DEPARTMENT OF THE INTERIOR
U.S. FISH AND WILDLIFE SERVICE
REGION 1

FY13 - ENVIRONMENTAL CONTAMINANTS PROGRAM
OFF-REFUGE INVESTIGATIONS SUB-ACTIVITY

WA - Investigation of Contaminants in Feeds and Fish at FWS Pacific Region National Fish Hatcheries and the Ramifications to Human and Ecological Health (Final Report for an investigation with completed funding)

DEC Project ID: 200710002.3
FFS #: 1F52

(Filename: fish feed final report FY13_1F52 final)

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August 2012

Congressional Districts OR02, WA03, WA06, & WA09

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LIST OF ACRONYMS/ABBREVIATIONS

Abbreviation or Acronym	Meaning
bdl	below detection limit
nd	not determined
OC	organochlorines
ng/g	nanograms/gram
ųg/g	micrograms/gram
SD	standard deviation
PCB	polychlorinated biphenyl
DDT	dichlorodiphenyltrichloroethane
NFH	National Fish Hatchery
WS NFH	Warm Springs National Fish Hatchery
QC NFH	Quilcene National Fish Hatchery
QN NFH	Quinault National Fish Hatchery
RET	Residual Effect Threshold
MS222	tricaine methanesulfonate
dioxins	polychlorinated dibenzo-p-dioxins
furans	polychlorinated dibenzofurans
PBDE	polybrominated diphenyl ether
DL	detection limits
Fed14	Fed for 14 days
Fed21	Fed for 21 days
Fast14	Fasted for 14 days
Fast21	Fasted for 21 days
HCH	hexachlorocyclohexanes
SAS	Statistical Analysis Software
MIDAS	Microarray data analysis system
GO	Gene Ontology
apoe	apolipoprotein e
pgds	prostaglandin d synthase
gk	glucokinase
gs-3	glutamine synthetase 3
col1a2	alpha 2 chain of type I collagen gene
cst	cystatin
h-fabp	fatty acid binding protein
pk	pyruvate kinase
rbp	retinol binding protein
cyp2k1	cytochrome p450 monooxygenase 2k1v2
cyp2k5	cytochrome p450 2K5
arnt	aryl hydrocarbon receptor nuclear translocator
ar	androgen receptor
thxl	hemopexin
id1	inhibitor of DNA binding/ differentiation 1
tf	transferrin
mt	metallothionein
hba	alpha globin
hbb	beta globin
g6pc	Glucose-6-phosphatase
pit-1	pituitary-specific transcription factor 1

I. OBJECTIVES:

1. Evaluate and compare overall contaminant levels, including congener specific PCBs, PBDEs, dioxins, furans, and OC pesticides in commercial feeds used at different Pacific Region NFHs.

2. Identify any temporal differences in contaminant levels found in various feed forms (starter and pelleted diets) by sampling different batches during the rearing period.

3. Evaluate contaminant levels and bioaccumulation rates of different commercial diets in various life-stage history classes of three NFH fish species to determine if they will pose a risk to the health of the fish itself or to fish eating birds and mammals, including humans, who may consume the NFH fish.

4. Assess the re-distribution of contaminants during smoltification (from approximately January through April).

5. Simulate the release of fish from the NFH by fasting fish for a period of time and monitoring the mobilization and re-distribution of contaminants from the fat to vital organs, as well as conduct a preliminary examination of the physiological impacts of the redistribution using cDNA microarray technology.

II. FINAL REPORT

CHAPTER 1: Contaminant residues in Pacific salmonids and feeds from three National Fish Hatcheries

ABSTRACT

Returning adult salmon and steelhead were sampled at three National Fish Hatcheries (NFHs); Warm Springs NFH (spring Chinook, *Oncorhynchus tshawytscha*), Quilcene NFH (coho, *O. kisutch*) and Quinault NFH (steelhead, *O. mykiss*). Fifteen males and 15 females from each hatchery were collected during spawning and a portion of muscle tissue, including skin, was sampled from the same area on each fish. Tissue samples were pooled so that there were three composite samples (five fish per composite) from each sex. Samples were taken on spawning days at each hatchery, August 30, 2006, October 24, 2006 and December 13, 2006, respectively. Samples were analyzed for congener specific polychlorinated biphenyls (PCBs), dioxins & furans, organochlorine pesticides, polybrominated diphenyl ethers (PBDEs) and metals. The levels of the contaminants varied by fish species and could be a result of migration route, diet, taxa-specific physiology and age at return. Eggs and unfed fry were also tested for the same contaminants. Eggs generally had contaminant levels higher than the adult females. Levels of contaminants in the unfed fry were generally lower than the eggs except for the fish at Quinault NFH. Feeds were collected throughout the rearing period for each species sampled and analyzed for the same contaminants as those in the fish.

INTRODUCTION

Adult salmon returning to hatcheries can be used for human consumption or stream nutrient enhancement. These fish are known to have a body burden of anthropogenic chemicals. The levels of these compounds can vary depending on species, age, sex and lipid composition. Adult salmon should be analyzed to determine their contaminant load because of these uses, such as human consumption (Hites et al. 2004a; Hites et al. 2004b; Foran et al. 2005; Ikonomou et al. 2007) and stream enhancement (Ewald et al. 1998; Missildine 2005; O'Toole et al. 2006). In addition, contaminants from the mother can affect development and survival of the offspring (Miller and Amrhein 1995, Carlson et al. 2000, Missildine et al. 2005). The contribution of contaminants from maternal transfer can be significant for the fish (Miller 1993, Miller and Amrhein 1995, Nyholm et al. 2008, Serrano et al. 2008). Variables that determine level of contaminants transferred from mothers to eggs include: the relative amounts of lipid in the eggs and mother, and the hydrophobicity of the organochlorine contaminants (Fisk and Johnston 1998). Nyholm et al. (2008) determined that maternal contaminants were at a higher level in the eggs than the mother. The transfer of contaminants depended on the physico-chemical properties of the compounds. Not only can the maternal contaminants affect the overall survival of fry, these juvenile hatchery fish can also be impacted by contaminants contributed by the hatchery environment. Fish can bioaccumulate potentially harmful levels of contaminants from food and water and the amount accumulated in the fish tissues is determined in part by the quantity and form of the contaminants (O'Toole et al. 2006). Fish health as well as water hardness and oxygen content, age, size and lipid content of the fish are also factors that affect contaminant bioaccumulation.

The objective of this work was not to compare between NFHs but to determine presence or absence of contaminants in returning adult fish and to determine possible transfer of contaminants to their eggs and unfed fry. Contaminants in the feed will also be examined and contaminant transfer from feed to fish will be evaluated.

MATERIALS AND METHODS

All tissue and feed sampling is summarized in Table 1-1. Adult salmonid muscle tissues were collected during spawning, 15 males and 15 females, at each hatchery. Spring Chinook were sampled at Warm Springs NFH, coho were sampled at Quilcene NFH and steelhead were sampled at Quinault NFH. For the muscle sample, a single piece of tissue was collected from each fish. A rectangular piece of muscle, approximately six grams including skin, was taken from the right side of each fish. The muscle sample was taken from the following area: beginning at the anterior most base of the dorsal fin to about 0.5 inches below this point and then forward about 2.5 inches. Three 30 g composite samples were made using six grams of tissue each from five of the sampled adults. Five muscle samples were composited for a total of three male and three female composite samples. The composite muscle samples were wrapped in clean aluminum foil that had been rinsed with hexane and stored frozen.

Egg samples, approximately six grams each, were collected from the corresponding females. They were taken directly from the females and put into chemically clean jars prior to fertilization. The six gram samples were later pooled so that eggs from the five females that were pooled were composited for three 30 g egg samples. During the sample collection period, all samples were

stored on ice in a cooler until they could be frozen.

Unfed swim-up fry with no yolk sack visible were collected from each facility immediately before stocking. Three composite samples, approximately two grams each, were taken. Each composite consisted of 10 fry. The fish were allowed to drip dry in the dip net then put into chemically clean jars. In total six adult samples, three of each sex; three egg samples and three fry samples were collected from the three hatcheries and analyzed.

Samples were analyzed by contract laboratories overseen by the U.S. Fish and Wildlife Service Analytical Control Facility. The Geochemical & Environmental Research Group Laboratory at Texas A&M University (College Station, TX) analyzed the tissues for congener specific PCBs and organochlorine pesticides, PBDEs (MacLeod et al. 1985; Wade et al. 1988; Brooks et al. 1989) and dioxins & furans (Tondeur 1987). The Alpha Woods Hole Laboratory (Raynham, MA) analyzed the samples for metals using inductively coupled plasma-mass spectrometry for all the metals except mercury. Mercury was analyzed for using the cold vapor atomic fluorescence spectroscopy. Lipid and moisture in the samples were analyzed using standard methods at the laboratory. The Geochemical & Environmental Research Group Laboratory used the lipid extraction method found in Qian et al. (1998).

Using Inductively Coupled Plasma-Mass Spectrometry, solid samples (approximately 1g) were digested by hot plate or microwave according to EPA Method 3050 (hot plate) or EPA Method 3051 (microwave) (US EPA 1999). An aliquot of digestate was nebulized into a spray chamber where a stream of argon carried the sample aerosol through a quartz torch and injected it into the radio frequency inductively coupled plasma. The ions produced in the plasma were introduced to the mass spectrometer for quantification against a multi-level initial calibration. For the mercury analysis using cold vapor atomic fluorescence spectroscopy, tissue samples (approximately 1g) were digested according to EPA Method 3051 (US EPA 1999). An aliquot of the digest was then prepared with HCl/BrCl and hydroxylamine hydrochloride solution. Mercury in the digested sample was reduced to elemental mercury, aerated from solution and introduced into the spectrophotometer. The emission was measured and compared to a multi-level initial calibration for quantification.

Data were evaluated to determine if tissue lipid content contributed to differences in contaminant concentrations found among life history stages. First, linear regression was used to determine if a relationship between tissue lipid content and contaminant concentration existed. If this relationship was deemed statistically significant, data were normalized for lipid content and evaluated using one-way analysis of variance (Hebert and Keenleyside 1995). If the relationship was found to be non-significant, data were evaluated to determine if a model without a covariate could accurately describe the data (Littell et al. 2006). When this model without a covariate was adequate, data were evaluated on a wet weight basis using analysis of variance. If a covariate was required, data were evaluated using analysis of covariance with lipid content (on a % wet weight basis) as the covariate. All analyses were performed using SAS® (version 9.2, Cary, North Carolina) with $\alpha=0.05$.

RESULTS AND DISCUSSION

Congener specific polychlorinated biphenyls (PCBs) were analyzed in all tissues. In this report we will consider the 12 dioxin-like PCBs. Of those 12, #105, 118, 149/123, 156, 157/173/201 and 167 were detected. The contaminant PCB #156 was found only in one composite of five adult male steelhead composite samples from Quinault NFH (Table 1-10). It was not found in any of the other tissue results covered in this study. The rest of the PCB compounds were generally found in the fillets of both male and female adults with few exceptions, in the eggs, and in the unfed fry (Tables 1-2, 1-6, 1-10). Multiple reasons could explain the differences seen in the data across species. Debruyn et al. (2004) noted the upriver migration of sockeye depleted the lipid stores of the fish while concentrating hydrophobic contaminants. The Chinook migrating to Warm Springs NFH show this trend with the adult males having about half as much lipid as the steelhead returning to Quinault and the coho returning to Quilcene NFHs (Tables 1-2, 1-6, 1-10). However, the contaminant compounds are somewhat higher in the Chinook, but not consistently.

Polybrominated diphenyl ethers (BDEs) were detected in adults, eggs and some unfed fry samples at all of the hatcheries. The predominant BDE found was #47 along with #99 and #100. These were also among the most predominant congeners found by Easton et al. (2002). The selective transport of the BDEs may be explained by the chemical properties of these hydrophobic contaminants (Nyholm et al. 2008). The BDE congeners were found in two egg and six fillet (male and female) samples, and one fry sample from Warm Springs NFH (Table 1-2); in three egg and six fillet (male and female) samples from Quilcene NFH (Table 1-6); and two egg, six fillet (male and female) and two fry samples from Quinault (Table 1-10).

Dioxins and furans were not very prevalent in the samples. These results are similar to what Shaw et al. (2006) found when they examined farmed Atlantic salmon from Maine and eastern Canada, wild Alaskan Chinook salmon and organically grown Norwegian salmon. They determined that dioxin/furans and dioxin like PCBs were not detected in >80% of their samples. Warm Springs NFH spring Chinook eggs contained detectable levels of 1,2,3,4,6,7,8-HpCDD, 1,2,3,7,8,9-HxCDD and OCDD in one composite egg sample, and 2,3,7,8-TCDF was detected in two egg samples (Table 1-2). Quilcene NFH had one adult male composite sample with OCDD (Table 1-6). Quinault NFH had 1,2,3,4,6,7,8-HpCDD; 1,2,3,4,6,7,8-HpCDF; 1,2,3,6,7,8,-HxCDD; OCDD and OCDF in one composited unfed fry sample. OCDD was present in one composite sample each of adult male and female fillets (Table 1-10).

Organochlorine pesticides were found in both the male and female fillets, however, with the exception of Warm Springs NFH, very few were passed on to the eggs possibly due to the size of the compounds. Mirex was found in both the male and female fillets but not detected in eggs or unfed fry. Serrano et al. (2008) determined that maternal transfer of larger compounds, for example PCBs with higher numbers of chlorine atoms, was decreased. The #105 and #118 PCBs were both passed to eggs at all three hatcheries. At Quilcene and Warm Springs NFHs #149/123 was also passed to the eggs. Generally the levels of the PCB contaminants in eggs were greater than in adult female fillets with the exception of Quinault NFH. A similar affect was seen in siscowet, a morphotype of the lake trout (Miller and Amrhein 1995).

Isosaari et al. (2004) showed that the variability in the accumulation of congener specific PCBs is determined by the chemical configurations of the compounds. Missildine et al. (2005) found that Chinook collected from hatcheries on Puget Sound had very different and higher levels of total PCBs than the Chinook collected at the Makah NFH where the fish come in directly from the ocean. O'Neill et al. (1998) found differences in PCB levels in two species, Chinook and coho as well as differences between sample sites, in-river and marine locations. Both Missildine and O'Neill concluded that location or region could explain the differences in PCB levels.

Easton et al. (2002) tested three species of salmon: Chinook, chum and sockeye. The contaminant loads seen in these fish were highly variable. This dissimilarity could be due to species-specific differences in migration area or the lipid concentrations in their flesh. Lipid concentrations differed among the adult fish we sampled. Comparing the females, the lipid content in their tissue samples ranged from 1.4% in the spring Chinook, 1.9% in the coho and 3.1% in the steelhead. The adult males had lipid levels of 5.2% in the spring Chinook, 10.9% in the coho and 11.6% in the steelhead. The Chinook lipid levels could be due to life history because these fish migrate to the Warm Springs NFH and are held two-three months prior to spawning resulting in a longer freshwater residence time and depleted lipid stores. Regardless of species, all females had lower lipid concentrations due to transference of lipid to eggs (egg lipids 12.2%, 4.3% and 8.6% in Warm Springs, Quilcene and Quinault NFH fish, respectively; Tables 1-2, 1-6 and 1-10). Differences in egg lipid content may result in variable contaminant loads as suggested by Miller (1993). In spite of the wide-ranging contaminant concentrations found in fillet and egg samples in this study, few relationships between tissue lipid content and contaminant concentrations were found.

It appears maternal transfer of contaminants to the egg may be the largest contributor of contaminants to these hatchery-reared fish. Contaminant levels decreased from egg to unfed fry to parr (Table 1-4, 1-8, 1-12). In contrast, the levels of some of the nonessential metals increased for each life stage (Table 1-3, 1-7, 1-11). These increases could be due to the feeds fed to the growing fish (Table 1-8, 1-9, 1-10). Organic contaminants in the feed tended to decrease as the feed size increased. These changes could partially be explained by the change in formulation as the feed size changed (Table 1-8, 1-9, 1-10).

Other variables, including the environment the fish encounter outside the hatchery, would have had an impact on their contaminant load. Researchers have concluded that 97- 99% of PCBs in returning Chinook salmon were accumulated by the fish in the marine environment (O'Neill et al.1998, Cullon et al. 2009). If comparing the adult returning fish on a lipid basis as O'Neill and Cullon did, Chinook had higher contaminant concentrations (Appendix A, C, E). This could be because they live longer, have a longer marine residence time, and grow to a larger size at sea. The adult Chinook sampled at Warm Springs NFH had a residence time of approximately 18 months in the hatchery and two years at sea. The Warm Springs spring Chinook are wide ranging from Monterey Bay, CA to northern Southeast Alaska. These fish have a highly oceanic distribution and generally don't spend much time in coastal waters (personal communication, Roger Sorenson, Manager, Warm Springs NFH).

The Quilcene coho have been captured from Alaska to the Oregon coast with the majority of

them being caught in Puget Sound and the Washington coast (personal communication Dave Zajac, biological technician, Western Washington Office, Lacey, WA). Further information about coho migrations can be found in Weitkamp et al. (1995). Coho salmon released from coastal hatcheries are recovered primarily in British Columbia (37-74%) and Washington (18-53%), with few recoveries from Oregon (3-16%) and almost none (<1%) from California or Alaska. Compared to Columbia River fish, Washington coastal hatchery coho salmon have much higher recovery rates from British Columbia and much lower recovery rates from Oregon and California.

Generally the steelhead from Quinault are captured in coastal gillnets near Queets, WA or collected at the Quinault NFH. Washington steelhead have been recovered as by-catch off of the Aleutian Islands so the possible migratory pattern for the steelhead is northerly. Data concerning the migration routes of the fish are based on coded wire tag recoveries. Coded wire tags should not be considered tracking tools (personal communication Dave Zajac, biological technician, Western Washington Office, Lacey, WA). Additional information about steelhead migrations include high seas recoveries of marked or tagged steelhead that show North American steelhead occur throughout most of the areas in the North Pacific Ocean that are known to be traveled by migrating steelhead, including the areas fished by the Japanese high seas salmon fisheries. The specific information obtained from tagged fish shows that stocks from different regions along the coast, including fish from summer and winter races or inland and coastal groups, intermingle extensively offshore. The general extent of steelhead marine distribution covers a broad area from the North American continent to the southeastern waters of the Sea of Okhotsk. The northern boundary of steelhead distribution closely follows the arc formed by the Aleutian Island chain and the Commander Islands. A few fish have been caught in the Bering Sea and in the northwestern Sea of Okhotsk. The southern boundary of steelhead distribution extends as far south as 39°N latitude, but generally lies between 40°N and 44°N latitude. North American steelhead have been found throughout most of this area except in waters west of l62°E longitude and along the southern fringe of the distribution, where the abundance of steelhead is relatively low (Light et al. 1988).

We used a modified bioaccumulation factor to examine contaminant load change over time as fish grew and developed from egg into parr (Tables 1-5, 1-9 and 1-13). The lipophilic contaminants appear to accumulate within lipid as fish develop from egg into unfed fry with a couple of exceptions. The sum of BHCs decreased in the egg to unfed fry at Warm Springs NFH and sum of DDTs decreased in the eggs to unfed fry at Quilcene NFH. Otherwise there was an increase in all of the reported contaminant groups. There was an overall decrease in contaminant concentration in the fry to parr at all three hatcheries with a few exceptions. At Warm Springs NFH, total chlorinated benzenes, BHCs, chlorinated pesticides and DDTs all increased, and total chlorinated pesticides increased at Quinault NFH. Kelly et al. (2008) also saw the persistent organic pollutants stay relatively low as the Pacific salmon fed commercial diets grew to smolts. When examining the contaminant load of Pacific salmon in the hatcheries and during their migration down the Columbia River and its tributaries, it was noted by Johnson et al. (2010) and Arkoosh et al. (2011) that the hatchery fish were less contaminated as they left the hatchery than when they reached the estuary. This suggests hatchery smolts accumulate contaminants as they emigrate down river.

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Table 1-1. Fish and feed samples taken at the three National Fish Hatcheries, Quilcene, Quinault and Warm Springs NFHs.

Hatcheries	Quilcene	Quinault	Warm Springs
Species	Coho	Winter-run steelhead	Spring Chinook
Rearing period (approximate)	18 months	18 months	18 months
Sampling date (adults & eggs)	10/24/06	12/13/06	08/30/06
Sampling date (fry)	02/13/07	03/15/07	01/08/07
Sampling date (fingerlings)	05/10/07	08/02/07	05/17/07
Sampling date (presmolts)	02/12/08	02/11/08	02/01/08
Sampling date (smolts at release)	04/24/08	04/25/08	04/08/08
Adult samples*	n=3 composites ea male & female	n=3 composites ea male & female	n=3 composites ea male & female
Egg samples	n=3 composites/5 fish each	n=3 composites/5 fish each	n=3 composites/5 fish each
Swim-up fry samples	n=3 composites/10 fish each	n=3 composites/10 fish each	n=3 composites/10 fish each
Fingerling samples	n=3 composites/10 fish each	n=3 composites/10 fish each	n=3 composites/10 fish each
Presmolt samples	n=3 composites/20 fish each	n=3 composites/20 fish each	n=3 composites/10 fish each
Smolt samples	n=3 composites/10 fish each	n=3 composites/10 fish each	n=3 composites/20 fish each

*Muscle from 15 males and 15 females, composites made=3 composites of five samples each. Eggs were also composited; eggs from the same composited 5 females.

