Data\Water Chemistry\[Ammonia Summary.xlsx]TAN Coversheet	

Action	Date	Initials
Created......	9-Mar-15	TJS TJS
Revised......	12-Mar-15	TJS TJS
Reviewed...	12 MAR-15	TJS
Certified...	3/13/15	JM

Total Ammonia Nitrogen Coversheet

Test Article: *Pseudomonas fluorescens* (*Pf*-CL145A) spray dried powder (SDP) and freeze dried powder (FDP)
Article Lot #s: SDP = MBI-401-110308AI-BD-3 and MBI-401 SDP 4655-12-Mix; FDP = 110607WB-FD-E
Exposure Dates: 12-May-11, 18-Oct-11, 17-Jan-12, and 19-Jan-12
Species Tested: PPB, FAM, HGE, WAS, BLS, MUC, and HIC

Data Explanation:

Species Abbreviations

- PPB - plain pocketbook (*Lampsilis cardium*)
- FAM - fatmucket (*Lampsilis siliquoidea*)
- HGE - Higgins eye (*Lampsilis higginsii*)
- WAS - washboard (Megalonaias nervosa)
- BLS - black sandshell (*Ligumia recta*)
- MUC - mucket (*Actinonaias ligamentina*)
- HIC - hickorynut (*Obovaria olivaria*)

For SDP treatments initiated on 12-May-11 (FAM, PPB, and HGE), total ammonia nitrogen was measured using a YSI 9000 photometer. For, all other treatments (SDP and FDP), total ammonia nitrogen analyses were conducted by the UMESC LTRM Water Quality Laboratory using the authomated phenate method.

pH means were calculated on hydrogen ion concentration; pH standard deviations were calculated on pH values.

TAN toxicity is determined according to U.S. Environmental Protection Agency, 2013, Ambient Water Quality Criteria for Ammonia—Freshwater 2013. Washington, D.C., Office of Water, EPA 822-R-13-001, 242 p. The EPA document sets the acute criterion magnitude at 17 mg TAN/L (1 hour average, pH 7, 20 °C) and a chronic criterion magnitude at 1.9 mg TAN/L (30-day rolling average, pH 7, 20 °C) with the stipulation that the chronic criterion cannot exceed a 4-day average of 4.8 mg/L.

Data anomalies and deviations:

For the PPB (SDP) exposures on 12-May-11, pH and temperature data were not measured on pooled samples used to measure total ammonia nitrogen. Therefore, the mean pH and temperature of each treatment group measured at the end of exposure period (24 hour) were used to calculate the TAN toxicity.

Item Number 3
Page 1 of 10

Plain Pocketbook (SDP) Total Ammonia Nitrogen

Treatment (mg/L)	Temperature (°C)[1]	pH[1]	Total Ammonia Nitrogen (mg NH_3-N/L)	Ammonia Toxicity
0	19.2	8.34	0.06	NT
50	19.2	8.25	0.08	NT
100	19	8.03	0.13	NT
200	19	8.09	0.24	NT
300	19.2	7.82	0.44	NT
300 HD	19.1	8.07	0.51	NT
Grand Mean	19.1	8.10	0.24	NT
SD	0.1	0.18	0.19	NA

NT = non-toxic levels of total ammonia nitrogen (below chronic and acute levels).

Note: Control water sample from 2B1 (HGE) was accidentally pooled with the Block 1 (PPB) Control group, therefore four control samples were pooled for determination of PPB control TAN.

Plain Pocketbook (SDP) End of Exposure pH and Temperature

Test Chamber	Treatment (mg/L)	pH	Temperature (°C)
1A3	0	8.23	19.1
1B1		8.35	19.5
1C3		8.41	19.0
	Mean	8.34	19.2
1A1	50	8.25	19.2
	Mean	8.25	19.2
1A4	100	8.03	19.0
	Mean	8.03	19.0
1C4	200	8.09	19.0
	Mean	8.09	19.0
1A5	300	7.85	19.0
1C2		7.87	19.5
1C5		7.74	19.1
	Mean	7.82	19.2
1A6	300 HD	8.07	19.1
	Mean	8.07	19.1

[1]pH and temperature data measured at the end of exposure period (24 hour) are used for making total ammonia nitrogen toxicity determinations since there was no termination pH and temperature data taken for PPB (SDP) exposure.

Item Number 3
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Study Number: AEH-11-PSEUDO-01
Lab Notebook 1
Data Source: File Folder: 10d
Forms: Water Quality - Form 3
Upon Termination (Hardness, Alkalinity, Conductivity, and Ammonia)

Test Article: MBI 401 (PF-CL 145A [SDP])
Article Lot #: MBI-401-110308AI-BD-3
Exposure Date: 12-May-11
Species Tested: HGE
Glochidia Lot # 111500

Higgins Eye (SDP) Total Ammonia Nitrogen

Treatment (mg/L)	Temperature (°C)	pH	Total Ammonia Nitrogen (mg NH_3-N/L)	Ammonia Toxicity
0	19.5	8.47	0.09	NT
50	19.6	7.99	0.17	NT
100	19.5	7.93	0.31	NT
200	19.6	7.40	0.53	NT
300	19.3	7.34	0.45	NT
300 HD	19.5	7.20	0.50	NT
Grand Mean	19.5	7.95	0.34	NT
SD	0.1	0.49	0.18	NA

NT = non-toxic levels of total ammonia nitrogen (below chronic and acute levels).
Note: Control water sample from 2B1 (HGE) was accidentally pooled with the Block 1 (PPB) Control group, therefore only two control samples were pooled for determination of HGE control TAN.

Item Number 3
Page 3 of 10

Fatmucket (SDP) Total Ammonia Nitrogen

Treatment (mg/L)	Temperature (°c)	pH	Total Ammonia Nitrogen (mg NH_3-N/L)	Ammonia Toxicity
0	20	8.45	0.10	NT
50	20	8.11	0.25	NT
100	19.8	7.61	0.24	NT
200	19.9	7.48	0.44	NT
300	19.7	7.25	0.63	NT
300 HD	19.7	7.17	0.55	NT
Grand Mean	19.9	7.93	0.37	NT
SD	0.1	0.50	0.20	NA

NT = non-toxic levels of total ammonia nitrogen (below chronic and acute levels).

Study Number: AEH-11-FSEUDO-01
Lab Notebook 1
Data Source: File Folder: 10d
Forms: Water Quality - Form 3
Upon Termination (Hardness, Alkalinity, Conductivity, Temperature, and pH)
LTRM Water Quality Laboratory Report

Test Article: MBI 401 (Pf-CL 145A [FDP])
Article Lot #: 110607WB-FB-E
Exposure Date: 18-Oct-11
Species Tested: PPB
Glochidia Lot # 115400

Plain Pocketbook (FDP) Total Ammonia Nitrogen

Treatment (mg/L)	Rep	Temperature (°C)	pH	Total Ammonia Nitrogen (mg NH_3-N/L)	Ammonia Toxicity
0	1	18.2	8.33	0.059	NT
	2	18.1	8.31	0.074	NT
	3	18.2	8.35	0.042	NT
	Mean	18.2	8.33	0.058	NT
	SD	0.1	0.02	0.016	NA
50	1	18.2	8.39	0.122	NT
	2	17.8	8.41	0.096	NT
	3	18.1	8.39	0.104	NT
	Mean	18.0	8.40	0.107	NT
	SD	0.2	0.01	0.013	NA
100	1	18.2	8.38	0.148	NT
	2	18.3	8.36	0.136	NT
	3	18.2	8.38	0.126	NT
	Mean	18.2	8.37	0.137	NT
	SD	0.1	0.01	0.011	NA
200	1	18.3	8.34	0.226	NT
	2	18.2	8.39	0.238	NT
	3	18.3	8.30	0.210	NT
	Mean	18.3	8.34	0.225	NT
	SD	0.1	0.05	0.014	NA
300	1	18.6	8.27	0.299	NT
	2	18.6	8.30	0.306	NT
	3	18.5	8.31	0.283	NT
	Mean	18.6	8.29	0.296	NT
	SD	0.1	0.02	0.012	NA
300 HD	1	18.2	8.34	0.181	NT
	2	18.2	8.31	0.204	NT
	3	18.2	8.28	0.193	NT
	Mean	18.2	8.31	0.193	NT
	SD	0.0	0.03	0.012	NA
	Grand Mean	18.2	8.34	0.169	NT
	SD	0.2	0.04	0.081	NA

NT = non-toxic levels of total ammonia nitrogen (below chronic and acute levels).

Item Number 3
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Study Number: AEH-11-PSEUDO-01
Lab Notebook 1
Data Source: File Folder: 10d
Forms: Water Quality - Form 3
Upon Termination (Hardness, Alkalinity, Conductivity, Temperature, and pH)
LTRM Water Quality Laboratory Report

Test Article: MBI 401 (Pf-CL 145A [FDP])
Article Lot #: 110607WB-FB-E
Exposure Date: 18-Oct-11
Species Tested: WAS
Glochidia Lot # 115500

Washboard (FDP) Total Ammonia Nitrogen

Treatment (mg/L)	Rep	Temperature (°C)	pH	Total Ammonia Nitrogen (mg NH_3-N/L)	Ammonia Toxicity
0	1	18.7	8.47	0.027	NT
	2	18.7	8.49	0.022	NT
	3	18.6	8.48	0.030	NT
	Mean	18.7	8.48	0.026	NT
	SD	0.1	0.01	0.004	NA
50	1	18.5	8.45	0.054	NT
	2	18.1	8.47	0.058	NT
	3	18.7	8.44	0.039	NT
	Mean	18.4	8.45	0.050	NT
	SD	0.3	0.02	0.010	NA
100	1	18.9	8.43	0.035	NT
	2	18.7	8.46	0.059	NT
	3	18.6	8.44	0.073	NT
	Mean	18.7	8.44	0.056	NT
	SD	0.2	0.02	0.019	NA
200	1	19.1	8.41	0.053	NT
	2	18.6	8.40	0.056	NT
	3	19.1	8.42	0.033	NT
	Mean	18.9	8.41	0.047	NT
	SD	0.3	0.01	0.013	NA
300	1	18.0	8.42	0.111	NT
	2	18.8	8.38	0.185	NT
	3	19.2	8.35	0.074	NT
	Mean	18.7	8.38	0.123	NT
	SD	0.6	0.04	0.057	NA
300 HD	1	18.6	8.42	0.096	NT
	2	18.3	8.45	0.100	NT
	3	18.6	8.40	0.063	NT
	Mean	18.5	8.42	0.086	NT
	SD	0.2	0.03	0.070	NA
	Grand Mean	18.7	8.43	0.065	NT
	SD	0.3	0.04	0.039	NA

NT = non-toxic levels of total ammonia nitrogen (below chronic and acute levels).

Item Number 3
Page 6 of 10

Stucy Number: AEH-11-PSEUDO-01
Lab Notebook 1
Data Source: File Folder: 10d
Forms: Water Quality - Form 3
Upon Termination (Hardness, Alkalinity, Conductivity, Temperature, and pH)
LTRM Water Quality Laboratory Report

Test Article: MBI 401 (PF-CL 145A [SDP])
Article Lot #: MBI-401 SDP 4655-12-Mix
Exposure Date: 18-Oct-11
Species Tested: BLS
Glochidia Lot # 120800

Black Sandshell (SDP) Total Ammonia Nitrogen

Treatment (mg/L)	Rep	Temperature (°C)	pH	Total Ammonia Nitrogen (mg NH_3-N/L)	Ammonia Toxicity
0	1	20.1	8.36	0.082	NT
	2	20.3	8.43	0.079	NT
	3	20.5	8.45	0.080	NT
	Mean	20.3	8.42	0.080	NT
	SD	0.2	0.05	0.002	NA
50	1	20.4	8.30	0.215	NT
	2	20.1	8.33	0.241	NT
	3	20.5	8.24	0.436	NT
	Mean	20.3	8.29	0.297	NT
	SD	0.2	0.05	0.121	NA
100	1	20.3	8.15	0.724	Chronic
	2	20.5	8.17	0.698	Chronic
	3	21.0	8.23	0.719	Chronic
	Mean	20.6	8.18	0.714	Chronic
	SD	0.4	0.04	0.014	NA
200	1	20.0	8.24	0.916	Chronic
	2	20.6	8.03	1.167	Chronic
	3	20.5	8.12	1.078	Chronic
	Mean	20.4	8.14	1.054	Chronic
	SD	0.3	0.11	0.127	NA
300	1	20.6	7.95	1.509	Chronic
	2	20.6	8.13	1.187	Chronic
	3	20.8	7.96	1.661	Chronic
	Mean	20.7	8.02	1.452	Chronic
	SD	0.1	0.10	0.242	NA
300 HD	1	20.3	8.28	0.245	NT
	2	20.2	8.16	0.197	NT
	3	18.4	8.46	0.247	NT
	Mean	19.6	8.32	0.230	NT
	SD	1.1	0.15	0.028	NA
	Grand Mean	20.3	8.25	0.638	Chronic
	SD	0.5	0.15	0.514	NA

NT = non-toxic levels of total ammonia nitrogen (below chronic and acute levels).

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Study Number: AEH-11-PSEUDO-01
Lab Notebook 1
Data Source: File Folder: 10d
Forms: Water Quality - Form 3
Upon Termination (Hardness, Alkalinity, Conductivity, Temperature, and pH)
LTRM Water Quality Laboratory Report

Test Article: MBI 401 (Pf-CL 145A [SDP])
Article Lot #: MBI-401 SDP 4655-12-Mix
Exposure Date: 17-Jan-12
Species Tested: MUC
Glochidia Lot # 120700

Mucket (SDP) Total Ammonia Nitrogen

Treatment (mg/L)	Rep	Temperature (°C)	pH	Total Ammonia Nitrogen (mg NH_3-N/L)	Ammonia Toxicity
0	1	19.8	8.60	0.037	NT
	2	19.8	8.57	0.052	NT
	3	20.1	8.56	0.110	NT
	Mean	19.9	8.58	0.066	NT
	SD	0.2	0.02	0.039	NA
50	1	19.9	8.42	0.183	NT
	2	20.1	8.36	0.533	Chronic
	3	20.1	8.39	0.088	NT
	Mean	20.0	8.39	0.268	NT
	SD	0.1	0.03	0.234	NA
100	1	20.4	8.29	0.694	Chronic
	2	20.2	8.25	0.430	NT
	3	19.2	8.43	0.212	NT
	Mean	19.9	8.33	0.445	NT
	SD	0.6	0.09	0.241	NA
200	1	20.1	8.27	0.861	Chronic
	2	19.9	8.28	0.777	Chronic
	3	19.9	8.33	0.921	Chronic
	Mean	20.0	8.29	0.853	Chronic
	SD	0.1	0.03	0.072	NA
300	1	20.7	8.30	1.136	Chronic
	2	19.8	8.21	0.916	Chronic
	3	19.9	8.25	1.088	Chronic
	Mean	20.1	8.25	1.047	Chronic
	SD	0.5	0.05	0.116	NA
300 HD	1	19.9	8.19	0.537	NT
	2	20.7	8.13	0.796	Chronic
	3	19.7	8.37	0.409	NT
	Mean	20.1	8.24	0.581	Chronic
	SD	0.5	0.12	0.197	NA
	Grand Mean	20.0	8.36	0.543	Chronic
	SD	0.4	0.13	0.371	NA

NT = non-toxic levels of total ammonia nitrogen (below chronic and acute levels).

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Hickorynut (SDP) Total Ammonia Nitrogen

Treatment (mg/L)	Rep	Temperature (°C)	pH	Total Ammonia Nitrogen (mg NH_3-N/L)	Ammonia Toxicity
0	1	20.1	8.42	0.082	NT
	2	20.0	8.47	0.084	NT
	3	19.7	8.48	0.032	NT
	Mean	19.9	8.46	0.066	NT
	SD	0.2	0.03	0.029	NA
50	1	20.0	8.45	0.265	NT
	2	20.1	8.29	0.266	NT
	3	19.2	8.45	0.098	NT
	Mean	19.8	8.40	0.210	NT
	SD	0.5	0.09	0.097	NA
100	1	19.0	8.42	0.094	NT
	2	19.9	8.35	0.533	Chronic
	3	19.8	8.27	0.473	NT
	Mean	19.6	8.35	0.367	NT
	SD	0.5	0.08	0.238	NA
200	1	19.7	8.33	0.213	NT
	2	20.7	8.03	0.254	NT
	3	20.4	8.13	0.263	NT
	Mean	20.3	8.18	0.243	NT
	SD	0.5	0.15	0.027	NA
300	1	20.6	8.05	0.231	NT
	2	20.1	8.08	0.192	NT
	3	20.3	7.98	0.482	NT
	Mean	20.3	8.04	0.302	NT
	SD	0.3	0.05	0.157	NA
300 HD	1	20.3	8.05	0.885	Chronic
	2	19.0	8.33	0.107	NT
	3	19.7	8.26	0.474	NT
	Mean	19.7	8.23	0.489	NT
	SD	0.7	0.15	0.389	NA

NT = non-toxic levels of total ammonia nitrogen (below chronic and acute levels).

Study Number: AEH-11-PSEUDO-01
Lab Notebook 1
Data Source: File Folder: 10d
Forms: Water Quality - Forms 2 and 3
During Exposure (Dissolved Oxygen, pH, Temperature)
Upon Termination (Hardness, Alkalinity, Conductivity, and Ammonia)
LTRM Water Quality Laboratory Report

Test Article: MBI 401 (Pf-CL 145A (SDP and FDP))
Article Lot #: MBI-401-110308AI-BD-3,MBI-401 SDP 4655-12-Mix, 110607WB-FB-E
Exposure Date: 12-May-11, 18-Oct-11, 17-Jan-12, 19-Jan-12
Species Tested: PPB, HGE, FAM, WAS, BLS, MUC, HIC
Glochidia Lot # 111400, 111500, 111600, 115400, 115500, 120700, 120800, 120900

Total Ammonia Nitrogen Summary

Treatment (mg/L)	Species (Formulation)	Mean Temperature (°C)	Mean pH	Mean Total Ammonia Nitrogen (mg NH_3-N/L)	Ammonia Toxicity
0	PPB (SDP)	19.2	8.34	0.060	NT
	HGE (SDP)	19.5	8.47	0.090	NT
	FAM (SDP)	20.0	8.45	0.100	NT
	PPB (FDP)	18.2	8.33	0.058	NT
	WAS (FDP)	18.7	8.48	0.026	NT
	BLS (SDP)	20.3	8.42	0.080	NT
	MUC (SDP)	19.9	8.58	0.066	NT
	HIC (SDP)	19.9	8.46	0.066	NT
	Mean	19.5	8.45	0.068	NT
	SD	0.7	0.08	0.023	NA
50	PPB (SDP)	19.2	8.25	0.080	NT
	HGE (SDP)	19.6	7.99	0.170	NT
	FAM (SDP)	20.0	8.11	0.250	NT
	PPB (FDP)	18.0	8.40	0.107	NT
	WAS (FDP)	18.4	8.45	0.050	NT
	BLS (SDP)	20.3	8.29	0.297	NT
	MUC (SDP)	20.0	8.39	0.268	NT
	HIC (SDP)	19.6	8.40	0.210	NT
	Mean	19.4	8.31	0.179	NT
	SD	0.8	0.16	0.092	NA
100	PPB (SDP)	19.0	8.03	0.130	NT
	HGE (SDP)	19.5	7.93	0.310	NT
	FAM (SDP)	19.8	7.61	0.240	NT
	PPB (FDP)	18.2	8.37	0.137	NT
	WAS (FDP)	18.7	8.44	0.056	NT
	BLS (SDP)	20.6	8.18	0.714	Chronic
	MUC (SDP)	19.9	8.33	0.445	NT
	HIC (SDP)	19.6	8.35	0.367	NT
	Mean	19.4	8.22	0.300	NT
	SD	0.7	0.28	0.212	NA
200	PPB (SDP)	19.0	8.09	0.240	NT
	HGE (SDP)	19.6	7.40	0.530	NT
	FAM (SDP)	19.9	7.48	0.440	NT
	PPB (FDP)	18.3	8.34	0.225	NT
	WAS (FDP)	18.9	8.41	0.047	NT
	BLS (SDP)	20.4	8.14	1.054	Chronic
	MUC (SDP)	20.0	8.29	0.853	Chronic
	HIC (SDP)	20.3	8.18	0.243	NT
	Mean	19.5	8.15	0.454	NT
	SD	0.7	0.39	0.345	NA
300	PPB (SDP)	19.2	7.82	0.440	NT
	HGE (SDP)	19.3	7.34	0.450	NT
	FAM (SDP)	19.7	7.25	0.630	NT
	PPB (FDP)	18.6	8.29	0.296	NT
	WAS (FDP)	18.7	8.38	0.123	NT
	BLS (SDP)	20.7	8.02	1.452	Chronic
	MUC (SDP)	20.1	8.25	1.047	Chronic
	HIC (SDP)	20.3	8.04	0.302	NT
	Mean	19.6	8.07	0.593	NT
	SD	0.8	0.43	0.445	NA
300 HD	PPB (SDP)	19.1	8.07	0.510	NT
	HGE (SDP)	19.5	7.20	0.500	NT
	FAM (SDP)	19.7	7.17	0.550	NT
	PPB (FDP)	18.2	8.31	0.193	NT
	WAS (FDP)	18.5	8.42	0.086	NT
	BLS (SDP)	19.6	8.32	0.230	NT
	MUC (SDP)	20.1	8.24	0.581	Chronic
	HIC (SDP)	19.7	8.23	0.489	NT
	Mean	19.3	8.17	0.392	NT
	SD	0.7	0.51	0.191	NA

NT = non-toxic levels of total ammonia nitrogen (below chronic and acute levels).

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Appendix 7. Glochidia Viability

Study Number: AEH-11-PSEUDO-01	
Lab Notebook: 1	
Data Source: File Folder: 11	
Forms: Initial Viability and Concentration Determination of Glochidia	
File Name: I:\AEH-11-PSEUDO-01\Data\Glochidia Viability Summary\[glochidia viability initial.xlsx]Initial Viability Coversheet	

Action	Date	Initials
Created......	13-Mar-15	TJS TJS
Revised......	13-Mar-15	TJS TJS
Reviewed...	13-MAR-15	TJS
Certified...	3/19/15	JA

Initial (Preexposure) Glochidia Viability

Test Article: *Pseudomonas fluorescens* (*Pf*-CL145A) spray dried powder (SDP) and freeze dried powder (FDP)
Article Lot #s: SDP = MBI-401-110308AI-BD-3 and MBI-401 SDP 4655-12-Mix; FDP = 110607WB-FD-E
Exposure Dates: 12-May-11, 18-Oct-11, 17-Jan-12, and 19-Jan-12
Species Tested: PPB, FAM, HGE, WAS, BLS, MUC, and HIC

Data Explanation:

Species Abbreviations

- PPB - plain pocketbook (*Lampsilis cardium*)
- FAM - fatmucket (*Lampsilis siliquoidea*)
- HGE - Higgins eye (*Lampsilis higginsii*)
- WAS - washboard (Megalonaias nervosa)
- BLS - black sandshell (*Ligumia recta*)
- MUC - mucket (*Actinonaias ligamentina*)
- HIC - hickorynut (*Obovaria olivaria*)

Glochidia "closed before salt" and "open after salt" are considered non-viable.

Data anomalies and deviations:

Washboard used four donor mussels for glochidia; all other species used three donor mussels.

Glochidia from black sandshell mussel #2 was evaluated three times for viability; the combined value of all three samples is reported.

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Initial (Preexposure) Viability Summary

Species	Formulation	Test Date	Mussel number	Total glochidia	Glochidia closed before salt	Glochidia open after salt	Percent Viability
PPB	SDP	12-May-11	1	467	14	22	92.3
			2	537	8	19	95.0
			3	927	10	45	94.1
			Grand Mean				93.8
			SD				1.4
HGE	SDP	12-May-11	1	129	32	17	62.0
			2	168	6	14	88.1
			3	357	13	14	92.4
			Grand Mean				80.8
			SD				16.5
FAM	SDP	12-May-11	1	548	22	48	87.2
			2	297	53	27	73.1
			3	456	34	55	80.5
			Grand Mean				80.3
			SD				7.1
PPB	FDP	18-Oct-11	1	212	4	12	92.5
			2	222	26	13	82.4
			3	150	15	16	79.3
			Grand Mean				84.7
			SD				6.9
WAS	FDP	18-Oct-11	1	124	1	1	98.4
			2	155	1	0	99.4
			3	51	4	1	90.2
			4	104	0	0	100.0
			Grand Mean				96.5
			SD				5.5
BLS	SDP	17-Jan-12	1	312	10	22	89.7
			2	1083	51	199	76.9
			3	405	12	41	86.9
			Grand Mean				84.5
			SD				6.7
MUC	SDP	17-Jan-12	1	161	6	8	91.3
			2	153	6	3	94.1
			3	287	3	18	92.7
			Grand Mean				92.7
			SD				1.4
HIC	SDP	19-Jan-12	1	366	15	39	85.2
			2	352	20	31	85.5
			3	309	27	12	87.4
			Grand Mean				86.0
			SD				1.2

```
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

FOOTNOTE1 'Performed by J. Luoma; SAS version' &SYSVER &SYSTIME &SYSDATE;

options ls=97 ps=57 formdlim='-' pageno = 1 nocenter nodate nosource2;

title1 h=2 'Effects of Pseudomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel spec
title2 h=2 'Statistical analysis of unadjusted glochidia viability following 24-h static exposure to \
title3 h=2 'Analysis Completion Date:18March2015 Analysis prepared by: JAL, SAS Version:';
title4 h=2 &SYSVER &SYSTIME &SYSDATE;
/************************************************************
* SAS ver 9.4       Analysis prepared by:  JAL              *
* Analysis completion date:  18March2015   JAL              *
************************************************************/

/***************************************************************************************************
* Variable Names:
* sps = three letter code for mussel species
*           BLS = Black sandshell (Ligumia recta)
*           FAM = Fatmucket (Lampsilis siliquoidea
*           WAS = Washboard (Megalonaias nervosa)
*           HGE = Higgins eye (Lampsilis higginsii)
*           PPB = Plain pocketbook (Lapsilis cardium)
*           HIC = Hickorynut (Obovaria olivaria)
*           MUC = Mucket (Actinonaias ligamentina)
* form = formulation (FDP [freeze dried powder] vs. SDP [spray dried powder])
* conc = concentration (in mg/L)
*           0 = control (0 mg/L)
*           50 = 50 mg/L active ingredient
*           100 = 100 mg/L active ingredient
*           200 = 200 mg/L active ingredient
*           300 = 300 mg/L active ingredient
*           400 = 300 mg/L heat deative
* cham = exposure chamber ID
*             i.e., 3A5 = test system (1, 2 or 3), Block ID (A or B), and Position in Block (1 - 6)
* time = time post exposure assessment occurred
* tot = total number of glochidia within sample
* cbs = number of glochidia closed prior to salt solution
* oas = number of glochidia open after salt solution
***************************************************************************************************

data glochidia; set Pseudo01.viability;
via_glo = tot - cbs - oas;
pctvia_glo = via_glo/tot*100;
run;

proc sort data=glochidia; by sps form conc; run;
proc print data=glochidia; run;
Title2 'Mean Unadjusted Viability of Glochidia by Species, Time, Form (SDP or FDP) and Treatment Grou
proc means data = glochidia mean std clm fw=8;
by sps form;
class conc time;
var tot cbs oas via_glo pctvia_glo;
run;

/***************************************************************************************************
```

```
* The exposure concentrations were renamed to place an alpha character in front of all concentrations,
* variable string to be first to set the variable character length (ie: 300HD vs 0).  SAS assumes the
* therefore, the control was given the last alpha character and thereby other concentrations will be (
* 50 mg/L = a
* 100 mg/L = b
* 200 mg/L = c
* 300 mg/L = d
* 300 mg/L HD (coded as 400 in dataset) = e
* 0 mg/L = f
**************************************************************************************************;
data glochidia2; set glochidia;
 If conc = '0' then conca = 'f';
 If conc = '50' then conca = 'a';
 If conc = '100' then conca = 'b';
 If conc = '200' then conca = 'c';
 If conc = '300' then conca = 'd';
 If conc = '400' then conca = 'e';
 run;
proc sort data=glochidia2; by form sps conca time; run;

proc glimmix data = glochidia2;
title2 'Overall Model: Glochidia Viability (unadjusted) - At 6, 12 and 24 h';
by form sps;
class conca time;
model via_glo/tot  = conca|time / d = bin link = logit noint s or;
  lsmeans conca / pdiff cl ilink or;
  lsmeans time / pdiff cl ilink or;
  lsmeans conca*time / pdiff cl ilink or;
  random _residual_;
  run;

quit;
```

```
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

FOOTNOTE1 'Performed by J. Luoma; SAS version' &SYSVER &SYSTIME &SYSDATE;
WARNING: The FOOTNOTE statement is ambiguous due to invalid options or unquoted text.

options ls=97 ps=57 formdlim='-' pageno = 1 nocenter nodate nosource2;

title1 h=2 'Effects of Pseudomonas fluorescens (Pf-CL145A) to glochidia from seven unionid
mussel species';
title2 h=2 'Statistical analysis of unadjusted glochidia viability following 24-h static
exposure to various concentrations of Pf-CL145A';
title3 h=2 'Analysis Completion Date:18March2015 Analysis prepared by: JAL, SAS Version:';
title4 h=2 &SYSVER &SYSTIME &SYSDATE;
WARNING: The TITLE statement is ambiguous due to invalid options or unquoted text.
/************************************************************
* SAS ver 9.4       Analysis prepared by:  JAL              *
* Analysis completion date:  18March2015                    *
************************************************************/

/*******************************************************************************************
*************
* Variable Names:
              *
* sps = three letter code for mussel species
              *
*             BLS = Black sandshell (Ligumia recta)
              *
*             FAM = Fatmucket (Lampsilis siliquoidea
              *
*             WAS = Washboard (Megalonaias nervosa)
              *
*             HGE = Higgins eye (Lampsilis higginsii)
              *
*             PPB = Plain pocketbook (Lapsilis cardium)
              *
*             HIC = Hickorynut (Obovaria olivaria)
              *
*             MUC = Mucket (Actinonaias ligamentina)
              *
* form = formulation (FDP [freeze dried powder] vs. SDP [spray dried powder])
              *
* conc = concentration (in mg/L)
              *
*             0 = control (0 mg/L)
              *
*             50 = 50 mg/L active ingredient
              *
*             100 = 100 mg/L active ingredient
              *
*             200 = 200 mg/L active ingredient
              *
*             300 = 300 mg/L active ingredient
              *
*             400 = 300 mg/L heat deative
              *
* cham = exposure chamber ID
```

```
122!             *
123  *              i.e., 3A5 = test system (1, 2 or 3), Block ID (A or B), and Position in Block
123! (1 - 6)      *
124  * time = time post exposure assessment occurred
124!             *
125  * tot = total number of glochidia within sample
125!             *
126  * cbs = number of glochidia closed prior to salt solution
126!             *
127  * oas = number of glochidia open after salt solution
127!             *
128  ******************************************************************************************
128! ************/
129
130  data glochidia; set Pseudo01.viability;
131  via_glo = tot - cbs - oas;
132  pctvia_glo = via_glo/tot*100;
133  run;

NOTE: There were 397 observations read from the data set PSEUDO01.VIABILITY.
NOTE: The data set WORK.GLOCHIDIA has 397 observations and 11 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


134
135  proc sort data=glochidia; by sps form conc; run;

NOTE: There were 397 observations read from the data set WORK.GLOCHIDIA.
NOTE: The data set WORK.GLOCHIDIA has 397 observations and 11 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.00 seconds
      cpu time            0.01 seconds


136  proc print data=glochidia; run;
NOTE: Writing HTML Body file: sashtml1.htm

NOTE: There were 397 observations read from the data set WORK.GLOCHIDIA.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.62 seconds
      cpu time            0.39 seconds


137  Title2 'Mean Unadjusted Viability of Glochidia by Species, Time, Form (SDP or FDP) and
137! Treatment Group';
138  proc means data = glochidia mean std clm fw=8;
139  by sps form;
140  class conc time;
141  var tot cbs oas via_glo pctvia_glo;
142  run;

NOTE: There were 397 observations read from the data set WORK.GLOCHIDIA.
NOTE: PROCEDURE MEANS used (Total process time):
      real time           0.35 seconds
```

```
      cpu time            0.34 seconds


143
144  /*********************************************************************************************
144! *************************************************
145  * The exposure concentrations were renamed to place an alpha character in front of all
145! concentrations.  SAS requires the longest          *
146  * variable string to be first to set the variable character length (ie: 300HD vs 0).  SAS
146! assumes the last entry to be the control,          *
147  * therefore, the control was given the last alpha character and thereby other concentrations
147!  will be compared to it.                           *
148  * 50 mg/L = a
148!                                                    *
149  * 100 mg/L = b
149!                                                    *
150  * 200 mg/L = c
150!                                                    *
151  * 300 mg/L = d
151!                                                    *
152  * 300 mg/L HD (coded as 400 in dataset) = e
152!                                                    *
153  * 0 mg/L = f
153!                                                    *
154  *********************************************************************************************
154! *************************************************/
155  data glochidia2; set glochidia;
156   If conc = '0' then conca = 'f';
157   If conc = '50' then conca = 'a';
158   If conc = '100' then conca = 'b';
159   If conc = '200' then conca = 'c';
160   If conc = '300' then conca = 'd';
161   If conc = '400' then conca = 'e';
162   run;

NOTE: Character values have been converted to numeric values at the places given by:
      (Line):(Column).
      156:12   157:12   158:12   159:12   160:12   161:12
NOTE: There were 397 observations read from the data set WORK.GLOCHIDIA.
NOTE: The data set WORK.GLOCHIDIA2 has 397 observations and 12 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


163  proc sort data=glochidia2; by form sps conca time; run;

NOTE: There were 397 observations read from the data set WORK.GLOCHIDIA2.
NOTE: The data set WORK.GLOCHIDIA2 has 397 observations and 12 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds


164
165  proc glimmix data = glochidia2;
```

```
title2 'Overall Model: Glochidia Viability (unadjusted) - At 6, 12 and 24 h';
by form sps;
class conca time;
model via_glo/tot  = conca|time / d = bin link = logit noint s or;
  lsmeans conca / pdiff cl ilink or;
  lsmeans time / pdiff cl ilink or;
  lsmeans conca*time / pdiff cl ilink or;
  random _residual_;
  run;



NOTE: The model does not contain an intercept. Columns of X are scaled only and not centered.
NOTE: Convergence criterion (ABSGCONV=0.00001) satisfied.
NOTE: The above message was for the following BY group:
      form=FDP sps=PPB
NOTE: The model does not contain an intercept. Columns of X are scaled only and not centered.
NOTE: Convergence criterion (ABSGCONV=0.00001) satisfied.
NOTE: The above message was for the following BY group:
      form=FDP sps=WAS
NOTE: The model does not contain an intercept. Columns of X are scaled only and not centered.
NOTE: Convergence criterion (ABSGCONV=0.00001) satisfied.
NOTE: The above message was for the following BY group:
      form=SDP sps=BLS
NOTE: The model does not contain an intercept. Columns of X are scaled only and not centered.
NOTE: Convergence criterion (GCONV=1E-8) satisfied.
NOTE: The above message was for the following BY group:
      form=SDP sps=FAM
NOTE: The model does not contain an intercept. Columns of X are scaled only and not centered.
NOTE: Convergence criterion (ABSGCONV=0.00001) satisfied.
NOTE: The above message was for the following BY group:
      form=SDP sps=HGE
NOTE: The model does not contain an intercept. Columns of X are scaled only and not centered.
NOTE: Convergence criterion (GCONV=1E-8) satisfied.
NOTE: The above message was for the following BY group:
      form=SDP sps=HIC
NOTE: The model does not contain an intercept. Columns of X are scaled only and not centered.
NOTE: Convergence criterion (GCONV=1E-8) satisfied.
NOTE: The above message was for the following BY group:
      form=SDP sps=MUC
NOTE: The model does not contain an intercept. Columns of X are scaled only and not centered.
NOTE: Convergence criterion (GCONV=1E-8) satisfied.
NOTE: The above message was for the following BY group:
      form=SDP sps=PPB
NOTE: PROCEDURE GLIMMIX used (Total process time):
      real time           1.42 seconds
      cpu time            1.29 seconds


quit;
```

Effects of Pseudomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel species
Statistical analysis of unadjusted glochidia viability following 24-h static exposure to various concentrations of Pf-CL145A
Analysis Completion Date:18March2015 Analysis prepared by: JAL, SAS Version:
9.3 08:43 18MAR15

3-18-15
JL

Obs	sps	form	conc	cham	time	tot	cbs	oas	F9	via_glo	pctvia_glo
1	BLS	SDP	0	1A5	6	241	8	28		205	85.0622
2	BLS	SDP	0	1B1	6	337	8	42		287	85.1632
3	BLS	SDP	0	1C6	6	314	8	33		273	86.9427
4	BLS	SDP	0	1A5	12	262	3	26		233	88.9313
5	BLS	SDP	0	1B1	12	231	6	18		207	89.6104
6	BLS	SDP	0	1C6	12	139	2	12		125	89.9281
7	BLS	SDP	0	1A5	24	612	12	83		517	84.4771
8	BLS	SDP	0	1B1	24	206	4	24		178	86.4078
9	BLS	SDP	0	1C6	24	572	26	74		472	82.5175
10	BLS	SDP	50	1A1	6	252	183	32		37	14.6825
11	BLS	SDP	50	1A3	6	290	284	3		3	1.0345
12	BLS	SDP	50	1C1	6	206	132	34		40	19.4175
13	BLS	SDP	50	1A1	12	223	157	22		44	19.7309
14	BLS	SDP	50	1A3	12	399	293	30		76	19.0476
15	BLS	SDP	50	1C1	12	248	221	3		24	9.6774
16	BLS	SDP	50	1A1	24	353	287	17		49	13.8810
17	BLS	SDP	50	1A3	24	137	98	16		23	16.7883
18	BLS	SDP	50	1C1	24	111	92	4		15	13.5135
19	BLS	SDP	100	1B2	6	166	164	2		0	0.0000
20	BLS	SDP	100	1B3	6	351	330	16		5	1.4245
21	BLS	SDP	100	1C5	6	144	136	8		0	0.0000
22	BLS	SDP	100	1B2	12	250	208	20		22	8.8000
23	BLS	SDP	100	1B3	12	198	155	19		24	12.1212
24	BLS	SDP	100	1C5	12	125	97	21		7	5.6000
25	BLS	SDP	100	1B2	24	181	166	12		3	1.6575
26	BLS	SDP	100	1B3	24	252	221	11		20	7.9365
27	BLS	SDP	100	1C5	24	222	179	18		25	11.2613
28	BLS	SDP	200	1A6	6	352	275	69		8	2.2727
29	BLS	SDP	200	1B5	6	168	138	25		5	2.9762
30	BLS	SDP	200	1B6	6	224	222	2		0	0.0000
31	BLS	SDP	200	1A6	12	242	235	5		2	0.8264
32	BLS	SDP	200	1B5	12	210	201	6		3	1.4286
33	BLS	SDP	200	1B6	12	304	280	20		4	1.3158
34	BLS	SDP	200	1A6	24	351	338	8		5	1.4245
35	BLS	SDP	200	1B5	24	302	256	38		8	2.6490
36	BLS	SDP	200	1B6	24	352	299	33		20	5.6818
37	BLS	SDP	300	1A2	6	159	117	42		0	0.0000
38	BLS	SDP	300	1B4	6	312	286	17		9	2.8846
39	BLS	SDP	300	1C2	6	250	234	15		1	0.4000
40	BLS	SDP	300	1A2	12	135	135	0		0	0.0000
41	BLS	SDP	300	1B4	12	93	86	7		0	0.0000

42	BLS	SDP	300	1C2	12	165	151	14		0	0.0000
43	BLS	SDP	300	1A2	24	284	270	14		0	0.0000
44	BLS	SDP	300	1B4	24	255	247	5		3	1.1765
45	BLS	SDP	300	1C2	24	383	373	6		4	1.0444
46	BLS	SDP	400	1A4	6	219	208	11		0	0.0000
47	BLS	SDP	400	1C3	6	254	213	41		0	0.0000
48	BLS	SDP	400	1C4	6	237	195	36		6	2.5316
49	BLS	SDP	400	1A4	12	281	268	13		0	0.0000
50	BLS	SDP	400	1C3	12	157	147	8		2	1.2739
51	BLS	SDP	400	1C4	12	133	119	12		2	1.5038
52	BLS	SDP	400	1A4	24	485	471	11		3	0.6186
53	BLS	SDP	400	1C3	24	381	367	8		6	1.5748
54	BLS	SDP	400	1C4	24	302	295	7		0	0.0000
55	FAM	SDP	0	3A2	6	273	36	46		191	69.9634
56	FAM	SDP	0	3B2	6	210	29	34		147	70.0000
57	FAM	SDP	0	3B3	6	265	32	29		204	76.9811
58	FAM	SDP	0	3A2	12	282	54	32		196	69.5035
59	FAM	SDP	0	3B2	12	328	55	79		194	59.1463
60	FAM	SDP	0	3B3	12	521	63	88		370	71.0173
61	FAM	SDP	0	3A2	24	307	48	55		204	66.4495
62	FAM	SDP	0	3B2	24	247	50	41		156	63.1579
63	FAM	SDP	0	3B3	24	244	19	33		192	78.6885
64	FAM	SDP	50	3B5	6	456	93	98		265	58.1140
65	FAM	SDP	50	3C5	6	276	57	71		148	53.6232
66	FAM	SDP	50	3C6	6	239	42	57		140	58.5774
67	FAM	SDP	50	3B5	12	430	146	36		248	57.6744
68	FAM	SDP	50	3C5	12	368	109	53		206	55.9783
69	FAM	SDP	50	3C6	12	207	33	42		132	63.7681
70	FAM	SDP	50	3B5	24	327	38	86		203	62.0795
71	FAM	SDP	50	3C5	24	334	64	76		194	58.0838
72	FAM	SDP	50	3C6	24	278	70	68		140	50.3597
73	FAM	SDP	100	3A1	6	237	126	37		74	31.2236
74	FAM	SDP	100	3A3	6	303	238	25		40	13.2013
75	FAM	SDP	100	3A5	6	351	119	83		149	42.4501
76	FAM	SDP	100	3A1	12	409	183	48		178	43.5208
77	FAM	SDP	100	3A3	12	221	64	53		104	47.0588
78	FAM	SDP	100	3A5	12	159	68	27		64	40.2516
79	FAM	SDP	100	3A1	24	112	41	20		51	45.5357
80	FAM	SDP	100	3A3	24	208	60	54		94	45.1923
81	FAM	SDP	100	3A5	24	313	146	60		107	34.1853
82	FAM	SDP	200	3B4	6	380	308	47		25	6.5789
83	FAM	SDP	200	3B6	6	526	407	53		66	12.5475
84	FAM	SDP	200	3C4	6	201	59	64		78	38.8060
85	FAM	SDP	200	3B4	12	198	127	40		31	15.6566
86	FAM	SDP	200	3B6	12	134	69	19		46	34.3284
87	FAM	SDP	200	3C4	12	214	172	23		19	8.8785

	FAM	SDP	200	3B4	24	271	190	43		38	14.0221
89	FAM	SDP	200	3B6	24	205	147	28		30	14.6341
90	FAM	SDP	200	3C4	24	271	193	61		17	6.2731
91	FAM	SDP	300	3B1	6	482	422	56		4	0.8299
92	FAM	SDP	300	3C1	6	501	224	121		156	31.1377
93	FAM	SDP	300	3C3	6	242	204	29		9	3.7190
94	FAM	SDP	300	3B1	12	298	233	55		10	3.3557
95	FAM	SDP	300	3C1	12	240	195	29		16	6.6667
96	FAM	SDP	300	3C3	12	206	173	26		7	3.3981
97	FAM	SDP	300	3B1	24	217	170	35		12	5.5300
98	FAM	SDP	300	3C1	24	176	145	23		8	4.5455
99	FAM	SDP	300	3C3	24	215	130	68		17	7.9070
100	FAM	SDP	400	3A4	6	216	155	56		5	2.3148
101	FAM	SDP	400	3A6	6	212	164	38		10	4.7170
102	FAM	SDP	400	3C2	6	205	166	39		0	0.0000
103	FAM	SDP	400	3A4	12	310	252	52		6	1.9355
104	FAM	SDP	400	3A6	12	298	229	54		15	5.0336
105	FAM	SDP	400	3C2	12	187	131	56		0	0.0000
106	FAM	SDP	400	3A4	24	281	250	29		2	0.7117
107	FAM	SDP	400	3A6	24	86	69	15		2	2.3256
108	FAM	SDP	400	3C2	24	336	293	36		7	2.0833
109	HGE	SDP	0	2B1	6	258	18	31		209	81.0078
110	HGE	SDP	0	2C3	6	248	18	76		154	62.0968
111	HGE	SDP	0	2C6	6	168	10	34		124	73.8095
112	HGE	SDP	0	2B1	12	254	29	57		168	66.1417
113	HGE	SDP	0	2C3	12	481	48	124		309	64.2412
114	HGE	SDP	0	2C6	12	210	13	38		159	75.7143
115	HGE	SDP	0	2C3	24	318	28	66		224	70.4403
116	HGE	SDP	0	2C3-R2	24	377	46	108		223	59.1512
117	HGE	SDP	0	2C6	24	557	61	70		426	76.4811
118	HGE	SDP	0	2C6-R2	24	408	44	73		291	71.3235
119	HGE	SDP	50	2A1	6	224	11	39		174	77.6786
120	HGE	SDP	50	2A2	6	616	26	88		502	81.4935
121	HGE	SDP	50	2A4	6	204	24	29		151	74.0196
122	HGE	SDP	50	2A1	12	225	38	48		139	61.7778
123	HGE	SDP	50	2A2	12	230	21	49		160	69.5652
124	HGE	SDP	50	2A4	12	339	36	50		253	74.6313
125	HGE	SDP	50	2A1	24	506	49	67		390	77.0751
126	HGE	SDP	50	2A2	24	514	55	68		391	76.0700
127	HGE	SDP	50	2A4	24	464	34	102		328	70.6897
128	HGE	SDP	100	2A3	6	261	47	51		163	62.4521
129	HGE	SDP	100	2C4	6	396	40	101		255	64.3939
130	HGE	SDP	100	2C5	6	414	103	51		260	62.8019
131	HGE	SDP	100	2A3	12	235	50	38		147	62.5532
132	HGE	SDP	100	2C4	12	273	35	60		178	65.2015
133	HGE	SDP	100	2C5	12	362	41	72		249	68.7845

	HGE	SDP	100	2A3	24	629	95	87		447	71.0652
135	HGE	SDP	100	2C4	24	423	87	69		267	63.1206
136	HGE	SDP	100	2C5	24	413	105	51		257	62.2276
137	HGE	SDP	200	2A6	6	492	120	62		310	63.0081
138	HGE	SDP	200	2B2	6	357	73	93		191	53.5014
139	HGE	SDP	200	2B5	6	512	121	117		274	53.5156
140	HGE	SDP	200	2A6	12	266	146	51		69	25.9398
141	HGE	SDP	200	2B2	12	344	65	73		206	59.8837
142	HGE	SDP	200	2B5	12	274	81	48		145	52.9197
143	HGE	SDP	200	2A6	24	296	83	56		157	53.0405
144	HGE	SDP	200	2B2	24	369	135	54		180	48.7805
145	HGE	SDP	200	2B5	24	441	272	56		113	25.6236
146	HGE	SDP	300	2B3	6	297	251	25		21	7.0707
147	HGE	SDP	300	2C1	6	279	148	48		83	29.7491
148	HGE	SDP	300	2C2	6	422	98	111		213	50.4739
149	HGE	SDP	300	2B3	12	240	107	60		73	30.4167
150	HGE	SDP	300	2C1	12	267	67	95		105	39.3258
151	HGE	SDP	300	2C2	12	215	52	63		100	46.5116
152	HGE	SDP	300	2B3	24	171	109	54		8	4.6784
153	HGE	SDP	300	2C1	24	354	287	34		33	9.3220
154	HGE	SDP	300	2C2	24	140	38	28		74	52.8571
155	HGE	SDP	400	2A5	6	327	216	60		51	15.5963
156	HGE	SDP	400	2B4	6	276	168	41		67	24.2754
157	HGE	SDP	400	2B6	6	139	102	37		0	0.0000
158	HGE	SDP	400	2A5	12	229	161	44		24	10.4803
159	HGE	SDP	400	2B4	12	192	143	32		17	8.8542
160	HGE	SDP	400	2B6	12	206	142	45		19	9.2233
161	HGE	SDP	400	2A5	24	137	89	42		6	4.3796
162	HGE	SDP	400	2B4	24	216	88	74		54	25.0000
163	HGE	SDP	400	2B6	24	330	229	60		41	12.4242
164	HIC	SDP	0	3A2	6	258	65	21		172	66.6667
165	HIC	SDP	0	3C1	6	156	8	12		136	87.1795
166	HIC	SDP	0	3C4	6	259	12	24		223	86.1004
167	HIC	SDP	0	3A2	12	257	6	22		229	89.1051
168	HIC	SDP	0	3C1	12	216	19	26		171	79.1667
169	HIC	SDP	0	3C4	12	144	5	8		131	90.9722
170	HIC	SDP	0	3A2	24	192	8	14		170	88.5417
171	HIC	SDP	0	3C1	24	376	22	34		320	85.1064
172	HIC	SDP	0	3C4	24	359	14	31		314	87.4652
173	HIC	SDP	50	3A5	6	267	16	21		230	86.1423
174	HIC	SDP	50	3B3	6	191	21	10		160	83.7696
175	HIC	SDP	50	3B6	6	274	26	13		235	85.7664
176	HIC	SDP	50	3A5	12	172	21	10		141	81.9767
177	HIC	SDP	50	3B3	12	197	24	8		165	83.7563
178	HIC	SDP	50	3B6	12	186	9	14		163	87.6344
179	HIC	SDP	50	3A5	24	223	9	8		206	92.3767

	HIC	SDP	50	3B3	24	3C3	18	22		263	86.7987
181	HIC	SDP	50	3B6	24	233	18	9		206	88.4120
182	HIC	SDP	100	3B1	6	232	39	32		161	69.3966
183	HIC	SDP	100	3C2	6	231	114	13		104	45.0216
184	HIC	SDP	100	3C5	6	292	81	11		200	68.4932
185	HIC	SDP	100	3B1	12	214	36	6		172	80.3738
186	HIC	SDP	100	3C2	12	195	18	6		171	87.6923
187	HIC	SDP	100	3C5	12	216	75	4		137	63.4259
188	HIC	SDP	100	3B1	24	404	36	21		347	85.8911
189	HIC	SDP	100	3C2	24	278	59	17		202	72.6619
190	HIC	SDP	100	3C5	24	257	51	10		196	76.2646
191	HIC	SDP	200	3A3	6	231	142	19		70	30.3030
192	HIC	SDP	200	3A4	6	176	90	18		68	38.6364
193	HIC	SDP	200	3C6	6	285	117	17		151	52.9825
194	HIC	SDP	200	3A3	12	134	86	3		45	33.5821
195	HIC	SDP	200	3A4	12	186	84	8		94	50.5376
196	HIC	SDP	200	3C6	12	138	41	5		92	66.6667
197	HIC	SDP	200	3A3	24	349	114	10		225	64.4699
198	HIC	SDP	200	3A4	24	203	78	12		113	55.6650
199	HIC	SDP	200	3C6	24	197	66	9		122	61.9289
200	HIC	SDP	300	3A6	6	293	192	13		88	30.0341
201	HIC	SDP	300	3B2	6	173	160	3		10	5.7803
202	HIC	SDP	300	3B5	6	266	226	10		30	11.2782
203	HIC	SDP	300	3A6	12	204	123	8		73	35.7843
204	HIC	SDP	300	3B2	12	200	107	12		81	40.5000
205	HIC	SDP	300	3B5	12	206	98	23		85	41.2621
206	H C	SDP	300	3A6	24	191	138	9		44	23.0366
207	HIC	SDP	300	3B2	24	140	98	4		38	27.1429
208	HIC	SDP	300	3B5	24	231	156	7		68	29.4372
209	HIC	SDP	400	3A1	6	362	262	25		75	20.7182
210	HIC	SDP	400	3B4	6	317	274	16		27	8.5174
211	HIC	SDP	400	3C3	6	246	209	7		30	12.1951
212	HIC	SDP	400	3A1	12	249	231	15		3	1.2048
213	HIC	SDP	400	3B4	12	338	313	22		3	0.8876
214	HIC	SDP	400	3C3	12	251	244	3		4	1.5936
215	HIC	SDP	400	3A1	24	290	287	2		1	0.3448
216	HIC	SDP	400	3B4	24	314	307	6		1	0.3185
217	HIC	SDP	400	3C3	24	226	221	3		2	0.8850
218	MUC	SDP	0	2B4	6	198	15	17		166	83.8384
219	MUC	SDP	0	2B5	6	132	2	12		118	89.3939
220	MUC	SDP	0	2C1	6	257	7	12		238	92.6070
221	MUC	SDP	0	2B4	12	178	2	11		165	92.6966
222	MUC	SDP	0	2B5	12	213	0	12		201	94.3662
223	MUC	SDP	0	2C1	12	254	6	5		243	95.6693
224	MUC	SDP	0	2B4	24	261	7	19		235	90.0383
225	MUC	SDP	0	2B5	24	361	1	20		340	94.1828