Table 1-2. Contaminants (mean ± SD) found in fish tissues collected from Warm Springs NFH. Warm Springs

Contaminant (ng/g wet weight)	Adult-male fillet	Adult-female fillet	Egg	Unfed Fry
PCB				
#105	0.7 ± 0.3	0.4 ± 0.2	1.0 ± 0.4	1.3 ± 0.6
#118	0.8 ± 0.5	0.4 ± 0.0	2.2 ± 1.1	1.0 ± 0.6
#149/123	1.2 ± 0.7	0.4 ±0.0	2.4 ± 1.2	1.0 ± 0.3
#156	bdl	bdl	bdl	bdl
#157/173/201	0.2 ± 0.1	0.1 ± 0.0	bdl	0.3 ± 0.1
#167	0.3 ± 0.2	bdl	1.4 ± 0.5	0.3
#189	bdl	bdl	bdl	bdl
PCB-total	67.7 ± 24.6	36.3 ± 11.3	92.9 ± 14.1	66.3 ± 14.3
PBDE				
#100	bdl	0.7	bdl	bdl
#47	2.0 ± 0.9	1.2 ± 0.5	2.1 ± 0.8	2.9
#99	1.0 ± 0.1	bdl	bdl	bdl
Dioxins/Furans				
1,2,3,4,6,7,8-HpCDD	bdl	bdl	0.04	bdl
1,2,3,7,8,9-HxCDD	bdl	bdl	0.04	bdl
2,3,7,8-TCDF	bdl	bdl	0.01 ± 0.0	bdl
OCDD	bdl	bdl	0.1	bdl
OCDF	bdl	bdl	0.1	bdl
Organochlorines				
Aldrin	bdl	bdl	bdl	bdl
α-BHC	bdl	bdl	bdl	bdl
α-chlordane	1.6 ± 0.8	0.9 ± 0.7	2.6 ± 1.3	1.6
β-BHC	0.3	bdl	2.3 ± 2.4	1.3
cis-nonachlor	1.0 ± 0.3	0.4 ± 0.2	1.1	bdl
Dieldrin	1.1 ± 0.4	0.4 ± 0.3	1.2 ± 0.3	bdl
Endosulfan II	bdl	bdl	bdl	bdl
Endrin	0.6 ± 0.2	0.3 ± 0.1	3.0 ± 1.3	1.2
γ-BHC	bdl	bdl	2.6	1.2
γ-chlordane	1.6 ± 0.2	0.8 ± 0.6	bdl	bdl
HCB	1.6 ± 0.4	3.4 ± 1.4	5.0 ± 0.9	2.0 ± 0.4
Heptachlor	0.3	0.2	bdl	bdl
Heptachlor epoxide	0.3 ± 0.0	0.3 ± 0.1	bdl	bdl
Mirex	0.6 ± 0.1	0.3 ± 0.1	bdl	bdl
o,p'-DDD	0.8 ± 0.3	bdl	1.2 ± 0.5	1.2
o,p'-DDE	bdl	bdl	bdl	bdl
o,p'-DDT	bdl	bdl	2.2 ± 0.4	bdl
Oxychlordane	0.5 ± 0.2	0.5 ± 0.5	bdl	bdl
p,p'-DDD	2.0 ± 1.1	0.9 ± 0.0	1.8 ± 0.68	2.1 ± 0.7
p,p'-DDE	17.2 ± 9.1	8.7 ± 1.7	16.1 ± 4.8	6.6 ± 0.7
p,p'-DDT	1.1 ± 0.3	0.4 ± 0.1	2.1 ± 1.2	1.7 ± 0.9

Pentachloro-anisole	0.5 ± 0.1	0.2 ± 0.0	1.5 ± 0.3	1.2 ± 0.2
Trans-nonachlor	3.6 ± 1.4	1.8 ± 0.9	2.5 ± 0.5	1.5 ± 0.3
Organophosphates and semi-volatiles				
Chlorpyrifos	bdl	bdl	bdl	1.6 ± 0.5
1,2,3,4-tetrachlorobenzene	0.9 ± 0.5	0.2	3.0 ± 1.7	2.5 ± 1.7
1,2,4,5-tetrachlorobenzene	1.5 ± 0.7	1.3 ± 0.4	15.0 ± 8.4	11.3 ± 11.2
Metals (µg/g wet weight)				
Al	50.7 ± 77.4	2.6 ± 1.2	bdl	5.2 ± 2.6
As	0.4 ± 0.0	0.4 ± 0.0	0.2 ± 0.0	bdl
B	bdl	bdl	bdl	bdl
Ba	0.1	0.1	bdl	0.1 ± 0.0
Cd	bdl	0.02	bdl	bdl
Cr	0.5 ± 0.0	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.0
Cu	0.6 ± 0.0	0.6 ± 0.1	3.0 ± 0.1	0.4 ± 0.0
Fe	6.4 ± 0.9	6.2 ± 1.4	16.4 ± 1.3	20.8 ± 0.8
Hg	0.1 ± 0.0	0.1 ± 0.0	0.01	bdl
Mg	183.0 ± 9.2	204.3± 16.6	673.3 ± 24.8	274.3 ± 9.6
Mn	0.1	bdl	0.3 ± 0.0	0.3 ± 0.0
Mo	bdl	bdl	bdl	bdl
Ni	0.1	bdl	bdl	bdl
Pb	bdl	bdl	bdl	bdl
Se	0.3 ± 0.0	0.3 ± 0.0	1.62 ± 0.1	1.0 ± 0.1
Sr	0.6 ± 0.0	0.8 ± 0.4	1.9 ± 0.0	3.9 ± 0.7
V	bdl	bdl	bdl	bdl
Zn	10.4± 1.0	11.2 ± 2.6	18.9 ± 1.3	14.9 ± 0.5
Moisture	74.5±2.3	77.0±0.9	55.3±0.7	79.1±1.3
Lipid (% wet weight)	5.2±3.1	1.4±0.2	12.2±3.7	3.6±0.8

Table 1-3. Nonessential metals (mean ± SD; µg/g wet weight) of eggs, fry and parr collected at Warm Springs NFH.

Hatchery	Contaminant	Age			One-way ANOVA
		Egg	Fry	Parr	P
Warm Springs	As	0.2 ± 0.0 [b]	bdl[1]	0.9 ± 0.0 [a]	<0.0001
	Cd	bdl	bdl	bdl	nd[2]
	Cu	3.0 ± 0.1 [a]	0.4 ± 0.0 [c]	0.7 ± 0.0 [b]	<0.0001
	Hg	0.01	bdl	0.02 ± 0.0	nd
	Pb	bdl	bdl	bdl	nd
	Zn	18.9 ± 1.3 [b]	14.9 ± 0.5 [b]	29.0 ± 4.4 [a]	0.0016
	∑Metals	22.1 ± 1.2 [b]	15.3 ± 0.5 [c]	30.6 ± 4.4 [a]	0.0013

[1]bdl=below detection limit
[2]nd=not determined

Table 1-4. Contaminants (mean ± SD) found in eggs, fry, and parr collected at Warm Springs NFH. The ∑DDTs correlated to tissue lipid content, so these data were evaluated as ng/g lipid using analysis of variance. All other data were analyzed as ng/g wet weight using analysis of variance as lipid content was not found to be a significant covariate for these parameters.

Contaminant	Age			P-value
	Egg	Fry	Parr	
∑PCBs	92.9 ± 14.1 [a]	66.3 ± 14.3 [ab]	58.0 ± 7.9 [b]	0.0324
∑BDEs	2.1 ± 0.8	2.9	0.9 ± 0.4	0.0812
∑Dioxins and Furans	0.2	bdl	0.1 ± 0.0	nd
∑Chlorinated Benzenes	18.0 ± 8.4	13.0 ± 13.0	9.3 ± 5.1	0.5561
∑BHCs	3.6 ± 0.6 [a]	1.2 ± 0.1 [b]	1.5 ± 0.4 [b]	0.0067
∑Chlordanes	5.5 ± 0.8 [a]	2.0 ± 1.1 [b]	4.6 ± 0.5 [a]	0.0054
∑Cyclodiene Pesticides	3.2 ± 1.9	1.2	1.1 ± 0.8	0.4015
∑Chlorinated Pesticides	1.5 ± 0.3	2.4 ± 1.2	2.9 ± 1.9	0.4606
∑DDTs*	211.7 ± 114.0	279.3 ± 47.2	202.4 ± 6.1	0.4101
Moisture (% wet weight)	55.3 ± 0.7 [b]	79.1 ± 1.3 [a]	77.6 ± 0.9 [a]	<0.0001
Lipid (% wet weight)	12.2 ± 3.7 [a]	3.6 ± 0.8 [b]	4.0 ± 0.5 [b]	0.0049

*ng/g lipid
[1]bdl=below detection limit
[2]nd=not determined

Table 1-5. Biomagnification of contaminants through time at Warm Springs NFH not factoring in feed (no feed involved in egg or fry sample). These numbers were calculated based on fry contaminant levels (ng/g lipid) divided by egg level and parr divided by fry. Values >1 represent concentration of contaminants and values <1 represent dilution.

	Change over time			Change over time*
Contaminant	Egg to Fry	Fry to Parr	P-value	Egg to Parr
∑PCBs	2.3 ± 0.4	0.8 ± 0.4	0.0482	1.7 ± 0.3
∑BDEs	4.4 ± 0.1	0.2 ± 0.1	0.0011	1.1 ± 0.2
∑Dioxins and Furans	nd	nd		nd
∑Chlorinated Benzenes	2.3 ± 1.1	0.6 ± 1.1	0.3319	2.3 ± 1.0
∑BHCs	0.9 ± 0.3	1.1 ± 0.2	0.6319	1.0 ± 0.2
∑Chlordanes	1.2 ± 0.4	1.9 ± 0.4	0.2700	2.3 ± 0.3
∑Cyclodiene Pesticides	1.4 ± 0.5	0.6 ± 0.4	0.4350	0.9 ± 0.4
∑Chlorinated Pesticides	5.1 ± 1.3	1.1 ± 1.3	0.1039	5.5 ± 1.8
∑DDTs	1.3 ± 0.1	0.7 ± 0.1	0.0102	1.0 ± 0.1

nd=not determined because of non-detect values
*Just for reference

Table 1-6. Contaminants (mean ± SD) found in fish tissues collected from Quilcene NFH. Quilcene

Contaminant (ng/g wet weight)	Adult-male fillet	Adult-female fillet	Egg	Unfed Fry
PCB				
#105	0.4 ± 0.1	0.4 ± 0.2	0.3 ± 0.1	1.2 ± 0.4
#118	2.4 ± 1.3	1.0 ± 0.1	1.7 ± 1.8	bdl
#149/123	2.0 ± 1.1	0.8 ± 0.1	0.5	bdl
#156	bdl	bdl	bdl	bdl
#157/173/201	0.9 ± 1.0	0.2 ± 0.1	bdl	2.4 ± 0.6
#167	0.3	bdl	bdl	bdl
#189	bdl	0.3	bdl	bdl
PCB-total	106.8 ± 29.1	47.7 ± 10.3	77.3 ± 30.9	83.2 ± 2.7
PBDE				
#100	1.7 ± 0.9	0.6 ± 0.0	bdl	bdl
#116	bdl	3.5	bdl	bdl
#15	bdl	10.9	bdl	bdl
#28	0.3	bdl	bdl	bdl
#47	5.5 ± 2.3	2.6 ± 0.2	4.4 ± 1.9	bdl
#49	1.0	bdl	bdl	bdl
#66	0.3	bdl	bdl	bdl
#99	1.8 ± 0.9	1.4 ± 0.3	bdl	bdl
Dioxins/Furans				
1,2,3,4,6,7,8-HpCDD	bdl	bdl	bdl	bdl
1,2,3,7,8,9-HxCDD	bdl	bdl	bdl	bdl
2,3,7,8-TCDF	bdl	bdl	bdl	bdl
OCDD	0.226	bdl	bdl	bdl
OCDF	0.0 ± 0.0	bdl	bdl	bdl
Organochlorines				
Aldrin	bdl	bdl	bdl	bdl
α-BHC	0.6 ± 0.7	0.1	bdl	bdl
α-chlordane	1.0 ± 0.6	0.3 ± 0.2	1.7 ± 0.3	bdl
β-BHC	1.9 ± 1.1	0.8 ± 0.3	8.1 ± 0.3	7.4 ± 3.8
cis-nonachlor	1.3 ± 0.4	0.7 ± 0.2	bdl	bdl
δ-BHC	0.3	0.1 ± 0.0	bdl	bdl
Dieldrin	2.3 ± 0.8	0.9 ± 0.1	bdl	bdl
Endosulfan II	1.1	bdl	bdl	bdl
Endrin	2.8 ± 2.7	0.3 ± 0.1	bdl	bdl
γ-BHC	0.8	0.1	bdl	bdl
γ-chlordane	1.8 ± 0.7	1.1 ± 0.1	1.5 ± 0.4	bdl
HCB	3.0 ± 0.1	1.2 ± 0.3	1.4 ± 0.2	bdl
Heptachlor	1.6 ± 0.5	0.8 ± 0.5	bdl	bdl
Heptachlor epoxide	0.5 ± 0.7	0.2 ± 0.1	bdl	bdl
Mirex	0.5 ± 0.4	0.3 ± 0.2	bdl	bdl
o,p'-DDD	1.2 ± 1.1	bdl	bdl	bdl

o,p'-DDE	0.6	bdl	2.9	bdl
o,p'-DDT	3.1	bdl	bdl	bdl
Oxychlordane	1.3 ± 0.6	1.2 ± 0.6	bdl	bdl
p,p'-DDD	4.0 ± 1.8	1.6 ± 0.2	bdl	bdl
p,p'-DDE	15.9 ± 3.7	5.0 ± 1.3	8.8 ± 0.9	4.1 ± 0.2
p,p'-DDT	2.0 ± 1.0	1.1 ± 0.2	1.1	1.5
Pentachloro-anisole	2.0 ± 0.4	0.6 ± 0.2	1.1 ± 0.1	2.0 ± 0.1
Trans-nonachlor	4.3 ± 1.8	1.3 ± 0.2	1.5	bdl
Organophosphates and semi-volatiles				
Chlorpyrifos	1.8 ± 0.2	1.8 ± 0.8	bdl	5.0
1,2,3,4-tetrachlorobenzene	2.7 ± 2.7	0.3 ± 0.1	3.5 ± 0.4	3.0 ± 0.5
1,2,4,5-tetrachlorobenzene	6.1 ± 3.5	2.4 ± 0.7	32.4 ± 5.3	40.3 ± 9.1
Metals (µg/g wet weight)				
Al	6.5 ± 0.7	4.1 ± 1.5	bdl	2.7 ± 1.6
As	0.7 ± 0.2	0.3 ± 0.0	0.2 ± 0.0	bdl
B	bdl	bdl	bdl	bdl
Ba	0.05	0.06 ± 0.01	bdl	bdl
Cd	bdl	bdl	bdl	bdl
Cr	3.0 ± 3.9	1.6 ± 0.9	0.6 ± 0.1	0.4 ± 0.1
Cu	0.6 ± 0.1	0.6 ± 0.0	4.7 ± 0.3	0.8 ± 0.1
Fe	22.2 ± 14.1	13.5 ± 5.1	13.9 ± 0.9	20.1 ± 5.2
Hg	0.06 ± 0.01	0.07 ± 0.01	bdl	bdl
Mg	187.3 ± 23.2	237.7 ± 14.7	626.7 ± 25.8	283.0 ± 37.3
Mn	0.4 ± 0.4	0.3 ± 0.1	0.9 ± 0.1	0.6 ± 0.2
Mo	0.9	0.2 ± 0.1	bdl	bdl
Ni	1.8 ± 2.9	0.8 ± 0.8	0.1	0.1
Pb	bdl	bdl	bdl	bdl
Se	0.3 ± 0.0	0.3 ± 0.0	1.9 ± 0.2	0.8 ± 0.2
Sr	1.7 ± 0.8	2.4 ± 0.5	3.5 ± 0.2	5.9 ± 1.4
V	0.1 ± 0.0	0.1	0.1 ± 0.0	bdl
Zn	8.4 ± 1.3	8.1 ± 1.5	25.8 ± 1.1	24.8 ± 3.8
Moisture				
	70.3±1.7	75.0±2.5	61.7±1.0	79.8±1.0
Lipid (% wet weight)	10.9±1.3	1.9±0.6	4.3±1.7	2.1±0.4

Table 1-7. Nonessential metals (mean ± SD; µg/g wet weight) of eggs, fry and parr collected at Quilcene NFH.

Hatchery	Contaminant	Age			One-way ANOVA P
		Egg	Fry	Parr	
Quilcene	As	0.2 ± 0.0 [b]	bdl	0.6 ± 0.0 [a]	<0.0001
	Cd	bdl	bdl	bdl	nd
	Cu	4.7 ± 0.3 [a]	0.8 ± 0.1 [b]	1.2± 0.1 [b]	<0.0001
	Hg	bdl	bdl	bdl	nd
	Pb	bdl	bdl	bdl	nd
	Zn	25.8 ± 1.1 [b]	24.8 ± 3.8 [b]	33.3 ± 1.2 [a]	0.0091
	∑Metals	30.7 ± 1.2 [ab]	25.6 ± 3.9 [b]	35.1 ± 1.1 [a]	0.0089

bdl=below detection limit
nd=not determined

Table 1-8. Contaminants (mean ± SD) found in eggs, fry and parr collected at Quilcene NFH. The ∑PCBs correlated to tissue lipid content, so these data were evaluated as ng/g lipid using analysis of variance. All other data were analyzed as ng/g wet weight using analysis of variance as lipid content was not found to be a significant covariate for these parameters.

Contaminant	Age			P-value
	Egg	Fry	Parr	
∑PCBs*	2102.2 ± 1389.0 [ab]	4122.2 ± 755.1[a]	383.3 ± 148.2 [b]	0.0106
∑BDEs	4.4 ± 1.9	bdl[1]	0.6 ± 0.2	0.0631
∑Dioxins and Furans	bdl	bdl	bdl	nd[2]
∑Chlorinated Benzenes	36.0 ± 5.5 [a]	43.3 ± 9.3 [a]	5.2 ± 1.8 [b]	0.0007
∑BHCs	9.1 ± 0.3 [a]	8.8 ± 3.9 [a]	1.5 ± 0.5 [b]	0.0105
∑Chlordanes	2.7 ± 2.3	bdl	1.3 ± 0.9	0.3915
∑Cyclodiene Pesticides	bdl	bdl	0.8 ± 0.2	nd
∑Chlorinated Pesticides	1.1 ± 0.1	3.7 ± 3.0	2.4 ± 0.1	0.2771
∑DDTs	10.1 ± 2.9 [a]	4.6 ± 1.0 [b]	4.8 ± 21.5 [b]	0.0128
Moisture (% wet weight)	61.7 ± 1.0 [c]	79.8 ± 1.0 [a]	76.8 ± 0.5 [b]	<0.0001
Lipid (% wet weight)	4.3 ± 1.7 [ab]	2.1 ± 0.4 [b]	4.9 ± 0.4 [b]	0.0322

*ng/g lipid
[1]bdl=below detection limit
[2]nd=not determined

Table 1-9. Biomagnification of contaminants through time at Quilcene NFH not factoring in feed (no feed involved in egg or fry sample). These numbers were calculated based on fry contaminant level (ng/g lipid) divided by egg level and parr divided by fry. Values >1 represent concentration of contaminants and values <1 represent dilution.

	Change over time			Change over time*
Contaminant	Egg to Fry	Fry to Parr	P-value	Egg to Parr
∑PCBs	2.0 ± 0.1	0.2 ± 0.1	0.0010	0.3 ± 0.1
∑BDEs	nd[1]	nd		nd
∑Dioxins and Furans	nd	nd		nd
∑Chlorinated Benzenes	2.2 ± 0.0	0.1 ± 0.0	<0.0001	0.1 ± 0.0
∑BHCs	1.9 ± 0.4	0.1 ± 0.4	0.0389	0.1 ± 0.4
∑Chlordanes	nd	nd		nd
∑Cyclodiene Pesticides	nd	nd		nd
∑Chlorinated Pesticides	5.8 ± 1.8	0.3 ± 1.8	0.0936	1.6 ± 1.5
∑DDTs	0.8 ± 0.1	0.4 ± 0.1	0.0109	0.4 ± 0.1

[1]nd=not determined because of non-detect values

*Just for reference

Table 1-10. Contaminants (mean ± SD) found in fish tissues collected from Quinault NFH.

Quinault

Contaminant (ng/g wet weight)	Adult-male fillet	Adult-female fillet	Egg	Unfed Fry
PCB				
#105	0.4 ± 0.2	0.4 ± 0.1	0.3	3.0
#118	1.3 ± 0.3	0.9 ± 0.4	0.5 ± 0.1	bdl
#149/123	0.9 ± 0.4	0.5 ± 0.4	bdl	1.5 ± 0.0
#156	0.6	bdl	bdl	bdl
#157/173/201	0.3	0.7	bdl	5.6 ± 3.5
#167	1.6 ± 0.9	0.2 ± 0.0	bdl	bdl
#189	bdl	bdl	bdl	bdl
PCB-total	167.7 ± 138.3	44.8 ± 5.6	32.3 ± 2.5	104 ± 50.5
PBDE				
#100	bdl	bdl	bdl	33.7
#116	bdl	bdl	bdl	bdl
#115	bdl	bdl	bdl	bdl
#28	bdl	bdl	bdl	bdl
#47	1.5 ± 0.5	0.6 ± 0.1	3.1 ± 0.2	29.4
#49	bdl	bdl	bdl	0.6
#66	bdl	bdl	bdl	bdl
#99	bdl	bdl	bdl	34.5
Dioxins/Furans				
1,2,3,4,6,7,8-HpCDD	bdl	bdl	bdl	0.4
1,2,3,7,8,9-HxCDD	bdl	bdl	bdl	bdl
2,3,7,8-TCDF	bdl	bdl	bdl	0.3
1,2,3,6,7,8-HxCDD	bdl	bdl	bdl	0.3
OCDD	0.05	0.04	bdl	1.2
OCDF	bdl	bdl	bdl	0.9
Organochlorines				
Aldrin	0.2	bdl	bdl	bdl
α-BHC	0.8 ± 0.5	0.2 ± 0.1	0.6 ± 0.1	2.6 ± 1.0
α-chlordane	2.2 ± 0.9	1.0 ± 0.1	1.3 ± 0.5	bdl
β-BHC	11.7 ± 13.2	0.3 ± 0.1	1.2 ± 0.1	3.9 ± 1.3
cis-nonachlor	2.0 ± 0.5	1.4 ± 0.3	bdl	bdl
δ-BHC	0.8	0.2	bdl	bdl
Dieldrin	1.2 ± 0.2	0.6 ± 0.2	bdl	bdl
Endosulfan II	bdl	bdl	bdl	bdl
Endrin	4.5 ± 3.4	0.9 ± 0.2	1.9 ± 0.4	bdl
γ-BHC	1.7 ± 1.3	0.3	bdl	bdl
γ-chlordane	2.3 ± 1.2	0.7 ± 0.3	bdl	bdl
HCB	3.5 ± 1.5	1.9 ± 0.5	1.9 ± 0.2	bdl
Heptachlor	2.4 ± 2.0	2.0 ± 0.5	bdl	bdl
Heptachlor epoxide	0.7 ± 0.1	0.4 ± 0.0	0.7 ± 0.1	bdl

Mirex	0.9	0.9 ± 1.1	bdl	bdl
o,p'-DDD	1.9 ± 1.4	bdl	bdl	bdl
o,p'-DDE	0.3	bdl	bdl	bdl
o,p'-DDT	2.0 ± 2.1	1.0 ± 0.7	1.1 ± 0.2	3.5
Oxychlordane	1.3 ± 0.9	0.8 ± 0.1	bdl	bdl
p,p'-DDD	3.3 ± 0.2	2.4 ± 0.7	bdl	bdl
p,p'-DDE	7.3 ± 1.0	5.1 ± 1.5	4.5 ± 1.1	2.5 ± 0.5
p,p'-DDT	5.3 ± 1.9	1.9 ± 0.5	3.6 ± 0.9	bdl
Pentachloro-anisole	1.8 ± 1.3	0.9 ± 0.2	0.8 ± 0.1	2.3 ± 0.3
Trans-nonachlor	4.0 ± 0.5	2.0 ± 0.6	1.3 ± 0.3	bdl
Organophosphates and semi-volatiles				
Chlorpyrifos	2.2 ± 2.3	0.2 ± 0.0	bdl	bdl
1,2,3,4-tetrachlorobenzene	7.0 ± 4.7	bdl	2.1 ± 2.3	2.2
1,2,4,5-tetrachlorobenzene	11.8 ± 7.1	2.5 ± 0.6	9.0 ± 5.8	29.6 ± 6.3
Metals (µg/g wet weight)				
Al	2.1 ± 0.8	1.5 ± 0.5	bdl	bdl
As	0.8 ± 0.2	0.5 ± 0.1	0.1 ± 0.0	bdl
B	bdl	bdl	bdl	bdl
Ba	0.05 ± 0.0	0.05	bdl	0.2 ± 0.1
Cd	bdl	bdl	bdl	bdl
Cr	0.8 ± 0.2	0.4 ± 0.0	0.4 ± 0.0	0.8 ± 0.7
Cu	0.4 ± 0.0	0.4 ± 0.0	5.8 ± 0.5	0.9 ± 0.1
Fe	12	9.6 ± 1.6	14.5 ± 0.3	19.1 ± 5.1
Hg	0.1 ± 0.2	0.1 ± 0.0	bdl	bdl
Mg	242 ± 5.3	277.3 ± 25.4	579.7 ± 24.8	281.3 ± 35.0
Mn	0.2 ± 0.0	0.2 ± 0.0	0.4 ± 0.0	1.2 ± 0.9
Mo	bdl	bdl	bdl	bdl
Ni	0.1 ± 0.1	0.1 ± 0.0	bdl	0.7
Pb	bdl	bdl	bdl	0.1
Se	0.4 ± 0.0	0.4 ± 0.0	2.3 ± 0.2	1.2 ± 0.3
Sr	7.3 ± 0.8	5.8 ± 2.4	3.7 ± 0.3	4.1 ± 0.8
V	bdl	bdl	bdl	bdl
Zn	10.2 ± 0.2	7.8 ± 0.6	25.1 ± 2.4	20.9 ± 1.7
Moisture				
	62.3±0.7	71.2±1.4	60.5±1.5	nd
Lipid (% wet weight)	11.6±0.4	3.1±1.3	8.6±7.2	1.6±0.2

Table 1-11. Nonessential metals (mean ± SD; µg/g wet weight) of eggs, fry and parr collected at Quinault NFH.

Hatchery	Contaminant	Age			One-way ANOVA P
		Egg	Fry	Parr	
Quinault	As	0.1 ± 0.0 [b]	bdl	0.4 ± 0.0 [a]	<0.0001
	Cd	bdl	bdl	bdl	nd
	Cu	5.8 ± 0.5 [a]	0.9 ± 0.1 [b]	0.9 ± 0.1 [b]	<0.0001
	Hg	0.01	bdl	0.02 ± 0.0	nd
	Pb	bdl	0.1	bdl	nd
	Zn	25.1 ± 2.4	20.9 ± 1.7	25.0 ± 1.5	0.0588
	∑Metals	31.0 ± 3.0 [a]	21.8 ± 1.7 [b]	26.3 ± 1.5 [ab]	0.0052

bdl=below detection limit
nd=not determined

Table 1-12. Contaminants (mean ± SD) found in eggs, fry and parr collected at Quinault. The ∑DDTs correlated to tissue lipid content, so these data were evaluated as ng/g lipid using analysis of variance. Lipid content was found to be a significant covariate for ∑Chlorinated Pesticides, and these data were analyzed using analysis of covariance. All other data were analyzed as ng/g wet weight using analysis of variance as lipid content was not found to be a significant covariate for these parameters.

Contaminant	Age			p-value
	Egg	Fry	Parr	
∑PCBs	32.3 ± 2.5	104 ± 50.5	42.5 ± 3.8	0.0480
∑BDEs	3.1 ± 0.2	61.7 ± 38.5	bdl	0.1640
∑Dioxins and Furans	bdl	2.1	bdl	nd
∑Chlorinated Benzenes	11.1 ± 8.1 [b]	30.3 ± 5.6 [a]	8.0 ± 1.2 [b]	0.0061
∑BHCs	1.6 ± 0.3	5.6 ± 2.9	3.5 ± 0.9	0.0848
∑Chlordanes	3.3 ± 0.9	bdl	2.4 ± 1.5	0.4432
∑Cyclodiene Pesticides	1.9 ± 0.4	bdl	bdl	nd
∑Chlorinated Pesticides	0.8 ± 0.1 [b]	2.3 ± 0.3 [b]	7.4 ± 0.7 [a]	0.0005
∑DDTs*	136.7 ± 66.6	268.3 ± 212.3	115.4 ± 60.9	0.3592
Moisture (% wet weight)	60.5 ± 1.5 [b]	nd	78.0 ± 0.0 [a]	<0.0001
Lipid (% wet weight)	8.6 ± 7.2	1.6 ± 0.2	1.9 ± 2.4	0.1453

*ng/g lipid
bdl=below detection limit
nd=not determined

Table 1-13. Biomagnification of contaminants through time at Quinault NFH not factoring in feed (no feed involved in egg or fry sample). These numbers were calculated based on fry contaminant level (ng/g lipid) divided by egg level and parr divided by fry. Values >1 represent concentration of contaminants and values <1 represent dilution.

	Change over time			Change over time*
Contaminant	Egg to Fry	Fry to Parr	P-value	Egg to Parr
∑PCBs	12.2 ± 2.7	0.3 ± 2.7	0.0357	4.1 ± 2.2
∑BDEs	nd	nd		nd
∑Dioxins and Furans	nd	nd		nd
∑Chlorinated Benzenes	7.9 ± 0.8	0.2 ± 0.8	0.0027	1.8 ± 0.7
∑BHCs	12.5 ± 3.0	0.5 ± 3.0	0.0459	6.4 ± 2.5
∑Chlordanes	nd	nd		nd
∑Cyclodiene Pesticides	nd	nd		nd
∑Chlorinated Pesticides	9.4 ± 1.0	2.8 ± 0.8	0.0154	9.4 ± 1.2
∑DDTs	2.0 ± 0.6	0.4 ± 0.5	0.1643	2.0 ± 0.8

nd=not determined because of non-detect values
*Just for reference

Table1-14. Contaminants in feed fed during the fish sampling period at Warm Springs NFH.

Feed size	#0	#1	#2
Contaminant (ng/g wet weight)			
PCB #105	0.45	0.10	0.46
PCB #118	0.36	0.89	0.69
PCB #149/123	1.34	0.67	1.33
PCB #156	0.48	0.49	0.59
PCB #157/173/201	0.43	0.29	0.18
PCB #167	bdl	0.38	0.69
PCB #189	bdl	0.34	bdl
∑PCBs	86.5	48.80	70.7
∑BDEs	1.57	1.32	0.94
∑Dioxins and Furans	bdl	0.07	0.10
∑Chlorinated Benzenes	8.58	2.58	4.33
∑BHCs	0.47	0.26	0.38
∑Chlordanes	4.98	2.32	3.34
∑Cyclodiene Pesticides	3.30	0.86	1.92
∑Chlorinated Pesticides	2.99	0.75	1.53
∑DDTs	12.42	8.26	8.66
Metals (µg/g wet weight)			
As	3.43	3.02	4.67
Cd	0.40	0.37	0.36
Cu	13.50	12.80	12.6
Hg	0.09	0.05	0.06
Pb	0.07	0.08	0.18
Zn	165.00	155.00	172.00
Composition (% wet weight)			
Moisture	3.9	1.2	2.4
Lipid	13.7	13.4	15.6

Table1-15. Contaminants in feed fed during the fish sampling period at Quilcene NFH.

Feed size	#0	#1	#2
Contaminant (ng/g wet weight)			
PCB #105	0.23	0.66	0.29
PCB #118	bdl	bdl	1.35
PCB #149/123	1.12	1.88	1.45
PCB #156	1.12	2.18	0.02
PCB #157/173/201	0.50	0.78	0.16
PCB #167	bdl	bdl	0.16
PCB #189	bdl	bdl	bdl
∑PCBs	79.40	90.60	68.70
∑BDEs	0.63	0.98	5.52
∑Dioxins and Furans	0.30	0.14	0.08
∑Chlorinated Benzenes	3.98	6.01	2.42
∑BHCs	1.67	2.25	0.75
∑Chlordanes	2.48	2.49	2.42
∑Cyclodiene Pesticides	1.09	3.79	0.67
∑Chlorinated Pesticides	0.83	1.09	0.70
∑DDTs	11.66	16.34	9.53
Metals (µg/g wet weight)			
As	2.25	3.77	2.69
Cd	0.42	0.26	0.42
Cu	15.5	15.6	14.90
Hg	0.01	0.02	0.05
Pb	0.30	0.46	0.17
Zn	231.00	271.00	181.00
Composition (% wet weight)			
Moisture	2.7	4.0	0.5
Lipid	18.2	16.2	12.3

Table1-16. Contaminants in feed fed during the fish sampling period at Quinault NFH.

Feed size	#0	#1	#2	#3
Contaminant (ng/g wet weight)				
PCB #105	0.79	1.04	bdl	0.61
PCB #118	0.85	1.72	0.61	1.36
PCB #149/123	0.55	6.81	0.15	0.21
PCB #156	bdl	bdl	0.16	bdl
PCB #157/173/201	0.50	0.56	0.28	0.16
PCB #167	bdl	0.08	0.84	2.23
PCB #189	bdl	bdl	bdl	bdl
∑PCBs	140.00	269.00	49.90	41.60
∑BDEs	1.01	1.02	0.54	0.28
∑Dioxins and Furans	bdl	bdl	0.03	0.16
∑Chlorinated Benzenes	2.39	2.23	0.84	0.63
∑BHCs	1.36	0.84	0.25	1.15
∑Chlordanes	34.86	6.71	2.46	0.66
∑Cyclodiene Pesticides	2.33	2.63	1.28	1.44
∑Chlorinated Pesticides	4.35	2.93	2.21	0.33
∑DDTs	12.87	11.4	3.66	3.51
Metals (µg/g wet weight)				
As	2.05	2.38	2.15	2.14
Cd	0.58	0.44	0.43	0.25
Cu	12.30	20.10	18.30	32.70
Hg	0.03	0.04	0.02	0.01
Pb	0.15	0.30	0.26	0.39
Zn	164.00	214.00	217.00	273.00
Composition (% wet weight)				
Moisture	6.3	4.5	4.3	4.8
Lipid	13.6	15.7	11.8	10.3

APPENDICES

Appendix A. Contaminants (mean ± SD) found in fish tissues collected from Warm Springs NFH (on a lipid basis).

Warm Springs				
Contaminant (ng/g lipid)	Adult-male fillet	Adult-female fillet	Egg	Unfed Fry
PCB				
#105	15.7 ± 6.7	30.2 ± 12.1	8.6 ± 2.1	35.7 ± 8.7
#118	15.5 ± 4.1	26.9 ± 4.8	17.9 ± 6.0	27.4 ± 16.4
#149/123	22.3 ± 5.7	29.5 ± 3.6	19.4 ± 5.5	27.0 ± 8.8
#156	bdl	bdl	bdl	bdl
#157/173/201	6.5 ± 1.3	7.0 ± 2.1	bdl	7.8 ± 3.2
#167	6.0 ± 5.2	bdl	10.0 ± 3.3	9.8
#189	bdl	bdl	bdl	bdl
PCB-total	1413.6 ± 414.6	2574.2 ± 369.2	849.9 ± 440.5	1936.5 ± 792.3
BDE				
#100	bdl	54.2	bdl	bdl
#47	39.7 ± 10.7	91.1 ± 49.0	22.3 ± 16.0	98.6
#99	19.2 ± 12.3	bdl	bdl	bdl
Dioxins/Furans				
1,2,3,4,6,7,8-HpCDD	bdl	bdl	0.3	bdl
1,2,3,7,8,9-HxCDD	bdl	bdl	0.3	bdl
2,3,7,8-TCDF	bdl	bdl	0.05 ± 0.01	bdl
OCDD	bdl	bdl	0.7	bdl
OCDF	bdl	bdl	0.6	bdl
Organochlorines				
Aldrin	bdl	bdl	bdl	bdl
α-BHC	bdl	bdl	bdl	bdl
α-chlordane	31.0 ± 5.0	58.1 ± 40.0	22.6 ± 9.8	52.5
β-BHC	7.0	bdl	27.2 ± 32.3	28.1
cis-nonachlor	20.2 ± 4.2	26.7 ± 10.7	13.6	bdl
Dieldrin	23.3 ± 7.7	27.6 ± 14.0	11.6 ± 7.2	bdl
Endosulfan II	bdl	bdl	bdl	bdl
Endrin	13.8 ± 5.2	20.7 ± 1.7	26.4 ± 0.9	40.7
γ-BHC	bdl	bdl	17.9	39.3
γ-chlordane	37.7 ± 17.0	53.3 ± 30.2	bdl	bdl
HCB	70.4 ± 18.0	110.4 ± 16.3	43.4 ± 11.0	56.1 ± 2.6
Heptachlor	8.8	14.9	bdl	bdl
Heptachlor epoxide	7.0 ± 4.8	18.4 ± 5.8	bdl	bdl
Mirex	13.6 ± 4.4	21.4 ± 5.3	bdl	bdl
o,p'-DDD	14.9 ± 4.4	bdl	8.4 ± 3.3	35.8
o,p'-DDE	bdl	bdl	bdl	bdl
o,p'-DDT	bdl	bdl	20.6 ± 11.3	bdl
Oxychlordane	11.2 ± 3.8	34.6 ± 28.2	bdl	bdl

p,p'-DDD	38.1 ± 7.0	68.6 ± 7.8	16.0 ± 6.5	51.7 ± 6.5
p,p'-DDE	340.6 ± 115.6	634.4 ± 149.2	152.4 ± 100.5	187.1 ± 39.6
p,p'-DDT	24.2 ± 9.1	25.6 ± 5.5	17.2 ± 7.5	45.8 ± 12.9
Pentachloro-anisole	10.5 ± 4.2	18.5 ± 0.3	14.1 ± 7.7	35.8 ± 17.0
Trans-nonachlor	75.8 ± 25.4	126.3 ± 39.2	22.4 ± 8.4	42.9 ± 15.9

Appendix B. Warm Springs NFH adult male and female spring Chinook, egg and unfed fry contaminant analyses (mean ± SD).

Factor	Fillet-adult male	Fillet-adult female	Egg	Unfed Fry
Contaminant (ng/g lipid)				
∑PCBs	1413.6 ± 414.6	2574.2 ± 369.1	849.9 ± 440.5	1936.5 ± 792.3
∑BDEs	39.7 ± 10.7	91.1 ± 49.0	22.3 ± 16.0	98.6
∑Dioxins and Furans	BDL*	BDL	1.2	BDL
∑Chlorinated Benzenes	52.6 ± 40.7	94.2 ± 24.7	172.9 ± 131.7	404.8 ± 468.0
∑BHCs	7.0	BDL	36.1 ± 19.6	33.7 ± 7.9
∑Chlordanes	172.4 ± 49.9	287.7 ± 121.0	49.5 ± 20.6	60.5 ± 45.5
∑Cyclodiene Pesticides	37.1 ± 9.2	48.3 ± 15.6	29.2 ± 19.3	40.7
∑Chlorinated Pesticides	24.1 ± 8.0	26.6 ± 9.2	14.1 ± 7.7	70.8 ± 44.3
∑DDTs	412.8 ± 138.0	728.7 ± 149.0	211.7 ± 114.0	279.3 ± 47.2
Metals (µg/g wet weight)				
As	0.4 ± 0.03	0.4 ± 0.04	0.2 ± 0.0	BDL
Cd	BDL	0.02	BDL	BDL
Cu	0.6 ± 0.02	0.6 ± 0.1	3.0 ± 0.1	0.4 ± 0.0
Hg	0.1 ± 0.01	0.1 ± 0.02	0.01	BDL
Pb	BDL	BDL	BDL	BDL
Zn	10.4 ± 1.0	11.2 ± 2.6	18.9 ± 1.3	14.9 ± 0.5
Composition (% wet weight)				
Moisture	74.5 ± 2.3	77.0 ± 0.9	55.3 ± 0.7	79.1 ± 1.3
Lipid	5.2 ± 3.1	1.4 ± 0.2	12.2 ± 3.7	3.6 ± 0.8

*BDL=below detection limit

Appendix C. Contaminants (mean ± SD) found in fish tissues collected from Quilcene NFH (on a lipid basis).

Quilcene

Contaminant (ng/g lipid)	Adult-male fillet	Adult-female fillet	Egg	Unfed Fry
PCB				
#105	3.9 ± 0.5	20.8 ± 12.6	7.9 ± 3.4	57.2 ± 17.8
#118	21.6 ± 9.6	53.5 ± 13.1	51.3 ± 61.5	bdl
#149/123	17.6 ± 8.0	42.8 ± 8.7	14.2	bdl
#156	bdl	bdl	bdl	bdl
#157/173/201	7.9 ± 8.5	12.0 ± 3.8	bdl	4.8
#167	2.1	bdl	bdl	bdl
#189	bdl	10.8	bdl	bdl
PCB-total	969.9 ± 159.0	2627.0 ± 454.8	2101.2 ± 1389.0	4122.2 ± 755.1
BDE				
#100	15.0 ± 5.6	29.0 ± 4.2	bdl	bdl
#116	bdl	290.2	bdl	bdl
#15	bdl	893.4	bdl	bdl
#28	2.8	bdl	bdl	bdl
#47	49.7 ± 15.7	147.3 ± 48.6	119.8 ± 82.8	bdl
#49	8.7	bdl	bdl	bdl
#66	2.4	bdl	bdl	bdl
#99	16.1 ± 5.4	84.1± 43.6	bdl	bdl
Dioxins/Furans				
1,2,3,4,6,7,8-HpCDD	bdl	bdl	bdl	bdl
1,2,3,7,8,9-HxCDD	bdl	bdl	bdl	bdl
2,3,7,8-TCDF	bdl	bdl	bdl	bdl
OCDD	2.0	bdl	bdl	bdl
OCDF	0.4 ± 0.1	bdl	bdl	bdl
Organochlorines				
Aldrin	bdl	bdl	bdl	bdl
α-BHC	5.0 ± 6.4	4.6	bdl	bdl
α-chlordane	8.6 ± 3.4	15.1 ± 8.2	42.2 ± 26.4	bdl
β-BHC	16.9 ± 8.8	43.7 ± 2.8	208.5 ± 68.8	376.4 ± 233.2
cis-nonachlor	12.2 ± 2.7	39.4 ± 8.4	bdl	bdl
Dieldrin	20.7 ± 4.1	49.9 ± 21.0	bdl	bdl
Endosulfan II	8.8	bdl	bdl	bdl
Endrin	24.7 ± 20.6	16.6 ± 0.7	bdl	bdl
γ-BHC	6.8	5.0	bdl	bdl
γ-chlordane	16.0 ± 4.1	61.1 ± 25.2	47.5 ±17.5	bdl
HCB	28.2 ± 2.7	67.2 ± 8.3	35.2 ± 11.4	bdl
Heptachlor	15.1 ± 4.7	42.9 ± 23.3	bdl	bdl
Heptachlor epoxide	4.5 ± 6.1	10.8 ± 5.1	bdl	bdl
Mirex	4.5 ± 2.8	13.2 ± 8.7	bdl	bdl

o,p'-DDD	10.3 ± 8.1	bdl	bdl	bdl
o,p'-DDE	4.6	bdl	92.9	bdl
o,p'-DDT	28.0	bdl	bdl	bdl
Oxychlordane	11.5 ± 4.7	58.8 ± 14.2	bdl	bdl
p,p'-DDD	36.7 ± 14.4	91.0 ± 21.2	bdl	bdl
p,p'-DDE	144.4 ± 17.4	271.0 ± 49.9	225.9 ± 76.4	200.8 ± 25.3
p,p'-DDT	17.6 ± 8.4	68.1 ± 40.5	35.0	69.3
Pentachloro-anisole	18.1 ± 2.5	32.6 ± 5.7	29.6 ± 11.1	98.8 ± 12.9
Trans-nonachlor	38.9 ± 13.9	73.9 ± 18.8	49.8	bdl

Appendix D. Quilcene NFH adult male and female coho, egg and unfed fry contaminant analyses (mean ± SD).

Factor	Fillet-adult male	Fillet-adult female	Egg	Unfed Fry
Contaminant (ng/g lipid)				
∑PCBs	969.9 ± 159.0	2627.0 ± 454.8	2102.2 ± 1389.0	4122.2 ± 755.1
∑BDEs	75.1 ± 39.8	645.3 ± 755.5	56.4 ± 66.9	BDL
∑Dioxins and Furans	1.0 ± 0.9	BDL*	BDL	BDL
∑Chlorinated Benzenes	71.1 ± 23.3	149.2 ± 37.9	949.0 ± 422.9	2088.9 ± 106.8
∑BHCs	25.1 ± 12.6	51.1 ± 10.0	233.8 ± 76.9	447.0 ± 246.2
∑Chlordanes	87.2 ± 15.6	238.2 ± 64.5	76.4 ± 81.7	BDL
∑Cyclodiene Pesticides	45.3 ± 24.8	66.5 ± 21.2	BDL	BDL
∑Chlorinated Pesticides	40.6 ± 10.3	144.6 ± 41.5	29.6 ± 11.1	174.7 ± 131.4
∑DDTs	216.3 ± 43.2	430.0 ± 96.4	268.5 ± 145.8	223.9 ± 39.8
Metals (µg/g wet weight)				
As	0.7 ± 0.2	0.3 ± 0.0	0.2 ± 0.0	BDL
Cd	BDL	BDL	BDL	BDL
Cu	0.6 ± 0.1	0.6 ± 0.0	4.7 ± 0.3	0.8 ± 0.1
Hg	0.1 ± 0.01	0.1 ± 0.01	BDL	BDL
Pb	BDL	BDL	BDL	BDL
Zn	8.4 ± 1.3	8.1 ± 1.5	25.8 ± 1.1	24.8 ± 3.8
Composition (% wet weight)				
Moisture	70.3 ± 1.7	75.0 ± 2.5	61.7 ± 1.0	79.8 ± 1.0
Lipid	10.9 ± 1.3	1.9 ± 0.6	4.3 ± 1.7	2.1 ± 0.4

*BDL =below detection limit

Appendix E. Contaminants (mean ± SD) found in fish tissues collected from Quinault NFH (on a lipid basis).