	MUC	SDP	0	2C1	24	251	8	8		235	93.6255
227	MUC	SDP	50	2A4	6	349	51	36		262	75.0716
228	MUC	SDP	50	2B6	6	128	43	3		82	64.0625
229	MUC	SDP	50	2C3	6	295	59	12		224	75.9322
230	MUC	SDP	50	2A4	12	221	63	14		144	65.1584
231	MJC	SDP	50	2B6	12	113	53	4		56	49.5575
232	MUC	SDP	50	2C3	12	193	17	12		164	84.9741
233	MUC	SDP	50	2A4	24	328	49	14		265	80.7927
234	MUC	SDP	50	2B6	24	146	49	8		89	60.9589
235	MUC	SDP	50	2C3	24	194	19	9		166	85.5670
236	MUC	SDP	100	2A2	6	190	61	13		116	61.0526
237	MUC	SDP	100	2B2	6	154	26	7		121	78.5714
238	MUC	SDP	100	2C6	6	466	78	29		359	77.0386
239	MUC	SDP	100	2A2	12	210	91	10		109	51.9048
240	MUC	SDP	100	2B2	12	138	46	14		78	56.5217
241	MUC	SDP	100	2C6	12	215	26	7		182	84.6512
242	MUC	SDP	100	2A2	24	191	42	5		144	75.3927
243	MUC	SDP	100	2B2	24	209	87	11		111	53.1100
244	MUC	SDP	100	2C6	24	213	71	10		132	61.9718
245	MUC	SDP	200	2A5	6	116	48	8		60	51.7241
246	MUC	SDP	200	2A6	6	202	82	23		97	48.0198
247	MUC	SDP	200	2C2	6	399	291	21		87	21.8045
248	MUC	SDP	200	2A5	12	175	83	17		75	42.8571
249	MUC	SDP	200	2A6	12	179	92	11		76	42.4581
250	MUC	SDP	200	2C2	12	362	227	9		126	34.8066
251	MUC	SDP	200	2A5	24	251	170	16		65	25.8964
252	MUC	SDP	200	2A6	24	407	241	10		156	38.3292
253	MUC	SDP	200	2C2	24	497	355	14		128	25.7545
254	MUC	SDP	300	2B1	6	265	199	14		52	19.6226
255	MUC	SDP	300	2B3	6	251	232	6		13	5.1793
256	MUC	SDP	300	2C4	6	323	283	6		34	10.5263
257	MUC	SDP	300	2B1	12	247	212	4		31	12.5506
258	MUC	SDP	300	2B3	12	198	181	2		15	7.5758
259	MUC	SDP	300	2C4	12	116	102	2		12	10.3448
260	MUC	SDP	300	2B1	24	192	171	3		18	9.3750
261	MUC	SDP	300	2B3	24	297	281	9		7	2.3569
262	MUC	SDP	300	2C4	24	451	417	6		28	6.2084
263	MUC	SDP	400	2A1	6	292	272	4		16	5.4795
264	MUC	SDP	400	2A3	6	355	342	8		5	1.4085
265	MUC	SDP	400	2C5	6	280	259	10		11	3.9286
266	MUC	SDP	400	2A1	12	237	201	14		22	9.2827
267	MUC	SDP	400	2A3	12	127	100	11		16	12.5984
268	MUC	SDP	400	2C5	12	184	175	4		5	2.7174
269	MUC	SDP	400	2A1	24	292	278	6		8	2.7397
270	MUC	SDP	400	2A3	24	308	276	4		28	9.0909
271	MUC	SDP	400	2C5	24	157	149	2		6	3.8217

	PPB	FDP	0	3A6	6	214	17	16		181	84.5794
273	PPB	FDP	0	3B5	6	89	7	6		76	85.3933
274	PPB	FDP	0	3B6	6	125	18	11		96	76.8000
275	PPB	FDP	0	3A6	12	133	3	20		110	82.7068
276	PPB	FDP	0	3B5	12	314	8	61		245	78.0255
277	PPB	FDP	0	3B6	12	182	5	23		154	84.6154
278	PPB	FDP	0	3A6	24	573	21	74		478	83.4206
279	PPB	FDP	0	3B5	24	279	7	47		225	80.6452
280	PPB	FDP	0	3B6	24	278	17	34		227	81.6547
281	PPB	FDP	50	3A4	6	157	16	17		124	78.9809
282	PPB	FDP	50	3C1	6	74	27	5		42	56.7568
283	PPB	FDP	50	3C2	6	144	22	17		105	72.9167
284	PPB	FDP	50	3A4	12	290	24	62		204	70.3448
285	PPB	FDP	50	3C1	12	160	8	29		123	76.8750
286	PPB	FDP	50	3C2	12	606	34	70		502	82.8383
287	PPB	FDP	50	3A4	24	164	39	21		104	63.4146
288	PPB	FDP	50	3C1	24	368	33	45		290	78.8043
289	PPB	FDP	50	3C2	24	384	30	46		308	80.2083
290	PPB	FDP	100	3B1	6	259	70	21		168	64.8649
291	PPB	FDP	100	3B3	6	190	33	25		132	69.4737
292	PPB	FDP	100	3C6	6	161	47	13		101	62.7329
293	PPB	FDP	100	3B1	12	179	46	22		111	62.0112
294	PPB	FDP	100	3B3	12	130	20	18		92	70.7692
295	PPB	FDP	100	3C6	12	142	15	15		112	78.8732
296	PPB	FDP	100	3B1	24	467	85	60		322	68.9507
297	PPB	FDP	100	3B3	24	405	93	55		257	63.4568
298	PPB	FDP	100	3C6	24	288	38	46		204	70.8333
299	PPB	FDP	200	3A2	6	256	86	52		118	46.0938
300	PPB	FDP	200	3C3	6	299	141	42		116	38.7960
301	PPB	FDP	200	3C5	6	157	47	14		96	61.1465
302	PPB	FDP	200	3A2	12	249	161	38		50	20.0803
303	PPB	FDP	200	3C3	12	815	104	140		571	70.0613
304	PPB	FDP	200	3C5	12	200	151	20		29	14.5000
305	PPB	FDP	200	3A2	24	144	96	27		21	14.5833
306	PPB	FDP	200	3C3	24	592	157	98		337	56.9257
307	PPB	FDP	200	3C5	24	386	281	39		66	17.0984
308	PPB	FDP	300	3A3	6	259	63	64		132	50.9653
309	PPB	FDP	300	3B4	6	272	149	58		65	23.8971
310	PPB	FDP	300	3C4	6	655	218	128		309	47.1756
311	PPB	FDP	300	3A3	12	251	174	66		11	4.3825
312	PPB	FDP	300	3B4	12	223	145	35		43	19.2825
313	PPB	FDP	300	3C4	12	348	289	46		13	3.7356
314	PPB	FDP	300	3A3	24	199	132	45		22	11.0553
315	PPB	FDP	300	3B4	24	714	442	171		101	14.1457
316	PPB	FDP	300	3C4	24	440	330	62		48	10.9091
317	PPB	FDP	400	3A1	6	200	144	45		11	5.5000

	PPB	FDP	400	3A5	6	379	87	114		178	46.9657
319	PPB	FDP	400	3B2	6	418	321	60		37	8.8517
320	PPB	FDP	400	3A1	12	72	58	11		3	4.1667
321	PPB	FDP	400	3A5	12	48	38	7		3	6.2500
322	PPB	FDP	400	3B2	12	242	211	21		10	4.1322
323	PPB	FDP	400	3A1	24	370	277	78		15	4.0541
324	PPB	FDP	400	3A5	24	253	207	38		8	3.1621
325	PPB	FDP	400	3B2	24	267	198	49		20	7.4906
326	PPB	SDP	0	1A3	6	160	2	12		146	91.2500
327	PPB	SDP	0	1B1	6	312	3	18		291	93.2692
328	PPB	SDP	0	1C3	6	426	7	25		394	92.4883
329	PPB	SDP	0	1A3	12	626	39	54		533	85.1438
330	PPB	SDP	0	1B1	12	599	39	53		507	84.6411
331	PPB	SDP	0	1C3	12	604	18	52		534	88.4106
332	PPB	SDP	0	1A3	24	429	12	26		391	91.1422
333	PPB	SDP	0	1B1	24	514	15	22		477	92.8016
334	PPB	SDP	0	1C3	24	276	12	9		255	92.3913
335	PPB	SDP	50	1A1	6	189	16	10		163	86.2434
336	PPB	SDP	50	1B5	6	344	59	13		272	79.0698
337	PPB	SDP	50	1C1	6	447	129	12		306	68.4564
338	PPB	SDP	50	1A1	12	265	24	14		227	85.6604
339	PPB	SDP	50	1B5	12	226	37	14		175	77.4336
340	PPB	SDP	50	1C1	12	624	104	67		453	72.5962
341	PPB	SDP	50	1A1	24	459	55	28		376	81.9172
342	PPB	SDP	50	1B5	24	270	27	16		227	84.0741
343	PPB	SDP	50	1C1	24	339	78	12		249	73.4513
344	PPB	SDP	100	1A2	6	116	16	9		91	78.4483
345	PPB	SDP	100	1A4	6	536	70	42		424	79.1045
346	PPB	SDP	100	1B4	6	170	43	11		116	68.2353
347	PPB	SDP	100	1A2	12	353	321	32		0	0.0000
348	PPB	SDP	100	1A4	12	504	75	40		389	77.1825
349	PPB	SDP	100	1B4	12	354	40	28		286	80.7910
350	PPB	SDP	100	1A2	24	210	33	16		161	76.6667
351	PPB	SDP	100	1A4	24	310	34	125		151	48.7097
352	PPB	SDP	100	1B4	24	324	56	24		244	75.3086
353	PPB	SDP	200	1B2	6	252	60	21		171	67.8571
354	PPB	SDP	200	1C4	6	238	165	6		67	28.1513
355	PPB	SDP	200	1C6	6	145	47	10		88	60.6897
356	PPB	SDP	200	1B2	12	152	47	15		90	59.2105
357	PPB	SDP	200	1C4	12	380	116	36		228	60.0000
358	PPB	SDP	200	1C6	12	177	129	11		37	20.9040
359	PPB	SDP	200	1B2	24	187	50	13		124	66.3102
360	PPB	SDP	200	1C4	24	506	267	14		225	44.4664
361	PPB	SDP	200	1C6	24	242	64	16		162	66.9421
362	PPB	SDP	300	1A5	6	114	75	14		25	21.9298
363	PPB	SDP	300	1C2	6	512	464	31		17	3.3203

	PPB	SDP	300	1C5	6	268	234	22		12	4.4776
365	PPB	SDP	300	1A5	12	1177	938	109		130	11.0450
366	PPB	SDP	300	1C2	12	305	284	13		8	2.6230
367	PPB	SDP	300	1C5	12	460	403	30		27	5.8696
368	PPB	SDP	300	1A5	24	493	399	23		71	14.4016
369	PPB	SDP	300	1C2	24	226	218	4		4	1.7699
370	PPB	SDP	300	1C5	24	295	274	15		6	2.0339
371	PPB	SDP	400	1A6	6	147	108	30		9	6.1224
372	PPB	SDP	400	1B3	6	119	91	23		5	4.2017
373	PPB	SDP	400	1B6	6	210	192	18		0	0.0000
374	PPB	SDP	400	1A6	12	242	193	32		17	7.0248
375	PPB	SDP	400	1B3	12	225	171	37		17	7.5556
376	PPB	SDP	400	1B6	12	322	280	33		9	2.7950
377	PPB	SDP	400	1A6	24	204	147	40		17	8.3333
378	PPB	SDP	400	1B3	24	257	245	4		8	3.1128
379	PPB	SDP	400	1B6	24	322	293	20		9	2.7950
380	WAS	FDP	0	1A5	24	55	9	11		35	63.6364
381	WAS	FDP	C	1B2	24	140	49	19		72	51.4286
382	WAS	FDP	0	1B4	24	73	32	11		30	41.0959
383	WAS	FDP	50	1A1	24	55	16	14		25	45.4545
384	WAS	FDP	50	1C1	24	65	19	9		37	56.9231
385	WAS	FDP	50	1C2	24	56	9	11		36	64.2857
386	WAS	FDP	100	1A2	24	67	23	23		21	31.3433
387	WAS	FDP	100	1A6	24	99	15	37		47	47.4747
388	WAS	FDP	100	1C6	24	104	23	13		68	65.3846
389	WAS	FDP	200	'B5	24	54	16	21		17	31.4815
390	WAS	FDP	200	1C3	24	60	12	19		29	48.3333
391	WAS	FDP	200	1C4	24	64	14	6		44	68.7500
392	WAS	FDP	300	1A4	24	52	8	11		33	63.4615
393	WAS	FDP	300	1B1	24	55	24	18		13	23.6364
394	WAS	FDP	300	1C5	24	69	8	38		23	33.3333
395	WAS	FDP	400	1A3	24	80	62	5		13	16.2500
396	WAS	FDP	400	1B3	24	80	34	16		30	37.5000
397	WAS	FDP	400	1B6	24	83	37	24		22	26.5060

Performed by J. Luoma; SAS version 9.3 08:43 18MAR15

Effects of Pseudomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel species

Mean Unadjusted Viability of Glochidia by Species, Time, Form (SDP or FDP) and Treatment Group

The MEANS Procedure

sps=BLS form=SDP

conc	time	N Obs	Variable	Label	Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
0	6	3	tot	tot	297.3	50.1232	172.8	421.8
			cbs	cbs	8.0000	0	.	.
			oas	oas	34.3333	7.0946	16.7094	51.9573
			via_glo		255.0	43.8634	146.0	364.0
			pctvia_glo		85.7227	1.0577	83.0952	88.3503
	12	3	tot	tot	210.7	63.9713	51.7530	369.6
			cbs	cbs	3.6667	2.0817	-1.5045	8.8378
			oas	oas	18.6667	7.0238	1.2187	36.1147
			via_glo		188.3	56.3678	48.3079	328.4
			pctvia_glo		89.4899	0.5092	88.2250	90.7548
	24	3	tot	tot	463.3	223.8	-92.4995	1019.2
			cbs	cbs	14.0000	11.1355	-13.6622	41.6622
			oas	oas	60.3333	31.7857	-18.6268	139.3
			via_glo		389.0	184.1	-68.3580	846.4
			pctvia_glo		84.4675	1.9452	79.6354	89.2995
50	6	3	tot	tot	249.3	42.0634	144.8	353.8
			cbs	cbs	199.7	77.3585	7.4976	391.8
			oas	oas	23.0000	17.3494	-20.0982	66.0982
			via_glo		26.6667	20.5508	-24.3842	77.7176
			pctvia_glo		11.7115	9.5448	-11.9992	35.4222
	12	3	tot	tot	290.0	95.2208	53.4584	526.5
			cbs	cbs	223.7	68.0392	54.6479	392.7
			oas	oas	18.3333	13.8684	-16.1178	52.7844
			via_glo		48.0000	26.2298	-17.1583	113.2
			pctvia_glo		16.1520	5.6175	2.1972	30.1067
	24	3	tot	tot	200.3	132.9	-129.7	530.4
			cbs	cbs	159.0	110.9	-116.5	434.5
			oas	oas	12.3333	7.2342	-5.6374	30.3040
			via_glo		29.0000	17.7764	-15.1590	73.1590
			pctvia_glo		14.7276	1.7941	10.2709	19.1843
100	6	3	tot	tot	220.3	113.7	-62.0983	502.8
			cbs	cbs	210.0	104.9	-50.4912	470.5
			oas	oas	8.6667	7.0238	-8.7813	26.1147
			via_glo		1.6667	2.8868	-5.5044	8.8378
			pctvia_glo		0.4748	0.8224	-1.5682	2.5179
	12	3	tot	tot	191.0	62.7933	35.0128	347.0
			cbs	cbs	153.3	55.5188	15.4171	291.2
			oas	oas	20.0000	1.0000	17.5159	22.4841
			via_glo		17.6667	9.2916	-5.4149	40.7482
			pctvia_glo		8.8404	3.2608	0.7401	16.9407
	24	3	tot	tot	218.3	35.6417	129.8	306.9
			cbs	cbs	188.7	28.7460	117.3	260.1
			oas	oas	13.6667	3.7859	4.2619	23.0715
			via_glo		16.0000	11.5326	-12.6485	44.6485
			pctvia_glo		6.9517	4.8770	-5.1635	19.0670
200	6	3	tot	tot	248.0	94.3186	13.6996	482.3
			cbs	cbs	211.7	69.0821	40.0573	383.3
			oas	oas	32.0000	34.0441	-52.5702	116.6
			via_glo		4.3333	4.0415	-5.7062	14.3729
			pctvia_glo		1.7496	1.5555	-2.1145	5.6138
	12	3	tot	tot	252.0	47.7912	133.3	370.7
			cbs	cbs	238.7	39.6274	140.2	337.1
			oas	oas	10.3333	8.3865	-10.4999	31.1665
			via_glo		3.0000	1.0000	0.5159	5.4841

			pctvia_glo		1.1903	0.3201	0.3951	1.9854
	24	3	tot	tot	335.0	28.5832	264.0	406.0
			cbs	cbs	297.7	41.0163	195.8	399.6
			oas	oas	26.3333	16.0728	-13.5936	66.2603
			via_glo		11.0000	7.9373	-8.7172	30.7172
			pctvia_glo		3.2518	2.1917	-2.1928	8.6963
300	6	3	tot	tot	240.3	76.9567	49.1623	431.5
			cbs	cbs	212.3	86.5583	-2.6893	427.4
			oas	oas	24.6667	15.0444	-12.7056	62.0390
			via_g o		3.3333	4.9329	-8.9206	15.5873
			pctvia_glo		1.0949	1.5628	-2.7874	4.9771
	12	3	tot	tot	131.0	36.1663	41.1580	220.8
			cbs	cbs	124.0	33.8674	39.8687	208.1
			oas	oas	7.0000	7.0000	-10.3890	24.3890
			via_glc		0	0	.	.
			pctvia_glo		0	0	.	.
	24	3	tot	tot	307.3	67.1143	140.6	474.1
			cbs	cbs	296.7	67.0994	130.0	463.4
			oas	oas	8.3333	4.9329	-3.9206	20.5873
			via_glo		2.3333	2.0817	-2.8378	7.5045
			pctvia_glo		0.7403	0.6445	-0.8607	2.3413
400	6	3	tot	tot	236.7	17.5024	193.2	280.1
			cbs	cbs	205.3	9.2916	182.3	228.4
			oas	oas	29.3333	16.0728	-10.5936	69.2603
			via_glo		2.0000	3.4641	-6.6053	10.6053
			pctvia_glo		0.8439	1.4616	-2.7870	4.4748
	12	3	tot	tot	190.3	79.4313	-6.9850	387.7
			cbs	cbs	178.0	79.1896	-18.7180	374.7
			oas	oas	11.0000	2.6458	4.4276	17.5724
			via_glo		1.3333	1.1547	-1.5351	4.2018
			pctvia_glo		0.9259	0.8100	-1.0864	2.9381
	24	3	tot	tot	389.3	91.7842	161.3	617.3
			cbs	cbs	377.7	88.4835	157.9	597.5
			oas	oas	8.6667	2.0817	3.4955	13.8378
			via_glo		3.0000	3.0000	-4.4524	10.4524
			pctvia_glo		0.7311	0.7934	-1.2398	2.7021

sps=FAM form=SDP

conc	time	N Obs	Variable	Label	Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
0	6	3	tot	tot	249.3	34.2977	164.1	334.5
			cbs	cbs	32.3333	3.5119	23.6093	41.0573
			oas	oas	36.3333	8.7369	14.6297	58.0370
			via_glo		180.7	29.8719	106.5	254.9
			pctvia_glo		72.3148	4.0412	62.2760	82.3537
	12	3	tot	tot	377.0	126.8	61.9843	692.0
			cbs	cbs	57.3333	4.9329	45.0794	69.5873
			oas	oas	66.3333	30.0721	-8.3700	141.0
			via_glo		253.3	101.0	2.3330	504.3
			pctvia_glo		66.5557	6.4612	50.5052	82.6062
	24	3	tot	tot	266.0	35.5387	177.7	354.3
			cbs	cbs	39.0000	17.3494	-4.0982	82.0982
			oas	oas	43.0000	11.1355	15.3378	70.6622
			via_glo		184.0	24.9800	121.9	246.1
			pctvia_glo		69.4320	8.1836	49.1028	89.7612
50	6	3	tot	tot	323.7	116.1	35.2891	612.0
			cbs	cbs	64.0000	26.2107	-1.1110	129.[illegible]
			oas	oas	75.3333	20.8407	23.5623	127.[illegible]
			via_glo		184.3	69.9738	10.5088	358.2
			pctvia_g o		56.7715	2.7364	49.9740	63.5691
	12	3	tot	tot	335.0	115.1	49.0651	620.9
			cbs	cbs	96.0000	57.6108	-47.1131	239.1
			oas	oas	43.6667	8.6217	22.2492	65.0841

			via_glo		195.3	58.7310	49.4374	341.2
			pctvia_glo		59.1403	4.0966	48.9638	69.3167
	24	3	tot	tot	313.0	30.5123	237.2	388.8
			cbs	cbs	57.3333	17.0098	15.0786	99.5880
			oas	oas	76.6667	9.0185	54.2635	99.0699
			via_glo		179.0	34.0735	94.3569	263.6
			pctvia_glo		56.8410	5.9579	42.0407	71.6413
100	6	3	tot	tot	297.0	57.2364	154.8	439.2
			cbs	cbs	161.0	66.7757	-4.8801	326.9
			oas	oas	48.3333	30.6159	-27.7208	124.4
			via_glo		87.6667	55.7704	-50.8746	226.2
			pctvia_glo		28.9584	14.7554	-7.6961	65.6128
	12	3	tot	tot	263.0	130.2	-60.3962	586.4
			cbs	cbs	105.0	67.5796	-62.8770	272.9
			oas	oas	42.6667	13.7961	8.3952	76.9382
			via_glo		115.3	57.8389	-28.3464	259.0
			pctvia_glo		43.6104	3.4045	35.1531	52.0677
	24	3	tot	tot	211.0	100.5	-38.7392	460.7
			cbs	cbs	82.3333	55.9494	-56.6526	221.3
			oas	oas	44.6667	21.5716	-8.9201	98.2535
			via_glo		84.0000	29.3067	11.1931	156.8
			pctvia_glo		41.6378	6.4563	25.5994	57.6761
200	6	3	tot	tot	369.0	162.8	-35.3654	773.4
			cbs	cbs	258.0	179.3	-187.4	703.4
			oas	oas	54.6667	8.6217	33.2492	76.0841
			via_glo		56.3333	27.7909	-12.7031	125.4
			pctvia_glo		19.3108	17.1450	-23.2798	61.9014
	12	3	tot	tot	182.0	42.3320	76.8414	287.2
			cbs	cbs	122.7	51.6366	-5.6056	250.9
			oas	oas	27.3333	11.1505	-0.3660	55.0327
			via_glo		32.0000	13.5277	-1.6048	65.6048
			pctvia_glo		19.6211	13.1800	-13.1198	52.3621
	24	3	tot	tot	249.0	38.1051	154.3	343.7
			cbs	cbs	176.7	25.7358	112.7	240.6
			oas	oas	44.0000	16.5227	2.9553	85.0447
			via_glo		28.3333	10.5987	2.0046	54.6621
			pctvia_glo		11.6431	4.6607	0.0654	23.2208
300	6	3	tot	tot	408.3	144.4	49.7187	766.9
			cbs	cbs	283.3	120.5	-16.0164	582.7
			oas	oas	68.6667	47.2899	-48.8079	186.1
			via_glo		56.3333	86.3501	-158.2	270.8
			pctvia_glo		11.8955	16.7267	-29.6559	53.4470
	12	3	tot	tot	248.0	46.5188	132.4	363.6
			cbs	cbs	200.3	30.3536	124.9	275.7
			oas	oas	36.6667	15.9478	-2.9499	76.2833
			via_glo		11.0000	4.5826	-0.3837	22.3837
			pctvia_glo		4.4735	1.8995	-0.2451	9.1920
	24	3	tot	tot	202.7	23.1157	145.2	260.1
			cbs	cbs	148.3	20.2073	98.1357	198.5
			oas	oas	42.0000	23.3024	-15.8863	99.8863
			via_glo		12.3333	4.5092	1.1317	23.5349
			pctvia_glo		5.9941	1.7282	1.7011	10.2871
400	6	3	tot	tot	211.0	5.5678	197.2	224.8
			cbs	cbs	161.7	5.8595	147.1	176.2
			oas	oas	44.3333	10.1160	19.2038	69.4629
			via_glo		5.0000	5.0000	-7.4207	17.4207
			pctvia_glo		2.3439	2.3586	-3.5152	8.2031
	12	3	tot	tot	265.0	67.8159	96.5359	433.5
			cbs	cbs	204.0	64.2573	44.3760	363.6
			oas	oas	54.0000	2.0000	49.0317	58.9683
			via_glo		7.0000	7.5498	-11.7548	25.7548
			pctvia_glo		2.3230	2.6391	-3.9844	8.6304
	24	3	tot	tot	234.3	131.4	-92.0103	560.7

			cbs	cbs	204.0	118.9	-91.2991	499.3
			oas	oas	26.6667	10.6927	0.1046	53.2287
			via_glo		3.6667	2.8868	-3.5044	10.8378
			pctvia_glo		1.7069	0.8703	-0.4550	3.8688

sps=HGE form=SDP

conc	time	N Obs	Variable	Label	Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
0	6	3	tot	tot	224.7	49.3288	102.1	347.2
			cbs	cbs	15.3333	4.6188	3.8596	26.8071
			oas	oas	47.0000	25.1595	-15.4996	109.5
			via_glo		162.3	43.1084	55.2462	269.4
			pctvia_glo		72.3047	9.5449	48.5939	96.0155
	12	3	tot	tot	315.0	145.4	-46.2777	676.3
			cbs	cbs	30.0000	17.5214	-13.5256	73.5256
			oas	oas	73.0000	45.1774	-39.2270	185.2
			via_glo		212.0	84.1249	3.0221	421.0
			pctvia_glo		68.6991	6.1492	53.4235	83.9746
	24	4	tot	tot	415.0	101.8	253.1	576.9
			cbs	cbs	44.7500	13.5000	23.2685	66.2315
			oas	oas	79.2500	19.3800	48.4121	110.1
			via_glo		291.0	95.4603	139.1	442.9
			pctvia_glo		69.3490	7.3019	57.7301	80.9680
50	6	3	tot	tot	348.0	232.3	-229.1	925.1
			cbs	cbs	20.3333	8.1445	0.1012	40.5655
			oas	oas	52.0000	31.5753	-26.4374	130.4
			via_glo		275.7	196.3	-212.1	763.4
			pctvia_glo		77.7306	3.7372	68.4468	87.0143
	12	3	tot	tot	264.7	64.4231	104.6	424.7
			cbs	cbs	31.6667	9.2916	8.5851	54.7482
			oas	oas	49.0000	1.0000	46.5159	51.4841
			via_glo		184.0	60.6712	33.2843	334.7
			pctvia_glo		68.6581	6.4746	52.5743	84.7418
	24	3	tot	tot	494.7	26.8576	427.9	561.4
			cbs	cbs	46.0000	10.8167	19.1299	72.8701
			oas	oas	79.0000	19.9249	29.5039	128.5
			via_glo		369.7	36.0879	280.0	459.3
			pctvia_glo		74.6116	3.4335	66.0824	83.1408
100	6	3	tot	tot	357.0	83.6242	149.3	564.7
			cbs	cbs	63.3333	34.5302	-22.4444	149.1
			oas	oas	67.6667	28.8675	-4.0442	139.4
			via_glo		226.0	54.6168	90.3242	361.7
			pctvia_glo		63.2160	1.0350	60.6449	65.7871
	12	3	tot	tot	290.0	65.1844	128.1	451.9
			cbs	cbs	42.0000	7.5498	23.2452	60.7548
			oas	oas	56.6667	17.2434	13.8318	99.5015
			via_glo		191.3	52.2909	61.4357	321.2
			pctvia_glo		65.5131	3.1273	57.7443	73.2818
	24	3	tot	tot	488.3	121.9	185.5	791.2
			cbs	cbs	95.6667	9.0185	73.2635	118.1
			oas	oas	69.0000	18.0000	24.2855	113.7
			via_glo		323.7	106.9	58.0459	589.3
			pctvia_glo		65.4711	4.8651	53.3855	77.5568
200	6	3	tot	tot	453.7	84.3109	244.2	663.1
			cbs	cbs	104.7	27.4287	36.5300	172.8
			oas	oas	90.6667	27.5741	22.1687	159.2
			via_glo		258.3	61.0273	106.7	409.9
			pctvia_glo		56.6751	5.4846	43.0505	70.2996
	12	3	tot	tot	294.7	42.9108	188.1	401.3
			cbs	cbs	97.3333	42.8991	-9.2340	203.9
			oas	oas	57.3333	13.6504	23.4239	91.2428
			via_glo		140.0	68.6367	-30.5031	310.5
			pctvia_glo		46.2478	17.9285	1.7108	90.7847

	24	3	tot	tot	368.7	72.5006	188.6	548.8
			cbs	cbs	163.3	97.6337	-79.2021	405.9
			oas	oas	55.3333	1.1547	52.4649	58.2018
			via_glo		150.0	34.0441	65.4298	234.6
			pctvia_glo		42.4815	14.7540	5.8306	79.1325
300	6	3	tot	tot	332.7	77.8867	139.2	526.1
			cbs	cbs	165.7	78.0150	-28.1332	359.5
			oas	oas	61.3333	44.5234	-49.2689	171.9
			via_glo		105.7	97.9864	-137.7	349.1
			pctvia_glo		29.0979	21.7089	-24.8301	83.0259
	12	3	tot	tot	240.7	26.0064	176.1	305.3
			cbs	cbs	75.3333	28.4312	4.7063	146.0
			oas	oas	72.6667	19.3993	24.4761	120.9
			via_glo		92.6667	17.2143	49.9039	135.4
			pctvia_glo		38.7514	8.0628	18.7222	58.7806
	24	3	tot	tot	221.7	115.6	-65.6175	509.0
			cbs	cbs	144.7	128.3	-174.0	463.3
			oas	oas	38.6667	13.6137	4.8483	72.4850
			via_glo		38.3333	33.3217	-44.4423	121.1
			pctvia_glo		22.2858	26.5771	-43.7354	88.3071
400	6	3	tot	tot	247.3	97.2231	5.8177	488.8
			cbs	cbs	162.0	57.2364	19.8170	304.2
			oas	oas	46.0000	12.2882	15.4744	76.5256
			via_glo		39.3333	34.9905	-47.5878	126.3
			pctvia_glo		13.2906	12.3008	-17.2664	43.8476
	12	3	tot	tot	209.0	18.6815	162.6	255.4
			cbs	cbs	148.7	10.6927	122.1	175.2
			oas	oas	40.3333	7.2342	22.3626	58.3040
			via_glo		20.0000	3.6056	11.0433	28.9567
			pctvia_glo		9.5193	0.8525	7.4015	11.6371
	24	3	tot	tot	227.7	97.0275	-13.3630	468.7
			cbs	cbs	135.3	81.1193	-66.1781	336.8
			oas	oas	58.6667	16.0416	18.8171	98.5162
			via_glo		33.6667	24.8261	-28.0047	95.3380
			pctvia_glo		13.9346	10.3929	-11.8827	39.7519

sps=HIC form=SDP

conc	time	N Obs	Variable	Label	Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
0	6	3	tot	tot	224.3	59.1805	77.3208	371.3
			cbs	cbs	28.3333	31.8172	-50.7049	107.4
			oas	oas	19.0000	6.2450	3.4866	34.5134
			via_glo		177.0	43.7150	68.4060	285.6
			pctvia_glo		79.9822	11.5442	51.3048	108.7
	12	3	tot	tot	205.7	57.2043	63.5633	347.8
			cbs	cbs	10.0000	7.8102	-9.4017	29.4017
			oas	oas	18.6667	9.4516	-4.8125	42.1458
			via_glo		177.0	49.2747	54.5948	299.4
			pctvia_glo		86.4146	6.3460	70.6504	102.2
	24	3	tot	tot	309.0	101.7	56.4107	561.6
			cbs	cbs	14.6667	7.0238	-2.7813	32.1147
			oas	oas	26.3333	10.7858	-0.4601	53.1267
			via_glo		268.0	84.9235	57.0383	479.0
			pctvia_glo		87.0377	1.7571	82.6729	91.4026
50	6	3	tot	tot	244.0	46.0326	129.6	358.4
			cbs	cbs	21.0000	5.0000	8.5793	33.4207
			oas	oas	14.6667	5.6862	0.5413	28.7921
			via_glo		208.3	41.9325	104.2	312.5
			pctvia_glo		85.2261	1.2753	82.0581	88.3941
	12	3	tot	tot	185.0	12.5300	153.9	216.1
			cbs	cbs	18.0000	7.9373	-1.7172	37.7172
			oas	oas	10.6667	3.0551	3.0775	18.2558
			via_glo		156.3	13.3167	123.3	189.4

			pctvia_glo		84.4558	2.8930	77.2693	91.6424
	24	3	tot	tot	253.0	43.5890	144.7	361.3
			cbs	cbs	15.0000	5.1962	2.0920	27.9080
			oas	oas	13.0000	7.8102	-6.4017	32.4017
			via_glo		225.0	32.9090	143.2	306.8
			pctvia_glo		89.1958	2.8704	82.0653	96.3263
100	6	3	tot	tot	251.7	34.9333	164.9	338.4
			cbs	cbs	78.0000	37.6899	-15.3785	171.4
			oas	oas	18.6667	11.5902	-10.1251	47.4584
			via_glo		155.0	48.2804	35.0648	274.9
			pctvia_glo		60.9704	13.8195	26.6410	95.2999
	12	3	tot	tot	208.3	11.5902	179.5	237.1
			cbs	cbs	43.0000	29.1376	-29.3818	115.4
			oas	oas	5.3333	1.1547	2.4649	8.2018
			via_glo		160.0	19.9249	110.5	209.5
			pctvia_glo		77.1640	12.4475	46.2426	108.1
	24	3	tot	tot	313.0	79.5047	115.5	510.5
			cbs	cbs	48.6667	11.6762	19.6614	77.6719
			oas	oas	16.0000	5.5678	2.1689	29.8311
			via_glo		248.3	85.5005	35.9383	460.7
			pctvia_glo		78.2725	6.8394	61.2826	95.2624
200	6	3	tot	tot	230.7	54.5008	95.2793	366.1
			cbs	cbs	116.3	26.0064	51.7298	180.9
			oas	oas	18.0000	1.0000	15.5159	20.4841
			via_glo		96.3333	47.3533	-21.2987	214.0
			pctvia_glo		40.6406	11.4718	12.1431	69.1381
	12	3	tot	tot	152.7	28.9367	80.7839	224.5
			cbs	cbs	70.3333	25.4231	7.1789	133.5
			oas	oas	5.3333	2.5166	-0.9183	11.5849
			via_glo		77.0000	27.7308	8.1128	145.9
			pctvia_glo		50.2621	16.5440	9.1645	91.3597
	24	3	tot	tot	249.7	86.0775	35.8383	463.5
			cbs	cbs	86.0000	24.9800	23.9463	148.1
			oas	oas	10.3333	1.5275	6.5388	14.1279
			via_glo		153.3	62.2281	-1.2498	307.9
			pctvia_glo		60.6880	4.5317	49.4305	71.9454
300	6	3	tot	tot	244.0	62.9524	87.6177	400.4
			cbs	cbs	192.7	33.0051	110.7	274.7
			oas	oas	6.6667	5.1316	-4.0809	21.4143
			via_glo		42.6667	40.5134	-57.9741	143.3
			pctvia_glo		15.6976	12.7165	-15.8920	47.2871
	12	3	tot	tot	203.3	3.0551	195.7	210.9
			cbs	cbs	109.3	12.6623	77.8785	140.8
			oas	oas	14.3333	7.7675	-4.9621	33.6288
			via_glo		79.6667	6.1101	64.4883	94.8450
			pctvia_glo		39.1821	2.9672	31.8113	46.5530
	24	3	tot	tot	187.3	45.6107	74.0301	300.6
			cbs	cbs	130.7	29.6873	56.9194	204.4
			oas	oas	6.6667	2.5166	0.4151	12.9183
			via_glo		50.0000	15.8745	10.5655	89.4345
			pctvia_glo		26.5389	3.2427	18.4835	34.5943
400	6	3	tot	tot	308.3	58.4836	163.1	453.6
			cbs	cbs	248.3	34.5881	162.4	334.3
			oas	oas	16.0000	9.0000	-6.3572	38.3572
			via_glo		44.0000	26.8887	-22.7951	110.8
			pctvia_glo		13.8102	6.2587	-1.7373	29.3578
	12	3	tot	tot	279.3	50.8167	153.1	405.6
			cbs	cbs	262.7	44.0719	153.2	372.1
			oas	oas	13.3333	9.6090	-10.5368	37.2035
			via_glo		3.3333	0.5774	1.8991	4.7676
			pctvia_glo		1.2287	0.3536	0.3502	2.1071
	24	3	tot	tot	276.7	45.4899	163.7	389.7
			cbs	cbs	271.7	45.0037	159.9	383.5

			oas	oas	3.6667	2.0817	-1.5045	6.8378
			via_glo		1.3333	0.5774	-0.1009	2.7676
			pctvia_glo		0.5161	0.3197	-0.2782	1.3103

sps=MUC form=SDP

conc	time	N Obs	Variable	Label	Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
0	6	3	tot	tot	195.7	62.5327	40.3269	351.0
			cbs	cbs	8.0000	6.5574	-8.2896	24.2896
			oas	oas	13.6667	2.8868	6.4956	20.8378
			via_glo		174.0	60.3987	23.9614	324.0
			pctvia_glo		88.6131	4.4362	77.5931	99.6331
	12	3	tot	tot	215.0	38.0395	120.5	309.5
			cbs	cbs	2.6667	3.0551	-4.9225	10.2558
			oas	oas	9.3333	3.7859	-0.0715	18.7381
			via_glo		203.0	39.0384	106.0	300.0
			pctvia_glo		94.2440	1.4901	90.5424	97.9456
	24	3	tot	tot	291.0	60.8276	139.9	442.1
			cbs	cbs	5.3333	3.7859	-4.0715	14.7381
			oas	oas	15.6667	6.6583	-0.8735	32.2069
			via_glo		270.0	60.6218	119.4	420.6
			pctvia_glo		92.6155	2.2493	87.0280	98.2031
50	6	3	tot	tot	257.3	115.2	-28.8748	543.5
			cbs	cbs	51.0000	8.0000	31.1269	70.8731
			oas	oas	17.0000	17.0587	-25.3762	59.3762
			via_glo		189.3	94.8754	-46.3501	425.0
			pctvia_glo		71.6888	6.6186	55.2474	88.1302
	12	3	tot	tot	175.7	56.0476	36.4367	314.9
			cbs	cbs	44.3333	24.1937	-15.7671	104.4
			oas	oas	10.0000	5.2915	-3.1448	23.1448
			via_glo		121.3	57.4572	-21.3983	264.1
			pctvia_glo		66.5633	17.7500	22.4698	110.7
	24	3	tot	tot	222.7	94.3257	-11.6513	457.0
			cbs	cbs	39.0000	17.3205	-4.0265	82.0265
			oas	oas	10.3333	3.2146	2.3479	18.3187
			via_glo		173.3	88.2289	-45.8393	392.5
			pctvia_glo		75.7729	13.0495	43.3562	108.2
100	6	3	tot	tot	270.0	170.7	-154.0	694.0
			cbs	cbs	55.0000	26.5141	-10.8648	120.9
			oas	oas	16.3333	11.3725	-11.9175	44.5841
			via_glo		198.7	138.9	-146.3	543.7
			pctvia_glo		72.2209	9.7023	48.1190	96.3228
	12	3	tot	tot	187.7	43.0852	80.6371	294.7
			cbs	cbs	54.3333	33.2916	-28.3677	137.0
			oas	oas	10.3333	3.5119	1.6093	19.0573
			via_glo		123.0	53.3948	-9.6399	255.6
			pctvia_glo		64.3592	17.7243	20.3296	108.4
	24	3	tot	tot	204.3	11.7189	175.2	233.4
			cbs	cbs	66.6667	22.8108	10.0015	123.3
			oas	oas	8.6667	3.2146	0.6813	16.6521
			via_glo		129.0	16.7033	87.5067	170.5
			pctvia_glo		63.4915	11.2188	35.6225	91.3605
200	6	3	tot	tot	239.0	145.1	-121.4	599.4
			cbs	cbs	140.3	131.6	-186.5	467.2
			oas	oas	17.3333	8.1445	-2.8988	37.5655
			via_glo		81.3333	19.1398	33.7873	128.9
			pctvia_glo		40.5162	16.3103	-0.00078	81.0331
	12	3	tot	tot	238.7	106.8	-26.7101	504.0
			cbs	cbs	134.0	80.6660	-66.3854	334.4
			oas	oas	12.3333	4.1633	1.9910	22.6756
			via_glo		92.3333	29.1605	19.8947	164.8
			pctvia_glo		40.0406	4.5372	28.7697	51.3116
			tot	tot	385.0	124.5	75.8072	694.2

	24	3	cbs	cbs	255.3	93.3292	23.4908	487.2
			oas	oas	13.3333	3.0551	5.7442	20.9225
			via_glo		116.3	46.6083	0.5519	232.1
			pctvia_glo		29.9934	7.2194	12.0594	47.9274
300	6	3	tot	tot	279.7	38.1750	184.8	374.5
			cbs	cbs	238.0	42.3202	132.9	343.1
			oas	oas	8.6667	4.6188	-2.8071	20.1404
			via_glo		33.0000	19.5192	-15.4884	81.4884
			pctvia_glo		11.7761	7.3023	-6.3639	29.9161
	12	3	tot	tot	187.0	66.1891	22.5771	351.4
			cbs	cbs	165.0	56.7186	24.1032	305.9
			oas	oas	2.6667	1.1547	-0.2018	5.5351
			via_glo		19.3333	10.2144	-6.0406	44.7072
			pctvia_glo		10.1571	2.4927	3.9648	16.3494
	24	3	tot	tot	313.3	130.3	-10.2759	636.9
			cbs	cbs	289.7	123.2	-16.4506	595.8
			oas	oas	6.0000	3.0000	-1.4524	13.4524
			via_glo		17.6667	10.5040	-8.4266	43.7600
			pctvia_glo		5.9801	3.5146	-2.7507	14.7109
400	6	3	tot	tot	309.0	40.2865	208.9	409.1
			cbs	cbs	291.0	44.6430	180.1	401.9
			oas	oas	7.3333	3.0551	-0.2558	14.9225
			via_glo		10.6667	5.5076	-3.0149	24.3482
			pctvia_glo		3.6055	2.0546	-1.4986	8.7095
	12	3	tot	tot	182.7	55.0121	46.0090	319.3
			cbs	cbs	158.7	52.4436	28.3895	288.9
			oas	oas	9.6667	5.1316	-3.0809	22.4143
			via_glo		14.3333	8.6217	-7.0841	35.7508
			pctvia_glo		8.1995	5.0288	-4.2927	20.6917
	24	3	tot	tot	252.3	82.9478	46.2796	458.4
			cbs	cbs	234.3	73.9076	50.7367	417.9
			oas	oas	4.0000	2.0000	-0.9683	8.9683
			via_glo		14.0000	12.1655	-16.2208	44.2208
			pctvia_glo		5.2174	3.3979	-3.2233	13.6582

sps=PPB form=FDP

conc	time	N Obs	Variable	Label	Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
0	6	3	tot	tot	142.7	64.3454	-17.1762	302.5
			cbs	cbs	14.0000	6.0828	-1.1104	29.1104
			oas	oas	11.0000	5.0000	-1.4207	23.4207
			via_glo		117.7	55.7524	-20.8300	256.2
			pctvia_glo		82.2576	4.7439	70.4731	94.0420
	12	3	tot	tot	209.7	93.6180	-22.8934	442.2
			cbs	cbs	5.3333	2.5166	-0.9183	11.5849
			oas	oas	34.6667	22.8546	-22.1073	91.4407
			via_glo		169.7	68.8501	-1.3664	340.7
			pctvia_glo		81.7825	3.3908	73.3594	90.2057
	24	3	tot	tot	376.7	170.0	-45.7122	799.0
			cbs	cbs	15.0000	7.2111	-2.9134	32.9134
			oas	oas	51.6667	20.4042	0.9797	102.4
			via_glo		310.0	145.5	-51.4314	671.4
			pctvia_glo		81.9068	1.4048	78.4171	85.3965
50	6	3	tot	tot	125.0	44.6430	14.1006	235.9
			cbs	cbs	21.6667	5.5076	7.9851	35.3482
			oas	oas	13.0000	6.9282	-4.2106	30.2106
			via_glo		90.3333	42.9224	-16.2918	197.0
			pctvia_glo		69.5514	11.4879	41.0139	98.0889
	12	3	tot	tot	352.0	229.4	-217.8	921.8
			cbs	cbs	22.0000	13.1149	-10.5792	54.5792
			oas	oas	53.6667	21.7332	-0.3217	107.7
			via_glo		276.3	199.6	-219.5	772.1
			pctvia_glo		76.6860	6.2489	61.1630	92.2091