Quinault

Contaminant (ng/g lipid)	Adult-male fillet	Adult-female fillet	Egg	Unfed Fry
PCB				
#105	3.5 ± 1.6	14.5 ± 7.7	9.3	200
#118	10.8 ± 2.3	31.1 ± 7.1	8.4 ± 4.5	bdl
#149/123	7.8 ± 3.6	13.2 ± 8.0	bdl	89.3 ± 12.3
#156	5.1	bdl	bdl	bdl
#157/173/201	2.9	41.9	bdl	353.0 ± 250.7
#167	13.8 ± 8.2	6.5 ± 0.7	bdl	bdl
#189	bdl	bdl	bdl	bdl
PCB-total	1467.2 ± 1258.7	1633.7 ± 713.9	545.4 ± 304.4	6621.8 ± 3579.1
BDE				
#100	bdl	bdl	bdl	2246.7
#116	bdl	bdl	bdl	bdl
#115	bdl	bdl	bdl	bdl
#28	bdl	bdl	bdl	bdl
#47	13.0 ± 4.6	24.1 ± 12.0	52.4 ± 49.2	1960.0
#49	bdl	bdl	bdl	1720.0
#66	bdl	bdl	bdl	bdl
#99	bdl	bdl	bdl	1906.1
Dioxins/Furans				
1,2,3,4,6,7,8-HpCDD	bdl	bdl	bdl	23.8
1,2,3,7,8,9-HxCDD	bdl	bdl	bdl	bdl
1,2,3,6,7,8-HxCDD	bdl	bdl	bdl	18.3
2,3,7,8-TCDF	bdl	bdl	bdl	18.1
OCDD	0.4	1.1	bdl	80
OCDF	bdl	bdl	bdl	60.3
Organochlorines				
Aldrin	1.9	bdl	bdl	bdl
α-BHC	7.3 ± 4.6	8.5 ± 7.7	10.6 ± 10.1	171.9 ± 70.0
α-chlordane	19.4 ± 8.8	38.4 ± 20.8	25.9 ± 22.5	bdl
β-BHC	102.5 ± 119.0	14.6 ± 9.1	21.7 ± 13.4	248.1 ± 102.8
cis-nonachlor	17.3 ± 4.3	49.5 ± 11.9	bdl	bdl
δ BHC	6.7	5.2	bdl	bdl
Dieldrin	10.0 ± 1.6	22.3 ± 12.6	bdl	bdl
Endosulfan II	bdl	bdl	bdl	bdl
Endrin	39.5 ± 31.3	36.6 ± 24.7	22.1 ± 12.6	bdl
γ-BHC	14.8 ± 11.5	9.8	bdl	bdl
γ-chlordane	19.8 ± 10.8	22.3 ± 1.1	bdl	bdl
HCB	30.1 ± 12.6	66.7 ± 14.1	31.8 ± 17.8	bdl
Heptachlor	20.2 ± 17.1	78.8 ± 57.8	bdl	bdl

Heptachlor epoxide	5.9 ± 0.9	13.8 ± 5.9	12.8 ± 9.3	bdl
Mirex	8.4	23.5 ± 22.3	bdl	bdl
o,p'-DDD	16.2 ± 11.9	bdl	bdl	bdl
o,p'-DDE	2.9	bdl	bdl	bdl
o,p'-DDT	17.8 ± 19.3	29.8 ± 13.2	16.3 ± 13.1	230.3
Oxychlordane	11.4 ± 7.5	30.2 ± 10.0	bdl	bdl
p,p'-DDD	28.8 ± 3.1	79.5 ± 10.1	bdl	bdl
p,p'-DDE	63.3 ± 10.3	171.7 ± 28.9	70.4 ± 35.4	153.2 ± 49.4
p,p'-DDT	45.1 ± 15.8	63.5 ± 15.4	55.4 ± 25.1	bdl
Pentachloro-anisole	15.2 ± 10.7	31.5 ± 14.3	14.5 ± 9.5	141.0 ± 36.0
Trans-nonachlor	34.1 ± 4.7	67.7 ± 10.0	22.7 ± 17.4	bdl

Appendix F. Quinault NFH adult male and female steelhead, egg and unfed fry contaminant analyses (mean ± SD).

Factor	Fillet-adult male	Fillet-adult female	Egg	Unfed Fry
Contaminant (ng/g lipid)				
∑PCBs	1467.2 ± 1258.7	1633.7 ± 713.9	545.4 ± 304.4	6621.8 ± 3579.1
∑BDEs	13.0 ± 4.6	24.1 ± 12.0	34.9 ± 46.1	4206.7
∑Dioxins and Furans	0.4	1.1	BDL*	140.3
∑Chlorinated Benzenes	143.4 ± 111.7	99.6 ± 64.0	241.4 ± 268.3	1907.8 ± 484.5
∑BHCs	124.5 ± 134.3	20.3 ± 16.1	28.8 ± 20.2	362.6 ± 210.6
∑Chlordanes	114.7 ± 1.8	270.5 ± 105.2	61.4 ± 49.2	BDL
∑Cyclodiene Pesticides	36.9 ± 32.8	58.9 ± 36.9	22.1 ± 12.6	BDL
∑Chlorinated Pesticides	37.3 ± 14.7	60.2 ± 24.1	14.5 ± 9.5	141.0 ± 36.0
∑DDTs	172.2 ± 37.5	344.5 ± 44.9	136.7 ± 66.6	268.3 ± 212.3
Metal (µg/g wet weight)				
As	0.8 ± 0.2	0.5 ± 0.1	0.1 ± 0.0	BDL
Cd	BDL	BDL	BDL	BDL
Cu	0.4 ± 0.02	0.4 ± 0.02	5.8 ± 0.5	0.9 ± 0.1
Hg	0.1 ± 0.03	0.1 ± 0.02	0.01	BDL
Pb	BDL	BDL	BDL	0.1
Zn	10.2 ± 0.2	7.8 ± 0.6	25.1 ± 2.4	20.9 ± 1.7
Composition (% wet weight)				
Moisture	62.3 ± 0.7	71.2 ± 1.4	60.5 ± 1.5	ndl[1]
Lipid	11.6 ± 0.4	3.1 ± 1.3	8.6 ± 7.2	1.6 ± 0.2

*BDL=below detection limit
[1]nd=not done

Appendix G. Warm Springs NFH fish food chemical concentrations.

Factor	#0	#1	#2	1.2 mm	2.0 mm
Contaminant (ng/g lipid)					
PCB #105	3.10	0.73	2.64	0.49	1.66
PCB #118	2.66	6.65	4.73	7.43	bdl
PCB #149/123	9.78	5.03	8.25	6.05	1.72
PCB #156	3.49	3.66	3.83	5.15	bdl
PCB #157/173/201	3.17	2.15	1.14	1.30	1.71
PCB #167	bdl	2.84	5.27	5.99	bdl
PCB #189	bdl	2.51	bdl	bdl	bdl
∑PCBs	511.60	284.33	501.95	433.82	298.19
∑BDEs	6.08	9.85	8.71	8.38	3.89
∑Dioxins and Furans	bdl	0.50	0.73	0.56	bdl
∑Chlorinated Benzenes	20.28	19.23	50.32	13.13	10.49
∑BHCs	1.65	1.96	3.45	4.63	5.28
∑Chlordanes	23.45	17.34	27.48	18.60	2.14
∑Cyclodiene Pesticides	13.86	6.45	17.15	10.01	12.87
∑Chlorinated Pesticides	13.43	5.58	13.35	5.21	6.65
∑DDTs	65.16	61.65	66.40	92.54	47.24
Metals (µg/g wet weight)					
As	3.43	3.02	4.67	4.26	1.75
Cd	0.40	0.37	0.36	0.31	0.44
Cu	13.50	12.80	12.6	11.00	10.42
Hg	0.09	0.05	0.06	0.05	bdl
Pb	0.07	0.08	0.18	0.13	0.22
Zn	165.00	155.00	172.00	170.00	85.05
Composition (% wet weight)					
Moisture	3.9	1.2	2.4	3.0	13.1
Lipid	13.7	13.4	15.6	13.6	10.8

Appendix H. Quilcene NFH fish food chemical concentrations.

Factor	#0	#1	#2	1.2 mm	2.0 mm	2.0 mm
Contaminant (ng/g lipid)						
PCB #105	1.26	4.06	2.33	0.94	1.37	1.47
PCB #118	bdl	bdl	10.98	4.17	bdl	bdl
PCB #149/123	6.15	11.60	11.79	3.77	12.22	5.61
PCB #156	6.16	13.47	0.12	bdl	bdl	bdl
PCB #157/173/201	2.75	4.80	1.29	1.36	bdl	bdl
PCB #167	bdl	bdl	1.33	4.42	bdl	bdl
PCB #189	bdl	bdl	bdl	0.27	bdl	bdl
∑PCBs	436.26	559.26	558.54	390.27	328.85	225.00
∑BDEs	3.45	6.05	44.88	12.57	bdl	bdl
∑Dioxins and Furans	1.65	0.87	0.62	1.98	bdl	bdl
∑Chlorinated Benzenes	21.84	37.09	19.67	18.94	16.89	20.38
∑BHCs	9.17	13.89	6.11	9.96	9.87	5.97
∑Chlordanes	13.62	15.36	19.71	12.18	16.64	5.00
∑Cyclodiene Pesticides	5.98	23.40	5.46	4.12	22.01	7.48
∑Chlorinated Pesticides	4.56	6.70	5.72	10.38	11.90	17.02
∑DDTs	64.07	100.86	77.48	79.44	27.21	27.74
Metals (µg/g wet weight)						
As	2.25	3.77	2.69	1.79	1.63	1.84
Cd	0.42	0.26	0.42	0.34	0.37	0.40
Cu	15.5	15.6	14.90	15.40	13.90	12.40
Hg	0.01	0.02	0.05	0.07	bdl	bdl
Pb	0.30	0.46	0.17	0.16	0.25	0.19
Zn	231.00	271.00	181.00	105.00	98.85	94.60
Composition (% wet weight)						
Moisture	2.7	4.0	0.5	4.5	4.3	4.6
Lipid	18.2	16.2	12.3	11.3	9.7	11.6

Appendix I. Quinault NFH fish food chemical concentrations.

Factor	#0	#1	#2	#3	#4	2.0 mm	3.0 mm
Contaminant (ng/g lipid)							
PCB #105	5.82	6.62	bdl	5.93	3.40	0.88	6.01
PCB #118	6.22	10.96	5.14	13.20	bdl	10.8	bdl
PCB #149/123	4.01	43.38	1.26	1.99	4.47	8.98	3.91
PCB #156	bdl	bdl	1.37	bdl	bdl	bdl	bdl
PCB #157/173/201	3.71	3.57	2.40	1.56	3.77	2.24	0.70
PCB #167	bdl	0.51	7.10	21.65	17.26	bdl	bdl
PCB #189	bdl	bdl	bdl	bdl	bdl	1.35	bdl
∑PCBs	1029.41	1713.38	422.88	403.88	436.19	389.25	202.13
∑BDEs	7.43	6.50	2.97	2.72	3.66	11.81	3.06
∑Dioxins and Furans	bdl	bdl	0.28	1.57	0.74	bdl	0.26
∑Chlorinated Benzenes	17.56	14.19	7.11	6.09	24.95	10.80	14.17
∑BHCs	9.99	5.34	2.10	11.17	5.65	9.32	1.19
∑Chlordanes	256.32	42.73	20.83	6.37	24.99	33.36	9.50
∑Cyclodiene Pesticides	17.10	16.78	10.87	13.98	19.53	11.02	4.30
∑Chlorinated Pesticides	31.95	18.66	18.75	3.18	21.97	18.86	20.83
∑DDTs	94.62	72.61	30.99	34.03	36.20	102.74	111.29
Metals (µg/g wet weight)							
As	2.05	2.38	2.15	2.14	2.00	2.11	2.05
Cd	0.58	0.44	0.43	0.25	0.23	0.20	0.22
Cu	12.30	20.10	18.30	32.70	8.67	8.08	7.43
Hg	0.03	0.04	0.02	0.01	0.01	bdl	bdl
Pb	0.15	0.30	0.26	0.39	0.15	2.13	0.28
Zn	164.00	214.00	217.00	273.00	80.40	126.00	108.00
Composition (% wet weight)							
Moisture	6.3	4.5	4.3	4.8	3.4	6.0	6.0
Lipid	13.6	15.7	11.8	10.3	9.6	14.1	12.4

CHAPTER 2: Tissue-specific contaminant burden and gene expression in Pacific salmonids after simulated release from three National Fish Hatcheries

INTRODUCTION

Over the past several decades it has become increasingly evident that feeds used in aquaculture worldwide contain significant concentrations of contaminants (Mac et al. 1979; Hilton et al. 1983; Rappe et al. 1998; Hites et al. 2004; Maule et al. 2007). Contaminants can enter fish feeds from a variety of sources, but generally reflect global contaminant inputs into oceans and eventually into marine food webs, which are the main sources of fish oil and fish meal used in fish feed (Horst et al. 1998). Organisms at higher trophic levels typically have higher levels of organochlorines (OCs) [e.g., polychlorinated biphenyl (PCBs), dioxins and furans, and many pesticides] due to biomagnification through the food web (Muir et al. 1992; Gobas et al. 1999). Diets that contain a high percentage of pelagic ocean fish meal and oil will likely contain higher amounts of contaminants of global concern, such as PCB congeners, dichlorodiphenyltrichloroethane (DDT) metabolites, and the heavy metal mercury. Hatchery-reared fish consuming feeds made from oils and meals derived from marine fish may accumulate these contaminants, thus placing some hatchery-reared fish at a higher trophic level than their wild counterparts that are consuming a natural diet. Feed contaminated with metals or OCs may disrupt endocrine function in hatchery-reared fingerlings and smolts, which could adversely affect their survival and reproductive success (Hilton et al. 1983; Macek 1968; Jørgensen et al. 2002). In a recent study (Maule et al. 2007), we found that all of the feed samples (collected from October 2001 to October 2003) at 11 cold-water U.S. Fish and Wildlife Service National Fish Hatcheries (NFH) across four regions in the United States contained measurable concentrations of at least one dioxin, furan, PCB congener, or DDT metabolite, and most contained more than one. The most commonly detected contaminants were PCBs (Maule et al. 2007). While contaminant concentrations were generally lower than previous reports, the ecological impacts of these compounds cannot be determined without a measure of their bioaccumulation in the fish and the fate of these compounds after the fish are released from the hatcheries (Maule et al. 2007).

Well-fed fish will accumulate lipophilic OCs in fat depots in muscle and viscera where the toxic effects are muted (Jørgensen et al. 1997; 1999). When fish stop feeding, however, the lipids are mobilized as an energy source, and the OCs are redeposited in vital organs (e.g., brain, liver, heart, and kidney). Recent work with Arctic char (*Salvelinus alpinus*) dramatically illustrates the impacts of this mobilization of OCs on physiological processes. Anadromous char normally feed for only 6 to 8 weeks in the ocean, in some locations accumulating OCs (Jørgensen et al. 1999; 2002). Upon returning to freshwater, the char fast for the remaining 10 months of the year. In the

earlier studies, char contaminated with PCBs were fasted or fed for five months, and physiological responses and the organ distribution of PCBs were measured. Contaminated char had impaired responses to stress and survival in saltwater. The fish also had reduced growth and reduced immune responses leading to decreased disease resistance (Jørgensen et al. 2002, 2004; Maule et al. 2005). Fasting played a significant role in these responses to PCB contamination and impacted metabolic processes in char liver (Vijayan et al. 2006). Disrupted hormone-receptor interactions in the brains of PCB-treated, fasted fish, but not fed fish, appeared to be a mechanism driving these physiological dysfunctions (Aluru et al. 2004). More recently, Wiseman et al. (2011) identified alterations in expression of several stress-related proteins in the livers of PCB-contaminated char in the wild. These results suggest strongly that PCBs and probably other OCs can reduce the fitness and survival of fish in the wild by altering a number of physiological systems, most likely by changing the expression of genes and their associated proteins.

In the Pacific Northwest, anadromous salmonids are raised in hatcheries for 6 to 18 months when they are released to migrate to the ocean. Survival of these hatchery fish to adulthood may be < 0.1% as compared to estimated survival of > 2% and as high as 10% in some wild salmon stocks (Williams and Smith 2001; Sandford et al. 2002). During the period of saltwater preadaption (i.e., smoltification) lipid depots in salmon are depleted (Sheridan et al. 1983; 1985; Sheridan 1989), and upon release from hatcheries, these salmon may not feed initially as they adjust to the new environment and food resources in rivers and streams (Olla et al. 1998). We hypothesize that OCs in fat depots would be mobilized and redeposited in organs where they could impair physiological functions necessary for survival (e.g., responding to stresses such as dam passage, the osmotic challenge of entering the saltwater, and resisting fish pathogens). If the availability of food is reduced—for example if poor ocean upwelling reduces nutrients available for the near-shore food web—more fats will be mobilized, which will increase the deposition of contaminants in the organs and lead to greater physiological dysfunction and reduced survival. The goal of the current study was to investigate more comprehensively the issue of contaminants in feeds and fish, including the mobilization and redeposition of contaminants to various fish tissues. Our objectives in this research were to (1) determine if contaminants shown to bioaccumulate in hatchery fish (Chapter 1, this report) were deposited in organs (brain and liver), as well as lipid tissues; (2) assess whether the contaminants in lipid tissues were mobilized and redeposited in organs in fasted as compared to fed fish; and (3) determine if there was differential expression of genes known to be affected by contaminant exposure in the livers of fasted fish as compared to fed fish.

METHODS

Fish maintenance, treatment and sampling.—

In this study, we used three species of Pacific salmon each raised in a different hatchery. Spring Chinook salmon (*Oncorhynchus tshawytscha*) were raised at Warm Springs NFH (WS NFH), Oregon; coho salmon (*O. kisutch*) were raised at Quilcene (QC NFH), Washington; and steelhead (*O. mykiss*) were raised at Quinault (QN NFH), Washington. Conditions under which these fish were raised are presented in Chapter 1. All fish were raised for about 18 months before the time in 2008 when they would normally have been released from the hatchery for their seaward migration—April 8 for WS NFH, April 24 for QC NFH and April 25 for QN NFH. At that time, we randomly collected fish from the general hatchery populations and put them into four smaller troughs, 100 fish per trough. Fish in half of the troughs continued to be fed as they had been in the hatchery and the rest of the fish were fasted for three weeks. After 14 and 21 days, we took 30 fish from each tank of fed and fasted fish, put them in a lethal dose of MS222 (200 mg/l), weighed (to the nearest 0.1 g), measured (to the nearest mm) and dissected them to remove livers and brains. A portion of liver was collected for gene expression analysis (see below). We also created three pools of 20 samples each of brains, remaining portions of livers and remaining carcasses to determine contaminant loads in each tissue. We assayed for the following congener-specific compounds: polychlorinated dibenzo-p-dioxins (dioxins), polychlorinated dibenzofurans (furans), PCBs, polybrominated diphenyl ethers (PBDEs), organochlorine pesticides (including dichlorodiphenyltrichloroethane (DDT) and metabolites) and metals (including mercury, cadmium, selenium and arsenic) as described in Chapter 1.

Contaminant data analyses.—

Results for each chemical assay contained many values that were below detection limits (< DL). As with our previous study (Maule et al. 2007), we did not consider those values in our analyses; that is, we did not prorate the value as a portion of DL nor did we include it as a zero. We used a variety of analyses in order to tease out the relations between the contaminants data and the four treatment groups—fish fed for 14 or 21 d, or fasted for 14 or 21 d (Fed14, Fed21, Fast14, Fast21). Because of the large dataset, we combined congeners and some classes of chemicals into the following groups: dioxins, furans, PCBs, PBDEs, chlorinated benzenes (tetrachlorobenzene 1,2,4,5; tetrachlorobenzene 1,2,3,4; pentachlorobenzene; hexachlorobenzene), hexachlorocyclohexanes (HCH) (α-HCH, β-HCH, γ-HCH, δ-HCH), chlordane-related compounds (heptachlor, hepatachlor epoxide, oxychlordane, α-chlordane, γ-chlordane, cis-nonachlor, trans-nonachlor), other cyclodiene pesticides (aldrin, dieldrin, endrin), and other chlorinated pesticides (pentachloroanisole, chlorpyrifos, mirex, endosulfan II). Some members of these classes were kept separate, such as DDT and metabolites. We also treated the metals separately. We ran one-way general linear models (i.e., ANOVAs for unequal sample sizes) on the tissue concentrations of the chemical groups to assess differences between the four treatments (Fed14, Fed21, Fast14, and Fast21). We also conducted canonical discriminatory analyses to determine the basic relations between the contaminant groups and tissues (brain, liver and carcass). Canonical discriminatory analyses were performed separately for the lipophilic OC contaminant groups and metals. We did not make any statistical comparisons between tissue contaminant loads and gene expression because of the need to pool differing numbers of samples for

the individual assays. For the canonical discriminatory analyses, all data were log (x+1) transformed and analyzed using Statistical Analysis Software, version 9.1 (SAS, Cary, North Carolina). All other analyses were conducted using GraphPad Prism (San Diego, California). We did not make statistical comparisons between hatcheries because of the large number of uncontrolled variables (e.g., species, water source and temperature, feed source, feeding regimen).

Tissue collection and RNA extraction.—

Portions of three livers (100-150 mg) were collected and immediately placed in RNAlater (Ambion, Austin, TX). Samples were stored overnight at 4°C to allow perfusion of the tissue. The RNAlater was then removed, and the tissues were transferred to -80°C for long-term storage. Total RNA was extracted from 20-30 mg of tissue using the Qiagen RNeasy kit with addition of DNase I treatment to remove genomic DNA per the manufacturer's instructions (Qiagen, Valencia, CA). RNA concentration was determined using a BioMate 3 spectrophotometer (Thermo Electron Corporation, Madison, WI), and RNA quality was checked by agarose gel electrophoresis.

Microarray analysis.—

A low density array comprising 147 genes from the rainbow trout (*O. mykiss)* genome was used to analyze gene expression in RNA samples from fed and fasted fish. See Wiseman et al. (2007) for array details and construction. An additional gene, *cyp1a1*, was added to the original array for this study, bringing the number of genes on the array to 148. Six samples each comprised of RNA from three fish were generated for each hatchery for each time point and treatment (Fed14, Fed21, Fast 21, Fast21). A control sample for each hatchery was made by pooling an equal amount of RNA from all samples from that hatchery. See Wiseman et al. (2007) for reverse transcription of cDNA. As previously described (Wiseman et al. 2007), lambda Q bacterial mRNA was spiked into the reverse transcription reaction as a positive control. After cDNA synthesis, samples were purified with a QIAquick PCR purification kit per the manufacturer's instructions (Qiagen, Valencia, CA). Sample was recovered by eluting twice with 50µl phosphate elution buffer (4 mM KPO_4, pH 8.5 in RNase-free H2O) and then ethanol precipitated with 3 M sodium acetate, pH 5.5. cDNA pellets were resuspended, labeled with Cy3 (control) or Cy5 (experimental samples), and purified as previously described (Wiseman et al. 2007). The Cy3 and Cy5 purified samples for an array were combined into a single tube and ethanol precipitated with 3 M sodium acetate, pH 5.5. The sample was resuspended in 26µl Dig Easy Hyb buffer (Roche Applied Science, Indianapolis, IN) with 2µl calf thymus DNA and 2µl yeast tRNA and then spread on the microarray surface. Arrays were covered with a Lifterslip coverslip (Thermo Scientific, Rockford, IL) and hybridized in a hybridization oven (VWR, Radnor, PA) at 37°C for 16-18 hrs. After hybridization, arrays were washed at 37°C three times in 2 x SSC with 0.1% SDS and at room temperature twice in 1 x SSC and twice in 0.1 x SSC. Slides were dried by centrifugation at 514 x g for 2 min.

Image and data analysis.—

Microarray slides were scanned with a GenePix 4000B scanner (Molecular Devices, Sunnyvale, CA) simultaneously at 532 nm (Cy3) and 635 nm (Cy5), and images were saved in TIFF format. GenePix Pro 6.0 software (Molecular Devices, Sunnyvale, CA) was used for finding spots, and spot identification was checked manually. Poorly labeled spots were flagged by the software and eliminated from the analysis. Microarray data analysis system (MIDAS) (Saeed et al. 2003) was used for data analysis. The data were analyzed and normalized as described (Wiseman et al. 2007) with the following modifications. The data were first calculated as the ratio of normalized gene intensity for the experimental sample to that of the control sample on the same slide. This ratio was divided by the average ratio for the lambda Q positive control spots on that array to give a normalized ratio that accounted for differences in dye intensity. Gene expression ratios from the six slides from the same treatment (Fed14, Fed21, Fast14 or Fast21) were averaged. Any gene that had fewer than four expression values from the six arrays (12 values possible because each gene was duplicated on the array) was eliminated from further analysis. The average gene expression ratio from the fasted treatment (by sample date) was divided by the average gene expression ratio from the fed treatment (from the same sample date) to generate a ratio describing the fold change for each gene between fed and fasted fish at each time point. Two-sample t-tests were used to determine the significance of changes in mean expression values between fed and fasted fish. Analyses were performed with GraphPad Prism software. Any genes that had an expression value for only the fed or fasted group could not be analyzed.

Gene ontology analysis.—

Genes with a positive or negative fold change of 2.0 or greater for either 14-d or 21-d samples were analyzed for function using the Gene Ontology (GO) database (http://www.geneontology.org/). We searched for each gene in the database and selected the GO biological process that best represented the function of that gene based on its functional description in the literature.

RESULTS

Fish condition.—

Fish that were fasted for 14 and 21 d were generally of less mass than those that were fed (Figure 1A). The change in mass combined with the general lack of difference in length between fed or fasted fish (Figure 1B), resulted in the fasted fish generally having lower condition factors than the fed fish (Figure 2A). While there appear to be large differences in lipid content between tissues, 2-way ANOVA indicated that there were no treatment effects. All species, however, did have tissue differences, with brain having proportionally higher lipid content than carcass or liver (Figure 2B).

Contaminants.—

We detected virtually no dioxins or furans in any tissue samples (data not shown). The most prevalent chemicals detected were PCB congeners (Figure 3) and DDT metabolites (Figure 4), which were detected in all tissues from some fish in each hatchery. There appeared to be

treatment effects in concentrations of PCBs and DDTs—most often the fasted fish had higher concentrations than the fed fish in carcass and liver. This was most obvious for PCBs in coho salmon from QN NFH (Figure 3) and DDTs in Chinook salmon from WS NFH (Figure 4). The only PBDE congener with significant concentrations was BDE47, which was found in livers of fish from all hatcheries (Figure 5). Where significant differences were found (Chinook salmon at WS NFH), fasted fish had higher concentrations of BDE47 than did fed fish. We also detected in fish from all hatcheries various concentrations of other OC contaminants grouped as follows: chlorinated benzenes, hexachlorocyclohexanes, chlordane-related compounds, other cyclodiene pesticides, and other chlorinated pesticides (Supplemental Figures 1 – 5). Mercury was found in some tissue samples from fish in each of the three hatcheries, especially in Chinook salmon from WS NFH (Figure 6). Again, where significant differences were found, organs from fasted fish had higher Hg concentrations than those from fed fish. There were very few Hg-positive tissue samples from steelhead and virtually none from coho salmon. Most other metals analyzed were found in all tissues of fish from the three hatcheries (Supplemental Figures 6 – 16). The one notable exception was cadmium, which was only detected in the livers of Chinook salmon from WS NFH (Supplemental Figure 7). As with the OC contaminants, there were treatment differences in tissue concentrations of metals, with the fasted fish most often having significantly higher concentrations than the fed fish (Supplemental Figures 6 – 16). Canonical correlation analyses confirmed that concentrations of both OCs and metals segregated based on tissue (Figure 7). Canonical correlation analyses also segregated OCs and metals based on whether fish were fed or fasted (Figure 8), but this was inconsistent with p-values generally < 0.10.

Gene expression.—

After eliminating from consideration genes on the array that did not meet quality control standards or were only represented for one treatment, 23 to 54 genes at each time point were included in the final analysis for each hatchery (Table 2-1). Of the genes that were analyzed, steelhead at QN NFH showed the greatest transcriptional changes (Tables 2-1 and 2-2), and spring Chinook salmon at WS NFH and coho salmon at QC NFH showed few transcriptional changes after 14 or 21 days of fasting (Tables 2-1, 2-3, and 2-4).

The transcriptional changes observed in fasted coho salmon as compared to fed fish from QC NFH were predominantly in genes involved in various metabolic pathways. In fasted fish at 14 d, genes involved in lipid metabolism [apolipoprotein e (apoe) and prostaglandin d synthase (pgds)] and toxin metabolism [cytochrome p450 monooxygenase 2k1v2 (cyp2k1)] were upregulated, but a gene involved in glucose metabolism [glucokinase (gk)] was downregulated as compared to fed fish. Expression of the alpha 2 chain of type I collagen gene, col1a2, which is critical for proper bone formation was also decreased, but fasting of fish carrying a contaminant load activated the beta-actin gene, actb, which is involved in cell structure and morphogenesis, the inducible and constitutive heat shock protein 70 genes, hsp 70 and hsc70a, which are part of the stress

response, the transferrin gene, tf, whose protein product binds free iron to maintain iron homeostasis, and vepγ, which encodes vitelline envelope protein gamma, an oocyte protein that is sensitive to estrogen and estrogen mimicking compounds (Salinas et al. 2010). At 21 d, fasted steelhead at QN NFH still showed changes primarily in metabolic pathways, including metabolic regulation [cystatin (cst)], lipid and fatty acid metabolism [apoe, pgds, and fatty acid binding protein (h-fabp)], glucose metabolism [pyruvate kinase (pk)], amino acid metabolism [glutamate dehydrogenase (gdh-3)], vitamin A metabolism [retinol binding protein (rbp)], toxin metabolism [cytochrome p450 2K5 (cyp2k5)], and xenobiotic metabolism [aryl hydrocarbon receptor nuclear translocator (arnt)]. Hsp 70 and vepγ were still upregulated at 21 d along with an additional vitelline envelope protein, vepβ. Other genes in fasted fish upregulated at 21 d included genes involved in steroid hormone signaling [androgen receptor (ar)], iron homeostasis [hemopexin (thxl)], and cell morphogenesis [inhibitor of DNA binding/ differentiation 1 (id1)].

Fasted spring Chinook salmon from WS NFH primarily showed changes in genes involved in metabolism and metal ion transport and homeostasis. At 14 d, pgds and tf were downregulated greater than 4-fold, and at 21 d, both genes were still downregulated greater than 3-fold. The remaining genes with altered expression in fasted fish at WS NFH were upregulated at 21 d. These included apoe, cyp2K5, rbp, and metallothionein (mt), a gene whose protein product plays a key role in divalent metal ion homeostasis and that is induced by heavy metal exposure (Hamilton and Mehrle 1986).

At 14 d, fasted coho salmon from QC NFH showed downregulation of alpha and beta globin (hba and hbb), components of the oxygen transport protein hemoglobin. Glucose-6-phosphatase (g6pc), a gene whose protein product aids in glucose homeostasis by hydrolyzing glucose-6-phosphate to generate free glucose, was also downregulated. By 21 d, expression of the genes downregulated in fasted fish at 14 d no longer differed from fed fish, but pgds was downregulated at this time point.

DISCUSSION

In Chapter 1, we demonstrated that fish accumulated contaminants contained in fish feeds that they received. Pursuant to the first objective in our present study, here we demonstrated that the compounds were deposited in organs (i.e., brain and liver), as well as the fish carcasses. While we did not detect dioxins or furans, we did find PCB congeners (Figure 3) and DDT metabolites

(Figure 4), which are legacy contaminants, in similar concentrations (expressed as ng / g of lipid) in tissues from the three species of salmonids, considered separately. In this study, we assayed for PBDE congeners because of the proliferation of these flame-retardants in the environment (Rayne et al. 2003). A few PBDE congeners were detected randomly across the tissue samples (data not shown), but only BDE47 was detected often and this only in livers of the three species (Figure 5). Similar to PCBs and DDTs, the other five classes of OCs were detected in all tissues of the three species (Supplemental Figures 1-5). Thus we conclude that, in general, the OCs were distributed throughout the tissues of all three species.

While we do not believe it is appropriate to compare between the three different species raised in different hatcheries, it is notable that mercury was detected in virtually all of the Chinook salmon, only a few of the coho salmon and at very low levels in one steelhead (Figure 6), reflecting the presence of this metal in the fish feeds from those hatcheries (Chapter 1). Most of the other metals were detected in all tissues (Supplemental Figures 7 – 16), with the exception of Cd (found only in livers of Chinook salmon; Supplemental Figure 7), Mo (found only in livers in all species; Supplemental Figure 12) and Ni (rarely found in livers; Supplemental Figure 13). Despite the similarities in tissue concentrations of the individual OC groupings and individual metals, when OCs and metals were analyzed separately by canonical analysis, there was significant separation based on tissue (Figure 7). Even though all of the canonical analyses were highly significant ($p < 0.0001$), there was no overlap in the distribution of metals in tissues of any of the three species, while the OC canonical distributions did overlap in the Chinook and coho salmon.

The second objective of our study was to determine if compounds would be released from the lipid stores and redistributed to organs in fish that were fasted for a short time as might happen upon release from a hatchery (Olla et al. 1998). This redistribution has been documented in fish fasted for several months (Jørgensen et al. 1997; 1999) and our results confirm that this is likely to happen in shorter fasts as fish learn to eat natural food after release from a hatchery. In the present study, fasted fish were generally of less mass but the same length as fed fish (Figure 1), resulting in lower condition factor (Figure 2A). The short fast to which we exposed fish did not result in significant lipid metabolism, as there was no treatment effect in percent lipids in tissue of any species (Figure 2B). It did appear, however, that redistribution of contaminants was beginning. While in most cases there were no differences in the tissue distribution of contaminants between fed and fasted groups, where there were differences, the concentrations of compounds were almost always higher in the Fast14 or Fast21 than in Fed14 or Fed21 fish (Figures 3-5; Supplemental Figures 1-16). The opposite effect (i.e., Fed > Fast) was true in only two instances (coho salmon carcasses, Figure 3 and Supplemental Figure 1). The separation of

fed and fasted groups based on OCs and metals was also evident visually based on canonical analyses (Figure 8).

The final objective of this study was to determine if gene expression in liver changed when contaminated fish were fasted. As expected, the majority of transcriptional changes in fasted fish at all three hatcheries were associated with various metabolic pathways (Salem et al. 2007, Drew et al. 2008), but changes in other genes that are known to be associated with contaminant exposure were also observed. At QN NFH *cyp2k1, cyp2k5,* and *arnt*, genes involved in metabolism of toxins and foreign chemicals were upregulated in livers of fasted steelhead (Romagnolo et al. 2006, Zhou et al. 2010). Two vitelline envelope protein genes, which are known to be activated by estrogen and estrogen mimicking compounds (Salinas et al. 2010), were also upregulated in fasted fish. The change of expression in genes that metabolize or respond to contaminant exposure suggested that fasting increased the exposure of the liver to foreign contaminants. This was supported by evidence of significantly increased levels of organochlorines, specifically PCBs and chlorinated pesticides, in the liver of fasted steelhead at QN NFH.

Like steelhead, fasted spring Chinook salmon from WS NFH also showed upregulation of *cyp2K5* and transcriptional changes in genes involved in other metabolic pathways. Two genes involved in metal ion homeostasis, *mt* and *tf*, also showed transcriptional changes in fasted fish. Interestingly, fasted fish from WS NFH showed the most significant accumulation of metals in livers of any fish from the three hatcheries, including significant increases in arsenic, cadmium, chromium, copper, iron, mercury, molybdenum, selenium, and zinc. Few significant differences were seen in contaminant load between livers of fed and fasted coho salmon at QC NFH. Similarly, few transcriptional changes were detected and none were in genes with a clear link to contaminant exposure. The results from our analysis of gene expression suggest that increased exposure to contaminants by redistribution of these contaminants to vital organs during fasting may influence expression of genes that respond to contaminant exposure.

This investigation confirmed that a relatively short period of fasting—similar to being released from a hatchery—had physiological effects on the three salmon species. These effects included the potential to lose mass and, as a result lipid metabolism, the redistribution of contaminants from lipids to organs (brain and liver). The combination of fasting and increased contaminant loads altered the expression of genes in the liver that are important for various metabolic pathways and genes known to result from contaminant exposure and metabolism of toxins. The changes in gene expression point to metabolic costs of fasting and contaminants that may reduce bioenergetic resources available for disease resistance, predator avoidance, and the process of smoltification or other activities necessary for survival. While this investigation did not explore alternative hatchery release strategies, the results suggest that an investigation of alternatives might be prudent. For example, the use of acclimation ponds or in-stream acclimation facilities where natural food items are available might eliminate or reduce the time required for fish to

adapt to these new food sources.

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Table 2-1. Number of genes included in the final analysis for each hatchery, and number of genes up -or downregulated two-fold or greater at each time point in fish fed food with contaminants and then fasted.

Hatchery	#of genes analyzed		# of genes upregulated		# of genes downregulated	
	14 d	21 d	14 d	21 d	14 d	21 d
Quinault	45	52	8	15	2	0
Warm Springs	44	54	0	4	2	2
Quilcene	23	35	0	0	3	1

Table 2-2. List of genes up- or down-regulated in steelhead from Quinault National Fish Hatchery fed food with contaminants and then fasted for 14 or 21 days. Only genes that showed a two-fold change or greater in fasted fish as compared to fed fish at 14 or 21 d are shown. The fold change is given at both time points for all genes shown. Fold changes of two or greater are in bold. na–gene eliminated at this time point based on quality control criteria.

Gene name	Accession #	GO biological process	GO number	14 d Fold change	p-value	21 d Fold change	p-value
a2type1 collagen	AB075699	Skeletal development	0001501	**-2.033**	0.470	na	–
AhR nuclear translocator a+b	U73841	Xenobiotic metabolism	0006805	1.549	0.313	**2.068**	0.093
androgen receptor alpha + beta	AB012096	Steroid hormone receptor signaling pathway	0030518	1.689	0.253	**2.317**	0.028
apolipoprotein e	AJ132620	Lipid metabolism	0006629	**3.729**	0.075	**2.807**	0.035
beta-actin	AJ438158	Cell morphogenesis	0000902	**2.136**	0.216	-1.944	0.079
cystatin	U33555	Regulation of metabolism	0019222	1.472	0.389	**2.856**	0.230
cytochrome p450 monooxygenase 2k1v2	L11528	Toxin metabolism	0009404	**2.369**	0.072	1.799	0.075
cytochrome p450 2K5	AF151524	Toxin metabolism	0009404	1.879	0.275	**2.253**	0.045
fatty acid binding protein	U95296	Fatty acid metabolism	0006631	1.412	0.448	**2.588**	0.030
glucokinase	AF053331	Regulation of glucose metabolism	0010906	**-3.056**	0.531	na	–
glutamate dehydrogenase	AF427344	Amino acid metabolism	0006520	na	–	**2.200**	0.143
hemopexin	Z68112	Iron homeostasis	0006879	1.402	0.499	**2.815**	0.047
hsp70 constitutive	S85730	Response to stress	006950	**2.215**	0.020	1.443	0.148
hsp70 induced	K02549	Response to stress	0006950	**4.492**	0.102	**2.056**	0.082
inhibitor of DNA binding/differentiation 1	Y08368	Cell morphogenesis in differentiation	0000904	-1.615	0.589	**2.184**	0.133
prostaglandin D synthase	AF281353	Lipid metabolism	0006629	**3.176**	0.080	**3.006**	0.141
pyruvate kinase	AY113695	Glucose metabolism	0006006	1.137	0.781	**2.231**	0.040
retinol binding protein	AF503212	Vitamin A metabolism	0006776	1.511	0.387	**3.308**	0.032
transferrin	D89083	Iron ion transport	0006826	**2.013**	0.433	1.211	0.539
vitelline envelope protein beta	AF231707	Binding of sperm to Zona Pellucida	0007339	1.486	0.364	**2.472**	0.036
vitelline envelope protein gamma	AF231708	Binding of sperm to Zona Pellucida	0007339	**4.124**	0.030	**2.531**	0.033

Table 2-3. List of genes up- or down-regulated in spring Chinook salmon from Warm Springs National Fish Hatchery fed food with contaminants and then fasted for 14 or 21 days. Only genes that showed a two-fold change or greater in fasted fish as compared to fed fish at 14 or 21 d are shown. The fold change is given at both time points for all genes shown. Fold changes of two or greater are in bold. na—gene eliminated at this time point based on quality control criteria.

Gene name	Accession #	GO biological process	GO number	14 d Fold change	p-value	21 d Fold change	p-value
apolipoprotein e	AJ132620	Lipid metabolism	0006629	1.797	0.045	**2.167**	0.003
cytochrome p450 2K5	AF151524	Toxin metabolism	0009404	1.855	0.100	**2.172**	0.017
metallothionein	M18103	Metal ion homeostasis	0006875	na	–	**2.458**	0.011
prostaglandin D synthase	AF281353	Lipid metabolism	0006629	**-4.320**	0.003	**-3.022**	0.010
retinol binding protein	AF503212	Vitamin A metabolism	0006776	1.753	0.053	**2.404**	0.001
transferrin	D89083	Iron ion transport	0006826	**-4.659**	0.029	**-4.447**	0.007

Table 2-4. List of genes up- or down-regulated in coho salmon from Quilcene National Fish Hatchery fed food with contaminants and then fasted for 14 or 21 days. Only genes that showed a two-fold change or greater in fasted fish as compared to fed fish at 14 or 21 d are shown. The fold change is given at both time points. Fold changes of two or greater are in bold. na—gene eliminated at this time point based on quality control criteria.

Gene name	Accession #	GO biological process	GO number	14 d Fold change	p-value	21 d Fold change	p-value
alpha-globin	D88113	Oxygen transport	0015671	**-2.529**	0.063	-1.277	0.541
beta-globin	D82926	Oxygen transport	0015671	**-2.091**	0.084	-1.007	0.987
glucose-6-phosphatase	AF120150	Glucose homeostasis	0042593	**-2.214**	0.005	1.048	0.886
prostaglandin D synthase	AF281353	Lipid metabolism	0006629	na	–	**-2.455**	0.136

Figure Captions

Figure 1. Mean (+ 1 SE) mass (A) and fork length (B) of spring Chinook salmon at Warm Springs National Fish Hatchery (NFH), steelhead at Quinault NFH and coho salmon at Quilcene NFH after 14 and 21 days of feeding (Fed14, Fed21) or fasting (Fast14, Fast21) beginning at the time the fish would normally have been released to emigrate. Bars within each graph without a letter in common differ ($P < 0.05$) based on one-way general linear models. Bars within each graph without letters do not differ. Sample sizes were 60.

Figure 2. Mean (+ 1 SE) condition factor (K-factor) (A) and tissue-specific percent lipids (B) of spring Chinook salmon at Warm Springs National Fish Hatchery (NFH), steelhead at Quinault NFH and coho salmon at Quilcene NFH after 14 and 21 days of feeding (Fed14, Fed21) or fasting (Fast14, Fast21) beginning at the time the fish would normally have been released to emigrate. Bars within each graph without a letter in common differ ($P < 0.05$) based on one-way general linear models. Bars within each graph without letters do not differ. Sample sizes for lipids were three 20-fish pools and for K-factor were 60 fish.

Figure 3. Mean (+ 1 SE) tissue-specific total polychlorinated biphenyl (PCBs) in spring Chinook salmon at Warm Springs National Fish Hatchery (NFH), steelhead at Quinault NFH and coho salmon at Quilcene NFH after 14 and 21 days of feeding (Fed14, Fed21) or fasting (Fast14, Fast21) beginning at the time the fish would normally have been released to emigrate. Bars within each graph without a letter in common differ ($P < 0.05$) based on one-way general linear models. Bars within each graph without letters do not differ. Sample sizes were three 20-fish pools.

Figure 4. Mean (+ 1 SE) tissue-specific total dichlorodiphenyltrichloroethane (DDT) and metabolites in spring Chinook salmon at Warm Springs National Fish Hatchery (NFH), steelhead at Quinault NFH and coho salmon at Quilcene NFH after 14 and 21 days of feeding (Fed14, Fed21) or fasting (Fast14, Fast21) beginning at the time the fish would normally have been released to emigrate. Bars within each graph without a letter in common differ ($P < 0.05$) based on one-way general linear models. Bars within each graph without letters do not differ. Sample sizes were three 20-fish pools.