	24	3	tot	tot	305.3	122.7	0.6304	610.0
			cbs	cbs	34.0000	4.5826	22.6163	45.3837
			oas	oas	37.3333	14.1539	2.1731	72.4936
			via_glo		234.0	112.9	-46.5846	5'4.6
			pctvia_glo		74.1424	9.3170	50.9976	97.2872
100	6	3	tot	tot	203.3	50.3422	78.2765	328.4
			cbs	cbs	50.0000	18.6815	3.5925	96.4075
			oas	oas	19.6667	6.1101	4.4883	34.8450
			via_glo		133.7	33.5311	50.3708	217.0
			pctvia_glo		65.6905	3.4454	57.1317	74.2493
	12	3	tot	tot	150.3	25.5408	86.8864	213.8
			cbs	cbs	27.0000	16.6433	-14.3443	68.3443
			oas	oas	18.3333	3.5119	9.6093	27.0573
			via_glo		105.0	11.2694	77.0052	133.0
			pctvia_glo		70.5512	8.4331	49.6021	91.5003
	24	3	tot	tot	386.7	90.8974	160.9	612.5
			cbs	cbs	72.0000	29.7153	-1.8169	145.8
			oas	oas	53.6667	7.0946	36.0427	71.2906
			via_glo		261.0	59.1016	114.2	407.8
			pctvia_glo		67.7470	3.8328	58.2258	77.2681
200	6	3	tot	tot	237.3	72.8171	56.4456	418.2
			cbs	cbs	91.3333	47.2264	-25.9836	208.7
			oas	oas	36.0000	19.6977	-12.9318	84.9318
			via_glo		110.0	12.1655	79.7792	140.2
			pctvia_glo		48.6787	11.3973	20.3663	76.9912
	12	3	tot	tot	421.3	341.8	-427.8	1270.4
			cbs	cbs	138.7	30.4357	63.0601	214.3
			oas	oas	66.0000	64.7148	-94.7604	226.8
			via_glo		216.7	307.0	-546.1	979.4
			pctvia_glo		34.8806	30.5950	-41.1215	110.9
	24	3	tot	tot	374.0	224.2	-183.0	931.0
			cbs	cbs	178.0	94.2709	-56.1819	412.2
			oas	oas	54.6667	38.0044	-39.7415	149.1
			via_glo		141.3	170.9	-283.3	566.0
			pctvia_glo		29.5358	23.7536	-29.4715	88.5431
300	6	3	tot	tot	395.3	225.0	-163.5	954.2
			cbs	cbs	143.3	77.6552	-49.5729	336.2
			oas	oas	83.3333	38.7986	-13.0478	179.7
			via_glo		168.7	126.1	-144.5	481.8
			pctvia_glo		40.6793	14.6568	4.2697	77.0889
	12	3	tot	tot	274.0	65.5973	111.0	437.0
			cbs	cbs	202.7	76.1599	13.4749	391.9
			oas	oas	49.0000	15.7162	9.9587	88.0413
			via_glo		22.3333	17.9258	-22.1968	66.8634
			pctvia_glo		9.1335	8.7952	-12.7150	30.982'
	24	3	tot	tot	451.0	257.7	-189.1	1091.1
			cbs	cbs	301.3	157.0	-88.6156	691.3
			oas	oas	92.6667	68.3691	-77.1716	262.5
			via_glo		57.0000	40.2616	-43.0155	157.0
			pctvia_glo		12.0367	1.8279	7.4959	16.5774
400	6	3	tot	tot	332.3	116.3	43.5494	621.1
			cbs	cbs	184.0	122.0	-119.1	487.1
			oas	oas	73.0000	36.2905	-17.1506	163.2
			via_glo		75.3333	89.8573	-147.9	298.6
			pctvia_glo		20.4391	23.0337	-36.7798	77.6581
	12	3	tot	tot	120.7	105.8	-142.1	383.4
			cbs	cbs	102.3	94.6379	-132.8	337.4
			oas	oas	13.0000	7.2111	-4.9134	30.9134
			via_glo		5.3333	4.0415	-4.7062	15.3729
			pctvia_glo		4.8496	1.2129	1.8367	7.8626
	24	3	tot	tot	296.7	63.8931	137.9	455.4
			cbs	cbs	227.3	43.2474	119.9	334.8
			oas	oas	55.0000	20.6640	3.6678	106.3
			via_glo		14.3333	6.0277	-0.6403	29.3070

conc	time	N Obs	Variable	Label	Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
			pctvia_glo		4.9022	2.2855	-0.7754	10.5799

sps=PPB form=SDP

conc	time	N Obs	Variable	Label	Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
0	6	3	tot	tot	299.3	133.5	-32.1789	630.8
			cbs	cbs	4.0000	2.6458	-2.5724	10.5724
			oas	oas	18.3333	6.5064	2.1705	34.4961
			via_glo		277.0	124.6	-32.5020	586.5
			pctvia_glo		92.3358	1.0182	89.8065	94.8652
	12	3	tot	tot	609.7	14.3643	574.0	645.3
			cbs	cbs	32.0000	12.1244	1.8814	62.1186
			oas	oas	53.0000	1.0000	50.5159	55.4841
			via_glo		524.7	15.3080	486.6	562.7
			pctvia_glo		86.0651	2.0467	80.9808	91.1495
	24	3	tot	tot	406.3	120.6	106.7	705.9
			cbs	cbs	13.0000	1.7321	8.6973	17.3027
			oas	oas	19.0000	8.8882	-3.0795	41.0795
			via_glo		374.3	111.9	96.2726	652.4
			pctvia_glo		92.1117	0.8643	89.9646	94.2587
50	6	3	tot	tot	326.7	129.9	4.0506	649.3
			cbs	cbs	68.0000	57.0351	-73.6830	209.7
			oas	oas	11.6667	1.5275	7.8721	15.4612
			via_glo		247.0	74.7061	61.4198	432.6
			pctvia_glo		77.9232	8.9488	55.6932	100.2
	12	3	tot	tot	371.7	219.4	-173.3	916.7
			cbs	cbs	55.0000	42.9302	-51.6445	161.6
			oas	oas	31.6667	30.5996	-44.3469	107.7
			via_glo		285.0	147.8	-82.1485	652.1
			pctvia_glo		78.5634	6.6050	62.1557	94.9711
	24	3	tot	tot	356.0	95.6399	118.4	593.6
			cbs	cbs	53.3333	25.5408	-10.1136	116.8
			oas	oas	18.6667	8.3267	-2.0179	39.3512
			via_glo		284.0	80.4301	84.2006	483.8
			pctvia_glo		79.8142	5.6150	65.8659	93.7625
100	6	3	tot	tot	274.0	228.5	-293.6	841.6
			cbs	cbs	43.0000	27.0000	-24.0717	110.1
			oas	oas	20.6667	18.5023	-25.2955	66.6288
			via_glo		210.3	185.5	-250.4	671.0
			pctvia_glo		75.2627	6.0947	60.1225	90.4028
	12	3	tot	tot	403.7	86.8927	187.8	619.5
			cbs	cbs	145.3	153.1	-235.1	525.7
			oas	oas	33.3333	6.1101	18.1550	48.5[illegible]7
			via_glo		225.0	201.5	-275.7	725.7
			pctvia_glo		52.6578	45.6387	-60.7150	166.0
	24	3	tot	tot	281.3	62.1718	126.9	435.8
			cbs	cbs	41.0000	13.0000	8.7062	73.2938
			oas	oas	55.0000	60.7536	-95.9203	205.9
			via_glo		185.3	51.0523	58.5125	312.2
			pctvia_glo		66.8950	15.7636	27.7361	106.1
200	6	3	tot	tot	211.7	58.1578	67.1946	356.1
			cbs	cbs	90.6667	64.7019	-70.0617	251.4
			oas	oas	12.3333	7.7675	-6.9621	31.6288
			via_glo		108.7	54.9939	-27.9459	245.3
			pctvia_glo		52.2327	21.1608	-0.3337	104.8
	12	3	tot	tot	236.3	125.0	-74.2965	547.0
			cbs	cbs	97.3333	44.0719	-12.1474	206.8
			oas	oas	20.6667	13.4288	-12.6924	54.0257
			via_glo		[illegible]18.3	98.6019	-126.6	363.3
			pctvia_glo		46.7048	22.3477	-8.8099	102.2
	24	3	tot	tot	311.7	170.5	-112.0	735.3
			cbs	cbs	127.0	121.4	-174.7	428.7
			oas	oas	14.3333	1.5275	10.5388	18.1279

			via_glo		170.3	51.0131	43.6098	297.1
			pctvia_glo		59.2396	12.7978	27.4480	91.0312
300	6	3	tot	tot	298.0	200.7	-200.5	796.5
			cbs	cbs	257.7	195.6	-228.2	743.5
			oas	oas	22.3333	8.5049	1.2060	43.4607
			via_glo		18.0000	6.5574	1.7104	34.2896
			pctvia_glo		9.9092	10.4262	-15.9908	35.8093
	12	3	tot	tot	647.3	465.2	-508.3	1803.0
			cbs	cbs	541.7	348.4	-323.7	1407.0
			oas	oas	50.6667	51.2282	-76.5914	177.9
			via_g o		55.0000	65.6430	-108.1	218.1
			pctvia_glo		6.5125	4.2477	-4.0393	17.0644
	24	3	tot	tot	338.0	138.6	-6.2929	682.3
			cbs	cbs	297.0	92.6661	66.8047	527.2
			oas	oas	14.0000	9.5394	-9.6972	37.6972
			via_glo		27.0000	38.1182	-67.6910	121.7
			pctvia_glo		6.0685	7.2179	-11.8618	23.9988
400	6	3	tot	tot	158.7	46.6083	42.8852	274.4
			cbs	cbs	130.3	54.0771	-4.0016	264.7
			oas	oas	23.6667	6.0277	8.6930	38.6403
			via_glo		4.6667	4.5092	-6.5349	15.8683
			pctvia_glo		3.4414	3.1312	-4.3370	11.2198
	12	3	tot	tot	253.0	51.7977	134.3	391.7
			cbs	cbs	214.7	57.6397	71.4818	357.9
			oas	oas	34.0000	2.6458	27.4276	40.5724
			via_glo		14.3333	4.6188	2.8596	25.8071
			pctvia_glo		5.7918	2.6088	-0.6888	12.2724
	24	3	tot	tot	261.0	59.1016	114.2	407.8
			cbs	cbs	228.3	74.4133	43.4805	413.2
			oas	oas	21.3333	18.0370	-23.4731	66.1397
			via_glo		11.3333	4.9329	-0.9206	23.5873
			pctvia_glo		4.7471	3.1099	-2.9782	12.4724

sps=WAS form=FDP

conc	time	N Obs	Variable	Label	Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
0	24	3	tot	tot	89.3333	44.7921	-21.9364	200.6
			cbs	cbs	30.0000	20.0749	-19.8687	79.8687
			oas	oas	13.6667	4.6188	2.1929	25.1404
			via_glo		45.6667	22.9420	-11.3243	102 7
			pctvia_glo		52.0536	11.2832	24.0245	80.0827
50	24	3	tot	tot	58.6667	5.5076	44.9851	72.3482
			cbs	cbs	14.6667	5.1316	1.9191	27.4143
			oas	oas	11.3333	2.5166	5.0817	17.5849
			via_glo		32.6667	6.6583	16.1265	49.2069
			pctvia_glo		55.5544	9.4899	31.9802	79.1286
100	24	3	tot	tot	90.0000	20.0749	40.1313	139.9
			cbs	cbs	20.3333	4.6188	8.8596	31.8071
			oas	oas	24.3333	12.0554	-5.6140	54.2807
			via_glo		45.3333	23.5443	-13.1539	103.8
			pctvia_glo		48.0675	17.0284	5.7666	90.3685
200	24	3	tot	tot	59.3333	5.0332	46.8301	71.8366
			cbs	cbs	14.0000	2.0000	9.0317	18.9683
			oas	oas	15.3333	8.1445	-4.8988	35.5655
			via_glo		30.0000	13.5277	-3.6046	63.6048
			pctvia_glo		49.5216	18.6627	3.1610	95.8822
300	24	3	tot	tot	58.6667	9.0738	36.1262	81.2072
			cbs	cbs	13.3333	9.2376	-9.6141	36.2808
			oas	oas	22.3333	14.0119	-12.4742	57.1408
			via_glo		23.0000	10.0000	-1.8414	47.8414
			pctvia_glo		40.1437	20.7677	-11.4461	91.7336
400	24	3	tot	tot	81.0000	1.7321	76.6973	85.3027
			cbs	cbs	44.3333	15.3731	6.1443	82.5223

			oas	oas	15.0000	9.6394	-8.6972	38.6972
			via_glo		21.6667	8.5049	0.5393	42.7940
			pctvia_glo		26.7520	10.6271	0.3527	53.1513

Performed by J. Luoma; SAS version 9.3 08:43 18MAR15

Effects of Pseudomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel species

Overall Model: Glochidia Viability (unadjusted) - At 6, 12 and 24 h

The GLIMMIX Procedure

form=FDP sps=PPB

Model Information	
Data Set	WORK.GLOCHIDIA2
Response Variable (Events)	via_glo
Response Variable (Trials)	tot
Response Distribution	Binomial
Link Function	Logit
Variance Function	Default
Variance Matrix	Diagonal
Estimation Technique	Maximum Likelihood
Degrees of Freedom Method	Residual

Class Level Information		
Class	Levels	Values
conca	6	a b c d e f
time	3	6 12 24

Number of Observations Read	54
Number of Observations Used	54
Number of Events	7526
Number of Trials	15463

Dimensions	
Covariance Parameters	1
Columns in X	27
Columns in Z	0
Subjects (Blocks in V)	1
Max Obs per Subject	54

Optimization Information	
Optimization Technique	Newton-Raphson
Parameters in Optimization	18
Lower Boundaries	0
Upper Boundaries	0
Fixed Effects	Not Profiled

Iteration History					
Iteration	Restarts	Evaluations	Objective Function	Change	Max Gradient
0	0	4	644.13728715	.	40.2452
1	0	3	624.00657322	20.13071393	2.843922
2	0	3	623.9131154	0.09345782	0.020633
3	0	3	623.91310768	0.00000771	2.119E-6

Convergence criterion (ABSGCONV=0.00001) satisfied.

Fit Statistics	
-2 Log Likelihood	1247.83
AIC (smaller is better)	1283.83
AICC (smaller is better)	1303.37
BIC (smaller is better)	1319.63
CAIC (smaller is better)	1337.63
HQIC (smaller is better)	1297.63
Pearson Chi-Square	932.56
Pearson Chi-Square / DF	25.90

Parameter Estimates

Effect	conca	time	Estimate	Standard Error	DF	t Value	Pr > |t|
conca	a		1.1880	0.3974	36	2.99	0.0050
conca	b		0.7309	0.3191	36	2.29	0.0279
conca	c		-0.4985	0.3134	36	-1.59	0.1204
conca	d		-1.9333	0.4164	36	-4.64	<.0001
conca	e		-2.9805	0.7956	36	-3.75	0.0006
conca	f		1.5369	0.3967	36	3.87	0.0004
time		6	0.01211	0.7590	36	0.02	0.9874
time		12	-0.09191	0.6513	36	-0.14	0.8886
time		24	0	.	.	.	.
conca*time	a	6	-0.2423	1.0386	36	-0.23	0.8168
conca*time	a	12	0.1992	0.8529	36	0.23	0.8166
conca*time	a	24	0	.	.	.	.
conca*time	b	6	-0.09137	0.9309	36	-0.10	0.9223
conca*time	b	12	0.2009	0.8937	36	0.22	0.8234
conca*time	b	24	0	.	.	.	.
conca*time	c	6	0.3400	0.9059	36	0.38	0.7096
conca*time	c	12	0.6474	0.7774	36	0.83	0.4105
conca*time	c	24	0	.	.	.	.
conca*time	d	6	1.6256	0.9159	36	1.77	0.0844
conca*time	d	12	-0.3968	1.0092	36	-0.39	0.6965
conca*time	d	24	0	.	.	.	.
conca*time	e	6	1.7412	1.1651	36	1.49	0.1438
conca*time	e	12	-0.00144	1.6586	36	-0.00	0.9993
conca*time	e	24	0	.	.	.	.
conca*time	f	6	0	.	.	.	.
conca*time	f	12	0	.	.	.	.
conca*time	f	24	0	.	.	.	.
Residual			25.9044	.	.	.	.

Type III Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
conca	5	36	20.90	<.0001
time	2	36	2.60	0.0880

conca*time	10	36	1.60	0.1449

Odds Ratio Estimates

conca	time	_conca	time	Estimate	DF	95% Confidence Limits	
a		f		0.695	36	0.305	1.588
b		f		0.463	36	0.208	1.033
c		f		0.182	36	0.087	0.377
d		f		0.047	36	0.020	0.108
e		f		0.019	36	0.006	0.067
	6		24	1.776	36	1.024	3.081
	12		24	1.016	36	0.508	2.033

conca Least Squares Means

conca	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Mean	Standard Error Mean	Lower Mean	Upper Mean
a	1.1470	0.2683	36	4.27	0.0001	0.05	0.6028	1.6912	0.7590	0.04909	0.6463	0.8444
b	0.7408	0.2501	36	2.96	0.0054	0.05	0.2335	1.2481	0.6772	0.05468	0.5581	0.7770
c	-0.1959	0.1905	36	-1.03	0.3105	0.05	-0.5823	0.1904	0.4512	0.04717	0.3584	0.5475
d	-1.5503	0.2756	36	-5.63	<.0001	0.05	-2.1093	-0.9913	0.1750	0.03980	0.1082	0.2706
e	-2.4272	0.5244	36	-4.63	<.0001	0.05	-3.4907	-1.3636	0.08112	0.03909	0.02958	0.2037
f	1.5103	0.3060	36	4.93	<.0001	0.05	0.8896	2.1309	0.8191	0.04535	0.7088	0.8939

Differences of conca Least Squares Means

conca	_conca	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Odds Ratio	Lower Confidence Limit for Odds Ratio	Upper Confidence Limit for Odds Ratio
a	b	0.4062	0.3668	36	1.11	0.2755	0.05	-0.3378	1.1501	1.501	0.713	3.159
a	c	1.3429	0.3291	36	4.08	0.0002	0.05	0.6756	2.0103	3.830	1.965	7.466
a	d	2.6973	0.3847	36	7.01	<.0001	0.05	1.9172	3.4774	14.839	6.802	32.375
a	e	3.5742	0.5891	36	6.07	<.0001	0.05	2.3794	4.7689	35.664	10.799	117.784
a	f	-0.3633	0.4070	36	-0.89	0.3780	0.05	-1.1887	0.4622	0.695	0.305	1.588
b	c	0.9368	0.3144	36	2.98	0.0051	0.05	0.2991	1.5744	2.552	1.349	4.828
b	d	2.2911	0.3722	36	6.16	<.0001	0.05	1.5363	3.0459	9.886	4.647	21.030
b	e	3.1680	0.5810	36	5.45	<.0001	0.05	1.9896	4.3463	23.759	7.313	77.194
b	f	-0.7695	0.3952	36	-1.95	0.0594	0.05	-1.5710	0.03214	0.463	0.208	1.033
c	d	1.3543	0.3350	36	4.04	0.0003	0.05	0.6749	2.0338	3.874	1.964	7.643
c	e	2.23´2	0.5579	36	4.00	0.0003	0.05	1.0997	3.3628	9.311	3.003	28.869
c	f	-1.7062	0.3605	36	-4.73	<.0001	0.05	-2.4373	-0.9751	0.182	0.087	0.377
d	e	0.8769	0.5924	36	1.48	0.1475	0.05	-0.3246	2.0784	2.403	0.723	7.991
d	f	-3.0606	0.4118	36	-7.43	<.0001	0.05	-3.8958	-2.2253	0.047	0.020	0.108
e	f	-3.9374	0.6072	36	-6.48	<.0001	0.05	-5.1689	-2.7060	0.019	0.006	0.067

time Least Squares Means

time	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Mean	Standard Error Mean	Lower Mean	Upper Mean
6	0.2462	0.1926	36	1.29	0.2058	0.05	-0.1425	0.6389	0.5617	0.04742	0.4644	0.6545
12	-0.3098	0.2829	36	-1.09	0.2808	0.05	-0.8836	0.2640	0.4232	0.06906	0.2924	0.5656

24	-0.3261	0.1916	36	-1.70	0.0974	0.05	-0.7147	0.06251	0.4192	0.04665	0.3286	0.5156

Differences of time Least Squares Means

time	time	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Odds Ratio	Lower Confidence Limit for Odds Ratio	Upper Confidence Limit for Odds Ratio
6	12	0.5580	0.3423	36	1.63	0.1118	0.05	-0.1362	1.2522	1.747	0.873	3.498
6	24	0.5743	0.2717	36	2.11	0.0415	0.05	0.02328	1.1253	1.776	1.024	3.081
12	24	0.01630	0.3417	36	0.05	0.9622	0.05	-0.6767	0.7093	1.016	0.508	2.033

conca*time Least Squares Means

conca	time	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Mean	Standard Error Mean	Lower Mean	Upper Mean
a	6	0.9577	0.5871	36	1.63	0.1115	0.05	-0.2329	2.1484	0.7227	0.1177	0.4420	0.8955
a	12	1.2953	0.3813	36	3.40	0.0017	0.05	0.5220	2.0685	0.7850	0.06434	0.6276	0.8878
a	24	1.1880	0.3974	36	2.99	0.0050	0.05	0.3819	1.9940	0.7664	0.07116	0.5943	0.8802
b	6	0.6516	0.4342	36	1.50	0.1422	0.05	-0.2290	1.5323	0.6574	0.09780	0.4430	0.8223
b	12	0.8399	0.5222	36	1.61	0.1165	0.05	-0.2192	1.8990	0.6984	0.1100	0.4454	0.8698
b	24	0.7309	0.3191	36	2.29	0.0279	0.05	0.08382	1.3780	0.6750	0.06999	0.5209	0.7987
c	6	-0.1463	0.3825	36	-0.38	0.7043	0.05	-0.9221	0.6294	0.4635	0.09512	0.2845	0.6524
c	12	0.05698	0.2864	36	0.20	0.8434	0.05	-0.5239	0.6379	0.5142	0.07155	0.3719	0.6543
c	24	-0.4985	0.3134	36	-1.59	0.1204	0.05	-1.1341	0.1371	0.3779	0.07367	0.2434	0.5342
d	6	-0.2956	0.2988	36	-0.99	0.3292	0.05	-0.9016	0.3105	0.4266	0.07310	0.2887	0.5770
d	12	-2.4220	0.6488	36	-3.73	0.0007	0.05	-3.7379	-1.1062	0.08151	0.04857	0.02325	0.2486
d	24	-1.9333	0.4164	36	-4.64	<.0001	0.05	-2.7778	-1.0888	0.1264	0.04598	0.05853	0.2519
e	6	-1.2272	0.3850	36	-3.19	0.0030	0.05	-2.0080	-0.4464	0.2267	0.06749	0.1184	0.3902
e	12	-3.0739	1.3015	36	-2.36	0.0237	0.05	-5.7134	-0.4343	0.04420	0.05498	0.003291	0.3931
e	24	-2.9805	0.7956	36	-3.75	0.0006	0.05	-4.5941	-1.3669	0.04831	0.03658	0.01001	0.2031
f	6	1.5490	0.6471	36	2.39	0.0220	0.05	0.2365	2.8614	0.8248	0.09353	0.5589	0.9459
f	12	1.4450	0.5165	36	2.80	0.0082	0.05	0.3975	2.4924	0.8092	0.07974	0.5981	0.9236
f	24	1.5369	0.3967	36	3.87	0.0004	0.05	0.7323	2.3414	0.8230	0.05779	0.6753	0.9123

Differences of conca*time Least Squares Means

conca	time	_conca	time	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Odds Ratio	Lower Confidence Limit for Odds Ratio	Upper Confidence Limit for Odds Ratio
a	6	a	12	-0.3375	0.7000	36	-0.48	0.6326	0.05	-1.7573	1.0822	0.714	0.173	2.951
a	6	a	24	-0.2302	0.7090	36	-0.32	0.7473	0.05	-1.6681	1.2076	0.794	0.189	3.345
a	6	b	6	0.3061	0.7302	36	0.42	0.6776	0.05	-1.1748	1.7870	1.358	0.309	5.972
a	6	b	12	0.1178	0.7857	36	0.15	0.8817	0.05	-1.4757	1.7114	1.125	0.229	5.536
a	6	b	24	0.2268	0.6682	36	0.34	0.7362	0.05	-1.1263	1.5820	1.255	0.324	4.865
a	6	c	6	1.1041	0.7007	36	1.58	0.1239	0.05	-0.3170	2.5251	3.016	0.728	12.493
a	6	c	12	0.9008	0.6532	36	1.38	0.1764	0.05	-0.4241	2.2256	2.461	0.654	9.259
a	6	c	24	1.4562	0.6655	36	2.19	0.0352	0.05	0.1065	2.8059	4.290	1.[illegible]2	16.542
a	6	d	6	1.2533	0.6588	36	1.90	0.0651	0.05	-0.08273	2.5893	3.502	0.921	13.320
a	6	d	12	3.3798	0.8750	36	3.86	0.0004	0.05	1.6052	5.1543	29.364	4.979	173.178
a	6	d	24	2.8910	0.7198	36	4.02	0.0003	0.05	1.4313	4.3508	18.012	4.184	77.540

a	6	e	6	2.1849	0.7021	36	3.11	0.0036	0.05	0.7610	3.6087	8.890	2.140	36.919
a	6	e	12	4.0316	1.4278	36	2.82	0.0077	0.05	1.1359	6.9273	56.350	3.114	>999.999
a	6	e	24	3.9382	0.9888	36	3.98	0.0003	0.05	1.9329	5.9436	51.328	6.909	381.290
a	6	f	6	-0.5913	0.8738	36	-0.68	0.5029	0.05	-2.3633	1.1808	0.554	0.094	3.257
a	6	f	12	-0.4872	0.7819	36	-0.62	0.5371	0.05	-2.0731	1.0986	0.614	0.126	3.000
a	6	f	24	-0.5791	0.7086	36	-0.82	0.4191	0.05	-2.0161	0.8579	0.560	0.133	2.358
a	12	a	24	0.1073	0.5507	36	0.19	0.8466	0.05	-1.0096	1.2243	1.113	0.364	3.402
a	12	b	6	0.6436	0.5778	36	1.11	0.2727	0.05	-0.5283	1.8156	1.903	0.590	6.145
a	12	b	12	0.4554	0.6466	36	0.70	0.4858	0.05	-0.8560	1.7667	1.577	0.425	5.85'
a	12	b	24	0.5644	0.4972	36	1.14	0.2638	0.05	-0.4439	1.5727	1.758	0.642	4.819
a	12	c	6	1.4416	0.5401	36	2.67	0.0113	0.05	0.3463	2.5369	4.227	1.414	12.641
a	12	c	12	1.2383	0.4769	36	2.60	0.0135	0.05	0.2712	2.2054	3.450	1.311	9.074
a	12	c	24	1.7938	0.4935	36	3.63	0.0009	0.05	0.7928	2.7947	6.012	2.210	16.357
a	12	d	6	1.5908	0.4844	36	3.28	0.0023	0.05	0.6084	2.5733	4.908	1.837	13.108
a	12	d	12	3.7173	0.7525	36	4.94	<.0001	0.05	2.1911	5.2435	41.153	8.945	189.332
a	12	d	24	3.2286	0.5646	36	5.72	<.0001	0.05	2.0835	4.3736	25.244	8.033	79.330
a	12	e	6	2.5224	0.5418	36	4.66	<.0001	0.05	1.4235	3.6213	12.459	4.152	37.387
a	12	e	12	4.3691	1.3562	36	3.22	0.0027	0.05	1.6186	7.1196	78.974	5.046	>999.999
a	12	e	24	4.2758	0.8823	36	4.85	<.0001	0.05	2.4865	6.0651	71.936	12.019	430.552
a	12	f	6	-0.2537	0.7511	36	-0.34	0.7375	0.05	-1.7770	1.2696	0.776	0.169	3.559
a	12	f	12	-0.1497	0.6420	36	-0.23	0.8170	0.05	-1.4617	1.1523	0.861	0.234	3.165
a	12	f	24	-0.2416	0.5502	36	-0.44	0.6632	0.05	-1.3575	0.8743	0.785	0.257	2.397
a	24	b	6	0.5363	0.5886	36	0.91	0.3683	0.05	-0.6575	1.7301	1.710	0.518	5.641
a	24	b	12	0.3480	0.6562	36	0.53	0.5991	0.05	-0.9829	1.6790	1.416	0.374	5.360
a	24	b	24	0.4571	0.5097	36	0.90	0.3758	0.05	-0.5766	1.4907	1.579	0.562	4.440
a	24	c	6	1.3343	0.5516	36	2.42	0.0207	0.05	0.2156	2.4530	3.797	1.241	11.623
a	24	c	12	1.1310	0.4899	36	2.31	0.0268	0.05	0.1374	2.1245	3.099	1.147	8.369
a	24	c	24	1.6864	0.5061	36	3.33	0.0020	0.05	0.6600	2.7129	5.400	1.935	15.073
a	24	d	6	1.4835	0.4972	36	2.98	0.0051	0.05	0.4751	2.4919	4.408	1.608	12.085
a	24	d	12	3.6100	0.7608	36	4.74	<.0001	0.05	2.0669	5.1531	36.965	7.900	172.959
a	24	d	24	3.1213	0.5756	36	5.42	<.0001	0.05	1.9538	4.2887	22.675	7.056	72.871
a	24	e	6	2.4151	0.5533	36	4.36	0.0001	0.05	1.2929	3.5373	11.191	3.643	34.374
a	24	e	12	4.2618	1.3608	36	3.13	0.0034	0.05	1.5019	7.0217	70.938	4.490	>999.999
a	24	e	24	4.1685	0.8894	36	4.69	<.0001	0.05	2.3648	5.9722	64.616	10.641	392.353
a	24	f	6	-0.3610	0.7594	36	-0.48	0.6374	0.05	-1.9012	1.1792	0.697	0.149	3.252
a	24	f	12	-0.2570	0.6517	36	-0.39	0.6956	0.05	-1.5787	1.0647	0.773	0.206	2.900
a	24	f	24	-0.3489	0.5615	36	-0.62	0.5383	0.05	-1.4878	0.7899	0.705	0.226	2.203
b	6	b	12	-0.1883	0.6792	36	-0.28	0.7832	0.05	-1.5657	1.1891	0.828	0.209	3.284
b	6	b	24	-0.07926	0.5388	36	-0.15	0.8839	0.05	-1.1721	1.0135	0.924	0.310	2.755
b	6	c	6	0.7980	0.5787	36	1.38	0.1764	0.05	-0.3756	1.9715	2.221	0.687	7.182
b	6	c	12	0.5946	0.5202	36	1.14	0.2605	0.05	-0.4603	1.6496	1.8'2	0.631	5.205
b	6	c	24	1.1501	0.5355	36	2.15	0.0385	0.05	0.06409	2.2361	3.159	1.066	9.357
b	6	d	6	0.9472	0.5271	36	1.80	0.0807	0.05	-0.1218	2.0162	2.578	0.885	7.510
b	6	d	12	3.0737	0.7807	36	3.94	0.0004	0.05	1.4903	4.6570	21.621	4.439	105.317
b	6	d	24	2.5849	0.6016	36	4.30	0.0001	0.05	1.3648	3.8051	13.262	3.915	44.928
b	6	e	6	1.8788	0.5803	36	3.24	0.0026	0.05	0.7019	3.0557	6.546	2.017	21.236

	6	e	12	3.7255	1.3720	36	2.72	0.0101	0.05	0.9429	6.5081	41.491	2.567	670.525
b	6	e	24	3.6321	0.9064	36	4.01	0.0003	0.05	1.7939	5.4704	37.793	6.013	237.552
b	6	f	6	-0.8974	0.7793	36	-1.15	0.2571	0.05	-2.4779	0.6832	0.408	0.084	1.980
b	6	f	12	-0.7933	0.6748	36	-1.18	0.2474	0.05	-2.1618	0.5752	0.452	0.115	1.777
b	6	f	24	-0.8852	0.5881	36	-1.51	0.1410	0.05	-2.0781	0.3076	0.413	0.125	1.360
b	12	b	24	0.1090	0.6120	36	0.18	0.8596	0.05	-1.1321	1.3502	1.115	0.322	3.858
b	12	c	6	0.9862	0.6473	36	1.52	0.1363	0.05	-0.3266	2.2991	2.681	0.721	9.965
b	12	c	12	0.7829	0.5956	36	1.31	0.1970	0.05	-0.4250	1.9909	2.188	0.654	7.322
b	12	c	24	1.3384	0.6090	36	2.20	0.0345	0.05	0.1032	2.5736	3.813	1.109	13.113
b	12	d	6	1.1355	0.6017	36	1.89	0.0672	0.05	-0.08476	2.3557	3.113	0.919	10.546
b	12	d	12	3.2619	0.8329	36	3.92	0.0004	0.05	1.5728	4.9511	26.100	4.820	141.324
b	12	d	24	2.7732	0.6679	36	4.15	0.0002	0.05	1.4186	4.1278	16.010	4.131	62.042
b	12	e	6	2.0671	0.6488	36	3.19	0.0030	0.05	0.7513	3.3829	7.902	2.120	29.455
b	12	e	12	3.9138	1.4024	36	2.79	0.0084	0.05	1.0697	6.7579	50.087	2.914	860.813
b	12	e	24	3.8204	0.9517	36	4.01	0.0003	0.05	1.8903	5.7505	45.623	6.621	314.361
b	12	f	6	-0.7091	0.8316	36	-0.85	0.3995	0.05	-2.3955	0.9774	0.492	0.091	2.658
b	12	f	12	-0.6050	0.7345	36	-0.82	0.4155	0.05	-2.0946	0.8846	0.546	0.123	2.422
b	12	f	24	-0.6969	0.6558	36	-1.06	0.2950	0.05	-2.0270	0.6331	0.498	0.132	1.883
b	24	c	6	0.8772	0.4981	36	1.76	0.0867	0.05	-0.1330	1.8874	2.404	0.875	6.602
b	24	c	12	0.6739	0.4288	36	1.57	0.1248	0.05	-0.1957	1.5435	1.962	0.822	4.681
b	24	c	24	1.2294	0.4472	36	2.75	0.0093	0.05	0.3224	2.1364	3.419	1.380	8.469
b	24	d	6	1.0264	0.4371	36	2.35	0.0245	0.05	0.1399	1.9130	2.791	1.150	6.773
b	24	d	12	3.1529	0.7230	36	4.36	0.0001	0.05	1.6866	4.6192	23.404	5.401	101.417
b	24	d	24	2.6642	0.5246	36	5.08	<.0001	0.05	1.6003	3.7281	14.356	4.954	41.601
b	24	e	6	1.9580	0.5000	36	3.92	0.0004	0.05	0.9440	2.9721	7.085	2.570	19.533
b	24	e	12	3.8047	1.3400	36	2.84	0.0074	0.05	1.0870	6.5224	44.913	2.965	680.243
b	24	e	24	3.7114	0.8572	36	4.33	0.0001	0.05	1.9729	5.4499	40.911	7.191	232.732
b	24	f	6	-0.8181	0.7215	36	-1.13	0.2643	0.05	-2.2814	0.6452	0.441	0.102	1.906
b	24	f	12	-0.7141	0.6071	36	-1.18	0.2472	0.05	-1.9453	0.5172	0.490	0.143	1.677
b	24	f	24	-0.8060	0.5091	36	-1.58	0.1221	0.05	-1.8385	0.2265	0.447	0.159	1.254
c	6	c	12	-0.2033	0.4779	36	-0.43	0.6730	0.05	-1.1725	0.7658	0.816	0.310	2.151
c	6	c	24	0.3522	0.4945	36	0.71	0.4810	0.05	-0.6507	1.3550	1.422	0.522	3.877
c	6	d	6	0.1492	0.4854	36	0.31	0.7603	0.05	-0.8352	1.1336	1.161	0.434	3.107
c	6	d	12	2.2757	0.7532	36	3.02	0.0046	0.05	0.7482	3.8032	9.735	2.113	44.844
c	6	d	24	1.7870	0.5654	36	3.16	0.0032	0.05	0.6402	2.9337	5.971	1.897	18.797
c	6	e	6	1.0808	0.5427	36	1.99	0.0540	0.05	-0.01983	2.1815	2.947	0.980	8.859
c	6	e	12	2.9275	1.3565	36	2.16	0.0377	0.05	0.1763	5.6787	18.681	1.193	292.573
c	6	e	24	2.8342	0.8828	36	3.21	0.0028	0.05	1.0438	4.6246	17.016	2.840	101.958
c	6	f	6	-1.6953	0.7517	36	-2.26	0.0303	0.05	-3.2199	-0.1707	0.184	0.040	0.843
c	6	f	12	-1.5913	0.6427	36	-2.48	0.0181	0.05	-2.8948	-0.2878	0.204	0.055	0.750
c	6	f	24	-1.6832	0.5511	36	-3.05	0.0042	0.05	-2.8008	-0.5656	0.186	0.061	0.568
c	12	c	24	0.5555	0.4246	36	1.31	0.1991	0.05	-0.3056	1.4166	1.743	0.737	4.123
c	12	d	6	0.3525	0.4139	36	0.85	0.4000	0.05	-0.4869	1.1920	1.423	0.615	3.294
c	12	d	12	2.4790	0.7092	36	3.50	0.0013	0.05	1.0406	3.9174	11.929	2.831	50.267
c	12	d	24	1.9903	0.5054	36	3.94	0.0004	0.05	0.9652	3.0153	7.318	2.625	20.395
c	12	e	6	1.2841	0.4799	36	2.68	0.0111	0.05	0.3109	2.2573	3.612	1.365	9.557

	12	e	12	3.1308	1.3326	36	2.35	0.0244	0.05	0.4281	5.8336	22.893	1.534	341.569
c	12	e	24	3.0375	0.8456	36	3.59	0.0010	0.05	1.3225	4.7525	20.853	3.753	115.868
c	12	f	6	-1.4920	0.7077	36	-2.11	0.0420	0.05	-2.9273	-0.05675	0.225	0.054	0.945
c	12	f	12	-1.3880	0.5906	36	-2.35	0.0244	0.05	-2.5858	-0.1902	0.250	0.075	0.827
c	12	f	24	-1.4799	0.4893	36	-3.02	0.0046	0.05	-2.4722	-0.4875	0.228	0.084	0.614
c	24	d	6	-0.2029	0.4330	36	-0.47	0.6421	0.05	-1.0811	0.6753	0.816	0.339	1.965
c	24	d	12	1.9235	0.7205	36	2.67	0.0113	0.05	0.4623	3.3848	6.845	1.588	29.513
c	24	d	24	1.4348	0.5212	36	2.75	0.0092	0.05	0.3778	2.4918	4.199	1.459	12.083
c	24	e	6	0.7287	0.4964	36	1.47	0.1508	0.05	-0.2781	1.7354	2.072	0.757	5.671
c	24	e	12	2.5754	1.3387	36	1.92	0.0623	0.05	-0.1396	5.2904	13.136	0.870	198.415
c	24	e	24	2.4820	0.8551	36	2.90	0.0063	0.05	0.7478	4.2163	11.965	2.112	67.780
c	24	f	6	-2.0475	0.7190	36	-2.85	0.0072	0.05	-3.5057	-0.5892	0.129	0.030	0.555
c	24	f	12	-1.9434	0.6041	36	-3.22	0.0027	0.05	-3.1687	-0.7182	0.143	0.042	0.488
c	24	f	24	-2.0354	0.5056	36	-4.03	0.0003	0.05	-3.0607	-1.0100	0.131	0.047	0.364
d	6	d	12	2.1265	0.7143	36	2.98	0.0052	0.05	0.6778	3.5751	8.385	1.970	35.700
d	6	d	24	1.6377	0.5125	36	3.20	0.0029	0.05	0.5983	2.6772	5.144	1.819	14.545
d	6	e	6	0.9316	0.4873	36	1.91	0.0639	0.05	-0.05679	1.9200	2.539	0.945	6.821
d	6	e	12	2.7783	1.3354	36	2.08	0.0447	0.05	0.07006	5.4865	16.092	1.073	241.417
d	6	e	24	2.6849	0.8499	36	3.16	0.0032	0.05	0.9613	4.4086	14.657	2.615	82.153
d	6	f	6	-1.8445	0.7128	36	-2.59	0.0138	0.05	-3.2901	-0.3989	0.158	0.037	0.671
d	6	f	12	-1.7405	0.5967	36	-2.92	0.0061	0.05	-2.9507	-0.5303	0.175	0.052	0.588
d	6	f	24	-1.8324	0.4967	36	-3.69	0.0007	0.05	-2.8397	-0.8252	0.160	0.058	0.438
d	12	d	24	-0.4887	0.7709	36	-0.63	0.5301	0.05	-2.0523	1.0748	0.613	0.128	2.929
d	12	e	6	-1.1949	0.7544	36	-1.58	0.1220	0.05	-2.7249	0.3352	0.303	0.066	1.398
d	12	e	12	0.6518	1.4542	36	0.45	0.6567	0.05	-2.2975	3.6012	1.919	0.101	36.641
d	12	e	24	0.5585	1.0266	36	0.54	0.5898	0.05	-1.5236	2.6406	1.748	0.218	14.021
d	12	f	6	-3.9710	0.9164	36	-4.33	0.0001	0.05	-5.8295	-2.1125	0.019	0.003	0.121
d	12	f	12	-3.8670	0.8293	36	-4.66	<.0001	0.05	-5.5488	-2.1851	0.021	0.004	0.112
d	12	f	24	-3.9589	0.7605	36	-5.21	<.0001	0.05	-5.5012	-2.4166	0.019	0.004	0.089
d	24	e	6	-0.7061	0.5671	36	-1.25	0.2211	0.05	-1.8563	0.4440	0.494	0.156	1.559
d	24	e	12	1.1406	1.3665	36	0.83	0.4094	0.05	-1.6308	3.9119	3.128	0.196	49.995
d	24	e	24	1.0472	0.8980	36	1.17	0.2512	0.05	-0.7740	2.8684	2.850	0.461	17.610
d	24	f	6	-3.4823	0.7695	36	-4.53	<.0001	0.05	-5.0430	-1.9216	0.031	0.006	0.146
d	24	f	12	-3.3783	0.6634	36	-5.09	<.0001	0.05	-4.7238	-2.0327	0.034	0.009	0.131
d	24	f	24	-3.4702	0.5751	36	-6.03	<.0001	0.05	-4.6366	-2.3037	0.031	0.010	0.100
e	6	e	12	1.8467	1.3572	36	1.36	0.1821	0.05	-0.9059	4.5993	6.339	0.404	99.416
e	6	e	24	1.7533	0.8839	36	1.98	0.0550	0.05	-0.03923	3.5459	5.774	0.962	34.672
e	6	f	6	-2.7761	0.7530	36	-3.69	0.0007	0.05	-4.3033	-1.2490	0.062	0.014	0.287
e	6	f	12	-2.6721	0.6442	36	-4.15	0.0002	0.05	-3.9786	-1.3656	0.069	0.019	0.255
e	6	f	24	-2.7640	0.5528	36	-5.00	<.0001	0.05	-3.8852	-1.6429	0.063	0.021	0.193
e	12	e	24	-0.09335	1.5254	36	-0.06	0.9515	0.05	-3.1870	3.0003	0.911	0.041	20.092
e	12	f	6	-4.6228	1.4535	36	-3.18	0.0030	0.05	-7.5707	-1.6750	0.010	<0.001	0.187
e	12	f	12	-4.5188	1.4002	36	-3.23	0.0027	0.05	-7.3586	-1.6790	0.011	<0.001	0.187
e	12	f	24	-4.6107	1.3606	36	-3.39	0.0017	0.05	-7.3702	-1.8513	0.010	<0.001	0.157
e	24	f	6	-4.5295	1.0256	36	-4.42	<.0001	0.05	-6.6094	-2.4495	0.011	0.001	0.086
e	24	f	12	-4.4255	0.9486	36	-4.67	<.0001	0.05	-6.3492	-2.5017	0.012	0.002	0.082

	24	f	24	-4.5174	0.8890	36	-5.08	<.0001	0.05	-6.3204	-2.7143	0.011	0.002	0.066
f	6	f	12	0.1040	0.8280	36	0.13	0.9007	0.05	-1.5752	1.7832	1.110	0.207	5.949
f	6	f	24	0.01211	0.7590	36	0.02	0.9874	0.05	-1.5273	1.5515	1.012	0.217	4.719
f	12	f	24	-0.09191	0.6513	36	-0.14	0.8886	0.05	-1.4127	1.2289	0.912	0.243	3.417

Performed by J. Luoma; SAS version 9.3 08:43 18MAR15

Effects of Pseudomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel species

Overall Model: Glochidia Viability (unadjusted) - At 6, 12 and 24 h

The GLIMMIX Procedure

form=FDP sps=WAS

Model Information	
Data Set	WORK.GLOCHIDIA2
Response Variable (Events)	via_glo
Response Variable (Trials)	tot
Response Distribution	Binomial
Link Function	Logit
Variance Function	Default
Variance Matrix	Diagonal
Estimation Technique	Maximum Likelihood
Degrees of Freedom Method	Residual

Class Level Information		
Class	Levels	Values
conca	6	a b c d e f
time	1	24

Number of Observations Read	18
Number of Observations Used	18
Number of Events	595
Number of Trials	1311

Dimensions	
Covariance Parameters	1
Columns in X	13
Columns in Z	0
Subjects (Blocks in V)	1
Max Obs per Subject	18

Optimization Information	
Optimization Technique	Newton-Raphson
Parameters in Optimization	6
Lower Boundaries	0
Upper Boundaries	0
Fixed Effects	Not Profiled

Iteration History

Iteration	Restarts	Evaluations	Objective Function	Change	Max Gradient
0	0	4	79.355639344	.	1.385246
1	0	3	79.297918426	0.05772092	0.01404
2	0	3	79.297913227	0.00000520	1.521E-6

Convergence criterion (ABSGCONV=0.00001) satisfied.

Fit Statistics	
-2 Log Likelihood	158.60
AIC (smaller is better)	170.60
AICC (smaller is better)	178.23
BIC (smaller is better)	175.94
CAIC (smaller is better)	181.94
HQIC (smaller is better)	171.33
Pearson Chi-Square	74.96
Pearson Chi-Square / DF	6.25

Parameter Estimates

Effect	conca	time	Estimate	Standard Error	DF	t Value	Pr > |t|
conca	a		0.2283	0.3792	12	0.60	0.5585
conca	b		0.01482	0.3042	12	0.05	0.9620
conca	c		0.02247	0.3747	12	0.06	0.9532
conca	d		-0.4387	0.3859	12	-1.14	0.2778
conca	e		-1.0074	0.3622	12	-2.78	0.0166
conca	f		0.04478	0.3054	12	0.15	0.8859
time		24	0	.	.	.	.
conca*time	a	24	0	.	.	.	.
conca*time	b	24	0	.	.	.	.
conca*time	c	24	0	.	.	.	.
conca*time	d	24	0	.	.	.	.
conca*time	e	24	0	.	.	.	.
conca*time	f	24	0	.	.	.	.
Residual			6.2465	.	.	.	.

Type III Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
conca	5	12	1.61	0.2305
time	0	.	.	.
conca*time	0	.	.	.

Odds Ratio Estimates

conca	time	_conca	time	Estimate	DF	95% Confidence Limits	
a		f		1.201	12	0.416	3.471
b		f		0.970	12	0.379	2.482
c		f		0.978	12	0.341	2.804
d		f		0.617	12	0.211	1.802
e		f		0.349	12	0.124	0.980

conca Least Squares Means

conca	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Mean	Standard Error Mean	Lower Mean	Upper Mean
a	0.2283	0.3792	12	0.60	0.5585	0.05	-0.5980	1.0546	0.5568	0.09359	0.3548	0.7416
b	0.01482	0.3042	12	0.05	0.9620	0.05	-0.6480	0.6776	0.5037	0.07605	0.3434	0.6632
c	0.02247	0.3747	12	0.06	0.9532	0.05	-0.7939	0.8388	0.5056	0.09366	0.3113	0.6982

d	-0.4387	0.3859	12	-1.14	0.2778	0.05	-1.2795	0.4020	0.3920	0.09197	0.2176	0.5992
e	-1.0074	0.3622	12	-2.78	0.0166	0.05	-1.7966	-0.2182	0.2675	0.07097	0.1423	0.4457
f	0.04478	0.3054	12	0.15	0.8859	0.05	-0.6207	0.7102	0.5112	0.07632	0.3496	0.6705

Differences of conca Least Squares Means

conca	_conca	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Odds Ratio	Lower Confidence Limit for Odds Ratio	Upper Confidence Limit for Odds Ratio
a	b	0.2134	0.4862	12	0.44	0.6684	0.05	-0.8458	1.2727	1.238	0.429	3.571
a	c	0.2058	0.5331	12	0.39	0.7062	0.05	-0.9558	1.3673	1.228	0.385	3.925
a	d	0.6670	0.5410	12	1.23	0.2413	0.05	-0.5119	1.8458	1.948	0.599	6.333
a	e	1.2357	0.5244	12	2.36	0.0363	0.05	0.09304	2.3783	3.441	1.098	10.786
a	f	0.1835	0.4869	12	0.38	0.7129	0.05	-0.8775	1.2444	1.201	0.416	3.471
b	c	-0.00766	0.4826	12	-0.02	0.9876	0.05	-1.0592	1.0439	0.992	0.347	2.840
b	d	0.4535	0.4914	12	0.92	0.3742	0.05	-0.6171	1.5242	1.574	0.540	4.591
b	e	1.0222	0.4730	12	2.16	0.0516	0.05	-0.00839	2.0528	2.779	0.992	7.790
b	f	-0.02997	0.4311	12	-0.07	0.9457	0.05	-0.9692	0.9093	0.970	0.379	2.482
c	d	0.4612	0.5379	12	0.86	0.4080	0.05	-0.7107	1.6331	1.586	0.491	5.120
c	e	1.0299	0.5211	12	1.98	0.0716	0.05	-0.1056	2.1653	2.801	0.900	8.717
c	f	-0.02231	0.4834	12	-0.05	0.9639	0.05	-1.0755	1.0309	0.978	0.341	2.804
d	e	0.5687	0.5292	12	1.07	0.3037	0.05	-0.5845	1.7218	1.766	0.557	5.595
d	f	-0.4835	0.4921	12	-0.98	0.3453	0.05	-1.5558	0.5887	0.617	0.211	1.802
e	f	-1.0522	0.4738	12	-2.22	0.0464	0.05	-2.0845	-0.01989	0.349	0.124	0.980

time Least Squares Means

time	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Mean	Standard Error Mean	Lower Mean	Upper Mean
24	-0.1893	0.1444	12	-1.31	0.2143	0.05	-0.5038	0.1252	0.4528	0.03577	0.3766	0.5313

conca*time Least Squares Means

conca	time	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Mean	Standard Error Mean	Lower Mean	Upper Mean
a	24	0.2283	0.3792	12	0.60	0.5585	0.05	-0.5980	1.0546	0.5568	0.09359	0.3548	0.7416
b	24	0.01482	0.3042	12	0.05	0.9620	0.05	-0.6480	0.6776	0.5037	0.07605	0.3434	0.6632
c	24	0.02247	0.3747	12	0.06	0.9532	0.05	-0.7939	0.8388	0.5056	0.09366	0.3113	0.6982
d	24	-0.4387	0.3859	12	-1.14	0.2778	0.05	-1.2795	0.4020	0.3920	0.09197	0.2176	0.5992
e	24	-1.0074	0.3622	12	-2.78	0.0166	0.05	-1.7966	-0.2182	0.2675	0.07097	0.1423	0.4457
f	24	0.04478	0.3054	12	0.15	0.8859	0.05	-0.6207	0.7102	0.5112	0.07632	0.3496	0.6705

Differences of conca*time Least Squares Means

conca	time	_conca	time	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Odds Ratio	Lower Confidence Limit for Odds Ratio	Upper Confidence Limit for Odds Ratio
a	24	b	24	0.2134	0.4862	12	0.44	0.6684	0.05	-0.8458	1.2727	1.238	0.429	3.571
a	24	c	24	0.2058	0.5331	12	0.39	0.7062	0.05	-0.9558	1.3673	1.228	0.385	3.925
a	24	d	24	0.6670	0.5410	12	1.23	0.2413	0.05	-0.5119	1.8458	1.948	0.599	6.333
a	24	e	24	1.2357	0.5244	12	2.36	0.0363	0.05	0.09304	2.3783	3.441	1.098	10.786

a	24	f	24	0.1835	0.4869	12	0.38	0.7129	0.05	-0.8775	1.2444	1.201	0.416	3.471
b	24	c	24	-0.00766	0.4826	12	-0.02	0.9876	0.05	-1.0592	1.0439	0.992	0.347	2.840
b	24	d	24	0.4535	0.4914	12	0.92	0.3742	0.05	-0.6171	1.5242	1.574	0.540	4.591
b	24	e	24	1.0222	0.4730	12	2.16	0.0516	0.05	-0.00839	2.0528	2.779	0.992	7.790
b	24	f	24	-0.02997	0.4311	12	-0.07	0.9457	0.05	-0.9692	0.9093	0.970	0.379	2.482
c	24	d	24	0.4612	0.5379	12	0.86	0.4060	0.05	-0.7107	1.6331	1.586	0.491	5.120
c	24	e	24	1.0299	0.5211	12	1.98	0.0716	0.05	-0.1056	2.1653	2.801	0.900	8.717
c	24	f	24	-0.02231	0.4834	12	-0.05	0.9639	0.05	-1.0755	1.0309	0.978	0.341	2.804
d	24	e	24	0.5687	0.5292	12	1.07	0.3037	0.05	-0.5845	1.7218	1.766	0.557	5.595
d	24	f	24	-0.4835	0.4921	12	-0.98	0.3453	0.05	-1.5558	0.5887	0.617	0.211	1.802
e	24	f	24	-1.0522	0.4738	12	-2.22	0.0464	0.05	-2.0845	-0.01989	0.349	0.124	0.980

Performed by J. Luoma; SAS version 9.3 08:43 18MAR15

Effects of Pseudomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel species

Overall Model: Glochidia Viability (unadjusted) - At 6, 12 and 24 h

The GLIMMIX Procedure

form=SDP sps=BLS

Model Information	
Data Set	WORK.GLOCHIDIA2
Response Variable (Events)	via_glo
Response Variable (Trials)	tot
Response Distribution	Binomial
Link Function	Logit
Variance Function	Default
Variance Matrix	Diagonal
Estimation Technique	Maximum Likelihood
Degrees of Freedom Method	Residual

Class Level Information		
Class	Levels	Values
conca	6	a b c d e f
time	3	6 12 24

Number of Observations Read	54
Number of Observations Used	54
Number of Events	3005
Number of Trials	14012

Dimensions	
Covariance Parameters	1
Columns in X	27
Columns in Z	0
Subjects (Blocks in V)	1
Max Obs per Subject	54

Optimization Information	
Optimization Technique	Newton-Raphson
Parameters in Optimization	18
Lower Boundaries	0
Upper Boundaries	0
Fixed Effects	Not Profiled

Iteration History

Iteration	Restarts	Evaluations	Objective Function	Change	Max Gradient
0	0	4	184.62071378	.	18.79475
1	0	3	173.98809789	10.63261589	2.104118
2	0	3	173.64686027	0.34123762	0.045959
3	0	3	173.59835279	0.04850748	0.01659
4	0	3	173.58069969	0.01765310	0.006103

5	0	3	173.57420591	0.00649377	0.002245
6	0	3	173.57181704	0.00238887	0.000826
7	0	3	173.57093823	0.00087881	0.000304
8	0	3	173.57061494	0.00032329	0.000112
9	0	3	173.57049601	0.00011893	0.000041
10	0	3	173.57045225	0.00004375	0.000015
11	0	3	173.57043616	0.00001610	5.565E-6

Convergence criterion (ABSGCONV=0.00001) satisfied.

Fit Statistics	
-2 Log Likelihood	347.14
AIC (smaller is better)	383.14
AICC (smaller is better)	402.68
BIC (smaller is better)	418.94
CAIC (smaller is better)	436.94
HQIC (smaller is better)	396.95
Pearson Chi-Square	137.50
Pearson Chi-Square / DF	3.82

Parameter Estimates

Effect	conca	time	Estimate	Standard Error	DF	t Value	Pr > |t|
conca	a		-1.7763	0.2266	36	-7.84	<.0001
conca	b		-2.5373	0.2930	36	-8.66	<.0001
conca	c		-3.3828	0.3459	36	-9.78	<.0001
conca	d		-4.8730	0.7415	36	-6.57	<.0001
conca	e		-4.8581	0.6540	36	-7.43	<.0001
conca	f		1.6550	0.1428	36	11.59	<.0001
time		6	0.1407	0.2355	36	0.60	0.5541
time		12	0.4771	0.2901	36	1.64	0.1088
time		24	0	.	.	.	.
conca*time	a	6	-0.4866	0.4003	36	-1.22	0.2321
conca*time	a	12	-0.3185	0.4090	36	-0.78	0.4412
conca*time	a	24	0	.	.	.	.
conca*time	b	6	-2.4801	0.9545	36	-2.60	0.0135
conca*time	b	12	-0.2233	0.4994	36	-0.45	0.6575
conca*time	b	24	0	.	.	.	.
conca*time	c	6	-0.7873	0.6886	36	-1.14	0.2605
conca*time	c	12	-1.5131	0.7958	36	-1.90	0.0653
conca*time	c	24	0	.	.	.	.
conca*time	d	6	0.4683	0.9963	36	0.47	0.6412
conca*time	d	12	-13.1562	638.55	36	-0.02	0.9837
conca*time	d	24	0	.	.	.	.
conca*time	e	6	-0.04760	1.0607	36	-0.04	0.9645
conca*time	e	12	-0.5731	1.2139	36	-0.47	0.6397
conca*time	e	24	0	.	.	.	.
conca*time	f	6	0	.	.	.	.

conca*time	f	12	0	.	.	.	.
conca*time	f	24	0	.	.	.	.
Residual			3.8195	.	.	.	.

Type III Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
conca	5	36	164.07	<.0001
time	2	36	0.90	0.4153
conca*time	10	36	1.19	0.3275

Odds Ratio Estimates							
conca	time	_conca	time	Estimate	DF	95% Confidence Limits	
a		f		0.025	36	0.018	0.035
b		f		0.006	36	0.003	0.012
c		f		0.003	36	0.002	0.006
d		f		<0.001	36	<0.001	>999.999
e		f		0.001	36	<0.001	0.003
	6		24	0.660	36	0.353	1.236
	12		24	0.116	36	<0.001	>999.999

conca Least Squares Means												
conca	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Mean	Standard Error Mean	Lower Mean	Upper Mean
a	-1.8388	0.1232	36	-14.93	<.0001	0.05	-2.0886	-1.5889	0.1372	0.01458	0.1102	0.1695
b	-3.2325	0.3223	36	-10.03	<.0001	0.05	-3.8862	-2.5788	0.03796	0.01177	0.02011	0.07051
c	-3.9437	0.3070	36	-12.85	<.0001	0.05	-4.5663	-3.3211	0.01901	0.005724	0.01029	0.03485
d	-8.8964	212.85	36	-0.04	0.9669	0.05	-440.57	422.78	0.000137	0.02913	459E-194	1.0000
e	-4.8591	0.4751	36	-10.23	<.0001	0.05	-5.8226	-3.8956	0.007698	0.003629	0.002951	0.01993
f	1.8609	0.1151	36	16.17	<.0001	0.05	1.6275	2.0944	0.8654	0.01341	0.8358	0.8904

Differences of conca Least Squares Means												
conca	_conca	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Odds Ratio	Lower Confidence Limit for Odds Ratio	Upper Confidence Limit for Odds Ratio
a	b	1.3938	0.3451	36	4.04	0.0003	0.05	0.6940	2.0936	4.030	2.002	8.114
a	c	2.1049	0.3308	36	6.36	<.0001	0.05	1.4341	2.7758	8.207	4.196	16.052
a	d	7.0576	212.85	36	0.03	0.9737	0.05	-424.62	438.73	>999.999	<0.001	>999.999
a	e	3.0203	0.4908	36	6.15	<.0001	0.05	2.0249	4.0157	20.497	7.576	55.459
a	f	-3.6997	0.1686	36	-21.94	<.0001	0.05	-4.0416	-3.3578	0.025	0.018	0.035
b	c	0.7112	0.4451	36	1.60	0.1188	0.05	-0.1916	1.6139	2.036	0.826	5.023
b	d	5.6639	212.85	36	0.03	0.9789	0.05	-426.01	437.34	288.261	<0.001	>999.999
b	e	1.6265	0.5741	36	2.83	0.0075	0.05	0.4622	2.7908	5.086	1.588	16.295
b	f	-5.0935	0.3423	36	-14.88	<.0001	0.05	-5.7876	-4.3994	0.006	0.003	0.012
c	d	4.9527	212.85	36	0.02	0.9816	0.05	-426.72	436.63	141.553	<0.001	>999.999
c	e	0.9153	0.5656	36	1.62	0.1143	0.05	-0.2318	2.0625	2.498	0.793	7.866
c	f	-5.8047	0.3279	36	-17.70	<.0001	0.05	-6.4696	-5.1397	0.003	0.002	0.006
d	e	-4.0373	212.85	36	-0.02	0.9850	0.05	-435.72	427.64	0.018	<0.001	>999.999

d	f	-10.7573	212.85	36	-0.05	0.9600	0.05	-442.43	420.92	<0.001	<0.001	>999.999
e	f	-6.7200	0.4888	36	-13.75	<.0001	0.05	-7.7114	-5.7286	0.001	<0.001	0.003

time Least Squares Means

time	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Mean	Standard Error Mean	Lower Mean	Upper Mean
6	-3.0436	0.2465	36	-12.35	<.0001	0.05	-3.5435	-2.5438	0.04549	0.01070	0.02810	0.07284
12	-4.7824	106.42	36	-0.04	0.9644	0.05	-220.62	211.06	0.008307	0.8767	1.53E-96	1.0000
24	-2.6288	0.1867	36	-14.08	<.0001	0.05	-3.0074	-2.2501	0.06731	0.01172	0.04709	0.09534

Differences of time Least Squares Means

time	time	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Odds Ratio	Lower Confidence Limit for Odds Ratio	Upper Confidence Limit for Odds Ratio
6	12	1.7387	106.42	36	0.02	0.9871	0.05	-214.10	217.58	5.690	<0.001	>999.999
6	24	-0.4149	0.3092	36	-1.34	0.1880	0.05	-1.0419	0.2122	0.660	0.353	1.236
12	24	-2.1536	106.42	36	-0.02	0.9840	0.05	-217.99	213.69	0.116	<0.001	>999.999

conca*time Least Squares Means

conca	time	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Mean	Standard Error Mean	Lower Mean	Upper Mean
a	6	-2.1223	0.2312	36	-9.18	<.0001	0.05	-2.5912	-1.6533	0.1070	0.02208	0.06971	0.1607
a	12	-1.6177	0.1783	36	-9.07	<.0001	0.05	-1.9793	-1.2562	0.1655	0.02462	0.1214	0.2216
a	24	-1.7763	0.2266	36	-7.84	<.0001	0.05	-2.2358	-1.3168	0.1448	0.02805	0.09658	0.2113
b	6	-4.8767	0.8773	36	-5.56	<.0001	0.05	-6.6560	-3.0974	0.007564	0.006586	0.001285	0.04321
b	12	-2.2835	0.2818	36	-8.10	<.0001	0.05	-2.8551	-1.7120	0.09250	0.02365	0.05442	0.1529
b	24	-2.5373	0.2930	36	-8.66	<.0001	0.05	-3.1316	-1.9430	0.07328	0.01990	0.04182	0.1253
c	6	-4.0295	0.5468	36	-7.37	<.0001	0.05	-5.1385	-2.9204	0.01747	0.009388	0.005832	0.05115
c	12	-4.4188	0.6554	36	-6.74	<.0001	0.05	-5.7480	-3.0897	0.01190	0.007709	0.003179	0.04353
c	24	-3.3828	0.3459	36	-9.78	<.0001	0.05	-4.0844	-2.6813	0.03284	0.01099	0.01655	0.06409
d	6	-4.2641	0.6223	36	-6.85	<.0001	0.05	-5.5263	-3.0019	0.01387	0.008512	0.003965	0.04734
d	12	-17.5521	638.55	36	-0.03	0.9782	0.05	-1312.58	1277.48	2.384E-8	0.000015	0	1.0000
d	24	-4.8730	0.7415	36	-6.57	<.0001	0.05	-6.3768	-3.3692	0.007592	0.005587	0.001698	0.03327
e	6	-4.7650	0.8013	36	-5.95	<.0001	0.05	-6.3900	-3.1400	0.008451	0.006714	0.001675	0.04149
e	12	-4.9541	0.9806	36	-5.05	<.0001	0.05	-6.9428	-2.9653	0.007005	0.006821	0.000965	0.04902
e	24	-4.8581	0.6540	36	-7.43	<.0001	0.05	-6.1844	-3.5318	0.007705	0.005000	0.002057	0.02842
f	6	1.7957	0.1873	36	9.59	<.0001	0.05	1.4159	2.1755	0.8576	0.02287	0.8047	0.8980
f	12	2.1321	0.2525	36	8.44	<.0001	0.05	1.6200	2.6443	0.8940	0.02393	0.8348	0.9337
f	24	1.6550	0.1428	36	11.59	<.0001	0.05	1.3653	1.9447	0.8396	0.01924	0.7966	0.8749

Differences of conca*time Least Squares Means

conca	time	_conca	time	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Odds Ratio	Lower Confidence Limit for Odds Ratio	Upper Confidence Limit for Odds Ratio
a	6	a	12	-0.5045	0.2920	36	-1.73	0.0926	0.05	-1.0967	0.08762	0.604	0.334	1.092
a	6	a	24	-0.3459	0.3237	36	-1.07	0.2923	0.05	-1.0025	0.3106	0.708	0.367	1.364
a	6	b	6	2.7545	0.9073	36	3.04	0.0044	0.05	0.9144	4.5945	15.713	2.495	98.942

a	6	b	12	0.1613	0.3645	36	0.44	0.6608	0.05	-0.5780	0.9005	1.175	0.561	2.461
a	6	b	24	0.4151	0.3733	36	1.11	0.2735	0.05	-0.3419	1.1721	1.514	0.710	3.229
a	6	c	6	1.9072	0.5937	36	3.21	0.0028	0.05	0.7031	3.1113	6.734	2.020	22.450
a	6	c	12	2.2966	0.6950	36	3.30	0.0022	0.05	0.8871	3.7060	9.940	2.428	40.691
a	6	c	24	1.2606	0.4161	36	3.03	0.0045	0.05	0.4167	2.1045	3.527	1.517	8.203
a	6	d	6	2.1418	0.6639	36	3.23	0.0027	0.05	0.7953	3.4883	8.515	2.215	32.730
a	6	d	12	15.4298	638.55	36	0.02	0.9809	0.05	-1279.60	1310.46	>999.999	<0.001	>999.999
a	6	d	24	2.7508	0.7767	36	3.54	0.0011	0.05	1.1755	4.3260	15.654	3.240	75.640
a	6	e	6	2.6428	0.8339	36	3.17	0.0031	0.05	0.9514	4.3341	14.052	2.589	76.254
a	6	e	12	2.8318	1.0075	36	2.81	0.0079	0.05	0.7885	4.8751	16.976	2.200	130.969
a	6	e	24	2.7358	0.6936	36	3.94	0.0004	0.05	1.3291	4.1426	15.422	3.777	62.966
a	6	f	6	-3.9180	0.2975	36	-13.17	<.0001	0.05	-4.5214	-3.3145	0.020	0.011	0.036
a	6	f	12	-4.2544	0.3424	36	-12.43	<.0001	0.05	-4.9488	-3.5600	0.014	0.007	0.028
a	6	f	24	-3.7773	0.2718	36	-13.90	<.0001	0.05	-4.3285	-3.2261	0.023	0.013	0.040
a	12	a	24	0.1586	0.2883	36	0.55	0.5857	0.05	-0.4261	0.7433	1.172	0.653	2.103
a	12	b	6	3.2590	0.8953	36	3.64	0.0008	0.05	1.4433	5.0747	26.023	4.235	159.919
a	12	b	12	0.6658	0.3335	36	2.00	0.0535	0.05	-0.01049	1.3421	1.946	0.990	3.827
a	12	b	24	0.9196	0.3430	36	2.68	0.0110	0.05	0.2240	1.6152	2.508	1.251	5.029
a	12	c	6	2.4117	0.5752	36	4.19	0.0002	0.05	1.2452	3.5782	11.153	3.474	35.810
a	12	c	12	2.8011	0.6792	36	4.12	0.0002	0.05	1.4237	4.1785	16.463	4.152	65.271
a	12	c	24	1.7651	0.3892	36	4.54	<.0001	0.05	0.9758	2.5544	5.842	2.653	12.863
a	12	d	6	2.6464	0.6474	36	4.09	0.0002	0.05	1.3334	3.9593	14.102	3.794	52.421
a	12	d	12	15.9343	638.55	36	0.02	0.9802	0.05	-1279.10	1310.96	>999.999	<0.001	>999.999
a	12	d	24	3.2553	0.7626	36	4.27	0.0001	0.05	1.7086	4.8020	25.927	5.521	121.748
a	12	e	6	3.1473	0.8208	36	3.83	0.0005	0.05	1.4825	4.8120	23.273	4.404	122.982
a	12	e	12	3.3363	0.9967	36	3.35	0.0019	0.05	1.3150	5.3577	28.116	3.725	212.237
a	12	e	24	3.2404	0.6778	36	4.78	<.0001	0.05	1.8656	4.6151	25.543	6.460	100.995
a	12	f	6	-3.4134	0.2586	36	-13.20	<.0001	0.05	-3.9378	-2.8890	0.033	0.019	0.056
a	12	f	12	-3.7499	0.3091	36	-12.13	<.0001	0.05	-4.3768	-3.1230	0.024	0.013	0.044
a	12	f	24	-3.2728	0.2284	36	-14.33	<.0001	0.05	-3.7361	-2.8095	0.038	0.024	0.060
a	24	b	6	3.1004	0.9061	36	3.42	0.0016	0.05	1.2627	4.9381	22.207	3.535	139.505
a	24	b	12	0.5072	0.3616	36	1.40	0.1692	0.05	-0.2261	1.2405	1.661	0.798	3.458
a	24	b	24	0.7610	0.3704	36	2.05	0.0472	0.05	0.009803	1.5122	2.140	1.010	4.537
a	24	c	6	2.2531	0.5919	36	3.81	0.0005	0.05	1.0527	3.4536	9.518	2.865	31.614
a	24	c	12	2.6425	0.6934	36	3.81	0.0005	0.05	1.2362	4.0488	14.049	3.443	57.331
a	24	c	24	1.6065	0.4135	36	3.88	0.0004	0.05	0.7679	2.4452	4.985	2.155	11.533
a	24	d	6	2.4878	0.6623	36	3.76	0.0006	0.05	1.1446	3.8310	12.034	3.141	46.108
a	24	d	12	15.7758	638.55	36	0.02	0.9804	0.05	-1279.25	1310.81	>999.999	<0.001	>999.999
a	24	d	24	3.0967	0.7753	36	3.99	0.0003	0.05	1.5242	4.6692	22.125	4.592	106.607
a	24	e	6	2.9887	0.8327	36	3.59	0.0010	0.05	1.3000	4.6774	19.860	3.669	107.494
a	24	e	12	3.1777	1.0064	36	3.16	0.0032	0.05	1.1366	5.2189	23.993	3.116	184.734
a	24	e	24	3.0818	0.6921	36	4.45	<.0001	0.05	1.6781	4.4854	21.797	5.355	88.715
a	24	f	6	-3.5720	0.2939	36	-12.15	<.0001	0.05	-4.1681	-2.9759	0.028	0.015	0.051
a	24	f	12	-3.9084	0.3393	36	-11.52	<.0001	0.05	-4.5965	-3.2204	0.020	0.010	0.040
a	24	f	24	-3.4313	0.2678	36	-12.81	<.0001	0.05	-3.9745	-2.8881	0.032	0.019	0.056
b	6	b	12	-2.5932	0.9215	36	-2.81	0.0079	0.05	-4.4620	-0.7243	0.075	0.012	0.485

	6	b	24	-2.3394	0.9250	36	-2.53	0.0160	0.05	-4.2153	-0.4635	0.096	0.015	0.629
b	6	c	6	-0.8473	1.0338	36	-0.82	0.4179	0.05	-2.9439	1.2494	0.429	0.053	3.488
b	6	c	12	-0.4579	1.0951	36	-0.42	0.6783	0.05	-2.6788	1.7631	0.633	0.069	5.830
b	6	c	24	-1.4939	0.9431	36	-1.58	0.1219	0.05	-3.4065	0.4188	0.225	0.033	1.520
b	6	d	6	-0.6126	1.0757	36	-0.57	0.5725	0.05	-2.7942	1.5689	0.542	0.061	4.801
b	6	d	12	12.6754	638.55	36	0.02	0.9843	0.05	-1282.36	1307.71	>999.999	<0.001	>999.999
b	6	d	24	-0.00371	1.1487	36	-0.00	0.9974	0.05	-2.3334	2.3260	0.996	0.097	10.237
b	6	e	6	-0.1117	1.1882	36	-0.09	0.9256	0.05	-2.5214	2.2980	0.894	0.080	9.954
b	6	e	12	0.07734	1.3158	36	0.06	0.9535	0.05	-2.5912	2.7459	1.080	0.075	15.579
b	6	e	24	-0.01863	1.0943	36	-0.02	0.9865	0.05	-2.2379	2.2006	0.982	0.107	9.031
b	6	f	6	-6.6724	0.8971	36	-7.44	<.0001	0.05	-8.4918	-4.8530	0.001	<0.001	0.008
b	6	f	12	-7.0089	0.9130	36	-7.68	<.0001	0.05	-8.8604	-5.1573	<0.001	<0.001	0.006
b	6	f	24	-6.5317	0.8889	36	-7.35	<.0001	0.05	-8.3345	-4.7290	0.001	<0.001	0.009
b	12	b	24	0.2538	0.4065	36	0.62	0.5364	0.05	-0.5707	1.0783	1.289	0.565	2.940
b	12	c	6	1.7459	0.6152	36	2.84	0.0074	0.05	0.4983	2.9936	5.731	1.646	19.957
b	12	c	12	2.1353	0.7134	36	2.99	0.0050	0.05	0.6885	3.5821	8.460	1.991	35.949
b	12	c	24	1.0993	0.4462	36	2.46	0.0187	0.05	0.1944	2.0042	3.002	1.215	7.420
b	12	d	6	1.9806	0.6832	36	2.90	0.0063	0.05	0.5950	3.3661	7.247	1.813	28.965
b	12	d	12	15.2685	638.55	36	0.02	0.9811	0.05	-1279.76	1310.30	>999.999	<0.001	>999.999
b	12	d	24	2.5895	0.7932	36	3.26	0.0024	0.05	0.9807	4.1982	13.323	2.666	66.569
b	12	e	6	2.4815	0.8494	36	2.92	0.0060	0.05	0.7589	4.2041	11.959	2.136	66.958
b	12	e	12	2.6705	1.0203	36	2.62	0.0129	0.05	0.6013	4.7398	14.448	1.824	114.411
b	12	e	24	2.5746	0.7121	36	3.62	0.0009	0.05	1.1303	4.0188	13.125	3.097	55.632
b	12	f	6	-4.0792	0.3383	36	-12.06	<.0001	0.05	-4.7654	-3.3930	0.017	0.009	0.034
b	12	f	12	-4.4157	0.3784	36	-11.67	<.0001	0.05	-5.1831	-3.6483	0.012	0.006	0.026
b	12	f	24	-3.9386	0.3159	36	-12.47	<.0001	0.05	-4.5793	-3.2978	0.019	0.010	0.037
b	24	c	6	1.4921	0.6204	36	2.41	0.0214	0.05	0.2339	2.7504	4.447	1.264	15.648
b	24	c	12	1.8815	0.7179	36	2.62	0.0128	0.05	0.4256	3.3375	6.563	1.530	28.147
b	24	c	24	0.8455	0.4534	36	1.87	0.0703	0.05	-0.07394	1.7650	2.329	0.929	5.841
b	24	d	6	1.7268	0.6879	36	2.51	0.0167	0.05	0.3317	3.1219	5.622	1.393	22.688
b	24	d	12	15.0148	638.55	36	0.02	0.9814	0.05	-1280.02	1310.04	>999.999	<0.001	>999.999
b	24	d	24	2.3357	0.7973	36	2.93	0.0059	0.05	0.7187	3.9527	10.337	2.052	52.074
b	24	e	6	2.2277	0.8532	36	2.61	0.0131	0.05	0.4974	3.9580	9.278	1.644	52.351
b	24	e	12	2.4167	1.0235	36	2.36	0.0237	0.05	0.3411	4.4924	11.209	1.406	89.336
b	24	e	24	2.3208	0.7166	36	3.24	0.0026	0.05	0.8674	3.7741	10.183	2.381	43.560
b	24	f	6	-4.3330	0.3478	36	-12.46	<.0001	0.05	-5.0383	-3.6277	0.013	0.006	0.027
b	24	f	12	-4.6695	0.3868	36	-12.07	<.0001	0.05	-5.4540	-3.8849	0.009	0.004	0.021
b	24	f	24	-4.1923	0.3260	36	-12.86	<.0001	0.05	-4.8535	-3.5312	0.015	0.008	0.029
c	6	c	12	0.3894	0.8535	36	0.46	0.6510	0.05	-1.3417	2.1204	1.476	0.261	8.335
c	6	c	24	-0.6466	0.6471	36	-1.00	0.3243	0.05	-1.9589	0.6657	0.524	0.141	1.946
c	6	d	6	0.2346	0.8285	36	0.28	0.7786	0.05	-1.4456	1.9148	1.264	0.236	6.786
c	6	d	12	13.5226	638.55	36	0.02	0.9832	0.05	-1281.51	1308.55	>999.999	<0.001	>999.999
c	6	d	24	0.8435	0.9213	36	0.92	0.3660	0.05	-1.0250	2.7121	2.325	0.359	15.061
c	6	e	6	0.7356	0.9701	36	0.76	0.4532	0.05	-1.2318	2.7029	2.087	0.292	14.924
c	6	e	12	0.9246	1.1228	36	0.82	0.4156	0.05	-1.3525	3.2017	2.521	0.259	24.574
c	6	e	24	0.8286	0.8525	36	0.97	0.3375	0.05	-0.9003	2.5575	2.290	0.406	12.904

	6	f	6	-5.8252	0.5780	36	-10.08	<.0001	0.05	-6.9974	-4.6529	0.003	<0.001	0.010
c	6	f	12	-6.1616	0.6023	36	-10.23	<.0001	0.05	-7.3832	-4.9400	0.002	<0.001	0.007
c	6	f	24	-5.6845	0.5652	36	-10.06	<.0001	0.05	-6.8307	-4.5382	0.003	0.001	0.011
c	12	c	24	-1.0360	0.7411	36	-1.40	0.1707	0.05	-2.5389	0.4669	0.355	0.079	1.595
c	12	d	6	-0.1548	0.9038	36	-0.17	0.8650	0.05	-1.9877	1.6782	0.857	0.137	5.356
c	12	d	12	13.1332	638.55	36	0.02	0.9837	0.05	-1281.90	1308.16	>999.999	<0.001	>999.999
c	12	d	24	0.4542	0.9896	36	0.46	0.6490	0.05	-1.5528	2.4612	1.575	0.212	11.719
c	12	e	6	0.3462	1.0351	36	0.33	0.7400	0.05	-1.7532	2.4455	1.414	0.173	11.537
c	12	e	12	0.5352	1.1794	36	0.45	0.6527	0.05	-1.8568	2.9273	1.708	0.156	18.676
c	12	e	24	0.4392	0.9258	36	0.47	0.6381	0.05	-1.4384	2.3169	1.552	0.237	10.145
c	12	f	6	-6.2145	0.6816	36	-9.12	<.0001	0.05	-7.5969	-4.8322	0.002	<0.001	0.008
c	12	f	12	-6.5510	0.7023	36	-9.33	<.0001	0.05	-7.9754	-5.1266	0.001	<0.001	0.006
c	12	f	24	-6.0739	0.6707	36	-9.06	<.0001	0.05	-7.4342	-4.7135	0.002	<0.001	0.009
c	24	d	6	0.8812	0.7120	36	1.24	0.2239	0.05	-0.5628	2.3253	2.414	0.570	10.230
c	24	d	12	14.1692	638.55	36	0.02	0.9824	0.05	-1280.86	1309.20	>999.999	<0.001	>999.999
c	24	d	24	1.4902	0.8182	36	1.82	0.0769	0.05	-0.1693	3.1496	4.438	0.844	23.327
c	24	e	6	1.3822	0.8727	36	1.58	0.1220	0.05	-0.3878	3.1522	3.984	0.679	23.387
c	24	e	12	1.5712	1.0398	36	1.51	0.1395	0.05	-0.5377	3.6801	4.812	0.584	39.651
c	24	e	24	1.4752	0.7398	36	1.99	0.0538	0.05	-0.02521	2.9757	4.372	0.975	19.603
c	24	f	6	-5.1785	0.3934	36	-13.16	<.0001	0.05	-5.9763	-4.3808	0.006	0.003	0.013
c	24	f	12	-5.5150	0.4283	36	-12.88	<.0001	0.05	-6.3836	-4.6464	0.004	0.002	0.010
c	24	f	24	-5.0379	0.3743	36	-13.46	<.0001	0.05	-5.7969	-4.2788	0.006	0.003	0.014
d	6	d	12	13.2880	638.55	36	0.02	0.9835	0.05	-1281.74	1308.32	>999.999	<0.001	>999.999
d	6	d	24	0.6089	0.9681	36	0.63	0.5333	0.05	-1.3544	2.5722	1.838	0.258	13.095
d	6	e	6	0.5009	1.0146	36	0.49	0.6245	0.05	-1.5567	2.5585	1.650	0.211	12.917
d	6	e	12	0.6900	1.1614	36	0.59	0.5562	0.05	-1.6655	3.0455	1.994	0.189	21.020
d	6	e	24	0.5940	0.9028	36	0.66	0.5147	0.05	-1.2369	2.4249	1.811	0.290	11.301
d	6	f	6	-6.0598	0.6499	36	-9.32	<.0001	0.05	-7.3779	-4.7417	0.002	<0.001	0.009
d	6	f	12	-6.3962	0.6716	36	-9.52	<.0001	0.05	-7.7583	-5.0341	0.002	<0.001	0.007
d	6	f	24	-5.9191	0.6385	36	-9.27	<.0001	0.05	-7.2141	-4.6241	0.003	<0.001	0.010
d	12	d	24	-12.6791	638.55	36	-0.02	0.9843	0.05	-1307.71	1282.35	<0.001	<0.001	>999.999
d	12	e	6	-12.7871	638.55	36	-0.02	0.9841	0.05	-1307.82	1282.24	<0.001	<0.001	>999.999
d	12	e	12	-12.5980	638.55	36	-0.02	0.9844	0.05	-1307.63	1282.43	<0.001	<0.001	>999.999
d	12	e	24	-12.6940	638.55	36	-0.02	0.9842	0.05	-1307.72	1282.34	<0.001	<0.001	>999.999
d	12	f	6	-19.3478	638.55	36	-0.03	0.9760	0.05	-1314.38	1275.68	<0.001	<0.001	>999.999
d	12	f	12	-19.6842	638.55	36	-0.03	0.9756	0.05	-1314.71	1275.35	<0.001	<0.001	>999.999
d	12	f	24	-19.2071	638.55	36	-0.03	0.9762	0.05	-1314.24	1275.82	<0.001	<0.001	>999.999
d	24	e	6	-0.1080	1.0917	36	-0.10	0.9217	0.05	-2.3221	2.1061	0.898	0.098	8.216
d	24	e	12	0.08105	1.2294	36	0.07	0.9478	0.05	-2.4123	2.5744	1.084	0.090	13.123
d	24	e	24	-0.01493	0.9887	36	-0.02	0.9880	0.05	-2.0201	1.9902	0.985	0.133	7.317
d	24	f	6	-6.6687	0.7648	36	-8.72	<.0001	0.05	-8.2197	-5.1177	0.001	<0.001	0.006
d	24	f	12	-7.0051	0.7833	36	-8.94	<.0001	0.05	-8.5938	-5.4165	<0.001	<0.001	0.004
d	24	f	24	-6.5280	0.7551	36	-8.64	<.0001	0.05	-8.0595	-4.9966	0.001	<0.001	0.007
e	6	e	12	0.1890	1.2663	36	0.15	0.8822	0.05	-2.3792	2.7573	1.208	0.093	15.757
e	6	e	24	0.09307	1.0343	36	0.09	0.9288	0.05	-2.0045	2.1906	1.098	0.135	8.941
e	6	f	6	-6.5607	0.8228	36	-7.97	<.0001	0.05	-8.2295	-4.8919	0.001	<0.001	0.008

	6	f	12	-6.8972	0.8401	36	-8.21	<.0001	0.05	-8.6010	-5.1933	0.001	<0.001	0.006
e	6	f	24	-6.4200	0.8139	36	-7.89	<.0001	0.05	-8.0707	-4.7694	0.002	<0.001	0.008
e	12	e	24	-0.09598	1.1787	36	-0.08	0.9356	0.05	-2.4864	2.2945	0.908	0.083	9.919
e	12	f	6	-6.7498	0.9963	36	-6.76	<.0001	0.05	-8.7745	-4.7250	0.001	<0.001	0.009
e	12	f	12	-7.0862	1.0126	36	-7.00	<.0001	0.05	-9.1399	-5.0325	<0.001	<0.001	0.007
e	12	f	24	-6.6091	0.9910	36	-6.67	<.0001	0.05	-8.6188	-4.5993	0.001	<0.001	0.010
e	24	f	6	-6.6538	0.6803	36	-9.78	<.0001	0.05	-8.0334	-5.2742	0.001	<0.001	0.005
e	24	f	12	-6.9902	0.7010	36	-9.97	<.0001	0.05	-8.4120	-5.5685	<0.001	<0.001	0.004
e	24	f	24	-6.5131	0.6694	36	-9.73	<.0001	0.05	-7.8707	-5.1555	0.001	<0.001	0.006
f	6	f	12	-0.3364	0.3144	36	-1.07	0.2917	0.05	-0.9740	0.3011	0.714	0.378	1.351
f	6	f	24	0.1407	0.2355	36	0.60	0.5541	0.05	-0.3370	0.6183	1.151	0.714	1.856
f	12	f	24	0.4771	0.2901	36	1.64	0.1088	0.05	-0.1113	1.0655	1.611	0.895	2.902

Performed by J. Luoma; SAS version 9.3 08:43 18MAR15

Effects of Pseudomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel species

Overall Model: Glochidia Viability (unadjusted) - At 6, 12 and 24 h

The GLIMMIX Procedure

form=SDP sps=FAM

Model Information	
Data Set	WORK.GLOCHIDIA2
Response Variable (Events)	via_glo
Response Variable (Trials)	tot
Response Distribution	Binomial
Link Function	Logit
Variance Function	Default
Variance Matrix	Diagonal
Estimation Technique	Maximum Likelihood
Degrees of Freedom Method	Residual

Class Level Information		
Class	Levels	Values
conca	6	a b c d e f
time	3	6 12 24

Number of Observations Read	54
Number of Observations Used	54
Number of Events	5027
Number of Trials	15013

Dimensions	
Covariance Parameters	1
Columns in X	27
Columns in Z	0
Subjects (Blocks in V)	1
Max Obs per Subject	54

Optimization Information	
Optimization Technique	Newton-Raphson
Parameters in Optimization	18
Lower Boundaries	0
Upper Boundaries	0
Fixed Effects	Not Profiled

Iteration History					
Iteration	Restarts	Evaluations	Objective Function	Change	Max Gradient
0	0	4	460.01101939	.	77.99063
1	0	3	411.74220165	48.26881773	11.63871
2	0	3	410.4642484	1.27795325	0.487795
3	0	3	410.46192819	0.00232021	0.000989
4	0	3	410.46192818	0.00000001	4.089E-9

Convergence criterion (GCONV=1E-8) satisfied.

Fit Statistics	
-2 Log Likelihood	820.92
AIC (smaller is better)	856.92
AICC (smaller is better)	876.47
BIC (smaller is better)	892.73
CAIC (smaller is better)	910.73
HQIC (smaller is better)	870.73
Pearson Chi-Square	530.92
Pearson Chi-Square / DF	14.75

Parameter Estimates

Effect	conca	time	Estimate	Standard Error	DF	t Value	Pr > |t|
conca	a		0.2895	0.2533	36	1.14	0.2605
conca	b		-0.4134	0.3118	36	-1.33	0.1933
conca	c		-2.0526	0.4425	36	-4.64	<.0001
conca	d		-2.7365	0.6515	36	-4.20	0.0002
conca	e		-4.1417	1.1671	36	-3.55	0.0011
conca	f		0.8082	0.2944	36	2.75	0.0094
time		6	0.1592	0.4307	36	0.37	0.7136
time		12	-0.09110	0.3819	36	-0.24	0.8128
time		24	0	.	.	.	.
conca*time	a	6	-0.1688	0.5582	36	-0.30	0.7640
conca*time	a	12	0.1370	0.5199	36	0.26	0.7937
conca*time	a	24	0	.	.	.	.
conca*time	b	6	-0.6162	0.6019	36	-1.02	0.3128
conca*time	b	12	0.2573	0.5648	36	0.46	0.6514
conca*time	b	24	0	.	.	.	.
conca*time	c	6	0.1796	0.6959	36	0.26	0.7978
conca*time	c	12	0.5988	0.7266	36	0.82	0.4153
conca*time	c	24	0	.	.	.	.
conca*time	d	6	0.7450	0.8433	36	0.88	0.3829
conca*time	d	12	-0.2426	1.0188	36	-0.24	0.8131
conca*time	d	24	0	.	.	.	.
conca*time	e	6	0.2641	1.5983	36	0.17	0.8697
conca*time	e	12	0.6257	1.4930	36	0.42	0.6776
conca*time	e	24	0	.	.	.	.
conca*time	f	6	0	.	.	.	.
conca*time	f	12	0	.	.	.	.
conca*time	f	24	0	.	.	.	.
Residual			14.7478	.	.	.	.

Type III Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
conca	5	36	37.58	<.0001

time	2	36	0.25	0.7796
conca*time	10	36	0.73	0.6883

Odds Ratio Estimates

conca	time	_conca	time	Estimate	DF	95% Confidence Limits	
a		f		0.589	36	0.378	0.916
b		f		0.262	36	0.162	0.421
c		f		0.074	36	0.042	0.132
d		f		0.034	36	0.016	0.072
e		f		0.010	36	0.003	0.033
	6		24	1.254	36	0.656	2.399
	12		24	1.148	36	0.594	2.220

conca Least Squares Means

conca	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Mean	Standard Error Mean	Lower Mean	Upper Mean
a	0.3016	0.1439	36	2.10	0.0432	0.05	0.009703	0.5935	0.5748	0.03518	0.5024	0.6442
b	-0.5103	0.1676	36	-3.05	0.0043	0.05	-0.8501	-0.1705	0.3751	0.03928	0.2994	0.4575
c	-1.7705	0.2322	36	-7.63	<.0001	0.05	-2.2413	-1.2996	0.1455	0.02686	0.09610	0.2142
d	-2.5463	0.3322	36	-7.66	<.0001	0.05	-3.2201	-1.8726	0.07267	0.02239	0.03842	0.1332
e	-3.8224	0.5860	36	-6.52	<.0001	0.05	-5.0108	-2.6340	0.02141	0.01228	0.006621	0.06698
f	0.8309	0.1649	36	5.04	<.0001	0.05	0.4965	1.1653	0.6965	0.03485	0.6216	0.7623

Differences of conca Least Squares Means

conca	_conca	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Odds Ratio	Lower Confidence Limit for Odds Ratio	Upper Confidence Limit for Odds Ratio
a	b	0.8119	0.2209	36	3.68	0.0008	0.05	0.3639	1.2599	2.252	1.439	3.525
a	c	2.0721	0.2732	36	7.59	<.0001	0.05	1.5181	2.6261	7.941	4.563	13.820
a	d	2.8480	0.3621	36	7.87	<.0001	0.05	2.1137	3.5822	17.252	8.279	35.954
a	e	4.1240	0.6034	36	6.83	<.0001	0.05	2.9003	5.3478	61.807	18.179	210.141
a	f	-0.5293	0.2189	36	-2.42	0.0208	0.05	-0.9731	-0.08542	0.589	0.378	0.918
b	c	1.2602	0.2863	36	4.40	<.0001	0.05	0.6795	1.8409	3.526	1.973	6.302
b	d	2.0360	0.3721	36	5.47	<.0001	0.05	1.2814	2.7906	7.660	3.602	16.292
b	e	3.3121	0.6095	36	5.43	<.0001	0.05	2.0760	4.5482	27.443	7.973	94.459
b	f	-1.3412	0.2351	36	-5.71	<.0001	0.05	-1.8180	-0.8645	0.262	0.162	0.421
c	d	0.7759	0.4053	36	1.91	0.0636	0.05	-0.04613	1.5979	2.172	0.955	4.943
c	e	2.0519	0.6303	36	3.26	0.0025	0.05	0.7736	3.3303	7.783	2.168	27.946
c	f	-2.6014	0.2848	36	-9.14	<.0001	0.05	-3.1789	-2.0238	0.074	0.042	0.132
d	e	1.2761	0.6736	36	1.89	0.0662	0.05	-0.09007	2.6422	3.583	0.914	14.044
d	f	-3.3772	0.3709	36	-9.11	<.0001	0.05	-4.1294	-2.6251	0.034	0.016	0.072
e	f	-4.6533	0.6087	36	-7.64	<.0001	0.05	-5.8879	-3.4187	0.010	0.003	0.033

time Least Squares Means

time	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Mean	Standard Error Mean	Lower Mean	Upper Mean
6	-1.1480	0.2008	36	-5.72	<.0001	0.05	-1.5552	-0.7407	0.2409	0.03672	0.1743	0.3228

12	-1.2361	0.2089	36	-5.92	<.0001	0.05	-1.6598	-0.8125	0.2251	0.03644	0.1598	0.3074
24	-1.3744	0.2489	36	-5.52	<.0001	0.05	-1.8792	-0.8696	0.2019	0.04011	0.1325	0.2953

Differences of time Least Squares Means

time	time	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Odds Ratio	Lower Confidence Limit for Odds Ratio	Upper Confidence Limit for Odds Ratio
6	12	0.08815	0.2898	36	0.30	0.7627	0.05	-0.4995	0.6758	1.092	0.607	1.966
6	24	0.2264	0.3198	36	0.71	0.4835	0.05	-0.4222	0.8750	1.254	0.656	2.399
12	24	0.1383	0.3249	36	0.43	0.6730	0.05	-0.5207	0.7973	1.148	0.594	2.220

conca*time Least Squares Means

conca	time	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Mean	Standard Error Mean	Lower Mean	Upper Mean
a	6	0.2799	0.2489	36	1.12	0.2683	0.05	-0.2249	0.7847	0.5695	0.06102	0.4440	0.6867
a	12	0.3354	0.2457	36	1.37	0.1806	0.05	-0.1628	0.8337	0.5831	0.05973	0.4594	0.6971
a	24	0.2895	0.2533	36	1.14	0.2605	0.05	-0.2241	0.8032	0.5719	0.06201	0.4442	0.6907
b	6	-0.8704	0.2821	36	-3.09	0.0039	0.05	-1.4424	-0.2983	0.2952	0.05868	0.1912	0.4260
b	12	-0.2471	0.2755	36	-0.90	0.3757	0.05	-0.8059	0.3117	0.4385	0.06784	0.3088	0.5773
b	24	-0.4134	0.3118	36	-1.33	0.1933	0.05	-1.0458	0.2190	0.3981	0.07472	0.2600	0.5545
c	6	-1.7139	0.3209	36	-5.34	<.0001	0.05	-2.3647	-1.0630	0.1527	0.04151	0.08590	0.2567
c	12	-1.5449	0.4317	36	-3.58	0.0010	0.05	-2.4205	-0.6693	0.1758	0.06256	0.08162	0.3367
c	24	-2.0526	0.4425	36	-4.64	<.0001	0.05	-2.9500	-1.1552	0.1138	0.04462	0.04974	0.2395
d	6	-1.8323	0.3182	36	-5.76	<.0001	0.05	-2.4776	-1.1871	0.1380	0.03784	0.07744	0.2338
d	12	-3.0702	0.6838	36	-4.49	<.0001	0.05	-4.4571	-1.6833	0.04435	0.02899	0.01146	0.1567
d	24	-2.7365	0.6515	36	-4.20	0.0002	0.05	-4.0577	-1.4152	0.06086	0.03723	0.01699	0.1954
e	6	-3.7184	1.0035	36	-3.71	0.0007	0.05	-5.7537	-1.6832	0.02370	0.02322	0.003161	0.1567
e	12	-3.6070	0.8493	36	-4.25	0.0001	0.05	-5.3295	-1.8846	0.02642	0.02184	0.004823	0.1319
e	24	-4.1417	1.1671	36	-3.55	0.0011	0.05	-6.5086	-1.7748	0.01565	0.01798	0.001486	0.1449
f	6	0.9674	0.3143	36	3.08	0.0040	0.05	0.3299	1.6049	0.7246	0.06273	0.5817	0.8327
f	12	0.7171	0.2432	36	2.95	0.0056	0.05	0.2238	1.2104	0.6720	0.05361	0.5557	0.7704
f	24	0.8082	0.2944	36	2.75	0.0094	0.05	0.2112	1.4053	0.6917	0.06278	0.5526	0.8030

Differences of conca*time Least Squares Means

conca	time	_conca	time	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Odds Ratio	Lower Confidence Limit for Odds Ratio	Upper Confidence Limit for Odds Ratio
a	6	a	12	-0.05557	0.3497	36	-0.16	0.8746	0.05	-0.7649	0.6537	0.946	0.465	1.923
a	6	a	24	-0.00967	0.3551	36	-0.03	0.9784	0.05	-0.7299	0.7105	0.990	0.482	2.035
a	6	b	6	1.1503	0.3762	36	3.06	0.0042	0.05	0.3873	1.9132	3.159	1.473	6.775
a	6	b	12	0.5270	0.3713	36	1.42	0.1644	0.05	-0.2260	1.2800	1.694	0.798	3.597
a	6	b	24	0.6932	0.3990	36	1.74	0.0908	0.05	-0.1159	1.5024	2.000	0.891	4.492
a	6	c	6	1.9937	0.4061	36	4.91	<.0001	0.05	1.1701	2.8174	7.343	3.222	16.733
a	6	c	12	1.8248	0.4983	36	3.66	0.0008	0.05	0.8141	2.8355	6.201	2.257	17.038
a	6	c	24	2.3325	0.5077	36	4.59	<.0001	0.05	1.3029	3.3621	10.304	3.680	28.850
a	6	d	6	2.1122	0.4040	36	5.23	<.0001	0.05	1.2930	2.9315	8.267	3.644	18.755
a	6	d	12	3.3500	0.7277	36	4.60	<.0001	0.05	1.8741	4.8260	28.504	6.515	124.705

a	6	d	24	3.0163	0.6974	36	4.33	0.0001	0.05	1.6020	4.4307	20.417	4.963	83.994
a	6	e	6	3.9983	1.0339	36	3.87	0.0004	0.05	1.9014	6.0952	54.506	6.695	443.729
a	6	e	12	3.8869	0.8850	36	4.39	<.0001	0.05	2.0920	5.6819	48.761	8.101	293.493
a	6	e	24	4.4216	1.1933	36	3.71	0.0007	0.05	2.0014	6.8417	83.227	7.400	936.079
a	6	f	6	-0.6875	0.4009	36	-1.71	0.0950	0.05	-1.5007	0.1256	0.503	0.223	1.134
a	6	f	12	-0.4372	0.3480	36	-1.26	0.2171	0.05	-1.1430	0.2685	0.646	0.319	1.308
a	6	f	24	-0.5283	0.3855	36	-1.37	0.1790	0.05	-1.3102	0.2535	0.590	0.270	1.289
a	12	a	24	0.04590	0.3529	36	0.13	0.8972	0.05	-0.6697	0.7615	1.047	0.512	2.142
a	12	b	6	1.2058	0.3741	36	3.22	0.0027	0.05	0.4472	1.9645	3.340	1.564	7.131
a	12	b	12	0.5826	0.3692	36	1.58	0.1233	0.05	-0.1661	1.3313	1.791	0.847	3.786
a	12	b	24	0.7488	0.3970	36	1.89	0.0673	0.05	-0.05630	1.5539	2.115	0.945	4.730
a	12	c	6	2.0493	0.4042	36	5.07	<.0001	0.05	1.2296	2.8690	7.762	3.420	17.619
a	12	c	12	1.8803	0.4968	36	3.79	0.0006	0.05	0.8729	2.8878	6.556	2.394	17.954
a	12	c	24	2.3881	0.5061	36	4.72	<.0001	0.05	1.3616	3.4145	10.892	3.903	30.402
a	12	d	6	2.1678	0.4020	36	5.39	<.0001	0.05	1.3525	2.9831	8.739	3.867	19.748
a	12	d	12	3.4056	0.7266	36	4.69	<.0001	0.05	1.9319	4.8793	30.133	6.903	131.541
a	12	d	24	3.0719	0.6963	36	4.41	<.0001	0.05	1.6598	4.4840	21.583	5.258	88.589
a	12	e	6	4.0539	1.0332	36	3.92	0.0004	0.05	1.9585	6.1492	57.621	7.089	468.357
a	12	e	12	3.9425	0.8841	36	4.46	<.0001	0.05	2.1494	5.7356	51.547	8.580	309.701
a	12	e	24	4.4771	1.1926	36	3.75	0.0006	0.05	2.0584	6.8959	87.983	7.833	988.238
a	12	f	6	-0.6319	0.3990	36	-1.58	0.1219	0.05	-1.4411	0.1772	0.532	0.237	1.194
a	12	f	12	-0.3817	0.3457	36	-1.10	0.2769	0.05	-1.0828	0.3195	0.683	0.339	1.376
a	12	f	24	-0.4728	0.3834	36	-1.23	0.2256	0.05	-1.2504	0.3049	0.623	0.286	1.356
a	24	b	6	1.1599	0.3791	36	3.06	0.0042	0.05	0.3911	1.9288	3.190	1.479	6.881
a	24	b	12	0.5367	0.3743	36	1.43	0.1602	0.05	-0.2223	1.2957	1.710	0.801	3.654
a	24	b	24	0.7029	0.4017	36	1.75	0.0887	0.05	-0.1118	1.5176	2.020	0.894	4.561
a	24	c	6	2.0034	0.4088	36	4.90	<.0001	0.05	1.1743	2.8325	7.414	3.236	16.988
a	24	c	12	1.8344	0.5005	36	3.66	0.0008	0.05	0.8193	2.8496	6.262	2.269	17.281
a	24	c	24	2.3422	0.5098	36	4.59	<.0001	0.05	1.3082	3.3762	10.404	3.699	29.258
a	24	d	6	2.1219	0.4067	36	5.22	<.0001	0.05	1.297[illegible]	2.9467	8.347	3.659	19.042
a	24	d	12	3.3597	0.7292	36	4.61	<.0001	0.05	1.8807	4.8387	28.781	6.558	126.303
a	24	d	24	3.0260	0.6990	36	4.33	0.0001	0.05	1.6084	4.4436	20.6[illegible]5	4.995	85.061
a	24	e	6	4.0080	1.0350	36	3.87	0.0004	0.05	1.9089	6.1070	55.036	6.746	449.007
a	24	e	12	3.8966	0.8863	36	4.40	<.0001	0.05	2.0991	5.6940	49.235	8.159	297.092
a	24	e	24	4.4312	1.1942	36	3.71	0.0007	0.05	2.0092	6.8532	84.035	7.458	946.941
a	24	f	6	-0.6778	0.4037	36	-1.68	0.1018	0.05	-1.4965	0.1408	0.508	0.224	1.151
a	24	f	12	-0.4276	0.3511	36	-1.22	0.2313	0.05	-1.1397	0.2846	0.652	0.320	1.329
a	24	f	24	-0.5187	0.3884	36	-1.34	0.1901	0.05	-1.3063	0.2689	0.595	0.271	1.309
b	6	b	12	-0.6233	0.3943	36	-1.58	0.1227	0.05	-1.4229	0.1764	0.536	0.241	1.193
b	6	b	24	-0.4570	0.4205	36	-1.09	0.2843	0.05	-1.3098	0.3957	0.633	0.270	1.485
b	6	c	6	0.8435	0.4273	36	1.97	0.0561	0.05	-0.02305	1.7100	2.324	0.977	5.529
b	6	c	12	0.6745	0.5157	36	1.31	0.1992	0.05	-0.3714	1.7204	1.963	0.690	5.587
b	6	c	24	1.1822	0.5247	36	2.25	0.0304	0.05	0.1180	2.2464	3.262	1.125	9.454
b	6	d	6	0.9620	0.4252	36	2.26	0.0298	0.05	0.09962	1.8243	2.617	1.105	6.198
b	6	d	12	2.1998	0.7397	36	2.97	0.0052	0.05	0.6995	3.7000	9.023	2.013	40.448
b	6	d	24	1.8661	0.7099	36	2.63	0.0125	0.05	0.4263	3.3059	6.463	1.532	27.272

	6	e	6	2.8481	1.0424	36	2.73	0.0097	0.05	0.7340	4.9622	17.254	2.083	142.901
b	6	e	12	2.7367	0.8949	36	3.06	0.0042	0.05	0.9217	4.5517	15.435	2.513	94.789
b	6	e	24	3.2713	1.2007	36	2.72	0.0099	0.05	0.8363	5.7064	26.346	2.308	300.773
b	6	f	6	-1.8378	0.4223	36	-4.35	0.0001	0.05	-2.6943	-0.9813	0.159	0.068	0.375
b	6	f	12	-1.5875	0.3724	36	-4.26	0.0001	0.05	-2.3429	-0.8321	0.204	0.096	0.435
b	6	f	24	-1.6786	0.4077	36	-4.12	0.0002	0.05	-2.5055	-0.8517	0.187	0.082	0.427
b	12	b	24	0.1662	0.4161	36	0.40	0.6919	0.05	-0.6777	1.0101	1.181	0.508	2.746
b	12	c	6	1.4667	0.4230	36	3.47	0.0014	0.05	0.6089	2.3245	4.335	1.838	10.222
b	12	c	12	1.2978	0.5122	36	2.53	0.0158	0.05	0.2591	2.3365	3.661	1.296	10.345
b	12	c	24	1.8055	0.5212	36	3.46	0.0014	0.05	0.7483	2.8626	6.083	2.114	17.507
b	12	d	6	1.5852	0.4209	36	3.77	0.0006	0.05	0.7316	2.4388	4.880	2.078	11.459
b	12	d	12	2.8230	0.7373	36	3.83	0.0005	0.05	1.3276	4.3183	16.828	3.773	75.059
b	12	d	24	2.4893	0.7073	36	3.52	0.0012	0.05	1.0548	3.9239	12.053	2.871	50.597
b	12	e	6	3.4713	1.0407	36	3.34	0.0020	0.05	1.3608	5.5819	32.179	3.899	265.564
b	12	e	12	3.3599	0.8929	36	3.76	0.0006	0.05	1.5491	5.1708	28.787	4.707	176.051
b	12	e	24	3.8946	1.1991	36	3.25	0.0025	0.05	1.4626	6.3265	49.134	4.317	559.212
b	12	f	6	-1.2145	0.4180	36	-2.91	0.0062	0.05	-2.0622	-0.3668	0.297	0.127	0.693
b	12	f	12	-0.9642	0.3675	36	-2.62	0.0127	0.05	-1.7096	-0.2189	0.381	0.181	0.803
b	12	f	24	-1.0553	0.4032	36	-2.62	0.0129	0.05	-1.8731	-0.2376	0.348	0.154	0.789
b	24	c	6	1.3005	0.4475	36	2.91	0.0062	0.05	0.3930	2.2080	3.671	1.481	9.097
b	24	c	12	1.1315	0.5326	36	2.12	0.0405	0.05	0.05143	2.2116	3.100	1.053	9.131
b	24	c	24	1.6392	0.5413	36	3.03	0.0045	0.05	0.5414	2.7371	5.151	1.718	15.442
b	24	d	6	1.4190	0.4455	36	3.19	0.0030	0.05	0.5155	2.3225	4.133	1.674	10.201
b	24	d	12	2.6568	0.7516	36	3.53	0.0011	0.05	1.1325	4.1811	14.251	3.103	65.436
b	24	d	24	2.3231	0.7223	36	3.22	0.0027	0.05	0.8583	3.7879	10.207	2.359	44.163
b	24	e	6	3.3051	1.0508	36	3.15	0.0033	0.05	1.1738	5.4363	27.250	3.234	229.588
b	24	e	12	3.1937	0.9047	36	3.53	0.0012	0.05	1.3588	5.0286	24.378	3.891	152.717
b	24	e	24	3.7283	1.2080	36	3.09	0.0039	0.05	1.2784	6.1783	41.609	3.591	482.148
b	24	f	6	-1.3808	0.4428	36	-3.12	0.0036	0.05	-2.2787	-0.4828	0.251	0.102	0.617
b	24	f	12	-1.1305	0.3955	36	-2.86	0.0070	0.05	-1.9325	-0.3285	0.323	0.145	0.720
b	24	f	24	-1.2216	0.4288	36	-2.85	0.0072	0.05	-2.0913	-0.3519	0.295	0.124	0.703
c	6	c	12	-0.1690	0.5379	36	-0.31	0.7553	0.05	-1.2600	0.9220	0.845	0.284	2.514
c	6	c	24	0.3388	0.5466	36	0.62	0.5393	0.05	-0.7698	1.4473	1.403	0.463	4.252
c	6	d	6	0.1185	0.4519	36	0.26	0.7947	0.05	-0.7980	1.0350	1.126	0.450	2.815
c	6	d	12	1.3563	0.7554	36	1.80	0.0810	0.05	-0.1757	2.8883	3.882	0.839	17.964
c	6	d	24	1.0226	0.7262	36	1.41	0.1677	0.05	-0.4502	2.4955	2.780	0.637	12.128
c	6	e	6	2.0046	1.0536	36	1.90	0.0651	0.05	-0.1322	4.1414	7.423	0.876	62.888
c	6	e	12	1.8932	0.9079	36	2.09	0.0442	0.05	0.05185	3.7345	6.641	1.053	41.869
c	6	e	24	2.4278	1.2104	36	2.01	0.0524	0.05	-0.02692	4.8826	11.334	0.973	131.973
c	6	f	6	-2.6812	0.4492	36	-5.97	<.0001	0.05	-3.5923	-1.7702	0.068	0.028	0.170
c	6	f	12	-2.4310	0.4027	36	-6.04	<.0001	0.05	-3.2476	-1.6143	0.088	0.039	0.199
c	6	f	24	-2.5221	0.4355	36	-5.79	<.0001	0.05	-3.4053	-1.6388	0.080	0.033	0.194
c	12	c	24	0.5077	0.6182	36	0.82	0.4169	0.05	-0.7461	1.7615	1.661	0.474	5.821
c	12	d	6	0.2874	0.5363	36	0.54	0.5953	0.05	-0.8002	1.3751	1.333	0.449	3.956
c	12	d	12	1.5253	0.8087	36	1.89	0.0674	0.05	-0.1149	3.1654	4.596	0.891	23.699
c	12	d	24	1.1916	0.7815	36	1.52	0.1361	0.05	-0.3935	2.7766	3.292	0.675	16.065