Figure 5. Mean (+ 1 SE) polybrominated diphenyl ether congener 47 (BDE47) in livers from spring Chinook salmon at Warm Springs National Fish Hatchery after 14 and 21 days of feeding (Fed14, Fed21) or fasting (Fast14, Fast21) beginning at the time the fish would normally have been released to emigrate. Bars within each graph without a letter in common differ ($P < 0.05$) based on one-way general linear models. Bars within each graph without letters do not differ. Sample sizes were three 20-fish pools.

Figure 6. Mean (+ 1 SE) tissue-specific mercury in spring Chinook salmon at Warm Springs National Fish Hatchery (NFH), steelhead at Quinault NFH and coho salmon at Quilcene NFH after 14 and 21 days of feeding (Fed14, Fed21) or fasting (Fast14, Fast21) beginning at the time the fish would normally have been released to emigrate. Lack of a bar means there were no samples > detection limit (DL). Bars within each graph without a letter in common differ ($P < 0.05$) based on one-way general linear models. Bars within each graph without letters do not differ. Sample sizes were three 20-fish pools.

Figure 7. Canonical discriminatory analyses of total organochlorines and metals found in various tissues in spring Chinook salmon at Warm Springs National Fish Hatchery (NFH), steelhead at Quinault NFH and coho salmon at Quilcene NFH after 14 and 21 days of feeding (Fed14, Fed21) or fasting (Fast14, Fast21) beginning at the time the fish would normally have been released to emigrate. Circles (drawn by eye) demonstrate the tissue-specific separation of contaminant concentrations. In all cases $P < 0.001$.

Figure 8. Canonical discriminatory analyses of total organochlorines and metals found in various tissues in spring Chinook salmon at Warm Springs National Fish Hatchery (NFH), steelhead at Quinault NFH and coho salmon at Quilcene NFH after 14 and 21 days of feeding (Fed14, Fed21) or fasting (Fast14, Fast21) beginning at the time the fish would normally have been released to emigrate. Circles (drawn by eye) demonstrate the separation of contaminant concentrations based on whether fish were fed or fasted.

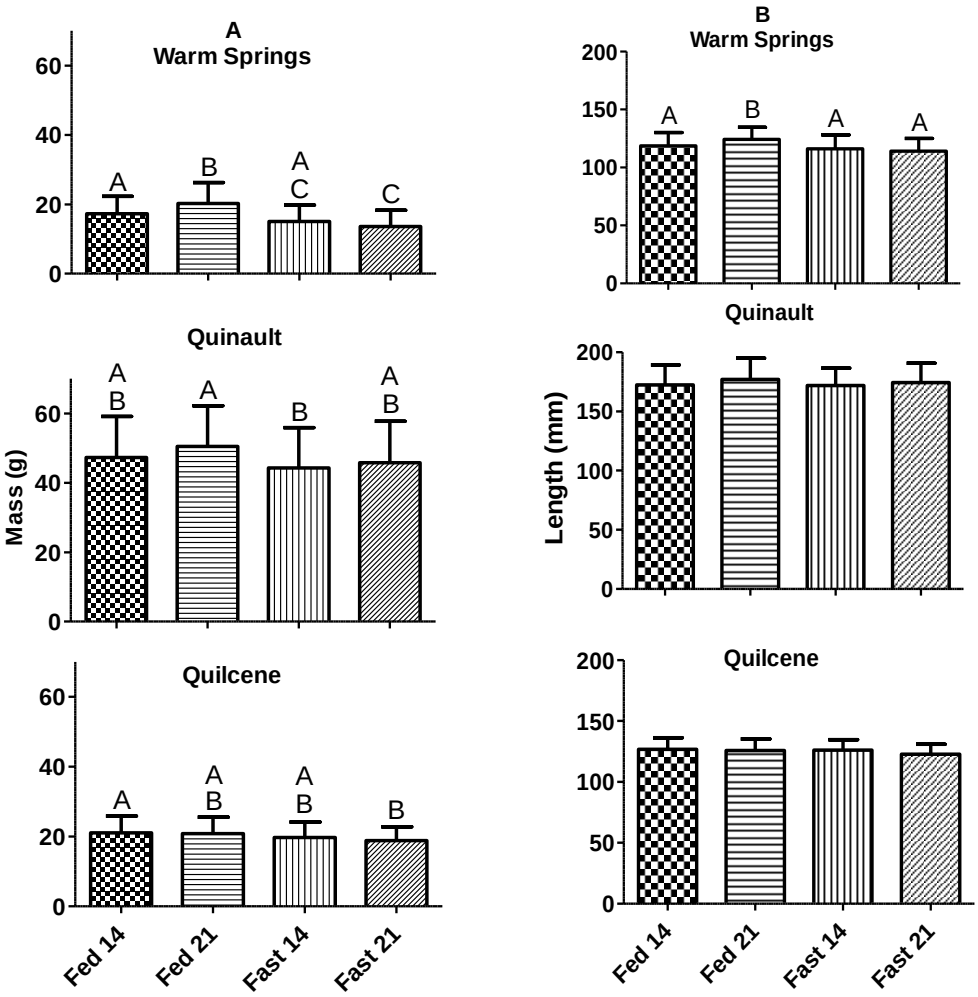


Figure 1. Mean (+ 1 SE) mass (A) and fork length (B) of spring Chinook salmon at Warm Springs National Fish Hatchery (NFH), steelhead at Quinault NFH and coho salmon at Quilcene NFH after 14 and 21 days of feeding (Fed14, Fed21) or fasting (Fast14, Fast21) beginning at the time the fish would normally have been released to emigrate. Bars within each graph without a letter in common differ ($P < 0.05$) based on one-way general linear models. Bars within each graph without letters do not differ. Sample sizes were 60.

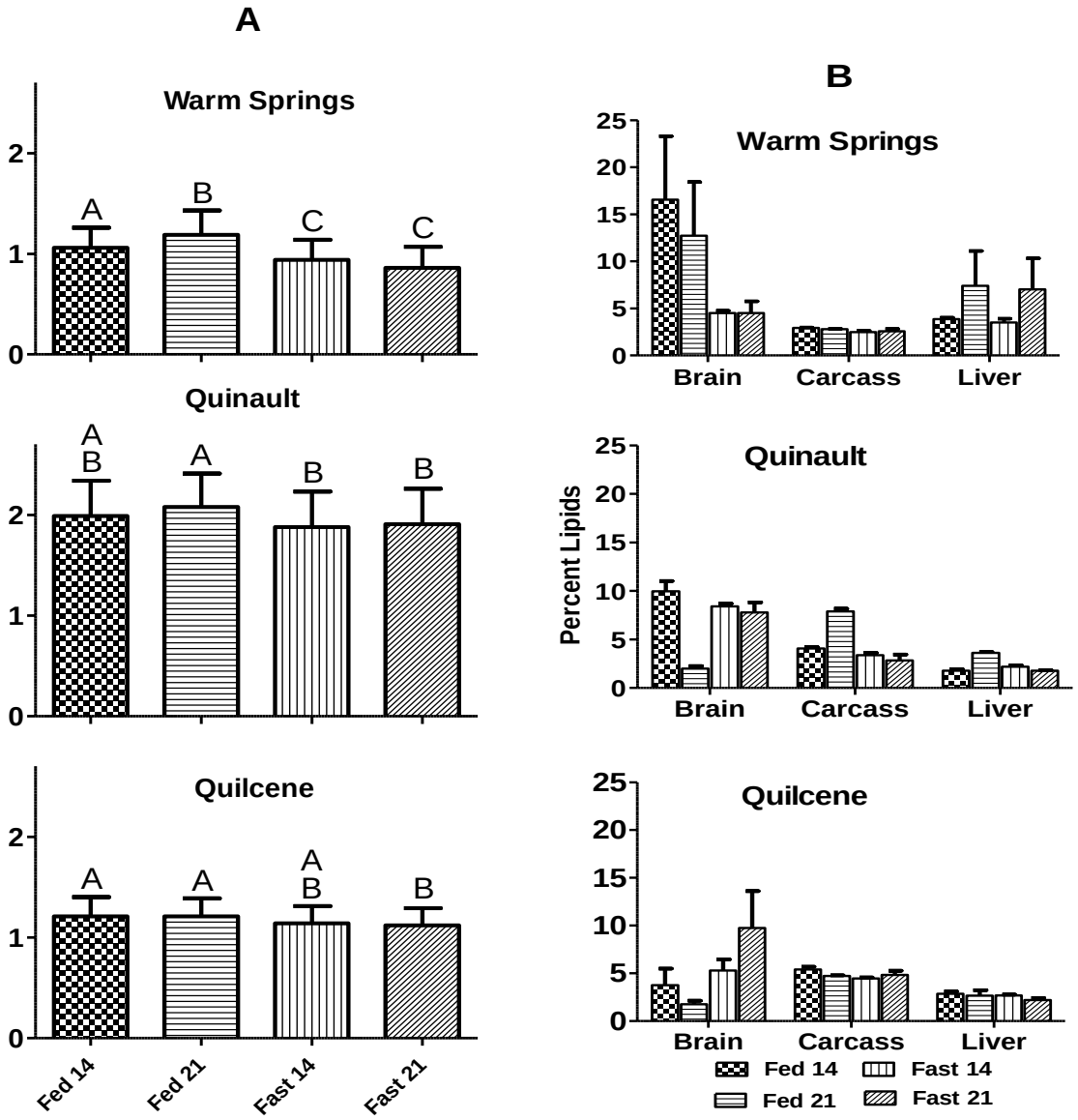


Figure 2. Mean (+ 1 SE) condition factor (K-factor) (A) and tissue-specific percent lipids (B) of spring Chinook salmon at Warm Springs National Fish Hatchery (NFH), steelhead at Quinault NFH and coho salmon at Quilcene NFH after 14 and 21 days of feeding (Fed14, Fed21) or fasting (Fast14, Fast21) beginning at the time the fish would normally have been released to emigrate. Bars within each graph without a letter in common differ ($P < 0.05$) based on one-way general linear models. Bars within each graph without letters do not differ. Sample sizes for lipids were three 20-fish pools and for K-factor were 60 fish.

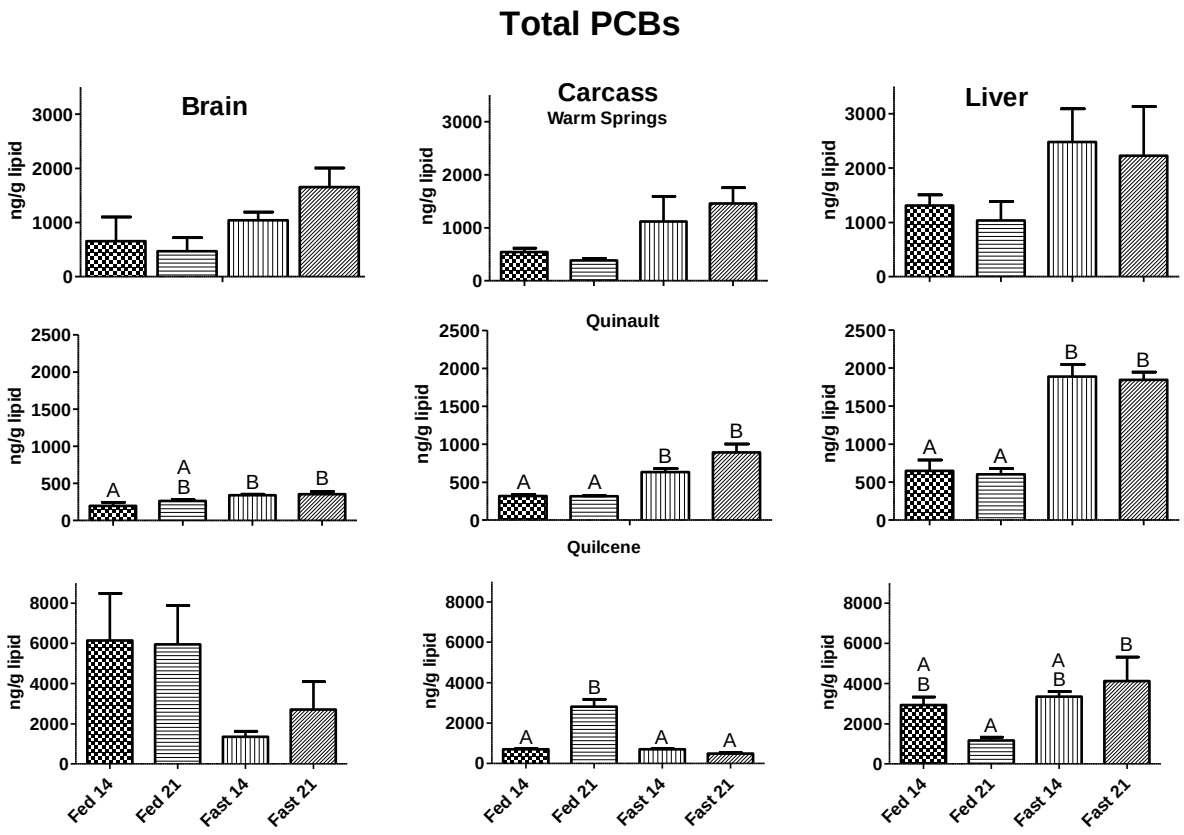


Figure 3. Mean (+ 1 SE) tissue-specific total polychlorinated biphenyl (PCBs) in spring Chinook salmon at Warm Springs National Fish Hatchery (NFH), steelhead at Quinault NFH and coho salmon at Quilcene NFH after 14 and 21 days of feeding (Fed14, Fed21) or fasting (Fast14, Fast21) beginning at the time the fish would normally have been released to emigrate. Bars within each graph without a letter in common differ ($P < 0.05$) based on one-way general linear models. Bars within each graph without letters do not differ. Sample sizes were three 20-fish pools.

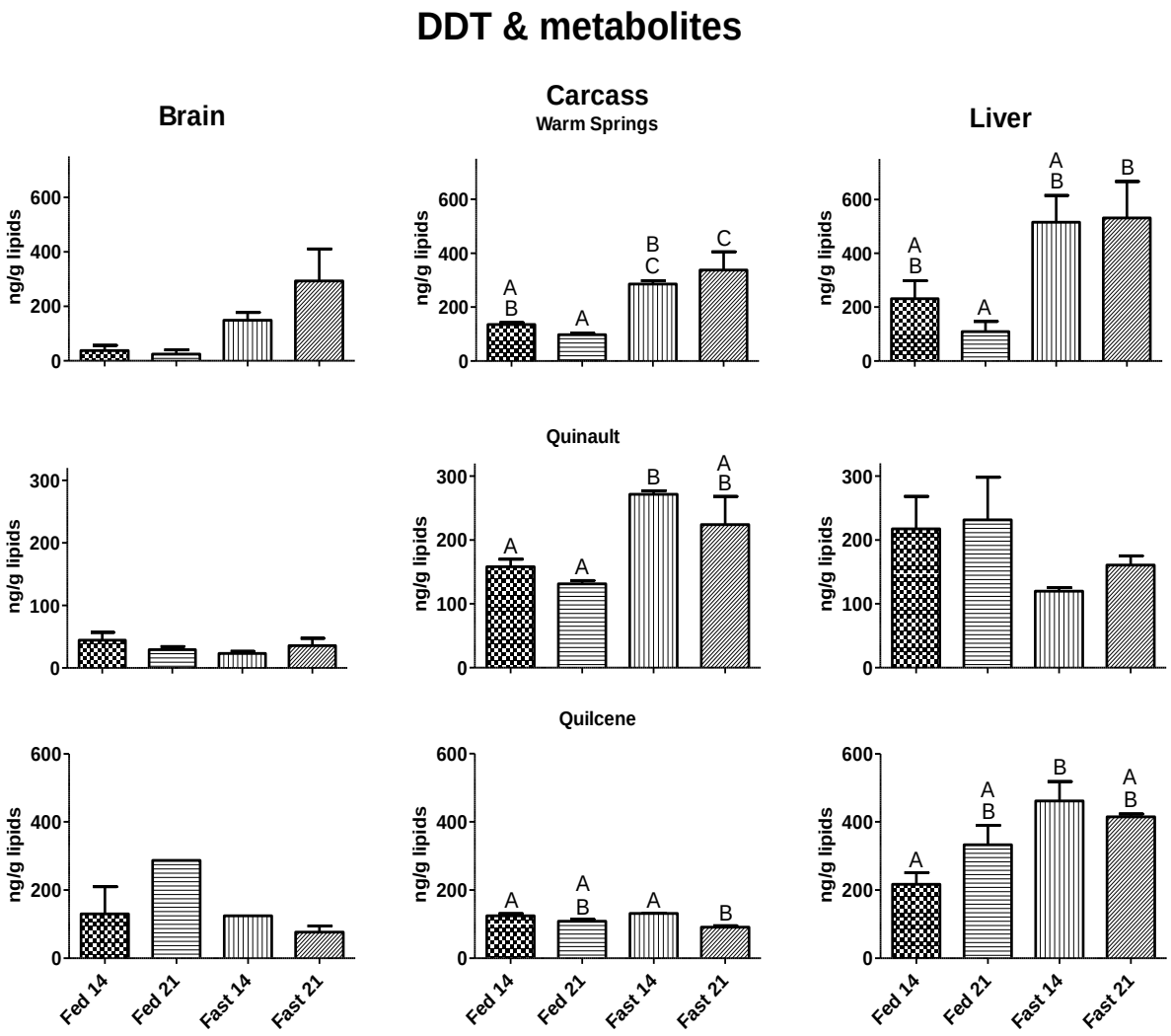


Figure 4. Mean (+ 1 SE) tissue-specific total dichlorodiphenyltrichloroethane (DDT) and metabolites in spring Chinook salmon at Warm Springs National Fish Hatchery (NFH), steelhead at Quinault NFH and coho salmon at Quilcene NFH after 14 and 21 days of feeding (Fed14, Fed21) or fasting (Fast14, Fast21) beginning at the time the fish would normally have been released to emigrate. Bars within each graph without a letter in common differ ($P < 0.05$) based on one-way general linear models. Bars within each graph without letters do not differ. Sample sizes were three 20-fish pools.

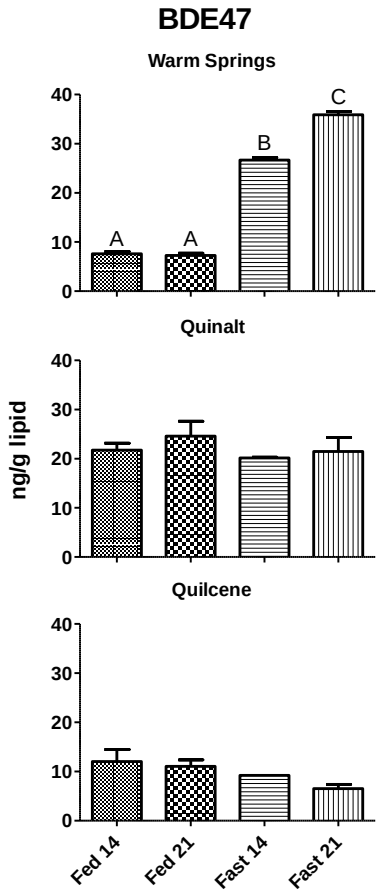


Figure 5. Mean (+ 1 SE) polybrominated diphenyl ether congener 47 (BDE47) in livers from spring Chinook salmon at Warm Springs National Fish Hatchery after 14 and 21 days of feeding (Fed14, Fed21) or fasting (Fast14, Fast21) beginning at the time the fish would normally have been released to emigrate. Bars within each graph without a letter in common differ ($P < 0.05$) based on one-way general linear models. Bars within each graph without letters do not differ. Sample sizes were three 20-fish pools.

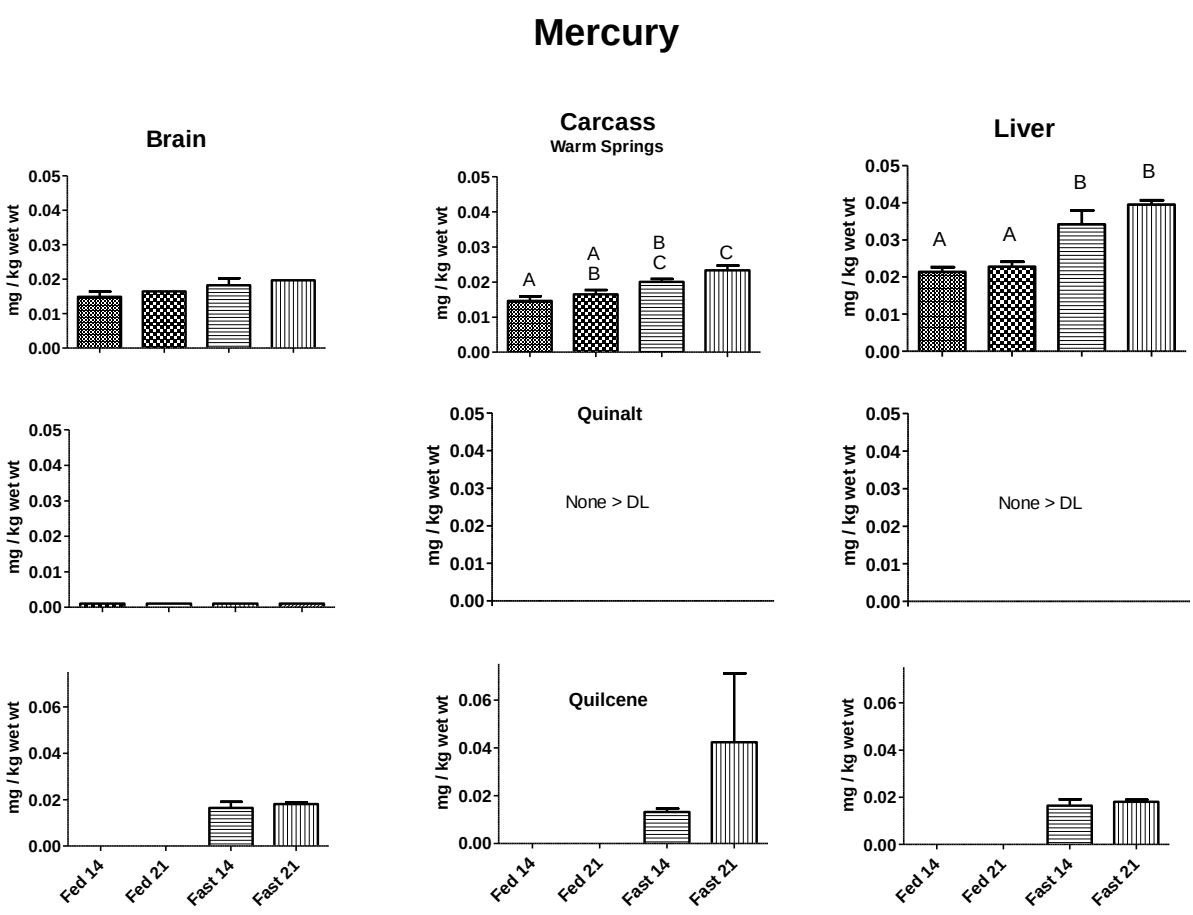


Figure 6. Mean (+ 1 SE) tissue-specific mercury in spring Chinook salmon at Warm Springs National Fish Hatchery (NFH), steelhead at Quinault NFH and coho salmon at Quilcene NFH after 14 and 21 days of feeding (Fed14, Fed21) or fasting (Fast14, Fast21) beginning at the time the fish would normally have been released to emigrate. Lack of a bar means there were no samples > detection limit (DL). Bars within each graph without a letter in common differ ($P < 0.05$) based on one-way general linear models. Bars within each graph without letters do not differ. Sample sizes were three 20-fish pools.