	12	e	6	2.1735	1.0925	36	1.99	0.0543	0.05	-0.04205	4.3891	8.789	0.959	80.570
c	12	e	12	2.0622	0.9527	36	2.16	0.0371	0.05	0.1299	3.9944	7.863	1.139	54.294
c	12	e	24	2.5968	1.2444	36	2.09	0.0440	0.05	0.07312	5.1205	13.421	1.076	167.412
c	12	f	6	-2.5123	0.5340	36	-4.70	<.0001	0.05	-3.5954	-1.4292	0.081	0.027	0.239
c	12	f	12	-2.2620	0.4956	36	-4.56	<.0001	0.05	-3.2670	-1.2570	0.104	0.038	0.284
c	12	f	24	-2.3531	0.5226	36	-4.50	<.0001	0.05	-3.4129	-1.2933	0.095	0.033	0.274
c	24	d	6	-0.2203	0.5450	36	-0.40	0.6885	0.05	-1.3256	0.8850	0.802	0.266	2.423
c	24	d	12	1.0176	0.8145	36	1.25	0.2196	0.05	-0.6344	2.6695	2.766	0.530	14.432
c	24	d	24	0.6839	0.7875	36	0.87	0.3909	0.05	-0.9133	2.2810	1.982	0.401	9.787
c	24	e	6	1.6658	1.0967	36	1.52	0.1375	0.05	-0.5585	3.8901	5.290	0.572	48.916
c	24	e	12	1.5544	0.9577	36	1.62	0.1133	0.05	-0.3878	3.4967	4.732	0.679	33.005
c	24	e	24	2.0891	1.2481	36	1.67	0.1028	0.05	-0.4422	4.6204	8.077	0.643	101.533
c	24	f	6	-3.0200	0.5428	36	-5.56	<.0001	0.05	-4.1208	-1.9192	0.049	0.016	0.147
c	24	f	12	-2.7697	0.5049	36	-5.49	<.0001	0.05	-3.7937	-1.7457	0.063	0.023	0.175
c	24	f	24	-2.8608	0.5315	36	-5.38	<.0001	0.05	-3.9387	-1.7830	0.057	0.019	0.168
d	6	d	12	1.2378	0.7542	36	1.64	0.1095	0.05	-0.2918	2.7675	3.448	0.747	15.919
d	6	d	24	0.9041	0.7250	36	1.25	0.2204	0.05	-0.5663	2.3745	2.470	0.568	10.746
d	6	e	6	1.8861	1.0528	36	1.79	0.0816	0.05	-0.2490	4.0212	6.594	0.780	55.766
d	6	e	12	1.7747	0.9070	36	1.96	0.0582	0.05	-0.06468	3.6141	5.899	0.937	37.118
d	6	e	24	2.3093	1.2097	36	1.91	0.0642	0.05	-0.1439	4.7626	10.068	0.866	117.053
d	6	f	6	-2.7997	0.4472	36	-6.26	<.0001	0.05	-3.7068	-1.8927	0.061	0.025	0.151
d	6	f	12	-2.5495	0.4005	36	-6.37	<.0001	0.05	-3.3617	-1.7372	0.078	0.035	0.176
d	6	f	24	-2.6406	0.4335	36	-6.09	<.0001	0.05	-3.5197	-1.7614	0.071	0.030	0.172
d	12	d	24	-0.3337	0.9445	36	-0.35	0.7259	0.05	-2.2492	1.5818	0.716	0.105	4.864
d	12	e	6	0.6483	1.2144	36	0.53	0.5967	0.05	-1.8146	3.1111	1.912	0.163	22.446
d	12	e	12	0.5369	1.0904	36	0.49	0.6254	0.05	-1.6746	2.7483	1.711	0.187	15.616
d	12	e	24	1.0715	1.3527	36	0.79	0.4335	0.05	-1.6718	3.8148	2.920	0.188	45.369
d	12	f	6	-4.0376	0.7526	36	-5.36	<.0001	0.05	-5.5640	-2.5112	0.018	0.004	0.081
d	12	f	12	-3.7873	0.7258	36	-5.22	<.0001	0.05	-5.2593	-2.3153	0.023	0.005	0.099
d	12	f	24	-3.8784	0.7445	36	-5.21	<.0001	0.05	-5.3883	-2.3684	0.021	0.005	0.094
d	24	e	6	0.9820	1.1964	36	0.82	0.4172	0.05	-1.4445	3.4085	2.670	0.236	30.219
d	24	e	12	0.8706	1.0704	36	0.81	0.4214	0.05	-1.3003	3.0414	2.388	0.272	20.935
d	24	e	24	1.4052	1.3366	36	1.05	0.3001	0.05	-1.3055	4.1159	4.076	0.271	61.309
d	24	f	6	-3.7039	0.7233	36	-5.12	<.0001	0.05	-5.1709	-2.2369	0.025	0.006	0.107
d	24	f	12	-3.4536	0.6954	36	-4.97	<.0001	0.05	-4.8639	-2.0433	0.032	0.008	0.130
d	24	f	24	-3.5447	0.7149	36	-4.96	<.0001	0.05	-4.9946	-2.0948	0.029	0.007	0.123
e	6	e	12	-0.1114	1.3147	36	-0.08	0.9329	0.05	-2.7777	2.5549	0.895	0.062	12.870
e	6	e	24	0.4233	1.5392	36	0.27	0.7849	0.05	-2.6983	3.5449	1.527	0.067	34.635
e	6	f	6	-4.6858	1.0516	36	-4.46	<.0001	0.05	-6.8186	-2.5531	0.009	0.001	0.078
e	6	f	12	-4.4356	1.0326	36	-4.30	0.0001	0.05	-6.5297	-2.3414	0.012	0.001	0.096
e	6	f	24	-4.5267	1.0458	36	-4.33	0.0001	0.05	-6.6477	-2.4057	0.011	0.001	0.090
e	12	e	24	0.5346	1.4434	36	0.37	0.7132	0.05	-2.3927	3.4620	1.707	0.091	31.879
e	12	f	6	-4.5744	0.9056	36	-5.05	<.0001	0.05	-6.4111	-2.7378	0.010	0.002	0.065
e	12	f	12	-4.3242	0.8835	36	-4.89	<.0001	0.05	-6.1159	-2.5324	0.013	0.002	0.079
e	12	f	24	-4.4153	0.8989	36	-4.91	<.0001	0.05	-6.2383	-2.5922	0.012	0.002	0.075
e	24	f	6	-5.1091	1.2086	36	-4.23	0.0002	0.05	-7.5603	-2.6578	0.006	<0.001	0.070

	24	f	12	-4.8588	1.1921	36	-4.08	0.0002	0.05	-7.2766	-2.4411	0.008	<0.001	0.087
e	24	f	24	-4.9499	1.2036	36	-4.11	0.0002	0.05	-7.3910	-2.5089	0.007	<0.001	0.081
f	6	f	12	0.2503	0.3974	36	0.63	0.5329	0.05	-0.5558	1.0563	1.284	0.574	2.876
f	6	f	24	0.1592	0.4307	36	0.37	0.7138	0.05	-0.7142	1.0326	1.173	0.490	2.808
f	12	f	24	-0.09110	0.3819	36	-0.24	0.8128	0.05	-0.8656	0.6834	0.913	0.421	1.981

Performed by J. Luoma; SAS version 9.3 08:43 18MAR15

Effects of Pseudomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel species

Overall Model: Glochidia Viability (unadjusted) - At 6, 12 and 24 h

The GLIMMIX Procedure

form=SDP sps=HGE

Model Information	
Data Set	WORK.GLOCHIDIA2
Response Variable (Events)	via_glo
Response Variable (Trials)	tot
Response Distribution	Binomial
Link Function	Logit
Variance Function	Default
Variance Matrix	Diagonal
Estimation Technique	Maximum Likelihood
Degrees of Freedom Method	Residual

Class Level Information		
Class	Levels	Values
conca	6	a b c d e f
time	3	6 12 24

Number of Observations Read	55
Number of Observations Used	55
Number of Events	9632
Number of Trials	17795

Dimensions	
Covariance Parameters	1
Columns in X	27
Columns in Z	0
Subjects (Blocks in V)	1
Max Obs per Subject	55

Optimization Information	
Optimization Technique	Newton-Raphson
Parameters in Optimization	18
Lower Boundaries	0
Upper Boundaries	0
Fixed Effects	Not Profiled

Iteration History

Iteration	Restarts	Evaluations	Objective Function	Change	Max Gradient
0	0	4	510.81807464	.	32.34472
1	0	3	494.62396182	16.19411283	1.732956
2	0	3	494.54769505	0.07626677	0.012895
3	0	3	494.54768856	0.00000649	1.39E-6

Convergence criterion (ABSGCONV=0.00001) satisfied.

Fit Statistics	
-2 Log Likelihood	989.10
AIC (smaller is better)	1025.10
AICC (smaller is better)	1044.10
BIC (smaller is better)	1061.23
CAIC (smaller is better)	1079.23
HQIC (smaller is better)	1039.07
Pearson Chi-Square	652.18
Pearson Chi-Square / DF	17.63

Parameter Estimates

Effect	conca	time	Estimate	Standard Error	DF	t Value	Pr > |t|
conca	a		1.0843	0.2508	37	4.32	0.0001
conca	b		0.6758	0.2320	37	2.91	0.0060
conca	c		-0.3769	0.2570	37	-1.47	0.1509
conca	d		-1.5650	0.4305	37	-3.64	0.0008
conca	e		-1.7513	0.4526	37	-3.87	0.0004
conca	f		0.8530	0.2251	37	3.79	0.0005
time		6	0.1041	0.4256	37	0.24	0.8081
time		12	-0.1312	0.3680	37	-0.36	0.7235
time		24	0	.	.	.	.
conca*time	a	6	0.1495	0.5887	37	0.25	0.8009
conca*time	a	12	-0.1285	0.5505	37	-0.23	0.8167
conca*time	a	24	0	.	.	.	.
conca*time	b	6	-0.2346	0.5530	37	-0.42	0.6739
conca*time	b	12	0.1177	0.5287	37	0.22	0.8251
conca*time	b	24	0	.	.	.	.
conca*time	c	6	0.5523	0.5477	37	1.01	0.3198
conca*time	c	12	0.4085	0.5305	37	0.77	0.4462
conca*time	c	24	0	.	.	.	.
conca*time	d	6	0.6962	0.6693	37	1.04	0.3050
conca*time	d	12	1.2280	0.6510	37	1.89	0.0671
conca*time	d	24	0	.	.	.	.
conca*time	e	6	-0.01823	0.7507	37	-0.02	0.9808
conca*time	e	12	-0.3635	0.8155	37	-0.45	0.6584
conca*time	e	24	0	.	.	.	.
conca*time	f	6	0	.	.	.	.
conca*time	f	12	0	.	.	.	.
conca*time	f	24	0	.	.	.	.
Residual			17.6264	.	.	.	.

Type III Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
conca	5	37	27.49	<.0001
time	2	37	1.34	0.2736

conca*time	10	37	0.80	0.6253

Odds Ratio Estimates

conca	time	_conca	time	Estimate	DF	95% Confidence Limits	
a		f		1.269	37	0.774	2.081
b		f		0.806	37	0.504	1.287
c		f		0.403	37	0.254	0.638
d		f		0.169	37	0.099	0.290
e		f		0.065	37	0.033	0.127
	6		24	1.343	37	0.922	1.956
	12		24	1.082	37	0.725	1.617

conca Least Squares Means

conca	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Mean	Standard Error Mean	Lower Mean	Upper Mean
a	1.0823	0.1733	37	6.25	<.0001	0.05	0.7312	1.4333	0.7469	0.03275	0.6751	0.8074
b	0.6278	0.1545	37	4.06	0.0002	0.05	0.3147	0.9409	0.6520	0.03506	0.5780	0.7193
c	-0.06567	0.1486	37	-0.44	0.6612	0.05	-0.3668	0.2355	0.4836	0.03712	0.4093	0.5586
d	-0.9326	0.2027	37	-4.60	<.0001	0.05	-1.3434	-0.5218	0.2824	0.04108	0.2070	0.3724
e	-1.8876	0.2803	37	-6.73	<.0001	0.05	-2.4556	-1.3196	0.1315	0.03202	0.07903	0.2109
f	0.8440	0.1719	37	4.91	<.0001	0.05	0.4958	1.1923	0.6993	0.03614	0.6215	0.7671

Differences of conca Least Squares Means

conca	_conca	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Odds Ratio	Lower Confidence Limit for Odds Ratio	Upper Confidence Limit for Odds Ratio
a	b	0.4545	0.2322	37	1.96	0.0579	0.05	-0.01596	0.9249	1.575	0.984	2.522
a	c	1.1479	0.2283	37	5.03	<.0001	0.05	0.6854	1.6105	3.152	1.985	5.005
a	d	2.0149	0.2667	37	7.56	<.0001	0.05	1.4745	2.5553	7.500	4.369	12.875
a	e	2.9699	0.3296	37	9.01	<.0001	0.05	2.3021	3.6376	19.490	9.995	38.002
a	f	0.2383	0.2441	37	0.98	0.3353	0.05	-0.2563	0.7328	1.269	0.774	2.081
b	c	0.6935	0.2144	37	3.23	0.0026	0.05	0.2590	1.1279	2.001	1.296	3.089
b	d	1.5604	0.2549	37	6.12	<.0001	0.05	1.0439	2.0769	4.761	2.840	7.980
b	e	2.5154	0.3201	37	7.86	<.0001	0.05	1.8668	3.1640	12.372	6.468	23.665
b	f	-0.2162	0.2311	37	-0.94	0.3556	0.05	-0.6846	0.2521	0.806	0.504	1.287
c	d	0.8670	0.2514	37	3.45	0.0014	0.05	0.3576	1.3763	2.380	1.430	3.960
c	e	1.8219	0.3173	37	5.74	<.0001	0.05	1.1790	2.4649	6.184	3.251	11.762
c	f	-0.9097	0.2272	37	-4.00	0.0003	0.05	-1.3701	-0.4493	0.403	0.254	0.638
d	e	0.9550	0.3460	37	2.76	0.0089	0.05	0.2540	1.6560	2.599	1.289	5.238
d	f	-1.7766	0.2658	37	-6.68	<.0001	0.05	-2.3152	-1.2381	0.169	0.099	0.290
e	f	-2.7316	0.3288	37	-8.31	<.0001	0.05	-3.3979	-2.0654	0.065	0.033	0.127

time Least Squares Means

time	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Mean	Standard Error Mean	Lower Mean	Upper Mean
6	0.1150	0.1308	37	0.88	0.3850	0.05	-0.1500	0.3800	0.5287	0.03259	0.4626	0.5939
12	-0.1009	0.1479	37	-0.68	0.4996	0.05	-0.4006	0.1989	0.4748	0.03689	0.4012	0.5496

24	-0.1800	0.1316	37	-1.37	0.1796	0.05	-0.4467	0.08665	0.4551	0.03264	0.3901	0.5216

Differences of time Least Squares Means

time	time	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Odds Ratio	Lower Confidence Limit for Odds Ratio	Upper Confidence Limit for Odds Ratio
6	12	0.2158	0.1974	37	1.09	0.2814	0.05	-0.1842	0.6159	1.241	0.832	1.851
6	24	0.2950	0.1855	37	1.59	0.1204	0.05	-0.08096	0.6709	1.343	0.922	1.956
12	24	0.07917	0.1980	37	0.40	0.6916	0.05	-0.3220	0.4803	1.082	0.725	1.617

conca*time Least Squares Means

conca	time	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Mean	Standard Error Mean	Lower Mean	Upper Mean
a	6	1.3379	0.3202	37	4.18	0.0002	0.05	0.6891	1.9867	0.7921	0.05272	0.6658	0.8794
a	12	0.8246	0.3237	37	2.55	0.0151	0.05	0.1688	1.4804	0.6952	0.06858	0.5421	0.8146
a	24	1.0843	0.2508	37	4.32	0.0001	0.05	0.5761	1.5924	0.7473	0.04736	0.6402	0.8310
b	6	0.5453	0.2662	37	2.05	0.0476	0.05	0.006019	1.0847	0.6331	0.06183	0.5015	0.7474
b	12	0.6623	0.3004	37	2.20	0.0338	0.05	0.05355	1.2710	0.6598	0.06744	0.5134	0.7809
b	24	0.6758	0.2320	37	2.91	0.0060	0.05	0.2057	1.1459	0.6628	0.05186	0.5512	0.7588
c	6	0.2795	0.2298	37	1.22	0.2316	0.05	-0.1861	0.7452	0.5694	0.05635	0.4536	0.6781
c	12	-0.09963	0.2828	37	-0.35	0.7266	0.05	-0.6726	0.4733	0.4751	0.07052	0.3379	0.6162
c	24	-0.3769	0.2570	37	-1.47	0.1509	0.05	-0.8976	0.1438	0.4069	0.06202	0.2895	0.5359
d	6	-0.7647	0.2855	37	-2.68	0.0110	0.05	-1.3431	-0.1863	0.3176	0.06187	0.2070	0.4536
d	12	-0.4682	0.3211	37	-1.46	0.1532	0.05	-1.1188	0.1824	0.3850	0.07603	0.2462	0.5455
d	24	-1.5650	0.4305	37	-3.64	0.0008	0.05	-2.4372	-0.6927	0.1729	0.06157	0.08038	0.3334
e	6	-1.6655	0.4215	37	-3.95	0.0003	0.05	-2.5194	-0.8115	0.1590	0.05636	0.07451	0.3076
e	12	-2.2460	0.5700	37	-3.94	0.0003	0.05	-3.4009	-1.0912	0.09569	0.04932	0.03227	0.2514
e	24	-1.7513	0.4526	37	-3.87	0.0004	0.05	-2.6683	-0.8344	0.1479	0.05703	0.06487	0.3027
f	6	0.9572	0.3612	37	2.65	0.0118	0.05	0.2253	1.6890	0.7226	0.07241	0.5561	0.8441
f	12	0.7219	0.2911	37	2.48	0.0178	0.05	0.1320	1.3117	0.6730	0.06407	0.5329	0.7878
f	24	0.8530	0.2251	37	3.79	0.0005	0.05	0.3969	1.3092	0.7012	0.04717	0.5979	0.7874

Differences of conca*time Least Squares Means

conca	time	_conca	time	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Odds Ratio	Lower Confidence Limit for Odds Ratio	Upper Confidence Limit for Odds Ratio
a	6	a	12	0.5133	0.4553	37	1.13	0.2669	0.05	-0.4093	1.4358	1.671	0.664	4.203
a	6	a	24	0.2536	0.4067	37	0.62	0.5368	0.05	-0.5705	1.0778	1.289	0.565	2.938
a	6	b	6	0.7926	0.4164	37	1.90	0.0648	0.05	-0.05114	1.6363	2.209	0.950	5.136
a	6	b	12	0.6756	0.4391	37	1.54	0.1324	0.05	-0.2140	1.5653	1.965	0.807	4.784
a	6	b	24	0.6621	0.3954	37	1.67	0.1025	0.05	-0.1391	1.4634	1.939	0.870	4.320
a	6	c	6	1.0584	0.3942	37	2.69	0.0108	0.05	0.2597	1.8570	2.882	1.297	6.405
a	6	c	12	1.4375	0.4272	37	3.37	0.0018	0.05	0.5720	2.3031	4.210	1.772	10.005
a	6	c	24	1.7148	0.4106	37	4.18	0.0002	0.05	0.8829	2.5467	5.556	2.418	12.765
a	6	d	6	2.1026	0.4290	37	4.90	<.0001	0.05	1.2334	2.9718	8.187	3.433	19.527
a	6	d	12	1.8061	0.4535	37	3.98	0.0003	0.05	0.8873	2.7249	6.087	2.428	15.256
a	6	d	24	2.9029	0.5365	37	5.41	<.0001	0.05	1.8158	3.9900	18.227	6.146	54.055

a	6	e	6	3.0034	0.5293	37	5.67	<.0001	0.05	1.9309	4.0758	20.153	6.896	58.900
a	6	e	12	3.5839	0.6538	37	5.48	<.0001	0.05	2.2593	4.9086	36.015	9.576	135.445
a	6	e	24	3.0893	0.5544	37	5.57	<.0001	0.05	1.9660	4.2126	21.961	7.142	67.529
a	6	f	6	0.3808	0.4827	37	0.79	0.4353	0.05	-0.5973	1.3588	1.463	0.550	3.891
a	6	f	12	0.6161	0.4328	37	1.42	0.1630	0.05	-0.2608	1.4929	1.852	0.770	4.450
a	6	f	24	0.4849	0.3914	37	1.24	0.2233	0.05	-0.3083	1.2780	1.624	0.735	3.589
a	12	a	24	-0.2597	0.4095	37	-0.63	0.5299	0.05	-1.0893	0.5700	0.771	0.336	1.768
a	12	b	6	0.2793	0.4191	37	0.67	0.5093	0.05	-0.5698	1.1284	1.322	0.566	3.091
a	12	b	12	0.1623	0.4416	37	0.37	0.7153	0.05	-0.7325	1.0571	1.176	0.481	2.878
a	12	b	24	0.1488	0.3982	37	0.37	0.7108	0.05	-0.6581	0.9557	1.160	0.518	2.601
a	12	c	6	0.5451	0.3970	37	1.37	0.1780	0.05	-0.2593	1.3494	1.725	0.772	3.855
a	12	c	12	0.9242	0.4298	37	2.15	0.0381	0.05	0.05339	1.7951	2.520	1.055	6.020
a	12	c	24	1.2015	0.4133	37	2.91	0.0061	0.05	0.3641	2.0389	3.325	1.439	7.682
a	12	d	6	1.5893	0.4316	37	3.68	0.0007	0.05	0.7148	2.4637	4.900	2.044	11.748
a	12	d	12	1.2928	0.4559	37	2.84	0.0074	0.05	0.3690	2.2166	3.643	1.446	9.176
a	12	d	24	2.3896	0.5386	37	4.44	<.0001	0.05	1.2983	3.4809	10.909	3.663	32.489
a	12	e	6	2.4901	0.5314	37	4.69	<.0001	0.05	1.4133	3.5668	12.062	4.110	35.403
a	12	e	12	3.0706	0.6555	37	4.68	<.0001	0.05	1.7425	4.3987	21.555	5.712	81.346
a	12	e	24	2.5760	0.5564	37	4.63	<.0001	0.05	1.4486	3.7033	13.144	4.257	40.582
a	12	f	6	-0.1325	0.4850	37	-0.27	0.7861	0.05	-1.1152	0.8501	0.876	0.328	2.340
a	12	f	12	0.1028	0.4353	37	0.24	0.8147	0.05	-0.7793	0.9848	1.108	0.459	2.677
a	12	f	24	-0.02843	0.3943	37	-0.07	0.9429	0.05	-0.8273	0.7704	0.972	0.437	2.161
a	24	b	6	0.5390	0.3657	37	1.47	0.1490	0.05	-0.2021	1.2800	1.714	0.817	3.596
a	24	b	12	0.4220	0.3913	37	1.08	0.2879	0.05	-0.3709	1.2150	1.525	0.690	3.370
a	24	b	24	0.4085	0.3417	37	1.20	0.2394	0.05	-0.2838	1.1008	1.505	0.753	3.006
a	24	c	6	0.8047	0.3402	37	2.37	0.0233	0.05	0.1155	1.4940	2.236	1.122	4.455
a	24	c	12	1.1839	0.3780	37	3.13	0.0034	0.05	0.4181	1.9497	3.267	1.519	7.027
a	24	c	24	1.4612	0.3591	37	4.07	0.0002	0.05	0.7336	2.1888	4.311	2.083	8.924
a	24	d	6	1.8489	0.3800	37	4.87	<.0001	0.05	1.0790	2.6189	6.353	2.942	13.720
a	24	d	12	1.5525	0.4074	37	3.81	0.0005	0.05	0.7270	2.3780	4.723	2.069	10.784
a	24	d	24	2.6493	0.4982	37	5.32	<.0001	0.05	1.6398	3.6588	14.144	5.154	38.813
a	24	e	6	2.7498	0.4904	37	5.61	<.0001	0.05	1.7560	3.7435	15.639	5.790	42.244
a	24	e	12	3.3303	0.6227	37	5.35	<.0001	0.05	2.0686	4.5920	27.947	7.914	98.693
a	24	e	24	2.8356	0.5174	37	5.48	<.0001	0.05	1.7873	3.8840	17.041	5.973	48.618
a	24	f	6	0.1271	0.4397	37	0.29	0.7741	0.05	-0.7638	1.0181	1.136	0.466	2.768
a	24	f	12	0.3624	0.3843	37	0.94	0.3517	0.05	-0.4162	1.1410	1.437	0.660	3.130
a	24	f	24	0.2312	0.3370	37	0.69	0.4969	0.05	-0.4516	0.9141	1.260	0.637	2.496
b	6	b	12	-0.1169	0.4014	37	-0.29	0.7724	0.05	-0.9302	0.6963	0.890	0.394	2.006
b	6	b	24	-0.1305	0.3531	37	-0.37	0.7139	0.05	-0.8459	0.5850	0.878	0.429	1.795
b	6	c	6	0.2658	0.3517	37	0.76	0.4545	0.05	-0.4468	0.9783	1.304	0.640	2.660
b	6	c	12	0.6450	0.3883	37	1.66	0.1052	0.05	-0.1419	1.4318	1.906	0.868	4.186
b	6	c	24	0.9223	0.3700	37	2.49	0.0173	0.05	0.1726	1.6719	2.515	1.188	5.322
b	6	d	6	1.3100	0.3903	37	3.36	0.0018	0.05	0.5192	2.1008	3.706	1.681	8.173
b	6	d	12	1.0135	0.4171	37	2.43	0.0201	0.05	0.1685	1.8586	2.755	1.183	6.415
b	6	d	24	2.1103	0.5061	37	4.17	0.0002	0.05	1.0848	3.1358	8.251	2.959	23.008
b	6	e	6	2.2108	0.4985	37	4.44	<.0001	0.05	1.2008	3.2208	9.123	3.323	25.048

	6	e	12	2.7914	0.6291	37	4.44	<.0001	0.05	1.5168	4.0659	16.303	4.557	58.319
b	6	e	24	2.2967	0.5250	37	4.37	<.0001	0.05	1.2329	3.3605	9.941	3.431	28.803
b	6	f	6	-0.4118	0.4487	37	-0.92	0.3646	0.05	-1.3209	0.4973	0.662	0.267	1.644
b	6	f	12	-0.1765	0.3945	37	-0.45	0.6571	0.05	-0.9758	0.6228	0.838	0.377	1.864
b	6	f	24	-0.3077	0.3486	37	-0.88	0.3831	0.05	-1.0141	0.3986	0.735	0.363	1.490
b	12	b	24	-0.01352	0.3796	37	-0.04	0.9718	0.05	-0.7826	0.7556	0.987	0.457	2.129
b	12	c	6	0.3827	0.3783	37	1.01	0.3182	0.05	-0.3837	1.1492	1.466	0.681	3.156
b	12	c	12	0.7619	0.4126	37	1.85	0.0728	0.05	-0.07404	1.5978	2.142	0.929	4.942
b	12	c	24	1.0392	0.3953	37	2.63	0.0124	0.05	0.2381	1.8402	2.827	1.269	6.298
b	12	d	6	1.4269	0.4144	37	3.44	0.0014	0.05	0.5872	2.2666	4.166	1.799	9.647
b	12	d	12	1.1305	0.4397	37	2.57	0.0143	0.05	0.2395	2.0214	3.097	1.271	7.549
b	12	d	24	2.2273	0.5260	37	4.24	0.0001	0.05	1.1636	3.2909	9.274	3.201	26.867
b	12	e	6	2.3277	0.5176	37	4.50	<.0001	0.05	1.2790	3.3764	10.255	3.593	29.266
b	12	e	12	2.9083	0.6443	37	4.51	<.0001	0.05	1.6028	4.2138	18.325	4.967	67.610
b	12	e	24	2.4136	0.5432	37	4.44	<.0001	0.05	1.3130	3.5142	11.174	3.717	33.590
b	12	f	6	-0.2949	0.4698	37	-0.63	0.5341	0.05	-1.2468	0.6570	0.745	0.287	1.929
b	12	f	12	-0.05959	0.4183	37	-0.14	0.8875	0.05	-0.9072	0.7881	0.942	0.404	2.199
b	12	f	24	-0.1908	0.3754	37	-0.51	0.6144	0.05	-0.9514	0.5699	0.826	0.386	1.768
b	24	c	6	0.3962	0.3266	37	1.21	0.2327	0.05	-0.2655	1.0580	1.486	0.767	2.881
b	24	c	12	0.7754	0.3658	37	2.12	0.0408	0.05	0.03430	1.5165	2.172	1.035	4.556
b	24	c	24	1.0527	0.3462	37	3.04	0.0043	0.05	0.3512	1.7542	2.865	1.421	5.779
b	24	d	6	1.4405	0.3679	37	3.92	0.0004	0.05	0.6951	2.1858	4.223	2.004	8.898
b	24	d	12	1.1440	0.3962	37	2.89	0.0064	0.05	0.3413	1.9467	3.139	1.407	7.005
b	24	d	24	2.2408	0.4890	37	4.58	<.0001	0.05	1.2499	3.2317	9.401	3.490	25.322
b	24	e	6	2.3413	0.4811	37	4.87	<.0001	0.05	1.3665	3.3161	10.394	3.921	27.552
b	24	e	12	2.9218	0.6154	37	4.75	<.0001	0.05	1.6749	4.1687	18.575	5.338	64.630
b	24	e	24	2.4271	0.5086	37	4.77	<.0001	0.05	1.3967	3.4576	11.326	4.042	31.740
b	24	f	6	-0.2814	0.4293	37	-0.66	0.5162	0.05	-1.1512	0.5885	0.755	0.316	1.801
b	24	f	12	-0.04607	0.3723	37	-0.12	0.9022	0.05	-0.8004	0.7082	0.955	0.449	2.030
b	24	f	24	-0.1773	0.3233	37	-0.55	0.5868	0.05	-0.8323	0.4778	0.838	0.435	1.613
c	6	c	12	0.3792	0.3644	37	1.04	0.3048	0.05	-0.3591	1.1175	1.461	0.698	3.057
c	6	c	24	0.6565	0.3448	37	1.90	0.0647	0.05	-0.04210	1.3550	1.928	0.959	3.877
c	6	d	6	1.0442	0.3665	37	2.85	0.0071	0.05	0.3016	1.7868	2.841	1.352	5.970
c	6	d	12	0.7477	0.3949	37	1.89	0.0661	0.05	-0.05235	1.5478	2.112	0.949	4.701
c	6	d	24	1.8445	0.4880	37	3.78	0.0006	0.05	0.8557	2.8333	6.325	2.353	17.002
c	6	e	6	1.9450	0.4800	37	4.05	0.0003	0.05	0.9723	2.9177	6.994	2.644	18.498
c	6	e	12	2.5256	0.6146	37	4.11	0.0002	0.05	1.2803	3.7708	12.498	3.598	43.414
c	6	e	24	2.0309	0.5076	37	4.00	0.0003	0.05	1.0025	3.0593	7.621	2.725	21.313
c	6	f	6	-0.6776	0.4281	37	-1.58	0.1220	0.05	-1.5450	0.1898	0.508	0.213	1.209
c	6	f	12	-0.4423	0.3709	37	-1.19	0.2407	0.05	-1.1939	0.3092	0.643	0.303	1.362
c	6	f	24	-0.5735	0.3217	37	-1.78	0.0829	0.05	-1.2254	0.07836	0.564	0.294	1.082
c	12	c	24	0.2773	0.3821	37	0.73	0.4726	0.05	-0.4969	1.0515	1.320	0.608	2.862
c	12	d	6	0.6650	0.4018	37	1.66	0.1064	0.05	-0.1491	1.4792	1.945	0.861	4.389
c	12	d	12	0.3686	0.4279	37	0.86	0.3945	0.05	-0.4983	1.2355	1.446	0.608	3.440
c	12	d	24	1.4654	0.5150	37	2.85	0.0072	0.05	0.4218	2.5089	4.329	1.525	12.292
c	12	e	6	1.5658	0.5075	37	3.09	0.0038	0.05	0.5375	2.5942	4.787	1.712	13.386

	12	e	12	2.1464	0.6363	37	3.37	0.0018	0.05	0.8572	3.4356	8.554	2.357	31.049
c	12	e	24	1.6517	0.5336	37	3.10	0.0037	0.05	0.5705	2.7330	5.216	1.769	15.378
c	12	f	6	-1.0568	0.4587	37	-2.30	0.0269	0.05	-1.9862	-0.1274	0.348	0.137	0.880
c	12	f	12	-0.8215	0.4058	37	-2.02	0.0502	0.05	-1.6438	0.000840	0.440	0.193	1.001
c	12	f	24	-0.9527	0.3614	37	-2.64	0.0122	0.05	-1.6850	-0.2203	0.386	0.185	0.802
c	24	d	6	0.3877	0.3841	37	1.01	0.3193	0.05	-0.3905	1.1660	1.474	0.677	3.209
c	24	d	12	0.09129	0.4113	37	0.22	0.8256	0.05	-0.7420	0.9246	1.096	0.476	2.521
c	24	d	24	1.1881	0.5014	37	2.37	0.0231	0.05	0.1722	2.2039	3.281	1.188	9.060
c	24	e	6	1.2886	0.4936	37	2.61	0.0130	0.05	0.2884	2.2887	3.628	1.334	9.862
c	24	e	12	1.8691	0.6252	37	2.99	0.0049	0.05	0.6023	3.1359	6.482	1.826	23.010
c	24	e	24	1.3744	0.5204	37	2.64	0.0120	0.05	0.3200	2.4289	3.953	1.377	11.347
c	24	f	6	-1.3341	0.4433	37	-3.01	0.0047	0.05	-2.2322	-0.4359	0.263	0.107	0.647
c	24	f	12	-1.0988	0.3883	37	-2.83	0.0075	0.05	-1.8856	-0.3119	0.333	0.152	0.732
c	24	f	24	-1.2300	0.3416	37	-3.60	0.0009	0.05	-1.9222	-0.5377	0.292	0.146	0.584
d	6	d	12	-0.2965	0.4296	37	-0.69	0.4945	0.05	-1.1670	0.5741	0.743	0.311	1.775
d	6	d	24	0.8003	0.5165	37	1.55	0.1298	0.05	-0.2463	1.8469	2.226	0.782	6.340
d	6	e	6	0.9008	0.5090	37	1.77	0.0850	0.05	-0.1306	1.9322	2.462	0.878	6.905
d	6	e	12	1.4814	0.6375	37	2.32	0.0257	0.05	0.1898	2.7730	4.399	1.209	16.006
d	6	e	24	0.9867	0.5351	37	1.84	0.0732	0.05	-0.09745	2.0708	2.682	0.907	7.931
d	6	f	6	-1.7218	0.4604	37	-3.74	0.0006	0.05	-2.6546	-0.7890	0.179	0.070	0.454
d	6	f	12	-1.4865	0.4077	37	-3.65	0.0008	0.05	-2.3127	-0.6604	0.226	0.099	0.517
d	6	f	24	-1.6177	0.3635	37	-4.45	<.0001	0.05	-2.3543	-0.8811	0.198	0.095	0.414
d	12	d	24	1.0968	0.5371	37	2.04	0.0483	0.05	0.008614	2.1850	2.995	1.009	8.890
d	12	e	6	1.1973	0.5298	37	2.26	0.0298	0.05	0.1237	2.2708	3.311	1.132	9.687
d	12	e	12	1.7778	0.6542	37	2.72	0.0099	0.05	0.4523	3.1033	5.917	1.572	22.272
d	12	e	24	1.2831	0.5549	37	2.31	0.0264	0.05	0.1588	2.4075	3.608	1.172	11.106
d	12	f	6	-1.4254	0.4833	37	-2.95	0.0055	0.05	-2.4046	-0.4461	0.240	0.090	0.640
d	12	f	12	-1.1901	0.4334	37	-2.75	0.0093	0.05	-2.0683	-0.3118	0.304	0.126	0.732
d	12	f	24	-1.3212	0.3922	37	-3.37	0.0018	0.05	-2.1158	-0.5267	0.267	0.121	0.591
d	24	e	6	0.1005	0.6024	37	0.17	0.8684	0.05	-1.1202	1.3212	1.106	0.326	3.748
d	24	e	12	0.6810	0.7143	37	0.95	0.3465	0.05	-0.7662	2.1283	1.976	0.465	8.400
d	24	e	24	0.1864	0.6246	37	0.30	0.7671	0.05	-1.0792	1.4519	1.205	0.340	4.271
d	24	f	6	-2.5221	0.5619	37	-4.49	<.0001	0.05	-3.6607	-1.3835	0.080	0.026	0.251
d	24	f	12	-2.2868	0.5197	37	-4.40	<.0001	0.05	-3.3398	-1.2339	0.102	0.035	0.291
d	24	f	24	-2.4180	0.4858	37	-4.98	<.0001	0.05	-3.4023	-1.4337	0.089	0.033	0.238
e	6	e	12	0.5805	0.7089	37	0.82	0.4180	0.05	-0.8557	2.0168	1.787	0.425	7.515
e	6	e	24	0.08588	0.6184	37	0.14	0.8903	0.05	-1.1671	1.3389	1.090	0.311	3.815
e	6	f	6	-2.6226	0.5550	37	-4.73	<.0001	0.05	-3.7473	-1.4980	0.073	0.024	0.224
e	6	f	12	-2.3873	0.5122	37	-4.66	<.0001	0.05	-3.4252	-1.3494	0.092	0.033	0.259
e	6	f	24	-2.5185	0.4778	37	-5.27	<.0001	0.05	-3.4866	-1.5504	0.081	0.031	0.212
e	12	e	24	-0.4947	0.7278	37	-0.68	0.5009	0.05	-1.9693	0.9800	0.610	0.140	2.664
e	12	f	6	-3.2032	0.6748	37	-4.75	<.0001	0.05	-4.5704	-1.8360	0.041	0.010	0.159
e	12	f	12	-2.9679	0.6400	37	-4.64	<.0001	0.05	-4.2647	-1.6711	0.051	0.014	0.188
e	12	f	24	-3.0991	0.6128	37	-5.06	<.0001	0.05	-4.3407	-1.8574	0.045	0.013	0.156
e	24	f	6	-2.7085	0.5790	37	-4.68	<.0001	0.05	-3.8817	-1.5353	0.067	0.021	0.215
e	24	f	12	-2.4732	0.5381	37	-4.60	<.0001	0.05	-3.5635	-1.3829	0.084	0.028	0.251

	24	f	24	-2.6044	0.5055	37	-5.15	<.0001	0.05	-3.6285	-1.5802	0.074	0.027	0.206
f	6	f	12	0.2353	0.4639	37	0.51	0.6150	0.05	-0.7047	1.1753	1.265	0.494	3.239
f	6	f	24	0.1041	0.4256	37	0.24	0.8081	0.05	-0.7582	0.9665	1.110	0.468	2.629
f	12	f	24	-0.1312	0.3680	37	-0.36	0.7235	0.05	-0.8769	0.6145	0.877	0.416	1.849

Performed by J. Luoma; SAS version 9.3 08:43 18MAR15

Effects of Pseudomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel species

Overall Model: Glochidia Viability (unadjusted) - At 6, 12 and 24 h

The GLIMMIX Procedure

form=SDP sps=HIC

Model Information	
Data Set	WORK.GLOCHIDIA2
Response Variable (Events)	via_glo
Response Variable (Trials)	tot
Response Distribution	Binomial
Link Function	Logit
Variance Function	Default
Variance Matrix	Diagonal
Estimation Technique	Maximum Likelihood
Degrees of Freedom Method	Residual

Class Level Information		
Class	Levels	Values
conca	6	a b c d e f
time	3	6 12 24

Number of Observations Read	54
Number of Observations Used	54
Number of Events	6966
Number of Trials	12978

Dimensions	
Covariance Parameters	1
Columns in X	27
Columns in Z	0
Subjects (Blocks in V)	1
Max Obs per Subject	54

Optimization Information	
Optimization Technique	Newton-Raphson
Parameters in Optimization	18
Lower Boundaries	0
Upper Boundaries	0
Fixed Effects	Not Profiled

Iteration History

Iteration	Restarts	Evaluations	Objective Function	Change	Max Gradient
0	0	4	290.37887854		11.89377
1	0	3	287.15844725	3.22043129	0.597349
2	0	3	287.1529168	0.00553045	0.001829
3	0	3	287.15291675	0.00000005	1.733E-8

Convergence criterion (GCONV=1E-8) satisfied.

Fit Statistics	
-2 Log Likelihood	574.31
AIC (smaller is better)	610.31
AICC (smaller is better)	629.85
BIC (smaller is better)	646.11
CAIC (smaller is better)	664.11
HQIC (smaller is better)	624.11
Pearson Chi-Square	298.77
Pearson Chi-Square / DF	8.30

Parameter Estimates

Effect	conca	time	Estimate	Standard Error	DF	t Value	Pr > |t|
conca	a		2.0839	0.3333	36	6.25	<.0001
conca	b		1.3456	0.2322	36	5.79	<.0001
conca	c		0.4648	0.2162	36	2.15	0.0384
conca	d		-1.0104	0.2747	36	-3.68	0.0008
conca	e		-5.3303	1.4439	36	-3.69	0.0007
conca	f		1.8774	0.2789	36	6.73	<.0001
time		6	-0.5585	0.3897	36	-1.43	0.1605
time		12	-0.05700	0.4358	36	-0.13	0.8967
time		24	0	.	.	.	.
conca*time	a	6	0.2395	0.5948	36	0.40	0.6896
conca*time	a	12	-0.3306	0.6444	36	-0.51	0.6110
conca*time	a	24	0	.	.	.	.
conca*time	b	6	-0.3149	0.5023	36	-0.63	0.5346
conca*time	b	12	-0.09147	0.5642	36	-0.16	0.8721
conca*time	b	24	0	.	.	.	.
conca*time	c	6	-0.2388	0.4979	36	-0.48	0.6344
conca*time	c	12	-0.3903	0.5560	36	-0.70	0.4872
conca*time	c	24	0	.	.	.	.
conca*time	d	6	0.01732	0.5531	36	0.03	0.9752
conca*time	d	12	0.6276	0.5679	36	1.11	0.2764
conca*time	d	24	0	.	.	.	.
conca*time	e	6	4.0958	1.5199	36	2.69	0.0106
conca*time	e	12	0.9709	1.7649	36	0.55	0.5856
conca*time	e	24	0	.	.	.	.
conca*time	f	6	0	.	.	.	.
conca*time	f	12	0	.	.	.	.
conca*time	f	24	0	.	.	.	.
Residual			8.2993	.	.	.	.