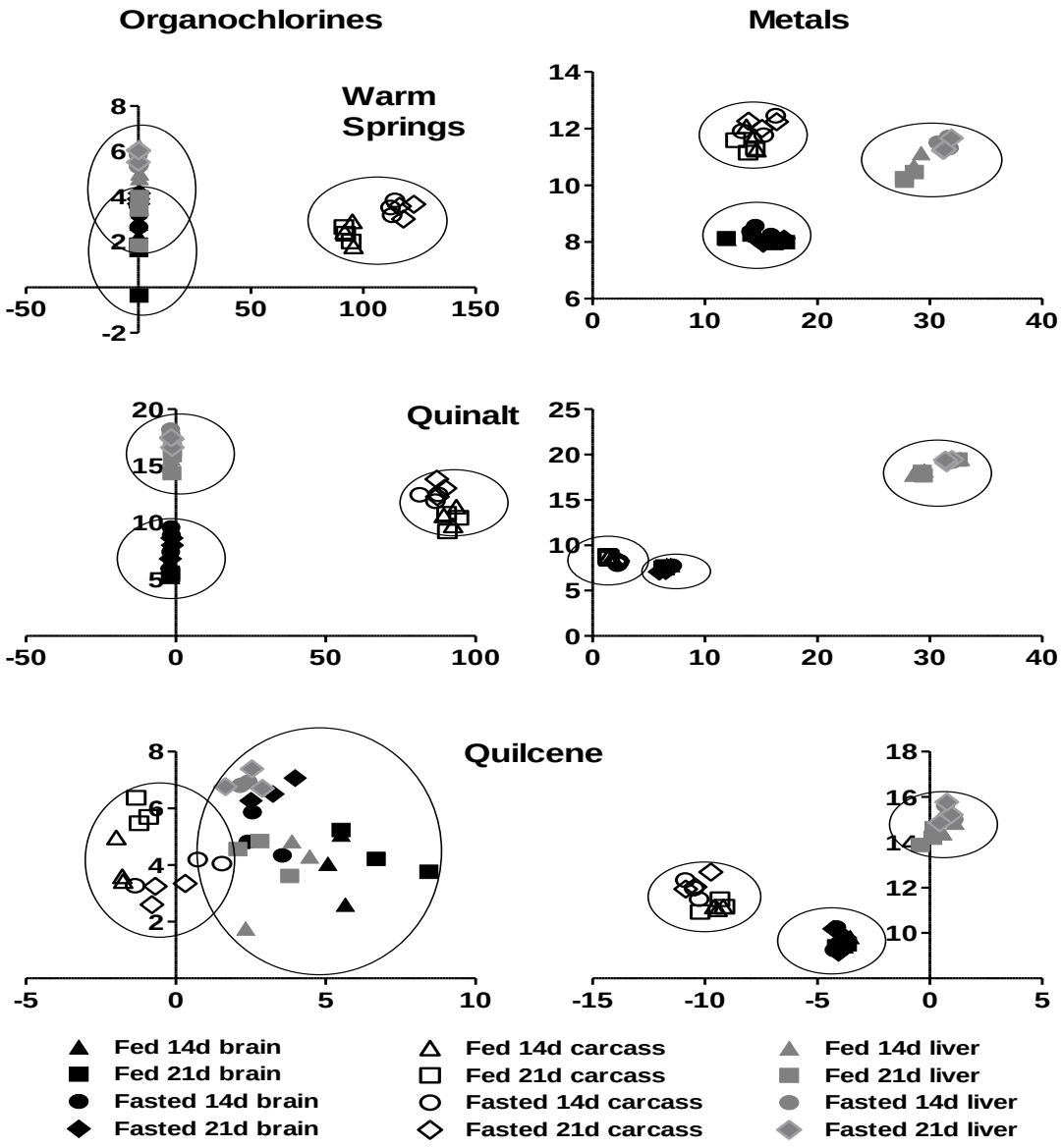


Figure 7. Canonical discriminatory analyses of total organochlorines and metals found in various tissues in spring Chinook salmon at Warm Springs National Fish Hatchery (NFH), steelhead at Quinault NFH and coho salmon at Quilcene NFH after 14 and 21 days of feeding (Fed14, Fed21) or fasting (Fast14, Fast21) beginning at the time the fish would normally have been released to emigrate. Circles (drawn by eye) demonstrate the tissue-specific separation of contaminant concentrations. In all cases $P < 0.001$.

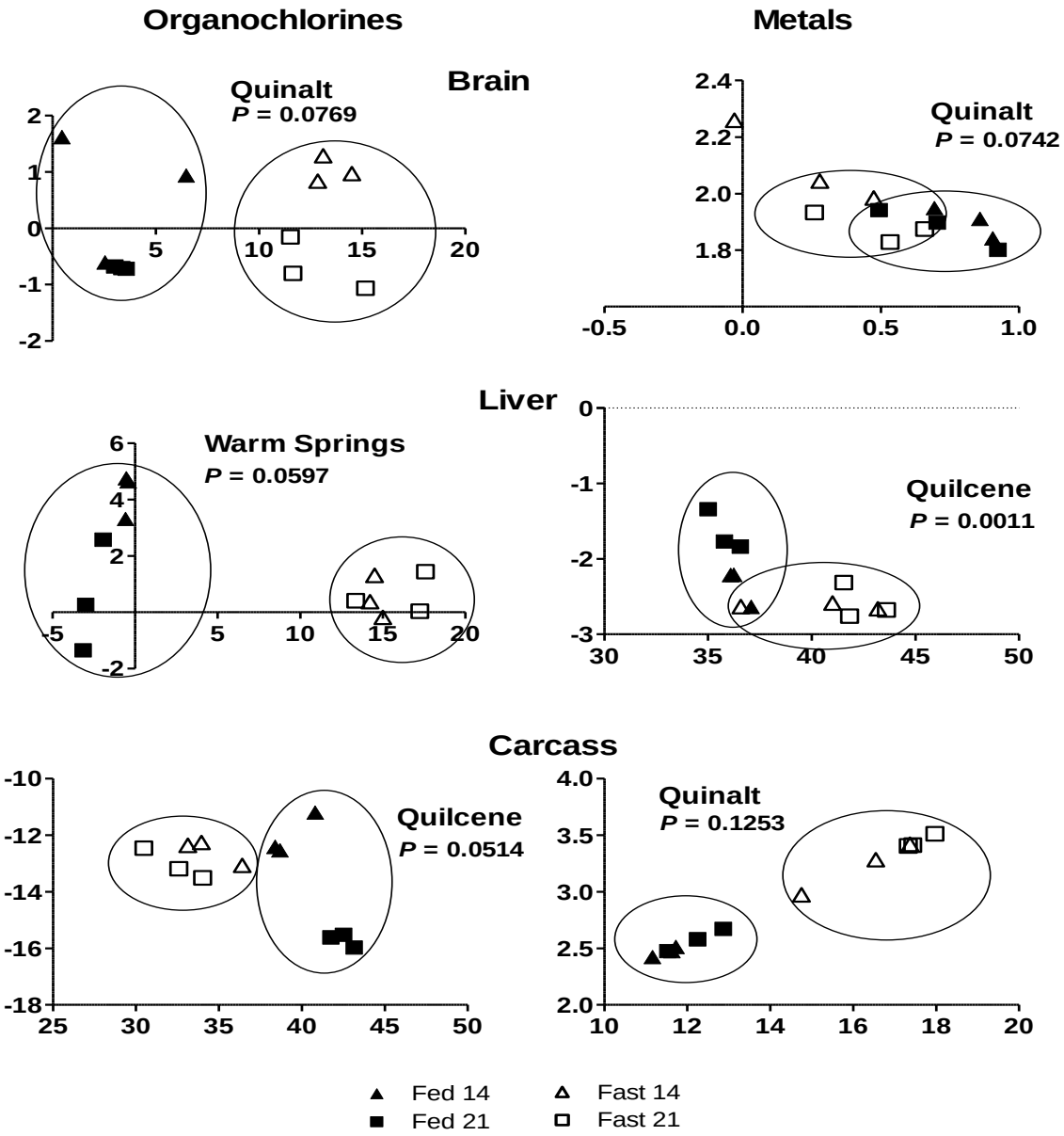
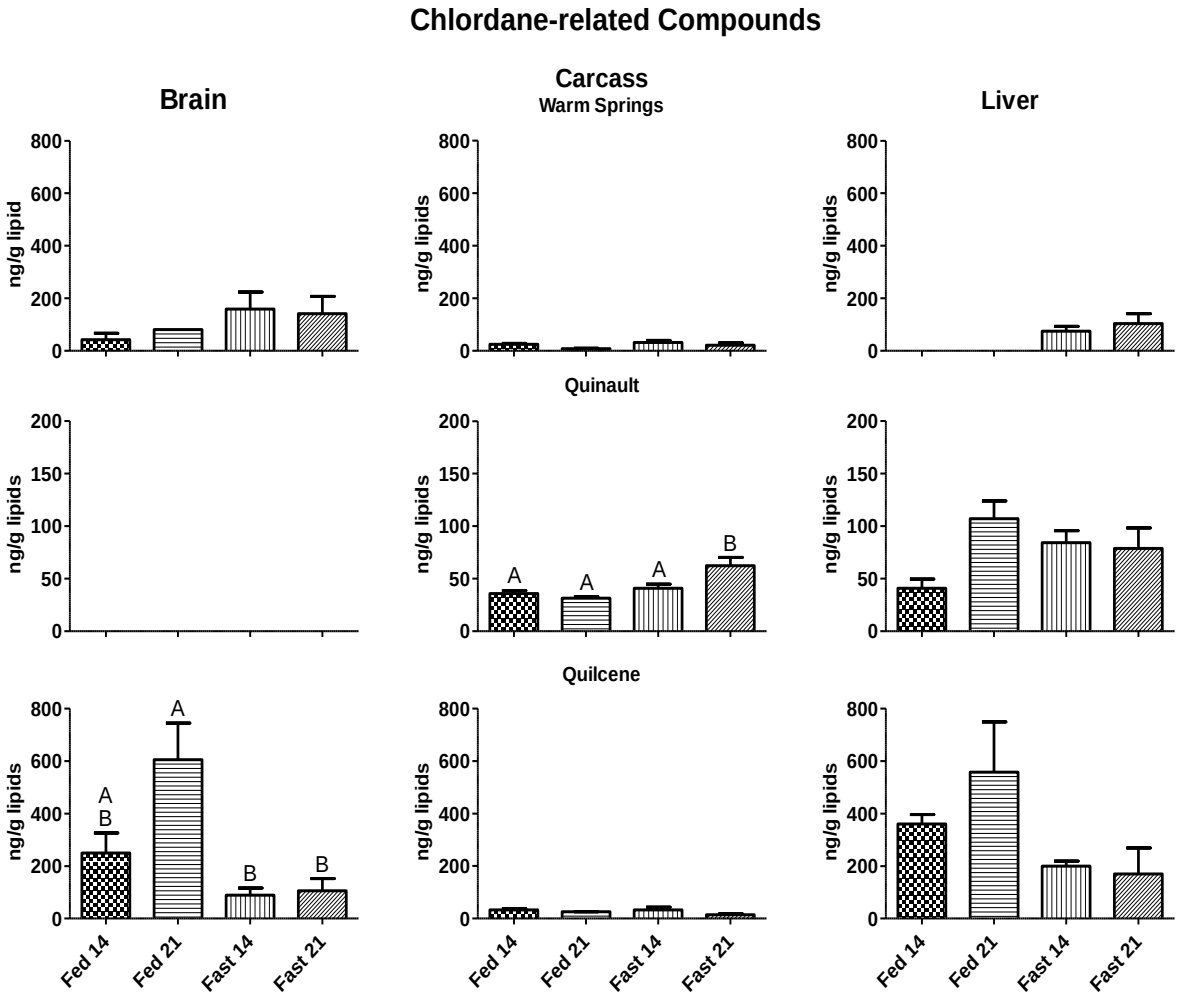
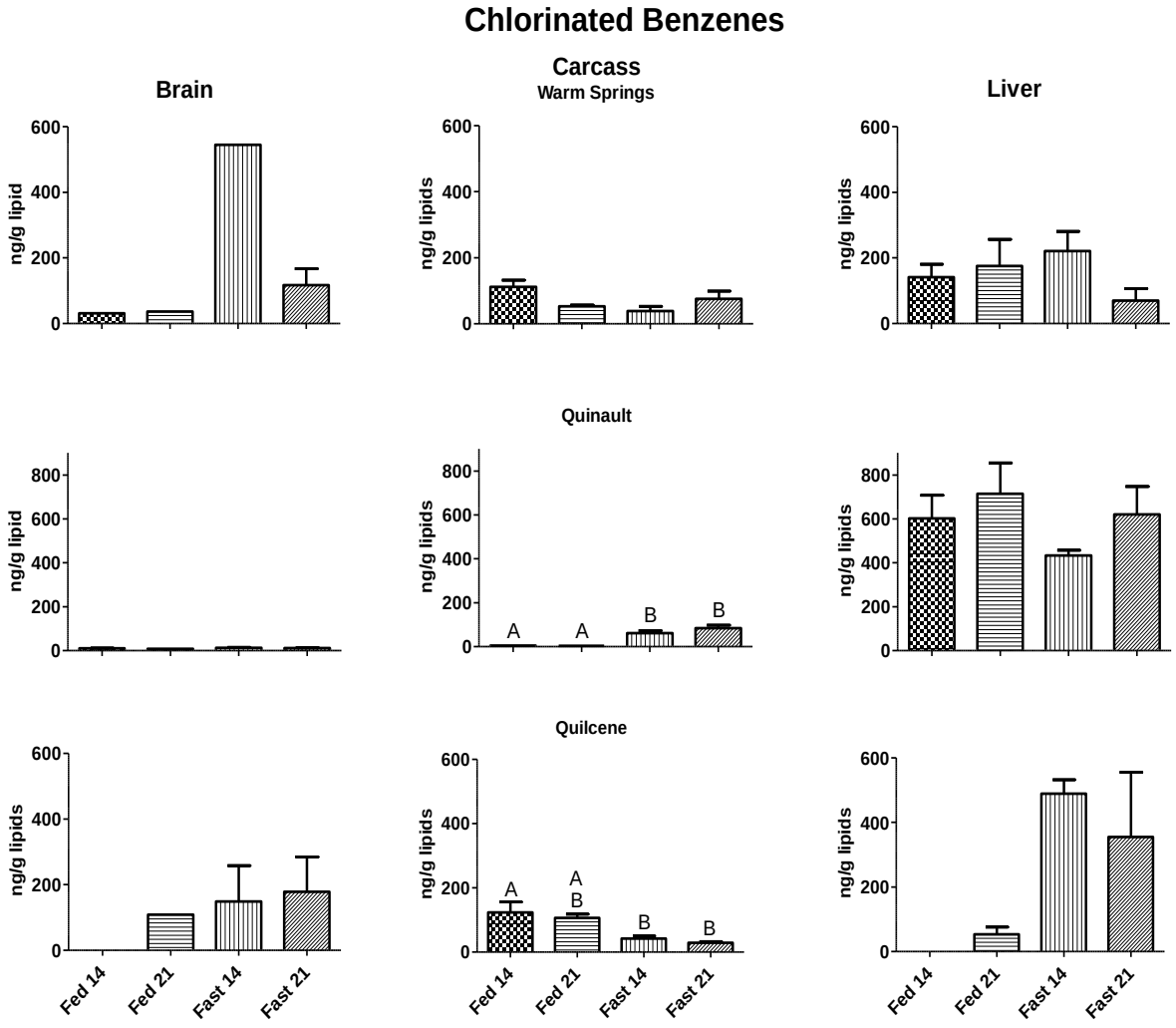


Figure 8. Canonical discriminatory analyses of total organochlorines and metals found in various tissues in spring Chinook salmon at Warm Springs National Fish Hatchery (NFH), steelhead at Quinault NFH and coho salmon at Quilcene NFH after 14 and 21 days of feeding (Fed14, Fed21) or fasting (Fast14, Fast21) beginning at the time the fish would normally have been released to emigrate. Circles (drawn by eye) demonstrate the separation of contaminant concentrations based on whether fish were fed or fasted.

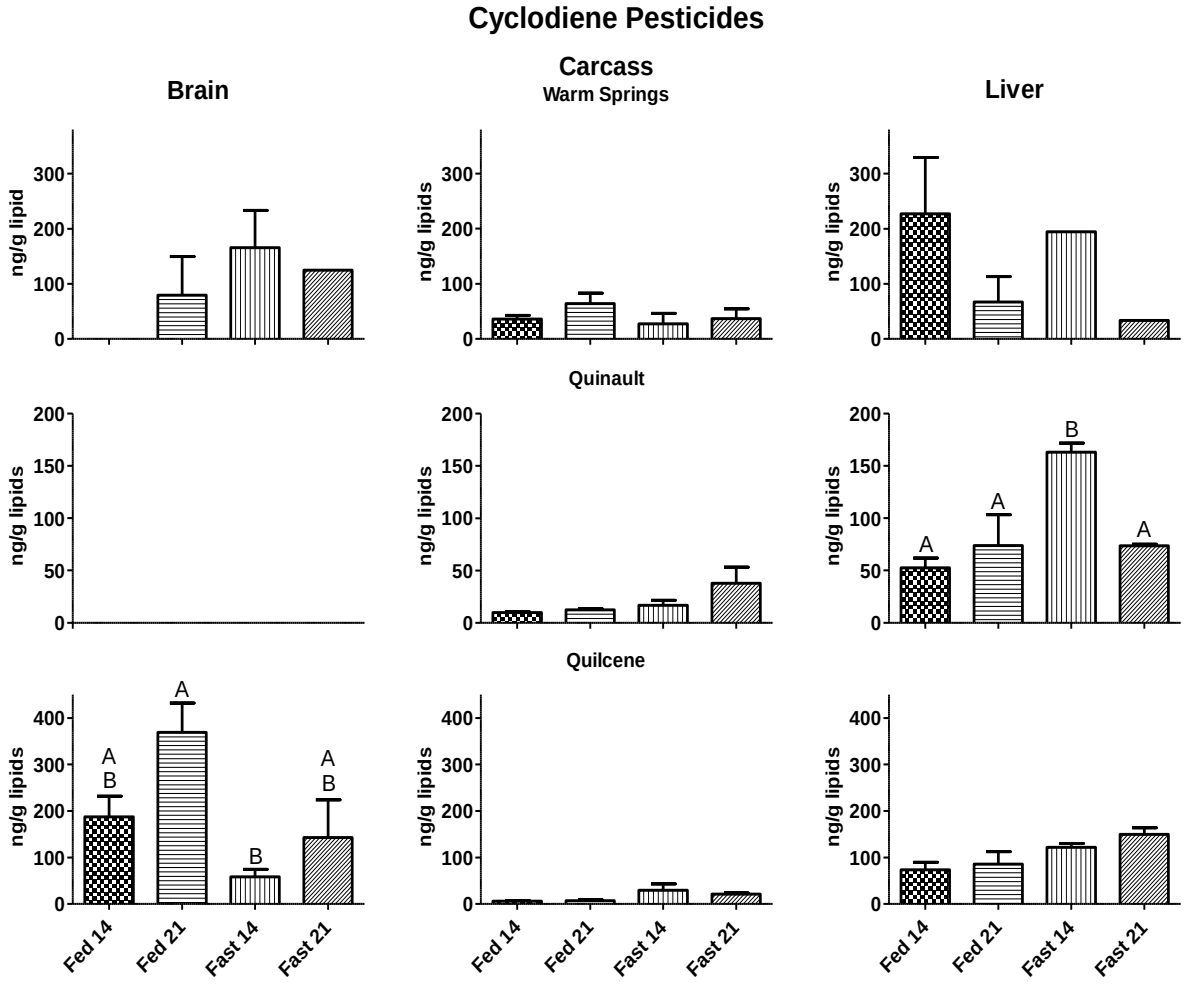
Supplemental Figures



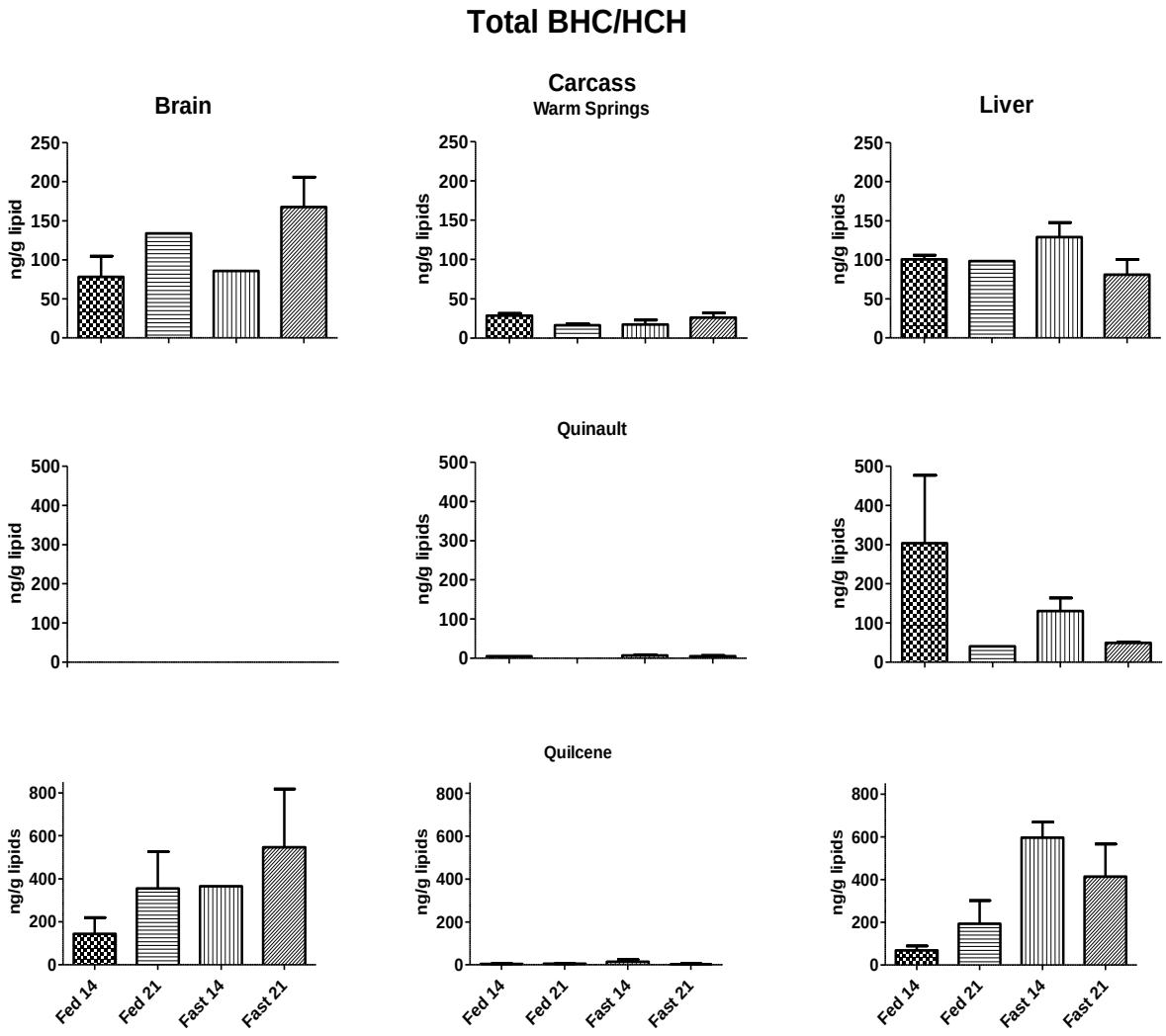
Supplemental Figure 1. Mean (+ 1 SE) tissue-specific total chlorinated benzenes (tetrachlorobenzene 1,2,4,5; tetrachlorobenzene 1,2,3,4; pentachlorobenzene; hexachlorobenzene) in spring Chinook salmon at Warm Springs National Fish Hatchery (NFH), steelhead at Quinault NFH and coho salmon at Quilcene NFH after 14 and 21 days of feeding (Fed14, Fed21) or fasting (Fast14, Fast21) beginning at the time the fish would normally have been released to emigrate. Lack of a bar means there were no samples > detection limit (DL). Bars within each graph without a letter in common differ ($P < 0.05$) based on one-way general linear models. Bars within each graph without letters do not differ. Sample sizes were three 20-fish pools.