Type III Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
conca	5	36	55.04	<.0001
time	2	36	0.04	0.9645

conca*time	10	36	2.55	0.0193

Odds Ratio Estimates

conca	time	_conca	time	Estimate	DF	95% Confidence Limits	
a		f		1.193	36	0.713	1.996
b		f		0.513	36	0.328	0.803
c		f		0.197	36	0.127	0.308
d		f		0.069	36	0.043	0.110
e		f		0.004	36	0.001	0.014
	6		24	1.078	36	0.608	1.909
	12		24	1.077	36	0.561	2.067

conca Least Squares Means

conca	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Mean	Standard Error Mean	Lower Mean	Upper Mean
a	1.8484	0.1874	36	9.86	<.0001	0.05	1.4683	2.2285	0.8639	0.02203	0.8128	0.9028
b	1.0049	0.1394	36	7.21	<.0001	0.05	0.7222	1.2876	0.7320	0.02735	0.6731	0.7837
c	0.04992	0.1369	36	0.36	0.7174	0.05	-0.2276	0.3275	0.5125	0.03419	0.4433	0.5811
d	-1.0006	0.1532	36	-6.53	<.0001	0.05	-1.3112	-0.6899	0.2688	0.03011	0.2123	0.3341
e	-3.8466	0.5772	36	-6.66	<.0001	0.05	-5.0171	-2.6760	0.02091	0.01181	0.006580	0.06440
f	1.6723	0.1713	36	9.76	<.0001	0.05	1.3249	2.0196	0.8419	0.02280	0.7900	0.8828

Differences of conca Least Squares Means

conca	_conca	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Odds Ratio	Lower Confidence Limit for Odds Ratio	Upper Confidence Limit for Odds Ratio
a	b	0.8434	0.2336	36	3.61	0.0009	0.05	0.3697	1.3172	2.324	1.447	3.733
a	c	1.7984	0.2321	36	7.75	<.0001	0.05	1.3278	2.2691	6.040	3.773	9.671
a	d	2.8489	0.2420	36	11.77	<.0001	0.05	2.3580	3.3398	17.269	10.570	28.214
a	e	5.6949	0.6068	36	9.38	<.0001	0.05	4.4642	6.9257	297.359	86.853	>999.999
a	f	0.1761	0.2539	36	0.69	0.4924	0.05	-0.3388	0.6910	1.193	0.713	1.996
b	c	0.9550	0.1954	36	4.89	<.0001	0.05	0.5588	1.3512	2.599	1.749	3.862
b	d	2.0055	0.2071	36	9.68	<.0001	0.05	1.5854	2.4255	7.430	4.881	11.308
b	e	4.8515	0.5938	36	8.17	<.0001	0.05	3.6473	6.0557	127.931	38.370	426.544
b	f	-0.6673	0.2208	36	-3.02	0.0046	0.05	-1.1152	-0.2195	0.513	0.328	0.803
c	d	1.0505	0.2054	36	5.11	<.0001	0.05	0.6339	1.4670	2.859	1.885	4.336
c	e	3.8965	0.5932	36	6.57	<.0001	0.05	2.6935	5.0995	49.230	14.783	163.943
c	f	-1.6223	0.2192	36	-7.40	<.0001	0.05	-2.0670	-1.1777	0.197	0.127	0.308
d	e	2.8460	0.5972	36	4.77	<.0001	0.05	1.6349	4.0571	17.219	5.129	57.807
d	f	-2.6728	0.2298	36	-11.63	<.0001	0.05	-3.1388	-2.2068	0.069	0.043	0.110
e	f	-5.5188	0.6020	36	-9.17	<.0001	0.05	-6.7398	-4.2978	0.004	0.001	0.014

time Least Squares Means

time	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Mean	Standard Error Mean	Lower Mean	Upper Mean
6	-0.02018	0.1071	36	-0.19	0.8516	0.05	-0.2373	0.1969	0.4950	0.02676	0.4410	0.5491
12	-0.02083	0.1879	36	-0.11	0.9123	0.05	-0.4018	0.3602	0.4948	0.04696	0.4009	0.5891

24	-0.09484	0.2609	36	-0.36	0.7163	0.05	-0.6239	0.4342	0.4763	0.06507	0.3489	0.6069

Differences of time Least Squares Means

time	time	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Odds Ratio	Lower Confidence Limit for Odds Ratio	Upper Confidence Limit for Odds Ratio
6	12	0.000653	0.2162	36	0.00	0.9976	0.05	-0.4379	0.4392	1.001	0.645	1.551
6	24	0.07467	0.2820	36	0.26	0.7927	0.05	-0.4972	0.6466	1.078	0.608	1.909
12	24	0.07401	0.3215	36	0.23	0.8192	0.05	-0.5780	0.7260	1.077	0.561	2.067

conca*time Least Squares Means

conca	time	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Mean	Standard Error Mean	Lower Mean	Upper Mean
a	6	1.7649	0.3014	36	5.86	<.0001	0.05	1.1537	2.3762	0.8538	0.03762	0.7602	0.9150
a	12	1.6963	0.3379	36	5.02	<.0001	0.05	1.0109	2.3816	0.8450	0.04425	0.7332	0.9154
a	24	2.0839	0.3333	36	6.25	<.0001	0.05	1.4079	2.7599	0.8893	0.03281	0.8034	0.9405
b	6	0.4722	0.2156	36	2.19	0.0351	0.05	0.03498	0.9093	0.6159	0.05099	0.5087	0.7129
b	12	1.1971	0.2730	36	4.38	<.0001	0.05	0.6434	1.7507	0.7680	0.04864	0.6555	0.8520
b	24	1.3455	0.2322	36	5.79	<.0001	0.05	0.8746	1.8165	0.7934	0.03806	0.7057	0.8601
c	6	-0.3325	0.2221	36	-1.50	0.1430	0.05	-0.7829	0.1178	0.4176	0.05401	0.3137	0.5294
c	12	0.01747	0.2692	36	0.06	0.9486	0.05	-0.5286	0.5635	0.5044	0.06730	0.3709	0.6373
c	24	0.4648	0.2162	36	2.15	0.0384	0.05	0.02625	0.9034	0.6142	0.05[illegible]24	0.5066	0.7116
d	6	-1.5515	0.2803	36	-5.53	<.0001	0.05	-2.1201	-0.9830	0.1749	0.04045	0.1072	0.2723
d	12	-0.4397	0.2389	36	-1.84	0.0740	0.05	-0.9243	0.04487	0.3918	0.05694	0.2841	0.5112
d	24	-1.0104	0.2747	36	-3.68	0.0008	0.05	-1.5676	-0.4532	0.2669	0.05375	0.1726	0.3886
e	6	-1.7930	0.2708	36	-6.62	<.0001	0.05	-2.3423	-1.2438	0.1427	0.03313	0.08768	0.2238
e	12	-4.4164	0.9165	36	-4.82	<.0001	0.05	-6.2752	-2.5577	0.01193	0.01081	0.001879	0.07191
e	24	-5.3303	1.4439	36	-3.69	0.0007	0.05	-8.2587	-2.4019	0.004819	0.006925	0.000259	0.08303
f	6	1.3189	0.2722	36	4.85	<.0001	0.05	0.7670	1.8709	0.7890	0.04531	0.6829	0.8666
f	12	1.8204	0.3349	36	5.44	<.0001	0.05	1.1413	2.4995	0.8606	0.04017	0.7579	0.9241
f	24	1.8774	0.2789	36	6.73	<.0001	0.05	1.3117	2.4431	0.8673	0.03210	0.7878	0.9201

Differences of conca*time Least Squares Means

conca	time	_conca	time	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Odds Ratio	Lower Confidence Limit for Odds Ratio	Upper Confidence Limit for Odds Ratio
a	6	a	12	0.06867	0.4528	36	0.15	0.8803	0.05	-0.8497	0.9870	1.071	0.428	2.683
a	6	a	24	-0.3190	0.4494	36	-0.71	0.4824	0.05	-1.2303	0.5924	0.727	0.292	1.808
a	6	b	6	1.2928	0.3706	36	3.49	0.0013	0.05	0.5413	2.0443	3.643	1.718	7.724
a	6	b	12	0.5679	0.4067	36	1.40	0.1711	0.05	-0.2569	1.3926	1.765	0.773	4.025
a	6	b	24	0.4194	0.3805	36	1.10	0.2776	0.05	-0.3522	1.1910	1.521	0.703	3.290
a	6	c	6	2.0974	0.3744	36	5.60	<.0001	0.05	1.3382	2.8567	8.145	3.812	17.404
a	6	c	12	1.7475	0.4041	36	4.32	0.0001	0.05	0.9278	2.5671	5.740	2.529	13.028
a	6	c	24	1.3001	0.3709	36	3.50	0.0012	0.05	0.5478	2.0524	3.670	1.729	7.787
a	6	d	6	3.3165	0.4116	36	8.06	<.0001	0.05	2.4817	4.1512	27.563	11.961	63.513
a	6	d	12	2.2047	0.3846	36	5.73	<.0001	0.05	1.4246	2.9847	9.067	4.156	19.781
a	6	d	24	2.7753	0.4078	36	6.81	<.0001	0.05	1.9482	3.6024	16.044	7.016	36.686

a	6	e	6	3.5579	0.4052	36	8.78	<.0001	0.05	2.7362	4.3797	35.091	15.428	79.815
a	6	e	12	6.1814	0.9648	36	6.41	<.0001	0.05	4.2247	8.1380	483.645	68.354	>999.999
a	6	e	24	7.0952	1.4750	36	4.81	<.0001	0.05	4.1037	10.0867	>999.999	60.566	>999.999
a	6	f	6	0.4460	0.4061	36	1.10	0.2794	0.05	-0.3776	1.2696	1.562	0.685	3.559
a	6	f	12	-0.05549	0.4505	36	-0.12	0.9027	0.05	-0.9692	0.8582	0.946	0.379	2.359
a	6	f	24	-0.1125	0.4107	36	-0.27	0.7857	0.05	-0.9453	0.7204	0.894	0.389	2.055
a	12	a	24	-0.3876	0.4747	36	-0.82	0.4195	0.05	-1.3503	0.5750	0.679	0.259	1.777
a	12	b	6	1.2241	0.4008	36	3.05	0.0042	0.05	0.4112	2.0370	3.401	1.509	7.668
a	12	b	12	0.4992	0.4344	36	1.15	0.2581	0.05	-0.3819	1.3803	1.647	0.683	3.976
a	12	b	24	0.3507	0.4100	36	0.86	0.3980	0.05	-0.4808	1.1823	1.420	0.618	3.262
a	12	c	6	2.0288	0.4044	36	5.02	<.0001	0.05	1.2087	2.8489	7.605	3.349	17.268
a	12	c	12	1.6788	0.4321	36	3.89	0.0004	0.05	0.8025	2.5551	5.359	2.231	12.872
a	12	c	24	1.2315	0.4012	36	3.07	0.0041	0.05	0.4178	2.0451	3.426	1.519	7.730
a	12	d	6	3.2478	0.4391	36	7.40	<.0001	0.05	2.3573	4.1383	25.734	10.563	62.694
a	12	d	12	2.1360	0.4139	36	5.16	<.0001	0.05	1.2966	2.9754	8.465	3.657	19.597
a	12	d	24	2.7066	0.4355	36	6.21	<.0001	0.05	1.8234	3.5899	14.979	6.193	36.231
a	12	e	6	3.4893	0.4331	36	8.06	<.0001	0.05	2.6110	4.3676	32.762	13.613	78.851
a	12	e	12	6.1127	0.9768	36	6.26	<.0001	0.05	4.1316	8.0937	451.549	62.279	>999.999
a	12	e	24	7.0266	1.4829	36	4.74	<.0001	0.05	4.0190	10.0341	>999.999	55.648	>999.999
a	12	f	6	0.3773	0.4339	36	0.87	0.3903	0.05	-0.5027	1.2573	1.458	0.605	3.516
a	12	f	12	-0.1242	0.4757	36	-0.26	0.7956	0.05	-1.0890	0.8407	0.883	0.337	2.318
a	12	f	24	-0.1812	0.4382	36	-0.41	0.6817	0.05	-1.0698	0.7075	0.834	0.343	2.029
a	24	b	6	1.6117	0.3969	36	4.06	0.0003	0.05	0.8067	2.4168	5.012	2.241	11.210
a	24	b	12	0.8868	0.4308	36	2.06	0.0468	0.05	0.01306	1.7606	2.427	1.013	5.816
a	24	b	24	0.7384	0.4062	36	1.82	0.0774	0.05	-0.08548	1.5622	2.093	0.918	4.769
a	24	c	6	2.4164	0.4005	36	6.03	<.0001	0.05	1.6041	3.2287	11.206	4.974	25.246
a	24	c	12	2.0664	0.4285	36	4.82	<.0001	0.05	1.1975	2.9354	7.897	3.312	18.829
a	24	c	24	1.6191	0.3973	36	4.08	0.0002	0.05	0.8133	2.4249	5.049	2.255	11.301
a	24	d	6	3.6354	0.4355	36	8.35	<.0001	0.05	2.7522	4.5187	37.919	15.677	91.717
a	24	d	12	2.5236	0.4101	36	6.15	<.0001	0.05	1.6919	3.3554	12.474	5.430	28.656
a	24	d	24	3.0943	0.4319	36	7.16	<.0001	0.05	2.2183	3.9703	22.071	9.191	53.000
a	24	e	6	3.8769	0.4295	36	9.03	<.0001	0.05	3.0059	4.7479	48.275	20.205	115.342
a	24	e	12	6.5003	0.9752	36	6.67	<.0001	0.05	4.5225	8.4782	665.357	92.065	>999.999
a	24	e	24	7.4142	1.4819	36	5.00	<.0001	0.05	4.4088	10.4196	>999.999	82.172	>999.999
a	24	f	6	0.7650	0.4303	36	1.78	0.0839	0.05	-0.1078	1.6377	2.149	0.898	5.143
a	24	f	12	0.2635	0.4725	36	0.56	0.5805	0.05	-0.6947	1.2217	1.301	0.499	3.393
a	24	f	24	0.2065	0.4346	36	0.48	0.6376	0.05	-0.6750	1.0879	1.229	0.509	2.968
b	6	b	12	-0.7249	0.3478	36	-2.08	0.0443	0.05	-1.4303	-0.01944	0.484	0.239	0.981
b	6	b	24	-0.8734	0.3168	36	-2.76	0.0091	0.05	-1.5159	-0.2308	0.418	0.220	0.794
b	6	c	6	0.8047	0.3095	36	2.60	0.0134	0.05	0.1770	1.4323	2.236	1.194	4.188
b	6	c	12	0.4547	0.3449	36	1.32	0.1957	0.05	-0.2448	1.1542	1.576	0.783	3.171
b	6	c	24	0.007357	0.3053	36	0.02	0.9809	0.05	-0.6119	0.6266	1.007	0.542	1.871
b	6	d	6	2.0237	0.3536	36	5.72	<.0001	0.05	1.3065	2.7409	7.566	3.693	15.500
b	6	d	12	0.9119	0.3218	36	2.83	0.0075	0.05	0.2592	1.5646	2.489	1.296	4.781
b	6	d	24	1.4825	0.3492	36	4.25	0.0001	0.05	0.7743	2.1907	4.404	2.169	8.942
b	6	e	6	2.2652	0.3461	36	6.54	<.0001	0.05	1.5632	2.9672	9.633	4.774	19.437

	6	e	12	4.8886	0.9415	36	5.19	<.0001	0.05	2.9791	6.7980	132.766	19.671	896.078
b	6	e	24	5.8025	1.4599	36	3.97	0.0003	0.05	2.8416	8.7633	331.112	17.144	>999.999
b	6	f	6	-0.8468	0.3472	36	-2.44	0.0198	0.05	-1.5509	-0.1426	0.429	0.212	0.867
b	6	f	12	-1.3483	0.3982	36	-3.39	0.0017	0.05	-2.1559	-0.5406	0.260	0.116	0.582
b	6	f	24	-1.4053	0.3525	36	-3.99	0.0003	0.05	-2.1202	-0.6903	0.245	0.120	0.501
b	12	b	24	-0.1485	0.3584	36	-0.41	0.6811	0.05	-0.8753	0.5784	0.862	0.417	1.783
b	12	c	6	1.5296	0.3519	36	4.35	0.0001	0.05	0.8159	2.2433	4.616	2.261	9.424
b	12	c	12	1.1796	0.3834	36	3.08	0.0040	0.05	0.4020	1.9572	3.253	1.495	7.080
b	12	c	24	0.7323	0.3483	36	2.10	0.0426	0.05	0.02595	1.4386	2.080	1.026	4.215
b	12	d	6	2.7486	0.3913	36	7.02	<.0001	0.05	1.9550	3.5422	15.621	7.064	34.541
b	12	d	12	1.6368	0.3628	36	4.51	<.0001	0.05	0.9010	2.3726	5.139	2.462	10.725
b	12	d	24	2.2074	0.3873	36	5.70	<.0001	0.05	1.4220	2.9929	9.092	4.145	19.944
b	12	e	6	2.9901	0.3845	36	7.78	<.0001	0.05	2.2102	3.7699	19.887	9.118	43.378
b	12	e	12	5.6135	0.9563	36	5.87	<.0001	0.05	3.6741	7.5529	274.097	39.411	>999.999
b	12	e	24	6.5274	1.4695	36	4.44	<.0001	0.05	3.5471	9.5076	683.586	34.712	>999.999
b	12	f	6	-0.1219	0.3855	36	-0.32	0.7537	0.05	-0.9037	0.6599	0.885	0.405	1.935
b	12	f	12	-0.6234	0.4320	36	-1.44	0.1577	0.05	-1.4996	0.2529	0.536	0.223	1.288
b	12	f	24	-0.6804	0.3903	36	-1.74	0.0898	0.05	-1.4719	0.1112	0.506	0.229	1.118
b	24	c	6	1.6780	0.3213	36	5.22	<.0001	0.05	1.0264	2.3297	5.355	2.791	10.274
b	24	c	12	1.3281	0.3555	36	3.74	0.0006	0.05	0.6070	2.0491	3.774	1.835	7.761
b	24	c	24	0.8807	0.3173	36	2.78	0.0087	0.05	0.2372	1.5242	2.413	1.268	4.592
b	24	d	6	2.8971	0.3640	36	7.96	<.0001	0.05	2.1588	3.6353	18.121	8.661	37.913
b	24	d	12	1.7853	0.3332	36	5.36	<.0001	0.05	1.1095	2.4610	5.961	3.033	11.717
b	24	d	24	2.3559	0.3597	36	6.55	<.0001	0.05	1.6264	3.0854	10.548	5.085	21.877
b	24	e	6	3.1385	0.3567	36	8.80	<.0001	0.05	2.4151	3.8620	23.070	11.190	47.562
b	24	e	12	5.7620	0.9454	36	6.09	<.0001	0.05	3.8445	7.6794	317.969	46.735	>999.999
b	24	e	24	6.6758	1.4625	36	4.56	<.0001	0.05	3.7098	9.6418	793.003	40.847	>999.999
b	24	f	6	0.02659	0.3578	36	0.07	0.9412	0.05	-0.6990	0.7522	1.027	0.497	2.122
b	24	f	12	-0.4749	0.4075	36	-1.17	0.2515	0.05	-1.3013	0.3515	0.622	0.272	1.421
b	24	f	24	-0.5319	0.3629	36	-1.47	0.1514	0.05	-1.2679	0.2042	0.587	0.281	1.226
c	6	c	12	-0.3500	0.3490	36	-1.00	0.3226	0.05	-1.0578	0.3578	0.705	0.347	1.430
c	6	c	24	-0.7973	0.3100	36	-2.57	0.0144	0.05	-1.4259	-0.1687	0.451	0.240	0.845
c	6	d	6	1.2190	0.3576	36	3.41	0.0016	0.05	0.4938	1.9443	3.384	1.638	6.989
c	6	d	12	0.1072	0.3262	36	0.33	0.7443	0.05	-0.5543	0.7688	1.113	0.574	2.157
c	6	d	24	0.6779	0.3532	36	1.92	0.0629	0.05	-0.03854	1.3943	1.970	0.962	4.032
c	6	e	6	1.4605	0.3502	36	4.17	0.0002	0.05	0.7502	2.1708	4.308	2.118	8.765
c	6	e	12	4.0839	0.9430	36	4.33	0.0001	0.05	2.1714	5.9964	59.378	8.771	401.988
c	6	e	24	4.9978	1.4609	36	3.42	0.0016	0.05	2.0350	7.9606	148.086	7.652	>999.999
c	6	f	6	-1.6514	0.3513	36	-4.70	<.0001	0.05	-2.3638	-0.9391	0.192	0.094	0.391
c	6	f	12	-2.1529	0.4018	36	-5.36	<.0001	0.05	-2.9678	-1.3380	0.116	0.051	0.262
c	6	f	24	-2.2099	0.3565	36	-6.20	<.0001	0.05	-2.9330	-1.4869	0.110	0.053	0.226
c	12	c	24	-0.4473	0.3453	36	-1.30	0.2034	0.05	-1.1477	0.2530	0.639	0.317	1.288
c	12	d	6	1.5690	0.3887	36	4.04	0.0003	0.05	0.7807	2.3573	4.802	2.183	10.562
c	12	d	12	0.4572	0.3600	36	1.27	0.2122	0.05	-0.2729	1.1873	1.580	0.761	3.278
c	12	d	24	1.0279	0.3847	36	2.67	0.0113	0.05	0.2477	1.8080	2.795	1.281	6.098
c	12	e	6	1.8105	0.3819	36	4.74	<.0001	0.05	1.0360	2.5850	6.113	2.818	13.263

	12	e	12	4.4339	0.9552	36	4.64	<.0001	0.05	2.4966	6.3712	84.259	12.141	584.738
c	12	e	24	5.3478	1.4688	36	3.64	0.0008	0.05	2.3689	8.3266	210.139	10.686	>999.999
c	12	f	6	-1.3015	0.3828	36	-3.40	0.0017	0.05	-2.0779	-0.5250	0.272	0.125	0.592
c	12	f	12	-1.8029	0.4297	36	-4.20	0.0002	0.05	-2.6744	-0.9315	0.165	0.069	0.394
c	12	f	24	-1.8599	0.3877	36	-4.80	<.0001	0.05	-2.6462	-1.0737	0.156	0.071	0.342
c	24	d	6	2.0163	0.3540	36	5.70	<.0001	0.05	1.2983	2.7344	7.511	3.663	15.400
c	24	d	12	0.9045	0.3223	36	2.81	0.0080	0.05	0.2510	1.5581	2.471	1.285	4.750
c	24	d	24	1.4752	0.3496	36	4.22	0.0002	0.05	0.7661	2.1842	4.372	2.151	8.884
c	24	e	6	2.2578	0.3466	36	6.52	<.0001	0.05	1.5550	2.9607	9.562	4.735	19.311
c	24	e	12	4.8812	0.9417	36	5.18	<.0001	0.05	2.9715	6.7910	131.792	19.521	889.791
c	24	e	24	5.7951	1.4600	36	3.97	0.0003	0.05	2.8341	8.7561	328.685	17.015	>999.999
c	24	f	6	-0.8541	0.3476	36	-2.46	0.0190	0.05	-1.5591	-0.1491	0.426	0.210	0.861
c	24	f	12	-1.3556	0.3986	36	-3.40	0.0017	0.05	-2.1640	-0.5472	0.258	0.115	0.579
c	24	f	24	-1.4126	0.3529	36	-4.00	0.0003	0.05	-2.1284	-0.6969	0.244	0.119	0.498
d	6	d	12	-1.1118	0.3683	36	-3.02	0.0046	0.05	-1.8588	-0.3648	0.329	0.156	0.694
d	6	d	24	-0.5412	0.3925	36	-1.38	0.1765	0.05	-1.3372	0.2549	0.582	0.263	1.290
d	6	e	6	0.2415	0.3898	36	0.62	0.5395	0.05	-0.5490	1.0320	1.273	0.578	2.807
d	6	e	12	2.8649	0.9584	36	2.99	0.0050	0.05	0.9212	4.8086	17.547	2.512	122.561
d	6	e	24	3.7788	1.4709	36	2.57	0.0145	0.05	0.7957	6.7618	43.762	2.216	864.202
d	6	f	6	-2.8705	0.3907	36	-7.35	<.0001	0.05	-3.6629	-2.0781	0.057	0.026	0.125
d	6	f	12	-3.3720	0.4367	36	-7.72	<.0001	0.05	-4.2576	-2.4863	0.034	0.014	0.083
d	6	f	24	-3.4290	0.3954	36	-8.67	<.0001	0.05	-4.2310	-2.6270	0.032	0.015	0.072
d	12	d	24	0.5706	0.3641	36	1.57	0.1258	0.05	-0.1678	1.3091	1.769	0.846	3.703
d	12	e	6	1.3533	0.3612	36	3.75	0.0006	0.05	0.6208	2.0857	3.870	1.860	8.051
d	12	e	12	3.9767	0.9471	36	4.20	0.0002	0.05	2.0558	5.8975	53.340	7.813	364.143
d	12	e	24	4.8906	1.4635	36	3.34	0.0020	0.05	1.9224	7.8588	133.028	6.837	>999.999
d	12	f	6	-1.7587	0.3622	36	-4.86	<.0001	0.05	-2.4932	-1.0242	0.172	0.083	0.359
d	12	f	12	-2.2602	0.4114	36	-5.49	<.0001	0.05	-3.0945	-1.4259	0.104	0.045	0.240
d	12	f	24	-2.3172	0.3673	36	-6.31	<.0001	0.05	-3.0620	-1.5723	0.099	0.047	0.208
d	24	e	6	0.7826	0.3858	36	2.03	0.0499	0.05	0.000275	1.5650	2.187	1.000	4.783
d	24	e	12	3.4060	0.9568	36	3.56	0.0011	0.05	1.4656	5.3465	30.146	4.330	209.867
d	24	e	24	4.3199	1.4698	36	2.94	0.0057	0.05	1.3390	7.3008	75.182	3.815	>999.999
d	24	f	6	-2.3293	0.3867	36	-6.02	<.0001	0.05	-3.1136	-1.5450	0.097	0.044	0.213
d	24	f	12	-2.8308	0.4331	36	-6.54	<.0001	0.05	-3.7092	-1.9524	0.059	0.024	0.142
d	24	f	24	-2.8878	0.3915	36	-7.38	<.0001	0.05	-3.6818	-2.0938	0.056	0.025	0.123
e	6	e	12	2.6234	0.9557	36	2.75	0.0094	0.05	0.6852	4.5616	13.783	1.984	95.734
e	6	e	24	3.5373	1.4691	36	2.41	0.0213	0.05	0.5578	6.5167	34.373	1.747	676.353
e	6	f	6	-3.1120	0.3839	36	-8.11	<.0001	0.05	-3.8906	-2.3333	0.045	0.020	0.097
e	6	f	12	-3.6134	0.4307	36	-8.39	<.0001	0.05	-4.4869	-2.7400	0.027	0.011	0.065
e	6	f	24	-3.6704	0.3888	36	-9.44	<.0001	0.05	-4.4589	-2.8820	0.025	0.012	0.056
e	12	e	24	0.9139	1.7102	36	0.53	0.5964	0.05	-2.5546	4.3823	2.494	0.078	80.025
e	12	f	6	-5.7354	0.9560	36	-6.00	<.0001	0.05	-7.6743	-3.7964	0.003	<0.001	0.022
e	12	f	12	-6.2368	0.9757	36	-6.39	<.0001	0.05	-8.2157	-4.2579	0.002	<0.001	0.014
e	12	f	24	-6.2938	0.9580	36	-6.57	<.0001	0.05	-8.2367	-4.3509	0.002	<0.001	0.013
e	24	f	6	-6.6492	1.4693	36	-4.53	<.0001	0.05	-9.6292	-3.6693	0.001	<0.001	0.025
e	24	f	12	-7.1507	1.4822	36	-4.82	<.0001	0.05	-10.1568	-4.1446	<0.001	<0.001	0.016

	24	f	24	-7.2077	1.4706	36	-4.90	<.0001	0.05	-10.1902	-4.2252	<0.001	<0.001	0.015
f	6	f	12	-0.5015	0.4315	36	-1.16	0.2528	0.05	-1.3766	0.3737	0.606	0.252	1.453
f	6	f	24	-0.5585	0.3897	36	-1.43	0.1605	0.05	-1.3488	0.2319	0.572	0.260	1.261
f	12	f	24	-0.05700	0.4358	36	-0.13	0.8967	0.05	-0.9409	0.8269	0.945	0.390	2.286

Performed by J. Luoma; SAS version 9.3 08:43 18MAR15

Effects of Pseudomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel species

Overall Model: Glochidia Viability (unadjusted) - At 6, 12 and 24 h

The GLIMMIX Procedure

form=SDP sps=MUC

Model Information	
Data Set	WORK.GLOCHIDIA2
Response Variable (Events)	via_glo
Response Variable (Trials)	tot
Response Distribution	Binomial
Link Function	Logit
Variance Function	Default
Variance Matrix	Diagonal
Estimation Technique	Maximum Likelihood
Degrees of Freedom Method	Residual

Class Level Information		
Class	Levels	Values
conca	6	a b c d e f
time	3	6 12 24

Number of Observations Read	54
Number of Observations Used	54
Number of Events	5942
Number of Trials	13218

Dimensions	
Covariance Parameters	1
Columns in X	27
Columns in Z	0
Subjects (Blocks in V)	1
Max Obs per Subject	54

Optimization Information	
Optimization Technique	Newton-Raphson
Parameters in Optimization	18
Lower Boundaries	0
Upper Boundaries	0
Fixed Effects	Not Profiled

Iteration History

Iteration	Restarts	Evaluations	Objective Function	Change	Max Gradient
0	0	4	321.3189661	.	8.466449
1	0	3	317.52390205	3.79506405	0.292219
2	0	3	317.51903284	0.00486921	0.00093
3	0	3	317.5190328	0.00000004	9.551E-9

Convergence criterion (GCONV=1E-8) satisfied.

Fit Statistics	
-2 Log Likelihood	635.04
AIC (smaller is better)	671.04
AICC (smaller is better)	690.58
BIC (smaller is better)	706.84
CAIC (smaller is better)	724.84
HQIC (smaller is better)	684.85
Pearson Chi-Square	355.52
Pearson Chi-Square / DF	9.88

Parameter Estimates

Effect	conca	time	Estimate	Standard Error	DF	t Value	Pr > |t|
conca	a		1.2566	0.2928	36	4.29	0.0001
conca	b		0.5379	0.2631	36	2.04	0.0483
conca	c		-0.8370	0.2014	36	-4.16	0.0002
conca	d		-2.8176	0.4444	36	-6.34	<.0001
conca	e		-2.8346	0.4989	36	-5.68	<.0001
conca	f		2.5539	0.4110	36	6.21	<.0001
time		6	-0.4706	0.5829	36	-0.81	0.4248
time		12	0.2744	0.6779	36	0.40	0.6880
time		24	0	.	.	.	.
conca*time	a	6	0.2380	0.7009	36	0.34	0.7362
conca*time	a	12	-0.7276	0.7956	36	-0.91	0.3665
conca*time	a	24	0	.	.	.	.
conca*time	b	6	0.9570	0.6868	36	1.39	0.1721
conca*time	b	12	-0.1694	0.7787	36	-0.22	0.8291
conca*time	b	24	0	.	.	.	.
conca*time	c	6	0.6457	0.6646	36	0.97	0.3378
conca*time	c	12	0.1021	0.7471	36	0.14	0.8920
conca*time	c	24	0	.	.	.	.
conca*time	d	6	1.2766	0.8065	36	1.58	0.1222
conca*time	d	12	0.3830	0.9202	36	0.42	0.6797
conca*time	d	24	0	.	.	.	.
conca*time	e	6	-0.02586	0.9531	36	-0.03	0.9785
conca*time	e	12	0.09685	0.9786	36	0.10	0.9217
conca*time	e	24	0	.	.	.	.
conca*time	f	6	0	.	.	.	.
conca*time	f	12	0	.	.	.	.
conca*time	f	24	0	.	.	.	.
Residual			9.8757	.	.	.	.

Type III Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
conca	5	36	72.78	<.0001
time	2	36	0.55	0.5814

conca*time	10	36	0.85	0.5879

Odds Ratio Estimates

conca	time	_conca	time	Estimate	DF	95% Confidence Limits	
a		f		0.232	36	0.124	0.436
b		f		0.173	36	0.093	0.322
c		f		0.043	36	0.024	0.079
d		f		0.008	36	0.004	0.017
e		f		0.005	36	0.002	0.011
	6		24	1.046	36	0.681	1.604
	12		24	1.248	36	0.797	1.956

conca Least Squares Means

conca	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Mean	Standard Error Mean	Lower Mean	Upper Mean
a	1.0280	0.1630	36	6.31	<.0001	0.05	0.6973	1.3587	0.7365	0.03164	0.6676	0.7955
b	0.7350	0.1526	36	4.82	<.0001	0.05	0.4255	1.0445	0.6759	0.03343	0.6048	0.7397
c	-0.6531	0.1334	36	-4.90	<.0001	0.05	-0.9236	-0.3827	0.3423	0.03002	0.2842	0.4055
d	-2.3297	0.2358	36	-9.88	<.0001	0.05	-2.8080	-1.8515	0.08869	0.01906	0.05689	0.1357
e	-2.8764	0.3014	36	-9.54	<.0001	0.05	-3.4877	-2.2650	0.05333	0.01522	0.02966	0.09406
f	2.4885	0.2646	36	9.40	<.0001	0.05	1.9518	3.0252	0.9233	0.01873	0.8756	0.9537

Differences of conca Least Squares Means

conca	_conca	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Odds Ratio	Lower Confidence Limit for Odds Ratio	Upper Confidence Limit for Odds Ratio
a	b	0.2930	0.2233	36	1.31	0.1978	0.05	-0.1599	0.7459	1.340	0.852	2.108
a	c	1.6811	0.2106	36	7.98	<.0001	0.05	1.2540	2.1083	5.372	3.504	8.234
a	d	3.3578	0.2867	36	11.71	<.0001	0.05	2.7763	3.9392	28.725	16.060	51.376
a	e	3.9044	0.3427	36	11.39	<.0001	0.05	3.2093	4.5994	49.618	24.762	99.425
a	f	-1.4605	0.3108	36	-4.70	<.0001	0.05	-2.0909	-0.8301	0.232	0.124	0.436
b	c	1.3882	0.2027	36	6.85	<.0001	0.05	0.9772	1.7992	4.008	2.657	6.045
b	d	3.0648	0.2809	36	10.91	<.0001	0.05	2.4951	3.6344	21.430	12.123	37.881
b	e	3.6114	0.3379	36	10.69	<.0001	0.05	2.9261	4.2966	37.017	18.656	73.451
b	f	-1.7535	0.3055	36	-5.74	<.0001	0.05	-2.3730	-1.1339	0.173	0.093	0.322
c	d	1.6766	0.2709	36	6.19	<.0001	0.05	1.1272	2.2260	5.347	3.087	9.263
c	e	2.2232	0.3296	36	6.74	<.0001	0.05	1.5547	2.8917	9.237	4.734	18.024
c	f	-3.1416	0.2963	36	-10.60	<.0001	0.05	-3.7427	-2.5406	0.043	0.024	0.079
d	e	0.5466	0.3827	36	1.43	0.1618	0.05	-0.2296	1.3228	1.727	0.795	3.754
d	f	-4.8182	0.3545	36	-13.59	<.0001	0.05	-5.5371	-4.0993	0.008	0.004	0.017
e	f	-5.3648	0.4011	36	-13.37	<.0001	0.05	-6.1784	-4.5513	0.005	0.002	0.011

time Least Squares Means

time	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Mean	Standard Error Mean	Lower Mean	Upper Mean
6	-0.3122	0.1485	36	-2.10	0.0426	0.05	-0.6133	-0.01107	0.4226	0.03623	0.3513	0.4972
12	-0.1349	0.1627	36	-0.83	0.4126	0.05	-0.4649	0.1951	0.4663	0.04050	0.3858	0.5486

24	-0.3568	0.1501	36	-2.38	0.0229	0.05	-0.6612	-0.05242	0.4117	0.03635	0.3405	0.4869

Differences of time Least Squares Means

time	time	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Odds Ratio	Lower Confidence Limit for Odds Ratio	Upper Confidence Limit for Odds Ratio
6	12	-0.1773	0.2203	36	-0.80	0.4262	0.05	-0.6240	0.2695	0.838	0.536	1.309
6	24	0.04463	0.2111	36	0.21	0.8338	0.05	-0.3835	0.4728	1.046	0.681	1.604
12	24	0.2219	0.2214	36	1.00	0.3228	0.05	-0.2270	0.6708	1.248	0.797	1.956

conca*time Least Squares Means

conca	time	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Mean	Standard Error Mean	Lower Mean	Upper Mean
a	6	1.0240	0.2565	36	3.99	0.0003	0.05	0.5038	1.5442	0.7358	0.04987	0.6233	0.8241
a	12	0.8034	0.2962	36	2.71	0.0102	0.05	0.2027	1.4041	0.6907	0.06327	0.5505	0.8028
a	24	1.2566	0.2928	36	4.29	0.0001	0.05	0.6628	1.8504	0.7784	0.05050	0.6599	0.8642
b	6	1.0243	0.2504	36	4.09	0.0002	0.05	0.5164	1.5322	0.7358	0.04868	0.6263	0.8223
b	12	0.6429	0.2787	36	2.31	0.0269	0.05	0.07773	1.2082	0.6554	0.06294	0.5194	0.7700
b	24	0.5379	0.2631	36	2.04	0.0483	0.05	0.004319	1.0715	0.6313	0.06124	0.5011	0.7449
c	6	-0.6619	0.2477	36	-2.67	0.0112	0.05	-1.1643	-0.1596	0.3403	0.05561	0.2379	0.4602
c	12	-0.4605	0.2411	36	-1.91	0.0642	0.05	-0.9495	0.02857	0.3869	0.05720	0.2790	0.5071
c	24	-0.8370	0.2014	36	-4.16	0.0002	0.05	-1.2454	-0.4286	0.3022	0.04246	0.2235	0.3945
d	6	-2.0115	0.3363	36	-5.98	<.0001	0.05	-2.6936	-1.3295	0.1180	0.03500	0.06335	0.2092
d	12	-2.1601	0.4358	36	-4.96	<.0001	0.05	-3.0439	-1.2763	0.1034	0.04040	0.04548	0.2182
d	24	-2.8176	0.4444	36	-6.34	<.0001	0.05	-3.7188	-1.9163	0.05638	0.02364	0.02369	0.1283
e	6	-3.3311	0.5654	36	-5.89	<.0001	0.05	-4.4777	-2.1845	0.03452	0.01884	0.01123	0.1012
e	12	-2.4634	0.4992	36	-4.93	<.0001	0.05	-3.4758	-1.4509	0.07847	0.03610	0.03001	0.1899
e	24	-2.8346	0.4989	36	-5.68	<.0001	0.05	-3.8465	-1.8227	0.05548	0.02615	0.02091	0.1391
f	6	2.0833	0.4133	36	5.04	<.0001	0.05	1.2450	2.9216	0.8893	0.04070	0.7764	0.9489
f	12	2.8283	0.5390	36	5.25	<.0001	0.05	1.7351	3.9215	0.9442	0.02841	0.8501	0.9806
f	24	2.5539	0.4110	36	6.21	<.0001	0.05	1.7203	3.3875	0.9278	0.02752	0.8482	0.9673

Differences of conca*time Least Squares Means

conca	time	_conca	time	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Odds Ratio	Lower Confidence Limit for Odds Ratio	Upper Confidence Limit for Odds Ratio
a	6	a	12	0.2206	0.3918	36	0.56	0.5769	0.05	-0.5740	1.0152	1.247	0.563	2.760
a	6	a	24	-0.2326	0.3893	36	-0.60	0.5538	0.05	-1.0221	0.5568	0.792	0.360	1.745
a	6	b	6	-0.00026	0.3585	36	-0.00	0.9994	0.05	-0.7273	0.7268	1.000	0.483	2.068
a	6	b	12	0.3811	0.3788	36	1.01	0.3211	0.05	-0.3871	1.1492	1.464	0.679	3.156
a	6	b	24	0.4861	0.3674	36	1.32	0.1942	0.05	-0.2591	1.2313	1.626	0.772	3.426
a	6	c	6	1.6859	0.3566	36	4.73	<.0001	0.05	0.9627	2.4091	5.397	2.619	11.124
a	6	c	12	1.4845	0.3521	36	4.22	0.0002	0.05	0.7705	2.1985	4.413	2.161	9.011
a	6	c	24	1.8610	0.3261	36	5.71	<.0001	0.05	1.1996	2.5224	6.430	3.319	12.458
a	6	d	6	3.0355	0.4230	36	7.18	<.0001	0.05	2.1777	3.8933	20.812	8.826	49.074
a	6	d	12	3.1841	0.5057	36	6.30	<.0001	0.05	2.1586	4.2097	24.147	8.659	67.336
a	6	d	24	3.8416	0.5131	36	7.49	<.0001	0.05	2.8010	4.8822	46.598	16.460	131.915

a	6	e	6	4.3551	0.6208	36	7.01	<.000'	0.05	3.0960	5.6142	77.874	22.108	274.298
a	6	e	12	3.4874	0.5613	36	6.21	<.0001	0.05	2.3491	4.6257	32.699	10.476	102.070
a	6	e	24	3.8586	0.5610	36	6.88	<.0001	0.05	2.7208	4.9964	47.400	15.193	147.882
a	6	f	6	-1.0593	0.4865	36	-2.18	0.0361	0.05	-2.0459	-0.07268	0.347	0.129	0.930
a	6	f	12	-1.8043	0.5969	36	-3.02	0.0046	0.05	-3.0149	-0.5936	0.165	0.049	0.552
a	6	f	24	-1.5299	0.4845	36	-3.16	0.0032	0.05	-2.5125	-0.5473	0.217	0.081	0.579
a	12	a	24	-0.4532	0.4165	36	-1.09	0.2837	0.05	-1.2978	0.3914	0.636	0.273	1.479
a	12	b	6	-0.2209	0.3879	36	-0.57	0.5726	0.05	-1.0075	0.5658	0.802	0.365	1.761
a	'2	b	12	0.1605	0.4067	36	0.39	0.6955	0.05	-0.6643	0.9852	1.174	0.515	2.678
a	12	b	24	0.2655	0.3961	36	0.67	0.5070	0.05	-0.5379	1.0689	1.304	0.584	2.912
a	12	c	6	1.4653	0.3861	36	3.80	0.0005	0.05	0.6823	2.2484	4.329	1.978	9.472
a	12	c	12	1.2639	0.3819	36	3.31	0.0021	0.05	0.4893	2.0385	3.539	1.631	7.679
a	12	c	24	1.6404	0.3581	36	4.58	<.0001	0.05	0.9141	2.3668	5.157	2.494	10.663
a	12	d	6	2.8149	0.4481	36	6.28	<.0001	0.05	1.9061	3.7238	16.692	6.727	41.421
a	12	d	12	2.9636	0.5269	36	5.62	<.0001	0.05	1.8950	4.0321	19.367	6.652	56.382
a	12	d	24	3.6210	0.5340	36	6.78	<.0001	0.05	2.5379	4.7040	37.373	12.653	110.389
a	12	e	6	4.1345	0.6383	36	6.48	<.0001	0.05	2.8401	5.4289	62.458	17.117	227.905
a	12	e	12	3.2668	0.5805	36	5.63	<.0001	0.05	2.0895	4.4440	26.226	8.081	85.115
a	12	e	24	3.6380	0.5802	36	6.27	<.0001	0.05	2.4613	4.8148	38.016	11.720	123.319
a	12	f	6	-1.2799	0.5085	36	-2.52	0.0164	0.05	-2.3112	-0.2486	0.278	0.099	0.780
a	12	f	12	-2.0249	0.6150	36	-3.29	0.0022	0.05	-3.2722	-0.7776	0.132	0.038	0.460
a	12	f	24	-1.7505	0.5066	36	-3.46	0.0014	0.05	-2.7780	-0.7230	0.174	0.062	0.485
a	24	b	6	0.2324	0.3853	36	0.60	0.5502	0.05	-0.5490	1.0137	1.262	0.578	2.756
a	24	b	12	0.6137	0.4042	36	1.52	0.1377	0.05	-0.2061	1.4335	1.847	0.814	4.193
a	24	b	24	0.7187	0.3936	36	1.83	0.0762	0.05	-0.07957	1.5170	2.052	0.924	4.559
a	24	c	6	1.9185	0.3835	36	5.00	<.0001	0.05	1.1408	2.6963	6.811	3.129	14.825
a	24	c	12	1.7171	0.3793	36	4.53	<.0001	0.05	0.9478	2.4864	5.568	2.580	12.017
a	24	c	24	2.0936	0.3553	36	5.89	<.0001	0.05	1.3730	2.8143	8.114	3.947	16.681
a	24	d	6	3.2681	0.4459	36	7.33	<.0001	0.05	2.3636	4.1725	26.263	10.632	64.875
a	24	d	12	3.4168	0.5250	36	6.51	<.0001	0.05	2.3520	4.4815	30.471	10.507	88.368
a	24	d	24	4.0742	0.5322	36	7.66	<.0001	0.05	2.9949	5.1534	58.802	19.984	173.023
a	24	e	6	4.5877	0.6367	36	7.21	<.0001	0.05	3.2964	5.8790	98.269	27.016	357.438
a	24	e	12	3.7200	0.5787	36	6.43	<.0001	0.05	2.5462	4.8937	41.263	12.759	133.449
a	24	e	24	4.0912	0.5785	36	7.07	<.0001	0.05	2.9180	5.2645	59.813	18.504	193.347
a	24	f	6	-0.8267	0.5065	36	-1.63	0.1114	0.05	-1.8540	0.2006	0.438	0.157	1.222
a	24	f	12	-1.5717	0.6134	36	-2.56	0.0147	0.05	-2.8157	-0.3277	0.208	0.060	0.721
a	24	f	24	-1.2973	0.5046	36	-2.57	0.0144	0.05	-2.3208	-0.2738	0.273	0.098	0.760
b	6	b	12	0.3813	0.3747	36	1.02	0.3156	0.05	-0.3786	1.1412	1.464	0.685	3.131
b	6	b	24	0.4864	0.3632	36	1.34	0.1890	0.05	-0.2503	1.2230	1.626	0.779	3.397
b	6	c	6	1.6862	0.3522	36	4.79	<.0001	0.05	0.9718	2.4006	5.399	2.643	11.029
b	6	c	12	1.4847	0.3477	36	4.27	0.0001	0.05	0.7797	2.1898	4.414	2.181	8.934
b	6	c	24	1.8613	0.3214	36	5.79	<.0001	0.05	1.2095	2.5130	6.432	3.352	12.342
b	6	d	6	3.0358	0.4193	36	7.24	<.0001	0.05	2.1854	3.8862	20.818	8.894	48.725
b	6	d	12	3.1844	0.5026	36	6.34	<.0001	0.05	2.1651	4.2038	24.153	8.715	66.938
b	6	d	24	3.8418	0.5101	36	7.53	<.0001	0.05	2.8073	4.8763	46.610	16.565	131.146
b	6	e	6	4.3554	0.6184	36	7.04	<.0001	0.05	3.1013	5.6094	77.894	22.226	272.992

	6	e	12	3.4876	0.5585	36	6.24	<.0001	0.05	2.3549	4.6203	32.708	10.537	101.529
b	6	e	24	3.8589	0.5583	36	6.91	<.0001	0.05	2.7267	4.9911	47.412	15.282	147.098
b	6	f	6	-1.0590	0.4833	36	-2.19	0.0350	0.05	-2.0392	-0.07886	0.347	0.130	0.924
b	6	f	12	-1.8040	0.5944	36	-3.04	0.0044	0.05	-3.0094	-0.5986	0.165	0.049	0.550
b	6	f	24	-1.5296	0.4813	36	-3.18	0.0030	0.05	-2.5058	-0.5535	0.217	0.082	0.575
b	12	b	24	0.1050	0.3833	36	0.27	0.7856	0.05	-0.6722	0.8823	1.111	0.511	2.417
b	12	c	6	1.3049	0.3729	36	3.50	0.0013	0.05	0.5487	2.0611	3.687	1.731	7.854
b	12	c	12	1.1034	0.3685	36	2.99	0.0050	0.05	0.3560	1.8508	3.014	1.428	6.365
b	12	c	24	1.4800	0.3438	36	4.30	0.0001	0.05	0.7826	2.1773	4.393	2.187	8.822
b	12	d	6	2.6545	0.4368	36	6.08	<.0001	0.05	1.7687	3.5403	14.217	5.863	34.477
b	12	d	12	2.8031	0.5173	36	5.42	<.0001	0.05	1.7540	3.8522	16.495	5.778	47.096
b	12	d	24	3.4605	0.5245	36	6.60	<.0001	0.05	2.3967	4.5243	31.833	10.987	92.231
b	12	e	6	3.9740	0.6303	36	6.30	<.0001	0.05	2.6957	5.2524	53.198	14.815	191.024
b	12	e	12	3.1063	0.5717	36	5.43	<.0001	0.05	1.9467	4.2658	22.338	7.006	71.225
b	12	e	24	3.4776	0.5715	36	6.08	<.0001	0.05	2.3185	4.6366	32.380	10.160	103.194
b	12	f	6	-1.4403	0.4985	36	-2.89	0.0065	0.05	-2.4514	-0.4293	0.237	0.086	0.651
b	12	f	12	-2.1854	0.6068	36	-3.60	0.0009	0.05	-3.4160	-0.9547	0.112	0.033	0.385
b	12	f	24	-1.9110	0.4966	36	-3.85	0.0005	0.05	-2.9181	-0.9038	0.148	0.054	0.405
b	24	c	6	1.1998	0.3613	36	3.32	0.0021	0.05	0.4670	1.9327	3.320	1.595	6.908
b	24	c	12	0.9984	0.3569	36	2.80	0.0082	0.05	0.2746	1.7222	2.714	1.316	5.597
b	24	c	24	1.3749	0.3313	36	4.15	0.0002	0.05	0.7030	2.0468	3.955	2.020	7.743
b	24	d	6	2.5494	0.4270	36	5.97	<.0001	0.05	1.6835	3.4154	12.800	5.384	30.429
b	24	d	12	2.6980	0.5090	36	5.30	<.0001	0.05	1.6657	3.7304	14.851	5.289	41.696
b	24	d	24	3.3554	0.5164	36	6.50	<.0001	0.05	2.3081	4.4028	28.658	10.055	81.677
b	24	e	6	3.8690	0.6236	36	6.20	<.0001	0.05	2.6043	5.1337	47.893	13.521	169.640
b	24	e	12	3.0012	0.5643	36	5.32	<.0001	0.05	1.8568	4.1457	20.111	6.403	63.162
b	24	e	24	3.3725	0.5641	36	5.98	<.0001	0.05	2.2285	4.5165	29.151	9.286	91.512
b	24	f	6	-1.5454	0.4900	36	-3.15	0.0032	0.05	-2.5391	-0.5517	0.213	0.079	0.576
b	24	f	12	-2.2904	0.5998	36	-3.82	0.0005	0.05	-3.5069	-1.0740	0.101	0.030	0.342
b	24	f	24	-2.0160	0.4880	36	-4.13	0.0002	0.05	-3.0058	-1.0263	0.133	0.050	0.358
c	6	c	12	-0.2014	0.3457	36	-0.58	0.5637	0.05	-0.9025	0.4996	0.818	0.406	1.648
c	6	c	24	0.1751	0.3192	36	0.55	0.5868	0.05	-0.4723	0.8225	1.191	0.624	2.276
c	6	d	6	1.3496	0.4177	36	3.23	0.0026	0.05	0.5025	2.1967	3.856	1.653	8.995
c	6	d	12	1.4982	0.5013	36	2.99	0.0050	0.05	0.4816	2.5148	4.474	1.619	12.364
c	6	d	24	2.1556	0.5087	36	4.24	0.0002	0.05	1.1238	3.1874	8.633	3.077	24.225
c	6	e	6	2.6692	0.6173	36	4.32	0.0001	0.05	1.4173	3.9210	14.428	4.126	50.451
c	6	e	12	1.8014	0.5573	36	3.23	0.0026	0.05	0.6712	2.9317	6.058	1.957	18.759
c	6	e	24	2.1727	0.5570	36	3.90	0.0004	0.05	1.0429	3.3024	8.782	2.838	27.179
c	6	f	6	-2.7452	0.4819	36	-5.70	<.0001	0.05	-3.7225	-1.7679	0.064	0.024	0.171
c	6	f	12	-3.4902	0.5932	36	-5.88	<.0001	0.05	-4.6933	-2.2871	0.030	0.009	0.102
c	6	f	24	-3.2158	0.4799	36	-6.70	<.0001	0.05	-4.1891	-2.2425	0.040	0.015	0.106
c	12	c	24	0.3765	0.3142	36	1.20	0.2385	0.05	-0.2606	1.0137	1.457	0.771	2.756
c	12	d	6	1.5510	0.4138	36	3.75	0.0006	0.05	0.7118	2.3903	4.716	2.038	10.917
c	12	d	12	1.6997	0.4980	36	3.41	0.0016	0.05	0.6896	2.7098	5.472	1.993	15.026
c	12	d	24	2.3571	0.5056	36	4.66	<.0001	0.05	1.3317	3.3824	10.560	3.787	29.443
c	12	e	6	2.8706	0.6147	36	4.67	<.0001	0.05	1.6240	4.1172	17.648	5.074	61.386

	12	e	12	2.0029	0.5544	36	3.61	0.0009	0.05	0.8765	3.1273	7.410	2.407	22.812
c	12	e	24	2.3741	0.5542	36	4.28	0.0001	0.05	1.2502	3.4980	10.742	3.491	33.050
c	12	f	6	-2.5438	0.4785	36	-5.32	<.0001	0.05	-3.5143	-1.5732	0.079	0.030	0.207
c	12	f	12	-3.2888	0.5905	36	-5.57	<.0001	0.05	-4.4864	-2.0912	0.037	0.011	0.124
c	12	f	24	-3.0144	0.4765	36	-6.33	<.0001	0.05	-3.9809	-2.0479	0.049	0.019	0.129
c	24	d	6	1.1745	0.3920	36	3.00	0.0049	0.05	0.3795	1.9695	3.237	1.462	7.167
c	24	d	12	1.3231	0.4801	36	2.76	0.0091	0.05	0.3495	2.2967	3.755	1.418	9.942
c	24	d	24	1.9805	0.4879	36	4.06	0.0003	0.05	0.9911	2.9700	7.247	2.694	19.492
c	24	e	6	2.4941	0.6002	36	4.16	0.0002	0.05	1.2769	3.7113	12.111	3.585	40.906
c	24	e	12	1.6263	0.5383	36	3.02	0.0046	0.05	0.5346	2.7181	5.085	1.707	15.151
c	24	e	24	1.9976	0.5380	36	3.71	0.0007	0.05	0.9064	3.0888	7.371	2.475	21.951
c	24	f	6	-2.9203	0.4598	36	-6.35	<.0001	0.05	-3.8528	-1.9878	0.054	0.021	0.137
c	24	f	12	-3.6653	0.5754	36	-6.37	<.0001	0.05	-4.8323	-2.4983	0.026	0.008	0.082
c	24	f	24	-3.3909	0.4577	36	-7.41	<.0001	0.05	-4.3192	-2.4626	0.034	0.013	0.085
d	6	d	12	0.1486	0.5505	36	0.27	0.7887	0.05	-0.9678	1.2650	1.160	0.380	3.543
d	6	d	24	0.8060	0.5573	36	1.45	0.1567	0.05	-0.3242	1.9362	2.239	0.723	6.933
d	6	e	6	1.3196	0.6578	36	2.01	0.0524	0.05	-0.01460	2.6537	3.742	0.986	14.207
d	6	e	12	0.4518	0.6019	36	0.75	0.4578	0.05	-0.7689	1.6726	1.571	0.464	5.326
d	6	e	24	0.8231	0.6017	36	1.37	0.1798	0.05	-0.3972	2.0434	2.278	0.672	7.717
d	6	f	6	-4.0948	0.5329	36	-7.68	<.0001	0.05	-5.1755	-3.0141	0.017	0.006	0.049
d	6	f	12	-4.8398	0.6353	36	-7.62	<.0001	0.05	-6.1283	-3.5513	0.008	0.002	0.029
d	6	f	24	-4.5654	0.5311	36	-8.60	<.0001	0.05	-5.6425	-3.4883	0.010	0.004	0.031
d	12	d	24	0.6574	0.6224	36	1.06	0.2979	0.05	-0.6049	1.9197	1.930	0.546	6.819
d	12	e	6	1.1709	0.7138	36	1.64	0.1096	0.05	-0.2768	2.6187	3.225	0.758	13.717
d	12	e	12	0.3032	0.6627	36	0.46	0.6500	0.05	-1.0407	1.6472	1.354	0.353	5.192
d	12	e	24	0.6745	0.6625	36	1.02	0.3154	0.05	-0.6691	2.0180	1.963	0.512	7.523
d	12	f	6	-4.2434	0.6006	36	-7.06	<.0001	0.05	-5.4616	-3.0253	0.014	0.004	0.049
d	12	f	12	-4.9884	0.6931	36	-7.20	<.0001	0.05	-6.3942	-3.5827	0.007	0.002	0.028
d	12	f	24	-4.7140	0.5990	36	-7.87	<.0001	0.05	-5.9290	-3.4991	0.009	0.003	0.030
d	24	e	6	0.5135	0.7191	36	0.71	0.4798	0.05	-0.9449	1.9720	1.671	0.389	7.185
d	24	e	12	-0.3542	0.6683	36	-0.53	0.5994	0.05	-1.7097	1.0013	0.702	0.181	2.722
d	24	e	24	0.01706	0.6681	36	0.03	0.9798	0.05	-1.3380	1.3721	1.017	0.262	3.944
d	24	f	6	-4.9008	0.6069	36	-8.08	<.0001	0.05	-6.1317	-3.6700	0.007	0.002	0.025
d	24	f	12	-5.6459	0.6986	36	-8.08	<.0001	0.05	-7.0626	-4.2291	0.004	<0.001	0.015
d	24	f	24	-5.3715	0.6053	36	-8.87	<.0001	0.05	-6.5991	-4.1438	0.005	0.001	0.016
e	6	e	12	-0.8677	0.7542	36	-1.15	0.2575	0.05	-2.3974	0.6619	0.420	0.091	1.939
e	6	e	24	-0.4965	0.7541	36	-0.66	0.5145	0.05	-2.0258	1.0328	0.609	0.132	2.809
e	6	f	6	-5.4144	0.7004	36	-7.73	<.0001	0.05	-6.8348	-3.9940	0.004	0.001	0.018
e	6	f	12	-6.1594	0.7811	36	-7.89	<.0001	0.05	-7.7436	-4.5751	0.002	<0.001	0.010
e	6	f	24	-5.8850	0.6990	36	-8.42	<.0001	0.05	-7.3026	-4.4674	0.003	<0.001	0.011
e	12	e	24	0.3713	0.7058	36	0.53	0.6021	0.05	-1.0602	1.8027	1.450	0.346	6.066
e	12	f	6	-4.5466	0.6481	36	-7.01	<.0001	0.05	-5.8611	-3.2322	0.011	0.003	0.039
e	12	f	12	-5.2917	0.7347	36	-7.20	<.0001	0.05	-6.7817	-3.8016	0.005	0.001	0.022
e	12	f	24	-5.0173	0.6467	36	-7.76	<.0001	0.05	-6.3287	-3.7058	0.007	0.002	0.025
e	24	f	6	-4.9179	0.6479	36	-7.59	<.0001	0.05	-6.2319	-3.6039	0.007	0.002	0.027
e	24	f	12	-5.6629	0.7345	36	-7.71	<.0001	0.05	-7.1525	-4.1733	0.003	<0.001	0.015

	24	f	24	-5.3885	0.6464	36	-8.34	<.0001	0.05	-6.6996	-4.0775	0.005	0.001	0.017
f	6	f	12	-0.7450	0.6793	36	-1.10	0.2800	0.05	-2.1226	0.6326	0.475	0.120	1.882
f	6	f	24	-0.4706	0.5829	36	-0.81	0.4248	0.05	-1.6528	0.7116	0.625	0.192	2.037
f	12	f	24	0.2744	0.6779	36	0.40	0.6880	0.05	-1.1004	1.6492	1.316	0.333	5.203

Performed by J. Luoma; SAS version 9.3 08:43 18MAR15

Effects of Pseudomonas fluorescens (Pf-CL145A) to glochidia from seven unionid mussel species

Overall Model: Glochidia Viability (unadjusted) - At 6, 12 and 24 h

The GLIMMIX Procedure

form=SDP sps=PPB

Model Information	
Data Set	WORK.GLOCHIDIA2
Response Variable (Events)	via_glo
Response Variable (Trials)	tot
Response Distribution	Binomial
Link Function	Logit
Variance Function	Default
Variance Matrix	Diagonal
Estimation Technique	Maximum Likelihood
Degrees of Freedom Method	Residual

Class Level Information		
Class	Levels	Values
conca	6	a b c d e f
time	3	6 12 24

Number of Observations Read	54
Number of Observations Used	54
Number of Events	9421
Number of Trials	18163

Dimensions	
Covariance Parameters	1
Columns in X	27
Columns in Z	0
Subjects (Blocks in V)	1
Max Obs per Subject	54

Optimization Information	
Optimization Technique	Newton-Raphson
Parameters in Optimization	18
Lower Boundaries	0
Upper Boundaries	0
Fixed Effects	Not Profiled

Iteration History

Iteration	Restarts	Evaluations	Objective Function	Change	Max Gradient
0	0	4	945.32974473	.	160.8709
1	0	3	795.60767544	149.72206929	47.7343
2	0	3	784.76560931	10.84206612	0.165926
3	0	3	784.7653506	0.00025872	0.000055
4	0	3	784.7653506	0.00000000	1.78E-10

Convergence criterion (GCONV=1E-8) satisfied.

Fit Statistics	
-2 Log Likelihood	1569.53
AIC (smaller is better)	1605.53
AICC (smaller is better)	1625.07
BIC (smaller is better)	1641.33
CAIC (smaller is better)	1659.33
HQIC (smaller is better)	1619.34
Pearson Chi-Square	1144.64
Pearson Chi-Square / DF	31.80

Parameter Estimates

Effect	conca	time	Estimate	Standard Error	DF	t Value	Pr > |t|
conca	a		1.3723	0.4296	36	3.19	0.0029
conca	b		0.6578	0.4094	36	1.61	0.1168
conca	c		0.1866	0.3704	36	0.50	0.6174
conca	d		-2.4440	0.6532	36	-3.74	0.0006
conca	e		-3.0924	0.9887	36	-3.13	0.0035
conca	f		2.4594	0.5996	36	4.10	0.0002
time		6	0.05853	0.9340	36	0.06	0.9504
time		12	-0.6393	0.7102	36	-0.90	0.3740
time		24	0	.	.	.	.
conca*time	a	6	-0.2993	1.1103	36	-0.27	0.7890
conca*time	a	12	0.4574	0.9211	36	0.50	0.6225
conca*time	a	24	0	.	.	.	.
conca*time	b	6	0.4787	1.1211	36	0.43	0.6719
conca*time	b	12	0.2121	0.8823	36	0.24	0.8114
conca*time	b	24	0	.	.	.	.
conca*time	c	6	-0.1916	1.1000	36	-0.17	0.8627
conca*time	c	12	0.4555	0.9061	36	0.50	0.6182
conca*time	c	24	0	.	.	.	.
conca*time	d	6	-0.3590	1.3877	36	-0.26	0.7973
conca*time	d	12	0.7065	1.0685	36	0.66	0.5127
conca*time	d	24	0	.	.	.	.
conca*time	e	6	-0.4627	2.0469	36	-0.23	0.8225
conca*time	e	12	0.8782	1.5047	36	0.58	0.5631
conca*time	e	24	0	.	.	.	.
conca*time	f	6	0	.	.	.	.
conca*time	f	12	0	.	.	.	.
conca*time	f	24	0	.	.	.	.
Residual			31.7956	.	.	.	.

Type III Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
conca	5	36	26.90	<.0001

time	2	36	0.17	0.8456
conca*time	10	36	0.33	0.9682

Odds Ratio Estimates

conca	time	_conca	time	Estimate	DF	95% Confidence Limits	
a		f		0.355	36	0.154	0.822
b		f		0.208	36	0.091	0.477
c		f		0.112	36	0.049	0.260
d		f		0.008	36	0.003	0.023
e		f		0.004	36	<0.001	0.021
	6		24	0.923	36	0.394	2.159
	12		24	0.829	36	0.428	1.607

conca Least Squares Means

conca	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Mean	Standard Error Mean	Lower Mean	Upper Mean
a	1.2314	0.2404	36	5.12	<.0001	0.05	0.7440	1.7189	0.7741	0.04204	0.6779	0.8480
b	0.6945	0.2335	36	2.97	0.0052	0.05	0.2208	1.1681	0.6670	0.05187	0.5550	0.7628
c	0.08100	0.2397	36	0.34	0.7374	0.05	-0.4051	0.5671	0.5202	0.05982	0.4001	0.6381
d	-2.5217	0.3747	36	-6.73	<.0001	0.05	-3.2817	-1.7617	0.07435	0.02579	0.03620	0.1466
e	-3.1475	0.6749	36	-4.66	<.0001	0.05	-4.5163	-1.7787	0.04119	0.02666	0.01081	0.1445
f	2.2658	0.3362	36	6.74	<.0001	0.05	1.5840	2.9477	0.9060	0.02863	0.8298	0.9502

Differences of conca Least Squares Means

conca	_conca	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Odds Ratio	Lower Confidence Limit for Odds Ratio	Upper Confidence Limit for Odds Ratio
a	b	0.5369	0.3351	36	1.60	0.1178	0.05	-0.1427	1.2166	1.711	0.867	3.376
a	c	1.1504	0.3394	36	3.39	0.0017	0.05	0.4620	1.8388	3.160	1.587	6.289
a	d	3.7531	0.4452	36	8.43	<.0001	0.05	2.8502	4.6560	42.654	17.292	105.217
a	e	4.3789	0.7164	36	6.11	<.0001	0.05	2.9259	5.8319	79.750	18.651	341.004
a	f	-1.0344	0.4133	36	-2.50	0.0170	0.05	-1.8726	-0.1962	0.355	0.154	0.822
b	c	0.6135	0.3346	36	1.83	0.0751	0.05	-0.06523	1.2922	1.847	0.937	3.641
b	d	3.2162	0.4416	36	7.28	<.0001	0.05	2.3207	4.1117	24.933	10.182	61.050
b	e	3.8419	0.7142	36	5.38	<.0001	0.05	2.3935	5.2904	46.616	10.952	198.416
b	f	-1.5713	0.4093	36	-3.84	0.0005	0.05	-2.4015	-0.7411	0.208	0.091	0.477
c	d	2.6027	0.4448	36	5.85	<.0001	0.05	1.7005	3.5049	13.500	5.477	33.277
c	e	3.2285	0.7162	36	4.51	<.0001	0.05	1.7759	4.6810	25.241	5.906	107.880
c	f	-2.1848	0.4129	36	-5.29	<.0001	0.05	-3.0222	-1.3474	0.112	0.049	0.260
d	e	0.6258	0.7720	36	0.81	0.4229	0.05	-0.9399	2.1914	1.870	0.391	8.948
d	f	-4.7875	0.5034	36	-9.51	<.0001	0.05	-5.8086	-3.7665	0.008	0.003	0.023
e	f	-5.4133	0.7540	36	-7.18	<.0001	0.05	-6.9425	-3.8841	0.004	<0.001	0.021

time Least Squares Means

time	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Mean	Standard Error Mean	Lower Mean	Upper Mean
6	-0.2238	0.3363	36	-0.67	0.5100	0.05	-0.9060	0.4583	0.4443	0.08304	0.2878	0.6126

12	-0.3311	0.2097	36	-1.58	0.1231	0.05	-0.7563	0.09420	0.4180	0.05101	0.3194	0.5235
24	-0.1434	0.2502	36	-0.57	0.5702	0.05	-0.6507	0.3640	0.4642	0.06222	0.3428	0.5900

Differences of time Least Squares Means

time	time	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Odds Ratio	Lower Confidence Limit for Odds Ratio	Upper Confidence Limit for Odds Ratio
6	12	0.1072	0.3964	36	0.27	0.7883	0.05	-0.6966	0.9111	1.113	0.498	2.467
6	24	-0.08045	0.4192	36	-0.19	0.8489	0.05	-0.9306	0.7697	0.923	0.394	2.159
12	24	-0.1877	0.3264	36	-0.57	0.5689	0.05	-0.8497	0.4743	0.829	0.428	1.607

conca*time Least Squares Means

conca	time	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Mean	Standard Error Mean	Lower Mean	Upper Mean
a	6	1.1315	0.4195	36	2.70	0.0106	0.05	0.2808	1.9822	0.7561	0.07735	0.5698	0.8789
a	12	1.1904	0.3993	36	2.98	0.0051	0.05	0.3805	2.0003	0.7668	0.07141	0.5940	0.8808
a	24	1.3723	0.4296	36	3.19	0.0029	0.05	0.5011	2.2435	0.7978	0.06931	0.6227	0.9041
b	6	1.1950	0.4657	36	2.57	0.0146	0.05	0.2506	2.1395	0.7676	0.08306	0.5623	0.8947
b	12	0.2306	0.3262	36	0.71	0.4842	0.05	-0.4310	0.8922	0.5574	0.08048	0.3939	0.7093
b	24	0.6578	0.4094	36	1.61	0.1168	0.05	-0.1724	1.4881	0.6588	0.09202	0.4570	0.8158
c	6	0.05356	0.4477	36	0.12	0.9054	0.05	-0.8544	0.9615	0.5134	0.1118	0.2985	0.7234
c	12	0.002821	0.4235	36	0.01	0.9947	0.05	-0.8562	0.8618	0.5007	0.1059	0.2981	0.7030
c	24	0.1866	0.3704	36	0.50	0.6174	0.05	-0.5646	0.9379	0.5465	0.09180	0.3625	0.7187
d	6	-2.7444	0.7916	36	-3.47	0.0014	0.05	-4.3499	-1.1389	0.06040	0.04493	0.01274	0.2425
d	12	-2.3767	0.4589	36	-5.18	<.0001	0.05	-3.3074	-1.4460	0.08496	0.03568	0.03532	0.1906
d	24	-2.4440	0.6532	36	-3.74	0.0006	0.05	-3.7686	-1.1193	0.07988	0.04801	0.02256	0.2461
e	6	-3.4965	1.5297	36	-2.29	0.0283	0.05	-6.5989	-0.3942	0.02941	0.04367	0.001360	0.4027
e	12	-2.8535	0.8843	36	-3.23	0.0027	0.05	-4.6470	-1.0600	0.05450	0.04557	0.009499	0.2573
e	24	-3.0924	0.9887	36	-3.13	0.0035	0.05	-5.0976	-1.0871	0.04342	0.04107	0.006074	0.2522
f	6	2.5179	0.7161	36	3.52	0.0012	0.05	1.0656	3.9703	0.9254	0.04944	0.7438	0.9815
f	12	1.8201	0.3806	36	4.78	<.0001	0.05	1.0481	2.5921	0.8606	0.04567	0.7404	0.9304
f	24	2.4594	0.5996	36	4.10	0.0002	0.05	1.2434	3.6755	0.9212	0.04350	0.7762	0.9753

Differences of conca*time Least Squares Means

conca	time	_conca	time	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Odds Ratio	Lower Confidence Limit for Odds Ratio	Upper Confidence Limit for Odds Ratio
a	6	a	12	-0.05888	0.5792	36	-0.10	0.9196	0.05	-1.2335	1.1157	0.943	0.291	3.052
a	6	a	24	-0.2408	0.6004	36	-0.40	0.6908	0.05	-1.4584	0.9769	0.786	0.233	2.656
a	6	b	6	-0.06350	0.6267	36	-0.10	0.9199	0.05	-1.3346	1.2076	0.938	0.263	3.345
a	6	b	12	0.9010	0.5314	36	1.70	0.0986	0.05	-0.1767	1.9787	2.462	0.838	7.233
a	6	b	24	0.4737	0.5861	36	0.81	0.4243	0.05	-0.7150	1.6624	1.606	0.489	5.272
a	6	c	6	1.0780	0.6135	36	1.76	0.0874	0.05	-0.1662	2.3222	2.939	0.847	10.198
a	6	c	12	1.1287	0.5961	36	1.89	0.0663	0.05	-0.08022	2.3377	3.092	0.923	10.357
a	6	c	24	0.9449	0.5596	36	1.69	0.1000	0.05	-0.1900	2.0798	2.573	0.827	8.003
a	6	d	6	3.8760	0.8959	36	4.33	0.0001	0.05	2.0590	5.6929	48.229	7.838	296.749
a	6	d	12	3.5083	0.6217	36	5.64	<.0001	0.05	2.2474	4.7692	33.391	9.463	117.823

a	6	d	24	3.5755	0.7762	36	4.61	<.0001	0.05	2.0012	5.1498	35.712	7.398	172.397
a	6	e	6	4.6280	1.5862	36	2.92	0.0060	0.05	1.4112	7.8449	102.314	4.101	>999.999
a	6	e	12	3.9851	0.9788	36	4.07	0.0002	0.05	2.0000	5.9701	53.789	7.389	391.548
a	6	e	24	4.2239	1.0740	36	3.93	0.0004	0.05	2.0457	6.4022	68.300	7.734	603.151
a	6	f	6	-1.3864	0.8299	36	-1.67	0.1035	0.05	-3.0696	0.2968	0.250	0.046	1.345
a	6	f	12	-0.6886	0.5664	36	-1.22	0.2320	0.05	-1.8373	0.4602	0.502	0.159	1.584
a	6	f	24	-1.3279	0.7318	36	-1.81	0.0779	0.05	-2.8119	0.1562	0.265	0.060	1.169
a	12	a	24	-0.1819	0.5865	36	-0.31	0.7583	0.05	-1.3714	1.0076	0.834	0.254	2.739
a	12	b	6	-0.00461	0.6135	36	-0.01	0.9940	0.05	-1.2488	1.2395	0.995	0.287	3.454
a	12	b	12	0.9598	0.5157	36	1.86	0.0709	0.05	-0.08596	2.0056	2.611	0.918	7.431
a	12	b	24	0.5326	0.5719	36	0.93	0.3579	0.05	-0.6272	1.6925	1.703	0.534	5.433
a	12	c	6	1.1369	0.5999	36	1.90	0.0661	0.05	-0.07984	2.3536	3.117	0.923	10.523
a	12	c	12	1.1876	0.5821	36	2.04	0.0487	0.05	0.007008	2.3682	3.279	1.007	10.678
a	12	c	24	1.0038	0.5447	36	1.84	0.0736	0.05	-0.1009	2.1085	2.729	0.904	8.236
a	12	d	6	3.9348	0.8866	36	4.44	<.0001	0.05	2.1366	5.7330	51.154	8.471	308.906
a	12	d	12	3.5672	0.6083	36	5.86	<.0001	0.05	2.3334	4.8009	35.416	10.313	121.622
a	12	d	24	3.6344	0.7656	36	4.75	<.0001	0.05	2.0817	5.1870	37.878	8.018	178.935
a	12	e	6	4.6869	1.5810	36	2.96	0.0053	0.05	1.4806	7.8933	108.519	4.396	>999.999
a	12	e	12	4.0439	0.9703	36	4.17	0.0002	0.05	2.0760	6.0119	57.051	7.973	408.241
a	12	e	24	4.2828	1.0663	36	4.02	0.0003	0.05	2.1201	6.4454	72.443	8.332	629.829
a	12	f	6	-1.3275	0.8199	36	-1.62	0.1142	0.05	-2.9904	0.3354	0.265	0.050	1.398
a	12	f	12	-0.6297	0.5517	36	-1.14	0.2612	0.05	-1.7486	0.4892	0.533	0.174	1.631
a	12	f	24	-1.2690	0.7204	36	-1.76	0.0866	0.05	-2.7301	0.1921	0.281	0.065	1.212
a	24	b	6	0.1773	0.6335	36	0.28	0.7812	0.05	-1.1076	1.4622	1.194	0.330	4.315
a	24	b	12	1.1417	0.5394	36	2.12	0.0413	0.05	0.04779	2.2357	3.132	1.049	9.353
a	24	b	24	0.7145	0.5934	36	1.20	0.2364	0.05	-0.4889	1.9179	2.043	0.613	6.807
a	24	c	6	1.3188	0.6204	36	2.13	0.0405	0.05	0.06043	2.5771	3.739	1.062	13.159
a	24	c	12	1.3695	0.6032	36	2.27	0.0293	0.05	0.1461	2.5929	3.933	1.157	13.369
a	24	c	24	1.1857	0.5672	36	2.09	0.0437	0.05	0.03531	2.3360	3.273	1.036	10.340
a	24	d	6	4.1167	0.9007	36	4.57	<.0001	0.05	2.2901	5.9433	61.358	9.876	381.206
a	24	d	12	3.7490	0.6286	36	5.96	<.0001	0.05	2.4742	5.0239	42.480	11.872	151.998
a	24	d	24	3.8163	0.7818	36	4.88	<.0001	0.05	2.2308	5.4017	45.434	9.307	221.790
a	24	e	6	4.8688	1.5889	36	3.06	0.0041	0.05	1.6465	8.0912	130.167	5.189	>999.999
a	24	e	12	4.2258	0.9831	36	4.30	0.0001	0.05	2.2319	6.2197	68.432	9.318	502.575
a	24	e	24	4.4647	1.0780	36	4.14	0.0002	0.05	2.2784	6.6510	86.894	9.761	773.574
a	24	f	6	-1.1456	0.8351	36	-1.37	0.1786	0.05	-2.8392	0.5480	0.318	0.058	1.730
a	24	f	12	-0.4478	0.5739	36	-0.78	0.4404	0.05	-1.6118	0.7162	0.639	0.200	2.047
a	24	f	24	-1.0871	0.7376	36	-1.47	0.1492	0.05	-2.5830	0.4088	0.337	0.076	1.505
b	6	b	12	0.9645	0.5686	36	1.70	0.0985	0.05	-0.1887	2.1176	2.623	0.828	8.311
b	6	b	24	0.5372	0.6200	36	0.87	0.3920	0.05	-0.7203	1.7947	1.711	0.487	6.018
b	6	c	6	1.1415	0.6460	36	1.77	0.0857	0.05	-0.1686	2.4516	3.131	0.845	11.607
b	6	c	12	1.1922	0.6295	36	1.89	0.0663	0.05	-0.08443	2.4689	3.294	0.919	11.809
b	6	c	24	1.0084	0.5950	36	1.69	0.0988	0.05	-0.1984	2.2152	2.741	0.820	9.163
b	6	d	6	3.9395	0.9184	36	4.29	0.0001	0.05	2.0768	5.8021	51.390	7.979	331.000
b	6	d	12	3.5718	0.6538	36	5.46	<.0001	0.05	2.2458	4.8977	35.579	9.448	133.986
b	6	d	24	3.6390	0.8022	36	4.54	<.0001	0.05	2.0121	5.2659	38.053	7.479	193.613

	6	e	6	4.6915	1.5990	36	2.93	0.0058	0.05	1.4486	7.9345	109.021	4.257	>999.999
b	6	e	12	4.0486	0.9995	36	4.05	0.0003	0.05	2.0216	6.0755	57.315	7.560	435.088
b	6	e	24	4.2874	1.0929	36	3.92	0.0004	0.05	2.0709	6.5040	72.778	7.932	667.777
b	6	f	6	-1.3229	0.8542	36	-1.55	0.1302	0.05	-3.0553	0.4095	0.266	0.047	1.506
b	6	f	12	-0.6251	0.6015	36	-1.04	0.3056	0.05	-1.8449	0.5947	0.535	0.158	1.813
b	6	f	24	-1.2644	0.7592	36	-1.67	0.1045	0.05	-2.8041	0.2753	0.282	0.061	1.317
b	12	b	24	-0.4272	0.5235	36	-0.82	0.4198	0.05	-1.4889	0.6344	0.652	0.226	1.886
b	12	c	6	0.1770	0.5539	36	0.32	0.7511	0.05	-0.9464	1.3005	1.194	0.388	3.671
b	12	c	12	0.2278	0.5346	36	0.43	0.6726	0.05	-0.8565	1.3120	1.256	0.425	3.714
b	12	c	24	0.04394	0.4936	36	0.09	0.9296	0.05	-0.9571	1.0450	1.045	0.384	2.843
b	12	d	6	2.9750	0.8562	36	3.47	0.0014	0.05	1.2385	4.7115	19.590	3.451	111.214
b	12	d	12	2.6073	0.5630	36	4.63	<.0001	0.05	1.4654	3.7492	13.563	4.329	42.488
b	12	d	24	2.6745	0.7301	36	3.66	0.0008	0.05	1.1938	4.1552	14.506	3.300	63.767
b	12	e	6	3.7271	1.5641	36	2.38	0.0226	0.05	0.5550	6.8992	41.558	1.742	991.478
b	12	e	12	3.0841	0.9426	36	3.27	0.0024	0.05	1.1724	4.9958	21.848	3.230	147.786
b	12	e	24	3.3230	1.0412	36	3.19	0.0029	0.05	1.2114	5.4346	27.742	3.358	229.190
b	12	f	6	-2.2874	0.7869	36	-2.91	0.0062	0.05	-3.8833	-0.6914	0.102	0.021	0.501
b	12	f	12	-1.5895	0.5013	36	-3.17	0.0031	0.05	-2.6062	-0.5728	0.204	0.074	0.564
b	12	f	24	-2.2288	0.6826	36	-3.27	0.0024	0.05	-3.6132	-0.8445	0.108	0.027	0.430
b	24	c	6	0.6043	0.6066	36	1.00	0.3259	0.05	-0.6261	1.8346	1.830	0.535	6.263
b	24	c	12	0.6550	0.5890	36	1.11	0.2735	0.05	-0.5396	1.8496	1.925	0.583	6.357
b	24	c	24	0.4712	0.5521	36	0.85	0.3991	0.05	-0.6485	1.5909	1.602	0.523	4.906
b	24	d	6	3.4022	0.8912	36	3.82	0.0005	0.05	1.5948	5.2097	30.031	4.927	183.034
b	24	d	12	3.0345	0.6150	36	4.93	<.0001	0.05	1.7873	4.2817	20.791	5.974	72.367
b	24	d	24	3.1018	0.7708	36	4.02	0.0003	0.05	1.5384	4.6651	22.237	4.657	106.178
b	24	e	6	4.1543	1.5835	36	2.62	0.0127	0.05	0.9428	7.3658	63.708	2.567	>999.999
b	24	e	12	3.5113	0.9745	36	3.60	0.0009	0.05	1.5350	5.4877	33.493	4.641	241.701
b	24	e	24	3.7502	1.0701	36	3.50	0.0012	0.05	1.5798	5.9205	42.529	4.854	372.611
b	24	f	6	-1.8601	0.8249	36	-2.26	0.0303	0.05	-3.5330	-0.1872	0.156	0.029	0.829
b	24	f	12	-1.1623	0.5590	36	-2.08	0.0448	0.05	-2.2960	-0.02861	0.313	0.101	0.972
b	24	f	24	-1.8016	0.7260	36	-2.48	0.0179	0.05	-3.2740	-0.3292	0.165	0.038	0.720
c	6	c	12	0.05074	0.6163	36	0.08	0.9348	0.05	-1.1992	1.3006	1.052	0.301	3.672
c	6	c	24	-0.1331	0.5811	36	-0.23	0.8201	0.05	-1.3115	1.0454	0.875	0.269	2.844
c	6	d	6	2.7980	0.9094	36	3.08	0.0040	0.05	0.9535	4.6424	16.411	2.595	103.795
c	6	d	12	2.4303	0.6411	36	3.79	0.0006	0.05	1.1301	3.7305	11.362	3.096	41.701
c	6	d	24	2.4975	0.7919	36	3.15	0.0032	0.05	0.8915	4.1035	12.152	2.439	60.551
c	6	e	6	3.5501	1.5939	36	2.23	0.0323	0.05	0.3176	6.7825	34.816	1.374	882.313
c	6	e	12	2.9071	0.9912	36	2.93	0.0058	0.05	0.8968	4.9173	18.303	2.452	136.638
c	6	e	24	3.1459	1.0854	36	2.90	0.0063	0.05	0.9447	5.3472	23.241	2.572	210.016
c	6	f	6	-2.4644	0.8445	36	-2.92	0.0060	0.05	-4.1772	-0.7516	0.085	0.015	0.472
c	6	f	12	-1.7666	0.5876	36	-3.01	0.0048	0.05	-2.9583	-0.5748	0.171	0.052	0.563
c	6	f	24	-2.4059	0.7483	36	-3.22	0.0028	0.05	-3.9235	-0.8882	0.090	0.020	0.411
c	12	c	24	-0.1838	0.5627	36	-0.33	0.7458	0.05	-1.3250	0.9573	0.832	0.266	2.605
c	12	d	6	2.7472	0.8978	36	3.06	0.0042	0.05	0.9264	4.5681	15.599	2.525	96.357
c	12	d	12	2.3796	0.6245	36	3.81	0.0005	0.05	1.1131	3.6461	10.800	3.044	38.324
c	12	d	24	2.4468	0.7785	36	3.14	0.0033	0.05	0.8680	4.0256	11.551	2.382	56.012

	12	e	6	3.4993	1.5872	36	2.20	0.0340	0.05	0.2603	6.7184	33.093	1.323	827.487
c	12	e	12	2.8563	0.9805	36	2 91	0.0061	0.05	0.8677	4.8450	17.398	2.382	127.097
c	12	e	24	3.0952	1.0756	36	2.88	0.0067	0.05	0.9137	5.2767	22.092	2.494	195.722
c	12	f	6	-2.5151	0.8320	36	-3.02	0.0046	0.05	-4.2025	-0.8278	0.081	0.015	0.437
c	ʻ2	f	12	-1.8173	0.5694	36	-3.19	0.0029	0.05	-2.9722	-0.6624	0.162	0.051	0.516
c	12	f	24	-2.4566	0.7341	36	-3.35	0.0019	0.05	-3.9454	-0.9678	0.086	0.019	0.380
c	24	d	6	2.9311	0.8740	36	3.35	0.0019	0.05	1.1585	4.7036	18.747	3.185	110.344
c	24	d	12	2.5634	0.5898	36	4.35	0.0001	0.05	1.3673	3.7594	12.980	3.925	42.924
c	24	d	24	2.6306	0.7509	36	3.50	0.0012	0.05	1.1077	4.1535	13.882	3.027	63.654
c	24	e	6	3.6831	1.5739	36	2.34	0.0249	0.05	0.4911	6.8752	39.771	1.634	967.923
c	24	e	12	3.0402	0.9588	36	3.17	0.0031	0.05	1.0957	4.9847	20.909	2.991	146.155
c	24	e	24	3.2790	1.0559	36	3.11	0.0037	0.05	1.1376	5.4204	26.550	3.119	225.966
c	24	f	6	-2.3313	0.8062	36	-2.89	0.0065	0.05	-3.9664	-0.6962	0.097	0.019	0.498
c	24	f	12	-1.6335	0.5311	36	-3.08	0.0040	0.05	-2.7107	-0.5563	0.195	0.066	0.573
c	24	f	24	-2.2728	0.7048	36	-3.22	0.0027	0.05	-3.7022	-0.8434	0.103	0.025	0.430
d	6	d	12	-0.3677	0.9150	36	-0.40	0.6902	0.05	-2.2234	1.4881	0.692	0.108	4.428
d	6	d	24	-0.3005	1.0263	36	-0.29	0.7714	0.05	-2.3819	1.7810	0.740	0.092	5.936
d	6	e	6	0.7521	1.7224	36	0.44	0.6650	0.05	-2.7411	4.2452	2.121	0.065	69.772
d	6	e	12	0.1091	1.1869	36	0.09	0.9273	0.05	-2.2980	2.5162	1.115	0.100	12.382
d	6	e	24	0.3480	1.2666	36	0.27	0.7851	0.05	-2.2208	2.9167	1.416	0.109	18.481
d	6	f	6	-5.2624	1.0675	36	-4.93	<.0001	0.05	-7.4273	-3.0974	0.005	<0.001	0.045
d	6	f	12	-4.5645	0.8784	36	-5.20	<.0001	0.05	-6.3460	-2.7831	0.010	0.002	0.062
d	6	f	24	-5.2038	0.9931	36	-5.24	<.0001	0.05	-7.2179	-3.1898	0.005	<0.001	0.041
d	12	d	24	0.06722	0.7983	36	0.08	0.9334	0.05	-1.5517	1.6862	1.070	0.212	5.399
d	12	e	6	1.1198	1.5970	36	0.70	0.4877	0.05	-2.1192	4.3587	3.064	0.120	78.157
d	12	e	12	0.4768	0.9963	36	0.48	0.6352	0.05	-1.5438	2.4974	1.611	0.214	12.151
d	12	e	24	0.7156	1.0900	36	0.66	0.5157	0.05	-1.4951	2.9264	2.045	0.224	18.660
d	12	f	6	-4.8947	0.8505	36	-5.75	<.0001	0.05	-6.6196	-3.1697	0.007	0.001	0.042
d	12	f	12	-4.1968	0.5962	36	-7.04	<.0001	0.05	-5.4060	-2.9877	0.015	0.004	0.050
d	12	f	24	-4.8361	0.7551	36	-6.41	<.0001	0.05	-6.3675	-3.3048	0.008	0.002	0.037
d	24	e	6	1.0526	1.6633	36	0.63	0.5309	0.05	-2.3208	4.4259	2.865	0.098	83.586
d	24	e	12	0.4096	1.0994	36	0.37	0.7117	0.05	-1.8201	2.6392	1.506	0.162	14.003
d	24	e	24	0.6484	1.1850	36	0.55	0.5876	0.05	-1.7549	3.0517	1.913	0.173	21.152
d	24	f	6	-4.9619	0.9692	36	-5.12	<.0001	0.05	-6.9276	-2.9962	0.007	<0.001	0.050
d	24	f	12	-4.2641	0.7560	36	-5.64	<.0001	0.05	-5.7973	-2.7309	0.014	0.003	0.065
d	24	f	24	-4.9034	0.8866	36	-5.53	<.0001	0.05	-6.7016	-3.1052	0.007	0.001	0.045
e	6	e	12	-0.6430	1.7669	36	-0.36	0.7181	0.05	-4.2265	2.9405	0.526	0.015	18.925
e	6	e	24	-0.4041	1.8214	36	-0.22	0.8257	0.05	-4.0981	3.2899	0.668	0.017	26.839
e	6	f	6	-6.0144	1.6890	36	-3.56	0.0011	0.05	-9.4399	-2.5890	0.002	<0.001	0.075
e	6	f	12	-5.3166	1.5763	36	-3.37	0.0018	0.05	-8.5136	-2.1197	0.005	<0.001	0.120
e	6	f	24	-5.9559	1.6430	36	-3.63	0.0009	0.05	-9.2881	-2.6238	0.003	<0.001	0.073
e	12	e	24	0.2389	1.3265	36	0.18	0.8581	0.05	-2.4515	2.9292	1.270	0.086	18.712
e	12	f	6	-5.3715	1.1379	36	-4.72	<.0001	0.05	-7.6793	-3.0636	0.005	<0.001	0.047
e	12	f	12	-4.6736	0.9628	36	-4.85	<.0001	0.05	-6.6262	-2.7210	0.009	0.001	0.066
e	12	f	24	-5.3129	1.0684	36	-4.97	<.0001	0.05	-7 4798	-3.1460	0.005	<0.001	0.043
e	24	f	6	-5.6103	1.2208	36	-4.60	<.0001	0.05	-8.0863	-3.1343	0.004	<0.001	0.044

	24	f	12	-4.9125	1.0595	36	-4.64	<.0001	0.05	-7.0612	-2.7638	0.007	<0.001	0.063
e	24	f	24	-5.5518	1.1563	36	-4.80	<.0001	0.05	-7.8970	-3.2066	0.004	<0.001	0.040
f	6	f	12	0.6978	0.8110	36	0.86	0.3952	0.05	-0.9469	2.3426	2.009	0.388	10.408
f	6	f	24	0.05853	0.9340	36	0.06	0.9504	0.05	-1.8357	1.9527	1.060	0.160	7.048
f	12	f	24	-0.6393	0.7102	36	-0.90	0.3740	0.05	-2.0797	0.8011	0.528	0.125	2.228

Performed by J. Luoma; SAS version 9.3 08:43 18MAR15

3/18/15
JA

Study Number	AEH-11-PSEUDO-01	Action		Date	Initials
Lab Notebook	1	Created......		7-Jun-12	TMS TJS
File Folder	12A	Revised......		16-Mar-15	JAL TJS
Raw Data Location	12A	Reviewed...		3-17-15	TJS
		Certified......		3-18-15	JA
File Name:	I:\AEH-11-PSEUDO-01\Data\Glochidia Viability Summary\[glochidia viability assessment 6h (adjusted).xlsx] Pocketbook SDP				

Glochidia Viability Assessment - 6 hour

Mussel species: Pocketbook Glochidia Lot number: 111400 Mean initial viability = 93.8%

Test Chemical: Pseudomonas florescens Pf-CL 145A (SDP) Chemical lot #: MBI-401-110308AI-BD-3

Exposure Date: 12-May-11

Test System Location: Block 1

Page 1 of 1

Species	Test Chamber	Treatment Group (mg/L)	Total Number Glochida Counted	Glochidia Closed before NaCl[1]	Glochidia Open after NaCl	Unadjusted 6h viability (%)	Adjusted 6 h viability (%)	Mean adjusted viability (%)	Standard Deviation (SD)
Pocketbook	1B1	0	312	3	18	93.3	99.4	98.4	1.1
Pocketbook	1C3	0	426	7	25	92.5	98.6		
Pocketbook	1A3	0	160	2	12	91.3	97.3		
Pocketbook	1B5	50	344	59	13	79.1	84.3	83.1	9.5
Pocketbook	1A1	50	189	16	10	86.2	91.9		
Pocketbook	1C1	50	447	129	12	68.5	73.0		
Pocketbook	1B4	100	170	43	11	68.2	72.7	80.2	6.5
Pocketbook	1A2	100	116	16	9	78.4	83.6		
Pocketbook	1A4	100	536	70	42	79.1	84.3		
Pocketbook	1C6	200	145	47	10	60.7	64.7	55.7	22.6
Pocketbook	1B2	200	252	60	21	67.9	72.3		
Pocketbook	1C4	200	238	165	6	28.2	30.0		
Pocketbook	1A5	300	114	75	14	21.9	23.4	10.6	11.1
Pocketbook	1C2	300	512	464	31	3.3	3.5		
Pocketbook	1C5	300	268	234	22	4.5	4.8		
Pocketbook	1A6	300HD	147	108	30	6.1	6.5	3.7	3.3
Pocketbook	1B3	300HD	119	91	23	4.2	4.5		
Pocketbook	1B6	300HD	210	192	18	0.0	0.0		

[1] If the number counted was glochidia open, the number of glochidia closed = total glochidia - open glochidia counted before NaCl.

Glochidia viability: (total # glochidia - (glochidia closed before + open after NaCl))/total glochidia *100

[2] Viability adjustments were calculated by dividing the observed viability by the mean initial viability for each species

Study Number	AEH-11-PSEUDO-01	Action		Date	Initials
Lab Notebook	1	Created......		7-Jun-12	TMS TS
File Folder	12A	Revised......		16-Mar-15	JAL TS
Raw data location	12A	Reviewed...		3-17-15	TS
		Certified......		3-18-15	JN
File Name:	I:\AEH-11-PSEUDO-01\Data\Glochidia Viability Summary\[glochidia viability assessment 6h (adjusted).xlsx]Fatmucket				

Glochidia Viability Assessment - 6 hour

Mussel species: Fatmucket Glochidia Lot number: 111600 Mean initial viability = 80.3%

Test Chemical: Pseudomonas florescens PF-CL 145A (SDP) Chemical lot #: MBI-4C1-110308AI-8D-3

Exposure Date: 12-May-11

Test System Location: Block 3

Species	Test Chamber	Treatment Group (mg/L)	Total Number Glochida Counted	Glochidia Closed before NaCl[1]	Glochidia Open after NaCl	Unadjusted 6h viability (%)	Adjusted 6 h viability (%)	Mean adjusted viability (%)	Standard Deviation (SD)
Fatmucket	3B2	0	210	29	34	70.0	87.2	90.1	5.0
Fatmucket	3B3	0	265	32	29	77.0	95.9		
Fatmucket	3A2	0	273	36	46	70.0	87.1		
Fatmucket	3B5	50	456	93	98	58.1	72.4	70.7	3.4
Fatmucket	3C5	50	276	57	71	53.6	66.8		
Fatmucket	3C6	50	239	42	57	58.6	72.9		
Fatmucket	3A1	100	237	126	37	31.2	38.9	36.1	18.4
Fatmucket	3A3	100	303	238	25	13.2	16.4		
Fatmucket	3A5	100	351	119	83	42.5	52.9		
Fatmucket	3B4	200	380	308	47	6.6	8.2	24.0	21.4
Fatmucket	3B6	200	526	407	53	12.5	15.6		
Fatmucket	3C4	200	201	59	64	38.8	48.3		
Fatmucket	3C1	300	501	224	121	31.1	38.8	14.8	20.8
Fatmucket	3C3	300	242	204	29	3.7	4.6		
Fatmucket	3B1	300	482	422	56	0.8	1.0		
Fatmucket	3A4	300HD	216	155	56	2.3	2.9	2.9	2.9
Fatmucket	3A6	300HD	212	164	38	4.7	5.9		
Fatmucket	3C2	300HD	205	166	39	0.0	0.0		

1 If the number counted was glochidia open, the number of glochidia closed = total glochidia - open glochidia counted before NaCl.

Glochidia viability: (total # glochidia - (glochidia closed before + open after NaCl))/total glochidia *100

[2] Viability adjustments were calculated by dividing the observed viability by the mean initial viability for each species

Study Number	AEH-11-PSEUDO-01	Action		Date	Initials
Lab Notebook	1	Created......		7-Jun-12	TMS TMS
File Folder	12A	Revised......		16-Mar-15	JAL JAL
Raw data location	12A	Reviewed...		3-17-15	TMS
		Certified......		3-18-15	JAL

File Name: I:\AEH-11-PSEUDO-01\Data\Glochidia Viability Summary\[glochidia viability assessment 6h (adjusted).xlsx]Higgins Eye

Glochidia Viability Assessment - 6 hour

Mussel species: Higgins Eye Glochidia Lot number: 111500

Test Chemical: Pseudomonas florescens Pf-CL 145A (SDP) Chemical lot #: MBI-401-110308AI-BD-3

Exposure Date: 12-May-11 Mean initial viability = 80.8%

Test System Location: Block 2

Species	Test Chamber	Treatment Group (mg/L)	Total Number Glochidia Counted	Glochidia Closed before NaCl[1]	Glochidia Open after NaCl	Unadjusted 6h viability (%)	Adjusted 6h Viability (%)[2]	Mean adjusted viability (%)	Standard Deviation (SD)
Higgins Eye	2C3	0	248	18	76	62.1	76.9	89.5	11.8
Higgins Eye	2C6	0	168	10	34	73.8	91.3		
Higgins Eye	2B1	0	258	18	31	81.0	100.3		
Higgins Eye	2A1	50	224	11	39	77.7	96.1	96.2	4.6
Higgins Eye	2A2	50	616	26	88	81.5	100.9		
Higgins Eye	2A4	50	204	24	29	74.0	91.6		
Higgins Eye	2A3	100	261	47	51	62.5	77.3	78.2	1.3
Higgins Eye	2C4	100	396	40	101	64.4	79.7		
Higgins Eye	2C5	100	414	103	51	62.8	77.7		
Higgins Eye	2A6	200	492	120	62	63.0	78.0	70.1	6.8
Higgins Eye	2B2	200	357	73	93	53.5	66.2		
Higgins Eye	2B5	200	512	121	117	53.5	66.2		
Higgins Eye	2C2	300	422	98	111	50.5	62.5	36.0	26.9
Higgins Eye	2C1	300	279	148	48	29.7	36.8		
Higgins Eye	2B3	300	297	251	25	7.1	8.8		
Higgins Eye	2A5	300HD	327	216	60	15.6	19.3	16.4	15.2
Higgins Eye	234	300HD	276	168	41	24.3	30.0		
Higgins Eye	236	300HD	139	102	37	0.0	0.0		

[1] If the number counted was glochidia open, the number of glochidia closed = total glochidia - open glochidia counted before NaCl.

[2] Viability adjustments were claculated by dividing the observed viability by the mean initial viability for each species

			Action		Date	Initials
Study Number	AEH-11-PSEUDO-01					
Lab Notebook	1		Created......		7-Jun-12	TMS TMS
File Folder	12A		Revised......		16-Mar-15	JAL TMS
Raw data location	12A		Reviewed......		3-17-15	TMS
			Certified......		3-18-15	JAL

File Name: I:\AEH-11-PSEUDO-01\Data\Glochidia Viability Summary\[glochidia viability assessment 6h (adjusted).xlsx]Pocketbook FDP

Glochidia Viability Assessment - 6 hour

Mussel species: Pocketbook Glochidia Lot number: 115400 Mean initial viability = 84.7%

Test Chemical: Pseudomonas florescens Pf-CL 145A (FDP) Chemical lot number: 110607WB-FD-E

Exposure Date: 18-Oct-11

Test System Location: Block 3

Species	Test Chamber	Treatment Group (mg/L)	Total Number Glochida Counted	Glochidia Closed before NaCl[1]	Glochidia Open after NaCl	Unadjusted 6h viability (%)	Adjusted 6h Viability (%)[2]	Mean adjusted viability (%)	Standard Deviation (SD)
Pocketbook	3A6	0	214	17	16	84.6	99.9	97.1	5.6
Pocketbook	3B5	0	89	7	6	85.4	100.8		
Pocketbook	3B6	0	125	18	11	76.8	90.7		
Pocketbook	3A4	50	157	16	17	79.0	93.2	82.1	13.6
Pocketbook	3C1	50	74	27	5	56.8	67.0		
Pocketbook	3C2	50	144	22	17	72.9	86.1		
Pocketbook	3B1	100	259	70	21	64.9	76.6	77.6	4.1
Pocketbook	3B3	100	190	33	25	69.5	82.0		
Pocketbook	3C6	100	161	47	13	62.7	74.1		
Pocketbook	3A2	200	256	86	52	46.1	54.4	57.5	13.5
Pocketbook	3C3	200	299	141	42	38.8	45.8		
Pocketbook	3C5	200	157	47	14	61.1	72.2		
Pocketbook	3A3	300	259	63	64	51.0	60.2	48.0	17.3
Pocketbook	3B4	300	272	149	58	23.9	28.2		
Pocketbook	3C4	300	655	218	128	47.2	55.7		
Pocketbook	3A1	300HD	200	144	45	5.5	6.5	24.1	27.2
Pocketbook	3A5	300HD	379	87	114	47.0	55.4		
Pocketbook	3B2	300HD	418	321	60	8.9	10.5		

[1] If the number counted was glochidia open, the number of glochidia closed = total glochidia - open glochidia counted before NaCl.

Glochidia viability: (total # glochidia - (glochidia closed before + open after NaCl))/total glochidia *100

[2] Viability adjustments were calculated by dividing the observed viability by the mean initial viability for each species

Study Number	AEH-11-PSEUDO-01	Action		Date	Initials
Lab Notebook	1	Created......		7-Jun-12	TMS TS
File Folder	12A	Revised......		16-Mar-15	JAL TS
Raw Data Location	12A	Reviewed...		3-17-15	TS
		Certified......		3-18-15	JA

File Name: I:\AEH-11-PSEUDO-01\Data\Glochidia Viability Summary\[glochidia viability assessment 6h (adjusted).xlsx]Black Sandshell

Glochidia Viability Assessment - 6 hour

Mussel species: Black Sandshell Glochidia Lot number: 120800 Mean initial viability = 84.5%

Test Chemical: Pseudomonas fluorescens Pf-CL 145A (SDP) Chemical lot #: MBI-401 SDP 4655-12-Mix

Exposure Date: 17-Jan-12

Test System Location: Block 1

Mussel Species	Test Chamber	Treatment Group (mg/L)	Total Number Glochida Counted	Glochidia Closed before NaCl[1]	Glochidia Open after NaCl	Unadjusted 6h viability (%)	Adjusted 6h Viability (%)[2]	Mean adjusted viability (%)	Standard Deviation (SD)
Black Sandshell	1A5	0	241	8	28	85.1	100.7	101.4	1.3
Black Sandshell	1B1	0	337	8	42	85.2	100.8		
Black Sandshell	1C6	0	314	8	33	86.9	102.9		
Black Sandshell	1A1	50	252	183	32	14.7	17.4	13.9	11.3
Black Sandshell	1A3	50	290	284	3	1.0	1.2		
Black Sandshell	1C1	50	206	132	34	19.4	23.0		
Black Sandshell	1B2	100	166	164	2	0.0	0.0	0.6	1.0
Black Sandshell	1B3	100	351	330	16	1.4	1.7		
Black Sandshell	1C5	100	144	136	8	0.0	0.0		
Black Sandshell	1A6	200	352	275	69	2.3	2.7	2.1	1.8
Black Sandshell	1B5	200	168	138	25	3.0	3.5		
Black Sandshell	1B6	200	224	222	2	0.0	0.0		
Black Sandshell	1A2	300	159	117	42	0.0	0.0	1.3	1.8
Black Sandshell	1B4	300	312	286	17	2.9	3.4		
Black Sandshell	1C2	300	250	234	15	0.4	0.5		
Black Sandshell	1A4	300HD	219	208	11	0.0	0.0	1.0	1.7
Black Sandshell	1C3	300HD	254	213	41	0.0	0.0		
Black Sandshell	1C4	300HD	237	195	36	2.5	3.0		

[1] If the number counted was glochidia open, the number of glochidia closed = total glochidia - open glochidia counted before NaCl.

Glochidia viability: (total # glochidia - (glochidia closed before + open after NaCl))/total glochidia *100

[2] Viability adjustments were calculated by dividing the observed viability by the mean initial viability for each species

Study Number	AEH-11-PSEUDO-01	Action		Date	Initials
Lab Notebook	1	Created......		7-Jun-12	TMS TS
File Folder	12A	Revised......		16-Mar-15	JAL TS
Raw Data Location	12A	Reviewed...		3-17-15	TYS
		Certified......		7-18-15	JS

File Name: I:\AEH-11-PSEUDO-01\Data\Glochidia Viability Summary\[glochidia viability assessment 6h (adjusted).xlsx]Mucket

Glochidia Viability Assessment - 6 hour

Mussel species: Mucket Glochidia Lot number: 120700 Mean initial viability = 92.7%

Test Chemical: Pseudomonas florescens Pf-CL 145A (SDP) Chemical lot #: MBI-401 SDP 4655-12-Mix

Exposure Date: 17-Jan-12

Test System Location: Block 2

Species	Test Chamber	Treatment Group (mg/L)	Total Number Glochida Counted	Glochidia Closed before NaCl[1]	Glochidia Open after NaCl	Unadjusted 6h viability (%)	Adjusted 6h Viability (%)[2]	Mean adjusted viability (%)	Standard Deviation (SD)
Mucket	2B4	0	198	15	17	83.8	90.4	95.6	4.8
Mucket	2B5	0	132	2	12	89.4	96.4		
Mucket	2C1	0	257	7	12	92.6	99.9		
Mucket	2A4	50	349	51	36	75.1	81.0	77.3	7.1
Mucket	2B6	50	128	43	3	64.1	69.1		
Mucket	2C3	50	295	59	12	75.9	81.9		
Mucket	2A2	100	190	61	13	61.1	65.9	77.9	10.5
Mucket	2B2	100	154	26	7	78.6	84.8		
Mucket	2C6	100	466	78	29	77.0	83.1		
Mucket	2A5	200	116	48	8	51.7	55.8	43.7	17.6
Mucket	2A6	200	202	82	23	48.0	51.8		
Mucket	2C2	200	399	291	21	21.8	23.5		
Mucket	2B1	300	265	199	14	19.6	21.2	12.7	7.9
Mucket	2B3	300	251	232	6	5.2	5.6		
Mucket	2C4	300	323	283	6	10.5	11.4		
Mucket	2A1	300HD	292	272	4	5.5	5.9	3.9	2.2
Mucket	2A3	300HD	355	342	8	1.4	1.5		
Mucket	2C5	300HD	280	259	10	3.9	4.2		

[1] If the number counted was glochidia open, the number of glochidia closed = total glochidia - open glochidia counted before NaCl.

Glochidia viability: (total # glochidia - (glochidia closed before + open after NaCl))/total glochidia *100

[2] Viability adjustments were calculated by dividing the observed viability by the mean initial viability for each species

Study Number	AEH-11-PSEUDO-01	Action		Date	Initials
Lab Notebook	1	Created......		7-Jun-12	TWS TJS
File Folder	12A	Revised......		17-Mar-15	TJS TJS
Raw Data Location	12A	Reviewed...		3-17-15	TJS
		Certified......		3-18-15	JA

File Name: I:\AEH-11-PSEUDO-01\Data\Glochidia Viability Summary\[glochidia viability assessment 6h (adjusted).xlsx]Hickorynut

Glochidia Viability Assessment - 6 hour

Mussel species: Hickorynut Glochidia Lot number: 120900 Mean initial viability = 86.0%

Test Chemical: Pseudomonas florescens Pf-CL 145A (SDP) Chemical lot number: MBI-401 SDP 4655-12-Mix

Exposure Date: 19-Jan-12

Test System Location: Block 3

Species	Test Chamber	Treatment Group (mg/L)	Total Number Glochida Counted	Glochidia Closed before NaCl[1]	Glochidia Open after NaCl	Unadjusted 6h viability (%)	Adjusted 6h Viability (%)[2]	Mean adjusted viability (%)	Standard Deviation (SD)
Hickorynut	3A2	0	258	65	21	66.7	77.5	93.0	13.4
Hickorynut	3C1	0	156	8	12	87.2	101.4		
Hickorynut	3C4	0	259	12	24	86.1	100.1		
Hickorynut	3A5	50	267	16	21	86.1	100.2	99.1	1.5
Hickorynut	3B3	50	191	21	10	83.8	97.4		
Hickorynut	3B6	50	274	26	13	85.8	99.7		
Hickorynut	3B1	100	232	39	32	69.4	80.7	70.9	16.1
Hickorynut	3C2	100	231	114	13	45.0	52.4		
Hickorynut	3C5	100	292	81	11	68.5	79.6		
Hickorynut	3A3	200	231	142	19	30.3	35.2	47.3	13.3
Hickorynut	3A4	200	176	90	18	38.6	44.9		
Hickorynut	3C6	200	285	117	17	53.0	61.6		
Hickorynut	3A6	300	293	192	13	30.0	34.9	18.3	14.8
Hickorynut	3B2	300	173	160	3	5.8	6.7		
Hickorynut	3B5	300	266	226	10	11.3	13.1		
Hickorynut	3A1	300HD	362	262	25	20.7	24.1	16.1	7.3
Hickorynut	3B4	300HD	317	274	16	8.5	9.9		
Hickorynut	3C3	300HD	246	209	7	12.2	14.2		

[1] If the number counted was glochidia open, the number of glochidia closed = total glochidia - open glochidia counted before NaCl.

Glochidia viability: (total # glochidia - (glochidia closed before + open after NaCl))/total glochidia *100

[2] Viability adjustments were calculated by dividing the observed viability by the mean initial viability for each species

Study Number	AEH-11-PSEUDO-01	Action		Date	Initials
Lab Notebook	1	Created......		7-Jun-12	TMS TK
File Folder	12A	Revised......		16-Mar-15	JAL TS
Raw Data Location	12A	Reviewed...		17-MAR-15	TS
		Certified......		3/15/15	JA
File Name:	I:\AEH-11-PSEUDO-01\Data\Glochidia Viability Summary\[Glochidia viability assessment 12h (adjusted).xlsx] Pocketbook SDP				

Glochidia Viability Assessment - 12 hour

Mussel species: Pocketbook Glochidia Lot number: 111400 Mean initial viability = 93.8%

Test Chemical: Pseudomonas florescens Pf-CL 145A (SDP) Chemical lot #: MBI-401-110308AI-BD-3

Exposure Date: 12-May-11

Test System Location: Block 1

Species	Test Chamber	Treatment Group (mg/L)	Total Number Glochida Counted	Glochidia Closed before NaCl[1]	Glochidia Open after NaCl	Unadjusted 12h viability (%)	Adjusted 12h viability (%)	Mean adjusted viability (%)	Standard Deviation (SD)
Pocketbook	1B1	0	599	39	53	84.6	90.2	91.8	2.2
Pocketbook	1C3	0	604	18	52	88.4	94.3		
Pocketbook	1A3	0	626	39	54	85.1	90.8		
Pocketbook	1B5	50	226	37	14	77.4	82.6	83.8	7.0
Pocketbook	1A1	50	265	24	14	85.7	91.3		
Pocketbook	1C1	50	624	104	67	72.6	77.4		
Pocketbook	1B4	100	354	40	28	80.8	86.1	56.1	48.7
Pocketbook	1A2	100	353	321	32	0.0	0.0		
Pocketbook	1A4	100	504	75	40	77.2	82.3		
Pocketbook	1C6	200	177	129	11	20.9	22.3	49.8	23.8
Pocketbook	1B2	200	152	47	15	59.2	63.1		
Pocketbook	1C4	200	380	116	36	60.0	64.0		
Pocketbook	1A5	300	1177	938	109	11.0	11.8	6.9	4.5
Pocketbook	1C2	300	305	284	13	2.6	2.8		
Pocketbook	1C5	300	460	403	30	5.9	6.3		
Pocketbook	1A6	300HD	242	193	32	7.0	7.5	6.2	2.8
Pocketbook	1B3	300HD	225	171	37	7.6	8.1		
Pocketbook	1B6	300HD	322	280	33	2.8	3.0		

[1] If the number counted was glochidia open, the number of glochidia closed = total glochidia - open glochidia counted before NaCl.

Glochidia viability: (total # glochidia - (glochidia closed before + open after NaCl))/total glochidia *100

[2] Viability adjustments were calculated by dividing the observed viability by the mean initial viability for each species

Study Number	AEH-11-PSEUDO-01	Action		Date	Initials
Lab Notebook	1	Created......		7-Jun-12	TMS TJS
File Folder	12A	Revised......		17-Mar-15	TJS TJS
Raw data location	12A	Reviewed...		17-MAR-15	TJS
		Certified......		3/18/15	JA
File Name:	I:\AEH-11-PSEUDO-01\Data\Glochidia Viability Summary\[Glochidia viability assessment 12h (adjusted).xlsx]Fatmuck				

Glochidia Viability Assessment - 12 hour

Mussel species: Fatmucket Glochidia Lot number: 111600 Mean initial viability = 80.3%

Test Chemical: Pseudomonas florescens Pf-CL 145A (SDP) Chemical lot #: MBI-401-110308AI-BD-3

Exposure Date: 12-May-11

Test System Location: Block 3

Species	Test Chamber	Treatment Group (mg/L)	Total Number Glochida Counted	Glochidia Closed before NaCl[1]	Glochidia Open after NaCl	Unadjusted 12h viability (%)	Adjusted 12h viability (%)	Mean adjusted viability (%)	Standard Deviation (SD)
Fatmucket	3B2	0	328	55	79	59.1	73.7	82.9	8.0
Fatmucket	3B3	0	521	63	88	71.0	88.4		
Fatmucket	3A2	0	282	54	32	69.5	86.6		
Fatmucket	3B5	50	430	146	36	57.7	71.8	73.6	5.1
Fatmucket	3C5	50	368	109	53	56.0	69.7		
Fatmucket	3C6	50	207	33	42	63.8	79.4		
Fatmucket	3A1	100	409	183	48	43.5	54.2	54.3	4.2
Fatmucket	3A3	100	221	64	53	47.1	58.6		
Fatmucket	3A5	100	159	68	27	40.3	50.1		
Fatmucket	3B4	200	198	127	40	15.7	19.5	24.4	16.4
Fatmucket	3B6	200	134	69	19	34.3	42.8		
Fatmucket	3C4	200	214	172	23	8.9	11.1		
Fatmucket	3C1	300	240	195	29	6.7	8.3	5.6	2.4
Fatmucket	3C3	300	206	173	26	3.4	4.2		
Fatmucket	3B1	300	298	233	55	3.4	4.2		
Fatmucket	3A4	300HD	310	252	52	1.9	2.4	2.9	3.2
Fatmucket	3A6	300HD	298	229	54	5.0	6.3		
Fatmucket	3C2	300HD	187	131	56	0.0	0.0		

1 If the number counted was glochidia open, the number of glochidia closed = total glochidia - open glochidia counted before NaCl.

Glochidia viability: (total # glochidia - (glochidia closed before + open after NaCl))/total glochidia *100

2 Viability adjustments were calculated by dividing the observed viability by the mean initial viability for each species

Study Number	AEH-11-PSEUDO-01	Action		Date	nitials
Lab Notebook	1	Created......		7-Jun-12	TMS TYS
File Folder	12A	Revised......		16-Mar-15	JAL TYS
Raw data location	12A	Reviewed...		17-MAR-15	TYS
		Certified......		3/15/17	JA

File Name: I:\AEH-11-PSEUDO-01\Data\Glochidia Viability Summary\[Glochidia viability assessment 12h (adjusted).xlsx]Higgins Eye

Glochidia Viability Assessment - 12 hour

Mussel species: Higgins Eye Glochidia Lot number: 111500

Test Chemical: Pseudomonas florescens Pf-CL 145A (SDP) Chemical lot #: MBI-401-110308AI-BD-3

Exposure Date: 12-May-11 Mean initial viability = 80.8%

Test System Location: Block 2

Species	Test Chamber	Treatment Group (mg/L)	Total Number Glochida Counted	Glochidia Closed before NaCl[1]	Glochidia Open after NaCl	Unadjusted 12h viability (%)	Adjusted 12h Viability (%)[2]	Mean adjusted viability (%)	Standard Deviation (SD)
Higgins Eye	2C3	0	481	48	174	64.2	79.5	85.0	7.6
Higgins Eye	2C6	0	210	13	38	75.7	93.7		
Higgins Eye	2B1	0	254	29	57	66.1	81.9		
Higgins Eye	2A1	50	225	38	48	61.8	76.5	85.0	8.0
Higgins Eye	2A2	50	230	21	49	69.6	86.1		
Higgins Eye	2A4	50	339	36	50	74.6	92.4		
Higgins Eye	2A3	100	235	50	38	62.6	77.4	81.1	3.9
Higgins Eye	2C4	100	273	35	60	65.2	80.7		
Higgins Eye	2C5	100	362	41	72	68.8	85.1		
Higgins Eye	2A6	200	266	146	51	25.9	32.1	57.2	22.2
Higgins Eye	2B2	200	344	65	73	59.9	74.1		
Higgins Eye	2B5	200	274	81	48	52.9	65.5		
Higgins Eye	2C2	300	215	52	63	46.5	57.6	48.0	10.0
Higgins Eye	2C1	300	267	67	95	39.3	48.7		
Higgins Eye	2B3	300	240	107	60	30.4	37.6		
Higgins Eye	2A5	300HD	229	161	44	10.5	13.0	11.8	1.1
Higgins Eye	2B4	300HD	192	143	32	8.9	11.0		
Higgins Eye	2B6	300HD	206	142	45	9.2	11.4		

[1] If the number counted was glochidia open, the number of glochidia closed = total glochidia - open glochidia counted before NaCl.

[2] Viability adjustments were claculated by dividing the observed viability by the mean initial viability for each species

Study Number	AEH-11-PSEUDO-01	Action		Date	Initials
Lab Notebook	1	Created......		7-Jun-12	TMS TS
File Folder	12A	Revised......		16-Mar-15	JAL TS
Raw data location	12A	Reviewed...		17-MAR-15	TS
		Certified......		3/18/15	J

File Name: I:\AEH-11-PSEUDO-01\Data\Glochidia Viability Summary\[Glochidia viability assessment 12h (adjusted).xlsx]Pocketbook FDP

Glochidia Viability Assessment - 12 hour

Mussel species: Pocketbook Glochidia Lot number: 115400 Mean initial viability = 84.7%

Test Chemical: Pseudomonas florescens Pf-CL 145A (FDP) Chemical lot number: 110607WB-FD-E

Exposure Date: 18-Oct-11

Test System Location: Block 3

Species	Test Chamber	Treatment Group (mg/L)	Total Number Glochida Counted	Glochidia Closed before NaCl[1]	Glochidia Open after NaCl	Unadjusted 12h viability (%)	Adjusted 12h Viability (%)[2]	Mean adjusted viability (%)	Standard Deviation (SD)
Pocketbook	3A6	0	133	3	20	82.7	97.6	96.6	4.0
Pocketbook	3B5	0	314	8	61	78.0	92.1		
Pocketbook	3B6	0	182	5	23	84.6	99.9		
Pocketbook	3A4	50	290	24	62	70.3	83.1	90.5	7.4
Pocketbook	3C1	50	160	8	29	76.9	90.8		
Pocketbook	3C2	50	606	34	70	82.8	97.8		
Pocketbook	3B1	100	179	46	22	62.0	73.2	83.3	10.0
Pocketbook	3B3	100	130	20	18	70.8	83.6		
Pocketbook	3C6	100	142	15	15	78.9	93.1		
Pocketbook	3A2	200	249	161	38	20.1	23.7	41.2	36.1
Pocketbook	3C3	200	815	104	140	70.1	82.7		
Pocketbook	3C5	200	200	151	20	14.5	17.1		
Pocketbook	3A3	300	251	174	66	4.4	5.2	10.8	10.4
Pocketbook	3B4	300	223	145	35	19.3	22.8		
Pocketbook	3C4	300	348	289	46	3.7	4.4		
Pocketbook	3A1	300HD	72	58	11	4.2	4.9	5.7	1.4
Pocketbook	3A5	300HD	48	38	7	6.3	7.4		
Pocketbook	3B2	300HD	242	211	21	4.1	4.9		

[1] If the number counted was glochidia open, the number of glochidia closed = total glochidia - open glochidia counted before NaCl.

Glochidia viability: (total # glochidia - (glochidia closed before + open after NaCl))/total glochidia *100

[2] Viability adjustments were calculated by dividing the observed viability by the mean initial viability for each species

			Action		Date	Initials
Study Number	AEH-11-PSEUDO-01		Created......		7-Jun-12	TMS TJS
Lab Notebook	1		Revised......		16-Mar-15	JAL TJS
File Folder	12A		Reviewed...		17-MAR-15	TJS
Raw Data Location	12A		Certified......		3/18/15	JL

File Name: I:\AEH-11-PSEUDO-01\Data\Glochidia Viability Summary\[Glochidia viability assessment 12h (adjusted).xlsx]Black Sandshell

Glochidia Viability Assessment - 12 hour

Mussel species: Black Sandshell Glochidia Lot number: 120800 Mean initial viability = 84.5%

Test Chemical: Pseudomonas florescens Pf-CL 145A (SDP) Chemical lot #: MBI-401 SDP 4655-12-Mix

Exposure Date: 17-Jan-12

Test System Location: Block 1

Mussel Species	Test Chamber	Treatment Group (mg/L)	Total Number Glochida Counted	Glochidia Closed before NaCl[1]	Glochidia Open after NaCl	Unadjusted 12h viability (%)	Adjusted 12h Viability (%)[2]	Mean adjusted viability (%)	Standard Deviation (SD)
Black Sandshell	1A5	0	262	3	26	88.9	105.2	105.9	0.6
Black Sandshell	1B1	0	231	6	18	89.6	106.0		
Black Sandshell	1C6	0	139	2	12	89.9	106.4		
Black Sandshell	1A1	50	223	157	22	19.7	23.4	19.1	6.6
Black Sandshell	1A3	50	399	293	30	19.0	22.5		
Black Sandshell	1C1	50	248	221	3	9.7	11.5		
Black Sandshell	1B2	100	250	208	20	8.8	10.4	10.5	3.9
Black Sandshell	1B3	100	198	155	19	12.1	14.3		
Black Sandshell	1C5	100	125	97	21	5.6	6.6		
Black Sandshell	1A6	200	242	235	5	0.8	1.0	1.4	0.4
Black Sandshell	1B5	200	210	201	6	1.4	1.7		
Black Sandshell	1B6	200	304	280	20	1.3	1.6		
Black Sandshell	1A2	300	135	135	0	0.0	0.0	0.0	0.0
Black Sandshell	1B4	300	93	86	7	0.0	0.0		
Black Sandshell	1C2	300	165	151	14	0.0	0.0		
Black Sandshell	1A4	300HD	281	268	13	0.0	0.0	1.1	1.0
Black Sandshell	1C3	300HD	157	147	8	1.3	1.5		
Black Sandshell	1C4	300HD	133	119	12	1.5	1.8		

[1] If the number counted was glochidia open, the number of glochidia closed = total glochidia - open glochidia counted before NaCl.

Glochidia viability: (total # glochidia - (glochidia closed before + open after NaCl))/total glochidia *100

[2] Viability adjustments were calculated by dividing the observed viability by the mean initial viability for each species

Study Number	AEH-11-PSEUDO-01	Action		Date	Initials
Lab Notebook	1	Created......		7-Jun-12	TMS TS
File Folder	12A	Revised......		16-Mar-15	JAL TS
Raw Data Location	12A	Reviewed...		17-MAR-15	TS
		Certified......		3/18/15	JS

File Name: I:\AEH-11-PSEUDO-01\Data\Glochidia Viability Summary\[Glochidia viability assessment 12h (adjusted).xlsx]Mucket

Glochidia Viability Assessment - 12 hour

Mussel species: Mucket Glochidia Lot number: 120700 Mean initial viability = 92.7%

Test Chemical: Pseudomonas florescens Pf-CL 145A (SDP) Chemical lot #: MBI-401 SDP 4655-12-Mix

Exposure Date: 17-Jan-12

Test System Location: Block 2

Species	Test Chamber	Treatment Group (mg/L)	Total Number Glochida Counted	Glochidia Closed before NaCl[1]	Glochidia Open after NaCl	Unadjusted 12h viability (%)	Adjusted 12h Viability (%)[2]	Mean adjusted viability (%)	Standard Deviation (SD)
Mucket	2B4	0	178	2	11	92.7	100.0	101.7	1.6
Mucket	2B5	0	213	0	12	94.4	101.8		
Mucket	2C1	0	254	6	5	95.7	103.2		
Mucket	2A4	50	221	63	14	65.2	70.3	71.8	19.1
Mucket	2B6	50	113	53	4	49.6	53.5		
Mucket	2C3	50	193	17	12	85.0	91.7		
Mucket	2A2	100	210	91	10	51.9	56.0	69.4	19.1
Mucket	2B2	100	138	46	14	56.5	61.0		
Mucket	2C6	100	215	26	7	84.7	91.3		
Mucket	2A5	200	175	83	17	42.9	46.2	43.2	4.9
Mucket	2A6	200	179	92	11	42.5	45.8		
Mucket	2C2	200	362	227	9	34.8	37.5		
Mucket	2B1	300	247	212	4	12.6	13.5	11.0	2.7
Mucket	2B3	300	198	181	2	7.6	8.2		
Mucket	2C4	300	116	102	2	10.3	11.2		
Mucket	2A1	300HD	237	201	14	9.3	10.0	8.8	5.4
Mucket	2A3	300HD	127	100	11	12.6	13.6		
Mucket	2C5	300HD	184	175	4	2.7	2.9		

[1] If the number counted was glochidia open, the number of glochidia closed = total glochidia - open glochidia counted before NaCl.

Glochidia viability: (total # glochidia - (glochidia closed before + open after NaCl))/total glochidia *100

[2] Viability adjustments were calculated by dividing the observed viability by the mean initial viability for each species

Study Number	AEH-11-PSEUDO-01	Action		Date	Initials
Lab Notebook	1	Created......		7-Jun-12	TMS TMS
File Folder	12A	Revised......		16-Mar-15	JAL TS
Raw Data Location	12A	Reviewed...		17-MAR-15	TS
		Certified......		3/18/15	JA

File Name: I:\AEH-11-PSEUDO-01\Data\Glochidia Viability Summary\[Glochidia viability assessment 12h (adjusted).xsx]Hickorynut

Glochidia Viability Assessment - 12 hour

Mussel species: Hickorynut Glochidia Lot number: 120903 Mean Initial viability = 86.0%

Test Chemical: Pseudomonas florescens Pf-CL 145A (SDP) Chemical lot number: MBI-401 SDP 4655-12-Mix

Exposure Date: 19-Jan-12

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Test System Location: Block 3

Species	Test Chamber	Treatment Group (mg/L)	Total Number Glochida Counted	Glochidia Closed before NaCl[1]	Glochidia Open after NaCl	Unadjusted 12h viability (%)	Adjusted 12h Viability (%)[2]	Mean adjusted viability (%)	Standard Deviation (SD)
Hickorynut	3A2	0	257	6	22	89.1	103.6	100.5	7.4
Hickorynut	3C1	0	216	19	26	79.2	92.1		
Hickorynut	3C4	0	144	5	8	91.0	105.8		
Hickorynut	3A5	50	172	21	10	82.0	95.3	98.2	3.4
Hickorynut	3B3	50	197	24	8	83.8	97.4		
Hickorynut	3B6	50	186	9	14	87.6	101.9		
Hickorynut	3B1	100	214	36	6	80.4	93.5	89.7	14.5
Hickorynut	3C2	100	195	18	6	87.7	102.0		
Hickorynut	3C5	100	216	75	4	63.4	73.8		
Hickorynut	3A3	200	134	86	3	33.6	39.0	58.4	19.2
Hickorynut	3A4	200	186	84	8	50.5	58.8		
Hickorynut	3C6	200	138	41	5	66.7	77.5		
Hickorynut	3A6	300	204	123	8	35.8	41.6	45.6	3.5
Hickorynut	3B2	300	200	107	12	40.5	47.1		
Hickorynut	3B5	300	206	98	23	41.3	48.0		
Hickorynut	3A1	300HD	249	231	15	1.2	1.4	1.4	0.4
Hickorynut	3B4	300HD	338	313	22	0.9	1.0		
Hickorynut	3C3	300HD	251	244	3	1.6	1.9		

[1]If the number counted was glochidia open, the number of glochidia closed = total glochidia - open glochidia counted before NaCl.

Glochidia viability: (total # glochidia - (glochidia closed before + open after NaCl))/total glochidia *100

[2] Viability adjustments were calculated by dividing the observed viability by the mean initial viability for each species

Study Number	AEH-11-PSEUDO-01	Action			Date	Initials
Lab Notebook	1	Created......			7-Jun-12	TMS TS
File Folder	12A	Revised......			16-Mar-15	JAL TS
Raw data locatio	12A	Reviewed...			17-MAR-15	TS
		Certified......			3/15/17	JR
File Name:	I:\AEH-11-PSEUDO-01\Data\Glochidia Viability Summary\[Glochidia viability assessment 24h (adjusted).xlsx]Higgins Eye					

Glochidia Viability Assessment - 24 hour

Mussel species: Higgins Eye Glochidia Lot number: 111500

Test Chemica : Pseudomonas florescens Pf-CL 145A (SDP) Chemical lot #: MBI-401-110308AI-BD-3

Exposure Date: 12-May-11 Mean initial viability = 80.8%

Test System Location: Block 2

Species	Test Chamber	Treatment Group (mg/L)	Total Number Glochida Counted	Glochidia Closed before NaCl[1]	Glochidia Open after NaCl	Unadjusted 24h viability (%)	Adjusted 24h Viability (%)[2]	Mean adjusted viability (%)	Standard Deviation (SD)
Higgins Eye	2C3	0	318	28	66	70.4	87.2	85.8	9.0
Higgins Eye	2C3-R2[4]	0	377	46	108	59.2	73.2		
Higgins Eye	2C6	0	557	61	70	76.5	94.7		
Higgins Eye	2C6-R2[4]	0	408	44	73	71.3	88.3		
Higgins Eye	2B1	0	lost[3]	-	-	-	-		
Higgins Eye	2A1	50	506		67	77.1	95.4	92.3	4.2
Higgins Eye	2A2	50	514	55	68	76.1	94.1		
Higgins Eye	2A4	50	464	34	102	70.7	87.5		
Higgins Eye	2A3	100	629	95	87	71.1	88.0	81.0	6.0
Higgins Eye	2C4	100	423	87	69	63.1	78.1		
Higgins Eye	2C5	100	413	105	51	62.2	77.0		
Higgins Eye	2A6	200	296	83	56	53.0	65.6	52.6	18.3
Higgins Eye	2B2	200	369	135	54	48.8	60.4		
Higgins Eye	2B5	200	441	272	56	25.6	31.7		
Higgins Eye	2C2	300	140	38	28	52.9	65.4	27.6	32.9
Higgins Eye	2C1	300	354	287	34	9.3	11.5		
Higgins Eye	2B3	300	171	109	54	4.7	5.8		
Higgins Eye	2A5	300HD	137	89	42	4.4	5.4	17.2	12.9
Higgins Eye	2B4	300HD	216	88	74	25.0	30.9		
Higgins Eye	2B6	300HD	330	229	60	12.4	15.4		

[1] If the number counted was glochidia open, the number of glochidia closed = total glochidia - open glochidia counted before NaCl.

[2] Viability adjustments were calculated by dividing the observed viability by the mean initial viability for each species

[3] Sample lost, the 2B1 chamber was accidentally pooled with control chambers from Block 1 before the 24 h glochidia sample collected.

[4] Two replicate samples were collected from the control samples 2C3 and 2C6 since sample 2B1 was lost.

Glochidia viability: (total # glochidia - (glochidia closed before + open after NaCl))/total glochidia *100

Study Number	AEH-11-PSEUDO-01	Action		Date	Initials
Lab Notebook	1	Created......		7-Jun-12	TMS TS
File Folder	12A	Revised......		16-Mar-15	JAL TS
Raw data location	12A	Reviewed...		17-MAR-15	TS
		Certified......		3/18/15	JM

File Name: I:\AEH-11-PSEUDO-01\Data\Glochidia Viability Summary\[Glochidia viability assessment 24h (adjusted).xlsx]Fatmuck

Glochidia Viability Assessment - 24 hour

Mussel species: Fatmucket Glochidia Lot number: 111600 Mean initial viability = 80.3%

Test Chemical: Pseudomonas florescens Pf-CL 145A (SDP) Chemical lot #: MBI-401-110308AI-BC-3

Exposure Date: 12-May-11

Test System Location: Block 3

Page 1 of 1

Species	Test Chamber	Treatment Group (mg/L)	Total Number Glochida Counted	Glochidia Closed before NaCl[1]	Glochidia Open after NaCl	Unadjusted 24h viability (%)	Adjusted 24h Viability (%)[2]	Mean adjusted viability (%)	Standard Deviation (SD)
Fatmucket	3B2	0	247	50	41	63.2	78.7	86.5	10.2
Fatmucket	3B3	0	244	19	33	78.7	98.0		
Fatmucket	3A2	0	307	48	55	66.4	82.8		
Fatmucket	3B5	50	327	38	86	62.1	77.3	70.8	7.4
Fatmucket	3C5	50	334	64	76	58.1	72.3		
Fatmucket	3C6	50	278	70	68	50.4	62.7		
Fatmucket	3A1	100	112	41	20	45.5	56.7	51.9	8.0
Fatmucket	3A3	100	208	60	54	45.2	56.3		
Fatmucket	3A5	100	313	146	60	34.2	42.6		
Fatmucket	3B4	200	271	190	43	14.0	17.5	14.5	5.8
Fatmucket	3B6	200	205	147	28	14.6	18.2		
Fatmucket	3C4	200	271	193	61	6.3	7.8		
Fatmucket	3C1	300	176	145	23	4.5	5.7	7.5	2.2
Fatmucket	3C3	300	215	130	68	7.9	9.8		
Fatmucket	3B1	300	217	170	35	5.5	6.9		
Fatmucket	3A4	300HD	281	250	29	0.7	0.9	2.1	1.1
Fatmucket	3A6	300HD	86	69	15	2.3	2.9		
Fatmucket	3C2	300HD	336	293	36	2.1	2.6		

1 If the number counted was glochidia open, the number of glochidia closed = total glochidia - open glochidia counted before NaCl.

Glochidia viability: (total # glochidia - (glochidia closed before + open after NaCl))/total glochidia *100

2 Viability adjustments were calculated by dividing the observed viability by the mean initial viability for each species

Study Number	AEH-11-PSEUDO-01	Action		Date	Initials
Lab Notebook	1	Created......		7-Jun-12	TMS TS
File Folder	12A	Revised......		16-Mar-15	JAL TS
Raw Data Location	12A	Reviewed...		17-MAR-15	TS
		Certified......		3/18/15	TA

File Name: I:\AEH-11-PSEUDO-01\Data\Glochidia Viability Summary\[Glochidia viability assessment 24h (adjusted).xlsx] Pocketbook SDP

Glochidia Viability Assessment - 24 hour

Mussel species: Pocketbook Glochidia Lot number: 111400 Mean Initial viability = 93.8%

Test Chemical: *Pseudomonas florescens* Pf-CL 145A (SDP) Chemical lot #: MBI-401-110308AI-BD-3

Exposure Date: 12-May-11

Test System Location: Block 1

Species	Test Chamber	Treatment Group (mg/L)	Total Number Glochida Counted	Glochidia Closed before NaCl[1]	Glochidia Open after NaCl	Unadjusted 24h viability (%)	Adjusted 24h Viability (%)[2]	Mean adjusted viability (%)	Standard Deviation (SD)
Pocketbook	1B1	0	514	15	22	92.8	98.9	98.2	1.0
Pocketbook	1C3	0	276	12	9	92.4	98.5		
Pocketbook	1A3	0	367	9	24	91.0	97.0		
Pocketbook	1B5	50	270	27	16	84.1	89.6	85.1	6.0
Pocketbook	1A1	50	459	55	28	81.9	87.3		
Pocketbook	1C1	50	339	78	12	73.5	78.3		
Pocketbook	1B4	100	324	56	24	75.3	80.3	71.3	16.8
Pocketbook	1A2	100	210	33	16	76.7	81.7		
Pocketbook	1A4	100	310	34	125	48.7	51.9		
Pocketbook	1C6	200	242	64	16	66.9	71.4	63.2	13.6
Pocketbook	1B2	200	187	50	13	66.3	70.7		
Pocketbook	1C4	200	506	267	14	44.5	47.4		
Pocketbook	1A5	300	493	399	23	14.4	15.4	6.5	7.7
Pocketbook	1C2	300	226	218	4	1.8	1.9		
Pocketbook	1C5	300	295	274	15	2.0	2.2		
Pocketbook	1A6	300HD	204	147	40	8.3	8.9	5.1	3.3
Pocketbook	1B3	300HD	257	245	4	3.1	3.3		
Pocketbook	1B6	300HD	322	293	20	2.8	3.0		

[1] If the number counted was glochidia open, the number of glochidia closed = total glochidia - open glochidia counted before NaCl.

Glochidia viability: (total # glochidia - (glochidia closed before + open after NaCl))/total glochidia *100

[2] Viability adjustments were calculated by dividing the observed viability by the mean initial viability for each species

Study Number	AEH-11-PSEUDO-01	Action	Date	Initials
Lab Notebook	1	Created......	7-Jun-12	TMS TS
File Folder	12A	Revised......	16-Mar-15	JAL TS
Raw data location	12A	Reviewed...	17-MAR-15	TS
		Certified......	3/18/15	JTJ

File Name: I:\AEH-11-PSEUDO-01\Data\Glochidia Viability Summary\[Glochidia viability assessment 24h (adjusted).xlsx]Pocketbook FDP

Glochidia Viability Assessment - 24 hour

Mussel species: Pocketbook Glochidia Lot number: 115400 Mean initial viability = 84.7%

Test Chemical: Pseudomonas florescens Pf-CL 145A (FDP) Chemical lot number: 110607WB-FD-E

Exposure Date: 18-Oct-11

Test System Location: Block 3

Species	Test Chamber	Treatment Group (mg/L)	Total Number Glochida Counted	Glochidia Closed before NaCl[1]	Glochidia Open after NaCl	Unadjusted 24h viability (%)	Adjusted 24h Viability (%)[2]	Mean adjusted viability (%)	Standard Deviation (SD)
Pocketbook	3A6	0	573	21	74	83.4	98.5	96.7	1.7
Pocketbook	3B5	0	279	7	47	80.6	95.2		
Pocketbook	3B6	0	278	17	34	81.7	96.4		
Pocketbook	3A4	50	164	39	21	63.4	74.9	87.5	11.0
Pocketbook	3C1	50	368	33	45	78.8	93.0		
Pocketbook	3C2	50	384	30	46	80.2	94.7		
Pocketbook	3B1	100	467	85	60	69.0	81.4	80.0	4.5
Pocketbook	3B3	100	405	93	55	63.5	74.9		
Pocketbook	3C6	100	288	38	46	70.8	83.6		
Pocketbook	3A2	200	144	96	27	14.6	17.2	34.9	28.0
Pocketbook	3C3	200	592	157	98	56.9	67.2		
Pocketbook	3C5	200	386	281	39	17.1	20.2		
Pocketbook	3A3	300	199	132	45	11.1	13.1	14.2	2.2
Pocketbook	3B4	300	714	442	171	14.1	16.7		
Pocketbook	3C4	300	440	330	62	10.9	12.9		
Pocketbook	3A1	300HD	370	277	78	4.1	4.8	5.8	2.7
Pocketbook	3A5	300HD	253	207	38	3.2	3.7		
Pocketbook	3B2	300HD	267	198	49	7.5	8.8		

[1] If the number counted was glochidia open, the number of glochidia closed = total glochidia - open glochidia counted before NaCl.

Glochidia viability: (total # glochidia - (glochidia closed before + open after NaCl))/total glochidia *100

[2] Viability adjustments were calculated by dividing the observed viability by the mean initial viability for each species

Study Number	AEH-11-PSEUDO-01	Action		Date	Initials
Lab Notebook	1	Created......		30-May-12	TJS TJS
File Folder	12C	Revised......		16-Mar-15	JAL TJS
Raw Data location	12C	Reviewed...		17-MAR-15	TJS
		Certified......		3/18/15	JS
File Name:	I:\AEH-11-PSEUDO-01\Data\Glochicia Viability Summary\[glochidia viability assessment 24h.xlsx]Washboard				

Glochidia Viability Assessment - 24 hour

Mussel species: Washboard Glochidia Lot number: 115500 Mean initial viability = 96.5%

Test Chemical: Pseudomonas florescens Pf-CL 145A (FDP) Chemical lot number: 110607WB-FD-E

Exposure Date: 18-Oct-11

Test System Location: Block 1 Page 1 of 1

Species	Test Chamber	Treatment Group (mg/L)	Total Number Glochida Counted[2]	Glochidia Closed before NaCl[1,2]	Glochidia Open after NaCl[2]	Unadjusted 24h viability (%)	Adjusted 24h Viability (%)[2]	Mean adjusted viability (%)	Standard Deviation (SD)
Washboard	1A5	0	55	9	11	63.6	65.9	53.9	11.7
Washboard	1B2	0	140	49	19	51.4	53.3		
Washboard	1B4	0	73	32	11	41.1	42.6		
Washboard	1A1	50	55	16	14	45.5	47.1	57.6	9.8
Washboard	1C1	50	65	19	9	56.9	59.0		
Washboard	1C2	50	56	9	11	64.3	66.6		
Washboard	1A2	100	67	23	23	31.3	32.5	49.8	17.6
Washboard	1A6	100	99	15	37	47.5	49.2		
Washboard	1C6	100	104	23	13	65.4	67.8		
Washboard	1B5	200	54	16	21	31.5	32.6	51.3	19.3
Washboard	1C3	200	60	12	19	48.3	50.1		
Washboard	1C4	200	64	14	6	68.8	71.2		
Washboard	1A4	300	52	8	11	63.5	65.8	41.6	21.5
Washboard	1B1	300	55	24	18	23.6	24.5		
Washboard	1C5	300	69	8	38	33.3	34.5		
Washboard	1A3	300HD	80	62	5	16.3	16.8	27.7	11.0
Washboard	1B3	300HD	80	34	16	37.5	38.9		
Washboard	1B6	300HD	83	37	24	26.5	27.5		

1 If the number counted was glochidia open, the number of glochidia closed = total glochidia - open glochidia counted before NaCl.

2 Washboard samples were manual processed with a dissection scope, chambers with multiple replicates were pooled for one value.

Glochidia viability: (total # glochidia - (glochidia closed before + open after NaCl))/total glochidia *100

[3] Viability adjustments were calculated by dividing the observed viability by the mean initial viability for each species

			Action		Date	Initials
Study Number	AEH-11-PSEUDO-01		Created......		7-Jun-12	TMS TS
Lab Notebook	1		Revised......		16-Mar-15	JAL TS
File Folder	12A		Reviewed...		17-MAR-15	TS
Raw Data Location	12A		Certified......		3/18/15	

File Name: I:\AEH-11-PSEUDO-01\Data\Glochidia Viability Summary\[Glochidia viability assessment 24h (adjusted).xlsx]Black Sandshell

Glochidia Viability Assessment - 24 hour

Mussel species: Black Sandshell Glochidia Lot number: 120800 Mean initial viability = 84.5%

Test Chemical: Pseudomonas florescens Pf-CL 145A (SDP) Chemical lot #: MBI-401 SDP 4655-12-Mix

Exposure Date: 17-Jan-12

Test System Location: Block 1

Mussel Species	Test Chamber	Treatment Group (mg/L)	Total Number Glochida Counted	Glochidia Closed before NaCl[1]	Glochidia Open after NaCl	Unadjusted 24h viability (%)	Adjusted 24h Viability (%)[2]	Mean adjusted viability (%)	Standard Deviation (SD)
Black Sandshell	1A5	0	612	12	83	84.5	100.0	100.0	2.3
Black Sandshell	1B1	0	206	4	24	86.4	102.3		
Black Sandshell	1C6	0	572	26	74	82.5	97.7		
Black Sandshell	1A1	50	353	287	17	13.9	16.4	17.4	2.1
Black Sandshell	1A3	50	137	98	16	16.8	19.9		
Black Sandshell	1C1	50	111	92	4	13.5	16.0		
Black Sandshell	1B2	100	181	166	12	1.7	2.0	8.2	5.8
Black Sandshell	1B3	100	252	221	11	7.9	9.4		
Black Sandshell	1C5	100	222	179	18	11.3	13.3		
Black Sandshell	1A6	200	351	338	8	1.4	1.7	3.8	2.6
Black Sandshell	1B5	200	302	256	38	2.6	3.1		
Black Sandshell	1B6	200	352	299	33	5.7	6.7		
Black Sandshell	1A2	300	284	270	14	0.0	0.0	0.9	0.8
Black Sandshell	1B4	300	255	247	5	1.2	1.4		
Black Sandshell	1C2	300	383	373	6	1.0	1.2		
Black Sandshell	1A4	300HD	485	471	11	0.6	0.7	0.9	0.9
Black Sandshell	1C3	300HD	381	367	8	1.6	1.9		
Black Sandshell	1C4	300HD	302	295	7	0.0	0.0		

[1] If the number counted was glochidia open, the number of glochidia closed = total glochidia - open glochidia counted before NaCl.

Glochidia viability: (total # glochidia - (glochidia closed before + open after NaCl))/total glochidia *100

[2] Viability adjustments were calculated by dividing the observed viability by the mean initial viability for each species

Study Number	AEH-11-PSEUDO-01
Lab Notebook	1
File Folder	12A
Raw Data Location	12A

Action		Date	Initials
Created......		7-Jun-12	TMS TS
Revised......		16-Mar-15	JAL TS
Reviewed...		17-MAR-15	TS
Certified......		3/18/15	JT

File Name: I:\AEH-11-PSEUDO-01\Data\Glochidia Viability Summary\[Glochidia viability assessment 24h (adjusted).xlsx]Mucket

Glochidia Viability Assessment - 24 hour

Mussel species: Mucket Glochidia Lot number: 120700 Mean initial viability = 92.7%

Test Chemical: Pseudomonas florescens Pf-CL 145A (SDP) Chemical lot #: MBI-401 SDP 4655-12-Mix

Exposure Date: 17-Jan-12

Test System Location: Block 2

Species	Test Chamber	Treatment Group (mg/L)	Total Number Glochida Counted	Glochidia Closed before NaCl[1]	Glochidia Open after NaCl	Unadjusted 24h viability (%)	Adjusted 24h Viability (%)[2]	Mean adjusted viability (%)	Standard Deviation (SD)
Mucket	2B4	0	261	7	19	90.0	97.1	99.9	2.4
Mucket	2B5	0	361	1	20	94.2	101.6		
Mucket	2C1	0	251	8	8	93.6	101.0		
Mucket	2A4	50	328	49	14	80.8	87.2	81.7	14.1
Mucket	2B6	50	146	49	8	61.0	65.8		
Mucket	2C3	50	194	19	9	85.6	92.3		
Mucket	2A2	100	191	42	5	75.4	81.3	68.5	12.1
Mucket	2B2	100	209	87	11	53.1	57.3		
Mucket	2C6	100	213	71	10	62.0	66.9		
Mucket	2A5	200	251	170	16	25.9	27.9	32.4	7.8
Mucket	2A6	200	407	241	10	38.3	41.3		
Mucket	2C2	200	497	355	14	25.8	27.8		
Mucket	2B1	300	192	171	3	9.4	10.1	6.5	3.8
Mucket	2B3	300	297	281	9	2.4	2.5		
Mucket	2C4	300	451	417	6	6.2	6.7		
Mucket	2A1	300HD	292	278	6	2.7	3.0	5.6	3.7
Mucket	2A3	300HD	308	276	4	9.1	9.8		
Mucket	2C5	300HD	157	149	2	3.8	4.1		

[1] If the number counted was glochidia open, the number of glochidia closed = total glochidia - open glochidia counted before NaCl.

Glochidia viability: (total # glochidia - (glochidia closed before + open after NaCl))/total glochidia *100

[2] Viability adjustments were calculated by dividing the observed viability by the mean initial viability for each species

Study Number	AEH-11-PSEUDO-01	Action		Date	Initials
Lab Notebook	1	Created......		7-Jun-12	TMS TS
File Folder	12A	Revised......		16-Mar-15	JAL TS
Raw Data Location	12A	Reviewed...		17-MAR-15	TS
		Certified......		3/18/15	JR

File Name: I:\AEH-11-PSEUDO-01\Data\Glochidia Viability Summary\[Glochidia viability assessment 24h (adjusted).xlsx]Hickorynut

Glochidia Viability Assessment - 24 hour

Mussel species: Hickorynut Glochidia Lot number: 120900 Mean initial viability = 86.0%

Test Chemical: Pseudomonas florescens Pf-CL 145A (SDP) Chemical lot number: MBI-401 SDP 4655-12-Mix

Exposure Date: 19-Jan-12

Test System Location: Block 3

Species	Test Chamber	Treatment Group (mg/L)	Total Number Glochida Counted	Glochidia Closed before NaCl[1]	Glochidia Open after NaCl	Unadjusted 24h viability (%)	Adjusted 24h Viability (%)[2]	Mean adjusted viability (%)	Standard Deviation (SD)
Hickorynut	3A2	0	192	8	14	88.5	103.0	101.2	2.0
Hickorynut	3C1	0	376	22	34	85.1	99.0		
Hickorynut	3C4	0	359	14	31	87.5	101.7		
Hickorynut	3A5	50	223	9	8	92.4	107.4	103.7	3.3
Hickorynut	3B3	50	303	18	22	86.8	100.9		
Hickorynut	3B6	50	233	18	9	88.4	102.8		
Hickorynut	3B1	100	404	36	21	85.9	99.9	91.0	8.0
Hickorynut	3C2	100	278	59	17	72.7	84.5		
Hickorynut	3C5	100	257	51	10	76.3	88.7		
Hickorynut	3A3	200	349	114	10	64.5	75.0	70.6	5.3
Hickorynut	3A4	200	203	78	12	55.7	64.7		
Hickorynut	3C6	200	197	66	9	61.9	72.0		
Hickorynut	3A6	300	191	138	9	23.0	26.8	30.9	3.8
Hickorynut	3B2	300	140	98	4	27.1	31.6		
Hickorynut	3B5	300	231	156	7	29.4	34.2		
Hickorynut	3A1	300HD	290	287	2	0.3	0.4	0.6	0.4
Hickorynut	3B4	300HD	314	307	5	0.3	0.4		
Hickorynut	3C3	300HD	226	221	3	0.9	1.0		

[1] If the number counted was glochidia open, the number of glochidia closed = total glochidia - open glochidia counted before NaCl.

Glochidia viability: (total # glochidia - (glochidia closed before + open after NaCl))/total glochidia *100

[2] Viability adjustments were calculated by dividing the observed viability by the mean initial viability for each species

Study Number: AEH-11-PSEUDO-01	Action	Date	Initials
Lab Notebook: 1	Created......	17-Mar-15	TJS TJS
Data Source: File Folder: 12d	Revised......	18-Mar-15	TJS TJS
Forms: Glochidia Photomicrograph Counting Record	Reviewed...	18-MAR-15	TJS
	Certified...	2/12/15	Jn
File Name: I:\AEH-11-PSEUDO-01\Data\Glochidia Viability Summary\[glochidia distribution counts.xlsx]Distribution Coversheet			

Glochidia Distribution Counts

Test Article: *Pseudomonas fluorescens* (*Pf*-CL145A) spray dried powder (SDP) and freeze dried powder (FDP)
Article Lot #s: SDP = MBI-401-110308AI-BD-3 and MBI-401 SDP 4655-12-Mix; FDP = 110607WB-FD-E
Exposure Dates: 12-May-11, 18-Oct-11, 17-Jan-12, and 19-Jan-12
Species Tested: PPB, FAM, HGE, WAS, BLS, MUC, and HIC

Data Explanation:

Species Abbreviations

- PPB - plain pocketbook (*Lampsilis cardium*)
- FAM - fatmucket (*Lampsilis siliquoidea*)
- HGE - Higgins eye (*Lampsilis higginsii*)
- WAS - washboard (Megalonaias nervosa)
- BLS - black sandshell (*Ligumia recta*)
- MUC - mucket (*Actinonaias ligamentina*)
- HIC - hickorynut (*Obovaria olivaria*)

Throughout distribution of glochidia to test chambers, n=16 100 µL samples of glochidia were collected, photographed, and enumerated (from photomicrographs) to estimate the number of glochidia distributed to test chambers.

These enumerations were used to calculate the mean number of glochidia distributed to each chamber (5 x 100µL mean = estimated glochidia per 500 µL test chamber).

Data anomalies and deviations:

Hickorynut samples contained large numbers of glochidia, and therefore, some samples were divided and multiple photographs were captured for enumeration.

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Stucy Number: AEH-11-PSEUDO-01
Lab Notebook 1
Data Source: File Folder: 12d
Forms: Glochidia Photomicrograph Counting Record

Test Article: MBI 401 (Pf-CL 145A [SDP])
Article Lot #: MBI-401-110308AI-BD-3
Exposure Dates: 12-May-11
Species Tested: PPB

Plain pocketbook (SDP) Distribution Glochidia Count

Species	Formulation	Test date	Distribution round	Rep number	Total glochidia per 100 µL
PPB	SDP	12-May-11	1	1	689
				2	715
				3	450
			2	1	577
				2	1136
				3	1056
			3	1	679
				2	544
				3	667
			4	1	731
				2	787
				3	520
			5	1	249
				2	659
				3	770
				4	506
				Mean (per 100 µL)	671
				SD	215
	Glochidia Distribution Estimate			**Mean (per 500 µL)**	**3355**

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Study Number: AEH-11-PSEUDO-01
Lab Notebook 1
Data Source: File Folder: 12d
Forms: Glochidia Photomicrograph Counting Record

Test Article: MBI 401 (Pf-CL 145A [SDP])
Article Lot #: MBI-401-110308A -BD-3
Exposure Dates: 12-May-11
Species Tested: FAM

Fatmucket (SDP) Distribution Glochidia Count

Species	Formulation	Test date	Distribution round	Rep number	Total glochidia per 100 µL
FAM	SDP	12-May-11	1	1	677
				2	1211
				3	630
			2	1	552
				2	635
				3	413
			3	1	399
				2	329
				3	508
			4	1	481
				2	381
				3	350
			5	1	450
				2	436
				3	423
				4	771
				Mean (per 100 µL)	540
				SD	219
	Glochidia Distribution Estimate			Mean (per 500 µL)	2702

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Stucy Number: AEH-11-PSEUDO-01
Lab Notebook 1
Data Source: File Folder: 12d
Forms: Glochidia Photomicrograph Counting Record

Test Article: MBI 401 (Pf-CL 145A [SDP])
Article Lot #: MBI-401-110308AI-BD-3
Exposure Dates: 12-May-11
Species Tested: HGE

Higgins eye (SDP) Distribution Glochidia Count

Species	Formulation	Test date	Distribution round	Rep number	Total glochidia per 100 µL
HGE	SDP	12-May-11	1	1	1107
				2	823
				3	636
			2	1	843
				2	871
				3	812
			3	1	746
				2	640
				3	549
			4	1	802
				2	593
				3	357
			5	1	724
				2	684
				3	481
				4	298
				Mean (per 100 µL)	685
				SD	204
	Glochidia Distribution Estimate			**Mean (per 500 µL)**	**3427**

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Study Number: AEH-11-PSEUDO-01
Lab Notebook 1
Data Source: File Folder: 12d
Forms: Glochidia Photomicrograph Counting Record

Test Article: MBI 401 (Pf-CL 145A (FDP))
Article Lot #: 110607WB-FD-E
Exposure Dates: 18-Oct-11
Species Tested: PPB

Plain pocketbook (FDP) Distribution Glochidia Count

Species	Formulation	Test date	Distribution round	Rep number	Total glochidia per 100 µL
PPB	FDP	18-Oct-11	1	1	762
				2	1075
				3	501
				4	620
			2	1	986
				2	1023
				3	493
			3	1	1152
				2	968
				3	1221
			4	1	1278
				2	1033
				3	971
			5	1	718
				2	1126
				3	800
				Mean (per 100 µL)	920
				SD	245
	Glochidia Distribution Estimate			Mean (per 500 µL)	4602

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Washboard (FDP) Distribution Glochidia Count

Species	Formulation	Test date	Distribution round	Rep number	Total glochidia per 100 µL
WAS	FDP	18-Oct-11	1	1	115
				2	7
				3	139
				4	110
			2	1	215
				2	149
				3	255
			3	1	204
				2	179
				3	288
			4	1	201
				2	303
				3	171
			5	1	131
				2	152
				3	80
				Mean (per 100 µL)	169
				SD	76
	Glochidia Distribution Estimate			Mean (per 500 µL)	843

Study Number: AEH-11-PSEUDO-01
Lab Notebook 1
Data Source: File Folder: 12d
Forms: Glochidia Photomicrograph Counting Record

Test Article: MBI 401 (Pf-CL 145A [SDP])
Article Lot #: MBI-401 SDP 4655-12-Mix
Exposure Dates: 17-Jan-12
Species Tested: BLS

Black sandshell (SDP) Distribution Glochidia Count

Species	Formulation	Test date	Distribution round	Rep number	Total glochidia per 100 µL
PPB	SDP	17-Jan-12	1	1	322
				2	456
				3	382
			2	1	468
				2	546
				3	430
			3	1	309
				2	367
				3	235
			4	1	453
				2	348
				3	514
			5	1	604
				2	415
				3	324
				4	295
				Mean (per 100 µL)	404
				SD	100
	Glochidia Distribution Estimate			**Mean (per 500 µL)**	**2021**

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Study Number: AEH-11-PSEUDO-01
Lab Notebook 1
Data Source: File Folder: 12d
Forms: Glochidia Photomicrograph Counting Record

Test Article: MBI 401 (Pf-CL 145A (SDP))
Article Lot #: MBI-401 SDP 4655-12-Mix
Exposure Dates: 17-Jan-12
Species Tested: MUC

Mucket (SDP) Distribution Glochidia Count

Species	Formulation	Test date	Distribution round	Rep number	Total glochidia per 100 µL
MUC	SDP	17-Jan-12	1	1	97
				2	576
				3	727
			2	1	521
				2	754
				3	218
			3	1	531
				2	350
				3	780
			4	1	618
				2	470
				3	285
			5	1	309
				2	198
				3	278
				4	329
				Mean (per 100 µL)	440
				SD	212
	Glochidia Distribution Estimate			**Mean (per 500 µL)**	**2200**

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Study Number: AEH-11-PSEUDO-01
Lab Notebook 1
Data Source: File Folder: 12d
Forms: Glochidia Photomicrograph Counting Record

Test Article: MBI 401 (Pf-CL 145A [SDP])
Article Lot #: MBI-401 SDP 4655-12-Mix
Exposure Dates: 19-Jan-12
Species Tested: HIC

Hickorynut (SDP) Distribution Glochidia Count

Species	Formulation	Test date	Distribution round	Rep number	Total glochidia per 100 µL
HIC	SDP	19-Jan-12	1	1	779
				2	995
				3	2555
			2	1	593
				2	1105
				3	521
			3	1	359
				2	853
				3	708
			4	1	637
				2	748
				3	1067
			5	1	630
				2	545
				3	701
				4	603
				Mean (per 100 µL)	837
				SD	501
	Glochidia Distribution Estimate			**Mean (per 500 µL)**	**4187**

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Study Number: AEH-11-PSEUDO-01
Lab Notebook 1
Data Source: File Folder: 12d
Forms: Glochidia Photomicrograph Counting Record

Test Article: MBI 401 (Pf-CL 145A [SDP] and [FDP])
Article Lot #: MBI-401-110308AI-BD-3, MBI-401 SDP 4655-12-Mix, 110607WB-FD-E
Exposure Dates: 12-May-11, 18-Oct-11, 17-Jan-12, and 19-Jan-12
Species Tested: PPB, FAM, HGE, WAS, BLS, MUC, and HIC

Distribution Glochidia Count Summary

Species	Formulation	Test date	Mean glochidia per sample (100 µL)	Mean glochidia per chamber (500 µL)
PPB	SDP	12-May-11	671	**3355**
FAM	SDP	12-May-11	540	**2702**
HGE	SDP	12-May-11	685	**3427**
PPB	FDP	18-Oct-11	920	**4602**
WAS	FDP	18-Oct-11	169	**843**
BLS	SDP	17-Jan-12	404	**2021**
MUC	SDP	17-Jan-12	440	**2200**
HIC	SDP	19-Jan-12	837	**4187**

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ISSN 2331-1258 (online)
http://dx.doi.org/10.3133/ofr20151094