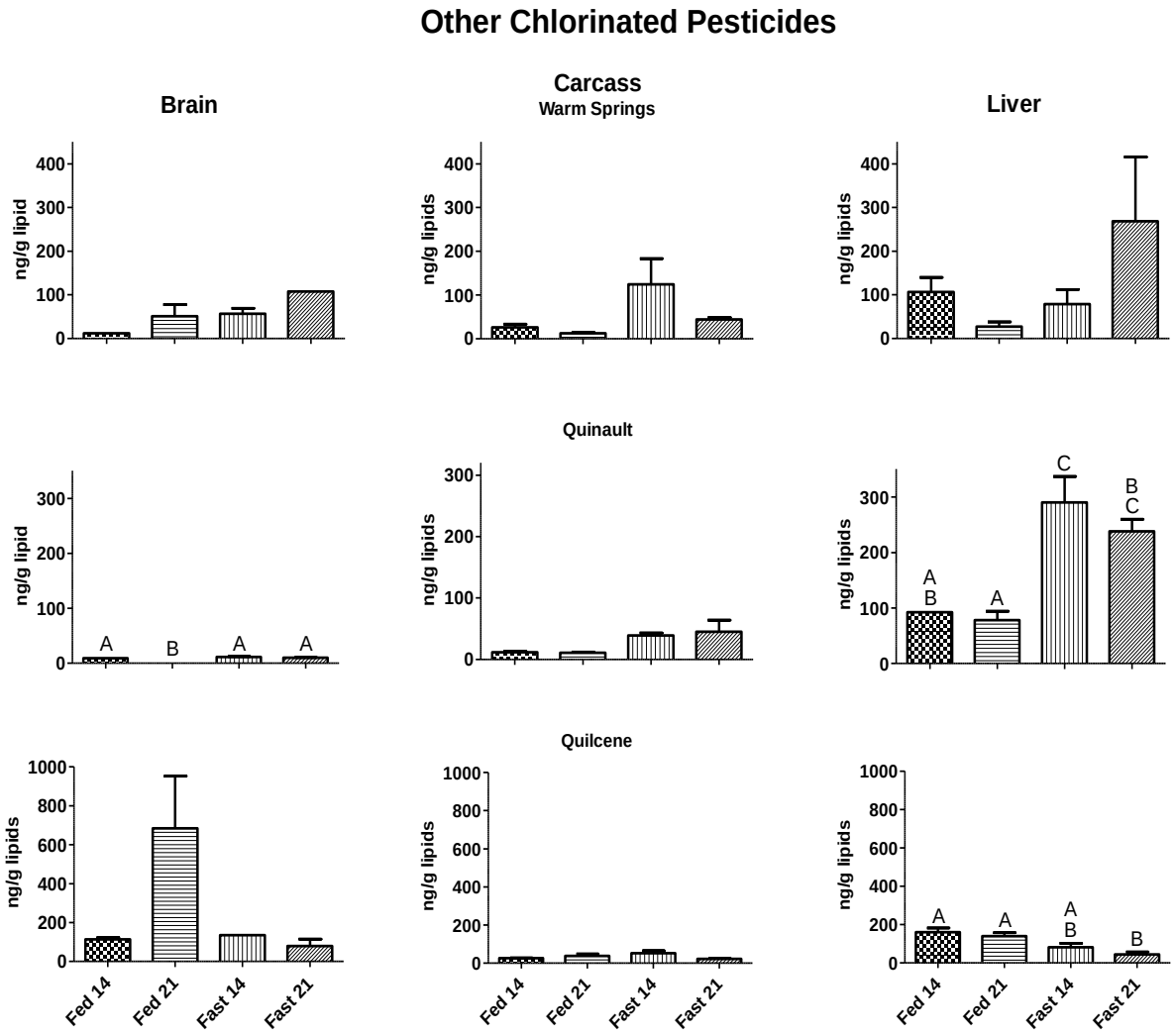
Supplemental Figure 2. Mean (+ 1 SE) tissue-specific total hexachlorocyclohexanes (HCH) (α-HCH, β-HCH, γ-HCH, δ-HCH) in spring Chinook salmon at Warm Springs National Fish Hatchery (NFH), steelhead at Quinault NFH and coho salmon at Quilcene NFH after 14 and 21 days of feeding (Fed14, Fed21) or fasting (Fast14, Fast21) beginning at the time the fish would normally have been released to emigrate. Lack of a bar means there were no samples > detection limit (DL). Bars within each graph without a letter in common differ ($P < 0.05$) based on one-way general linear models. Bars within each graph without letters do not differ. Sample sizes were three 20-fish pools.



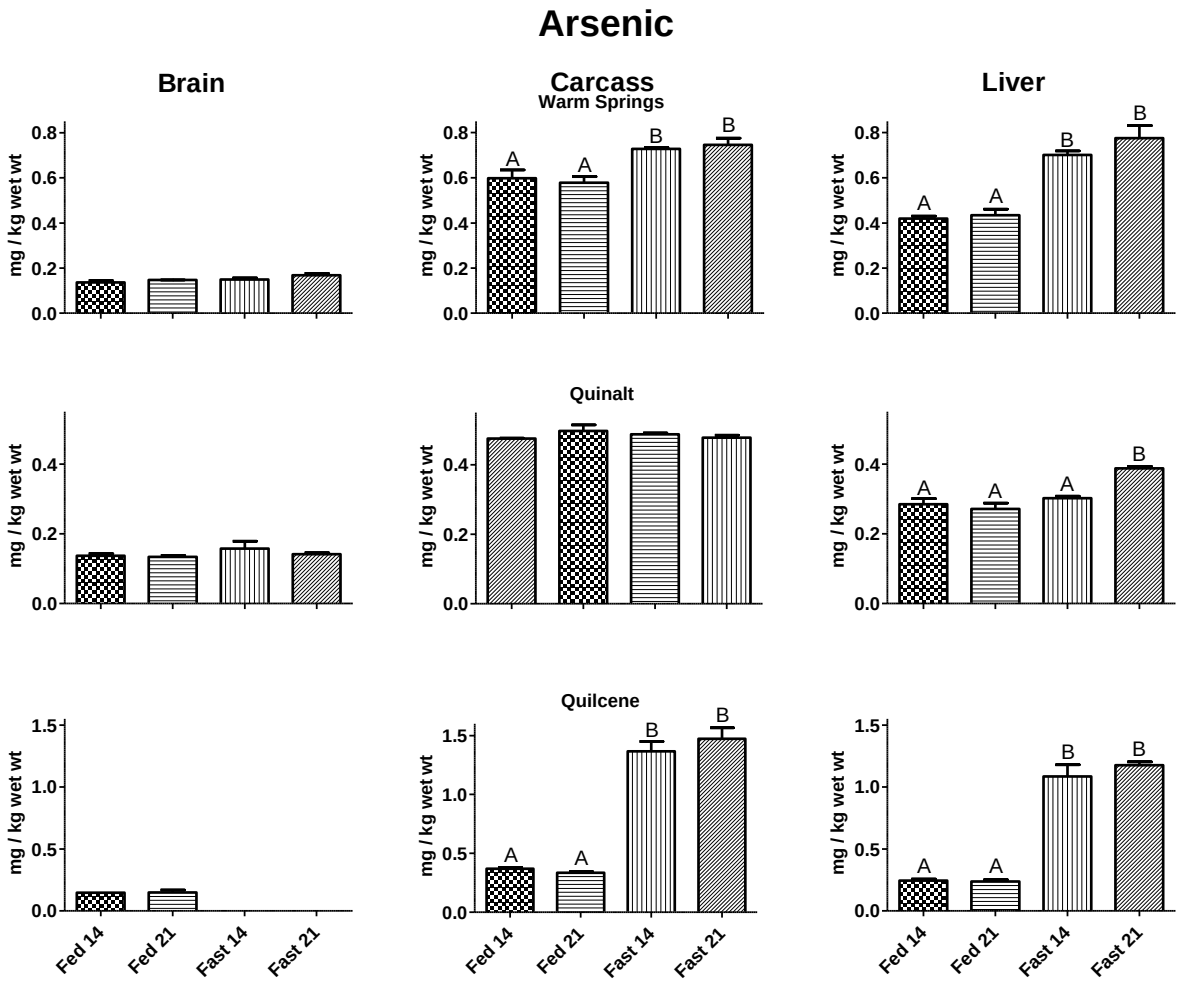
Supplemental Figure 3. Mean (+ 1 SE) tissue-specific total chlordane-related compounds (heptachlor, hepatachlor epoxide, oxychlordane, α-chlordane, γ-chlordane, cis-nonachlor, trans-nonachlor) in spring Chinook salmon at Warm Springs National Fish Hatchery (NFH), steelhead at Quinault NFH and coho salmon at Quilcene NFH after 14 and 21 days of feeding (Fed14, Fed21) or fasting (Fast14, Fast21) beginning at the time the fish would normally have been released to emigrate. Lack of a bar means there were no samples > detection limit (DL). Bars within each graph without a letter in common differ ($P < 0.05$) based on one-way general linear models. Bars within each graph without letters do not differ. Sample sizes were three 20-fish pools.



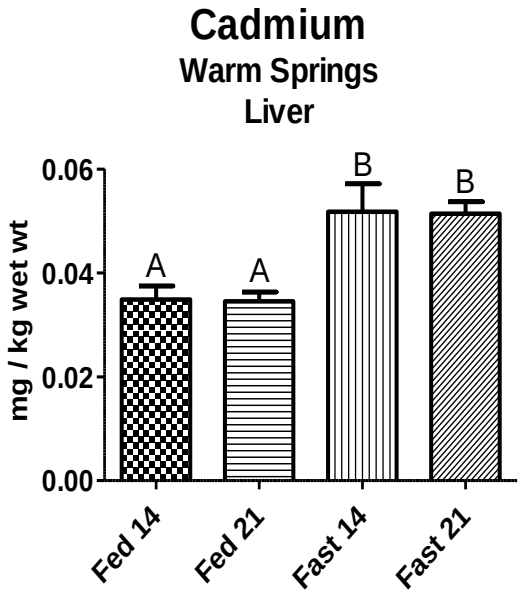
Supplemental Figure 4. Mean (+ 1 SE) tissue-specific total other cyclodiene pesticides (aldrin, dieldrin, endrin) in spring Chinook salmon at Warm Springs National Fish Hatchery (NFH), steelhead at Quinault NFH and coho salmon at Quilcene NFH after 14 and 21 days of feeding (Fed14, Fed21) or fasting (Fast14, Fast21) beginning at the time the fish would normally have been released to emigrate. Lack of a bar means there were no samples > detection limit (DL). Bars within each graph without a letter in common differ ($P < 0.05$) based on one-way general linear models. Bars within each graph without letters do not differ. Sample sizes were three 20-fish pools.



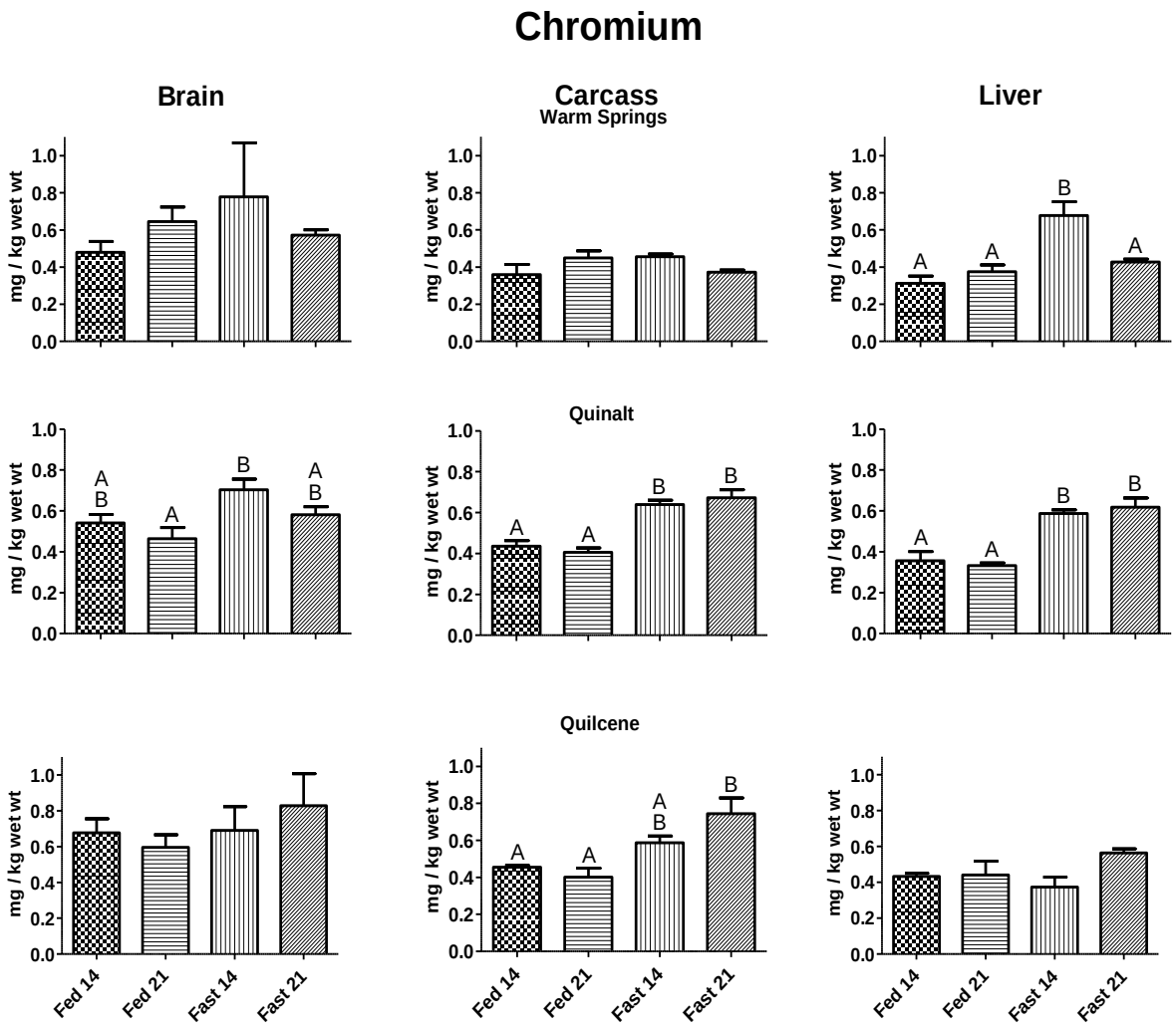
Supplemental Figure 5. Mean (+ 1 SE) tissue-specific total and other chlorinated pesticides (pentachloroanisole, chlorpyrifos, mirex, endosulfan II) in spring Chinook salmon at Warm Springs National Fish Hatchery (NFH), steelhead at Quinault NFH and coho salmon at Quilcene NFH after 14 and 21 days of feeding (Fed14, Fed21) or fasting (Fast14, Fast21) beginning at the time the fish would normally have been released to emigrate. Bars within each graph without a letter in common differ ($P < 0.05$) based on one-way general linear models. Bars within each graph without letters do not differ. Sample sizes were three 20-fish pools.



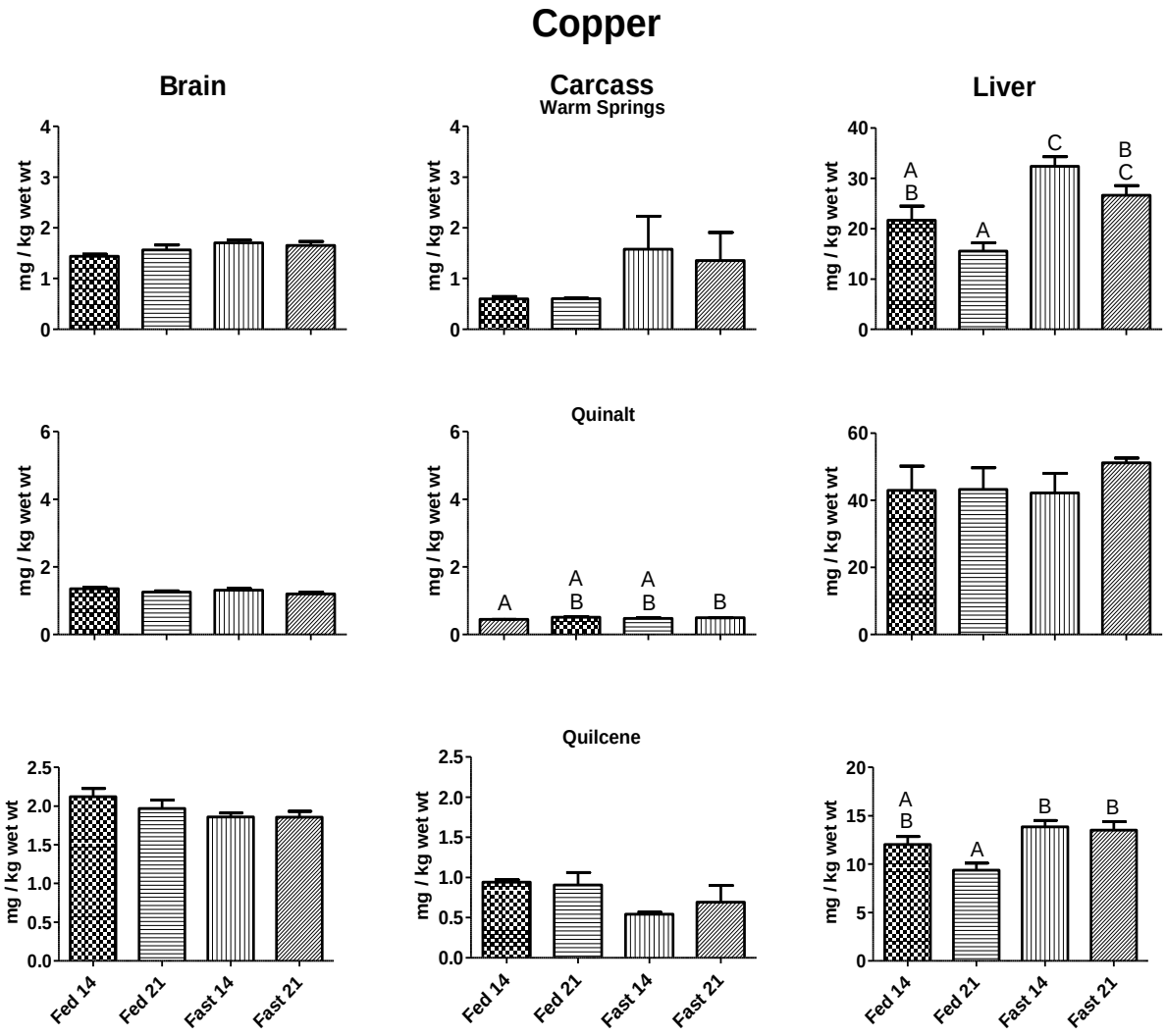
Supplemental Figure 6. Mean (+ 1 SE) tissue-specific arsenic in spring Chinook salmon at Warm Springs National Fish Hatchery (NFH), steelhead at Quinault NFH and coho salmon at Quilcene NFH after 14 and 21 days of feeding (Fed14, Fed21) or fasting (Fast14, Fast21) beginning at the time the fish would normally have been released to emigrate. Lack of a bar means there were no samples > detection limit (DL). Bars within each graph without a letter in common differ ($P < 0.05$) based on one-way general linear models. Bars within each graph without letters do not differ. Sample sizes were three 20-fish pools.



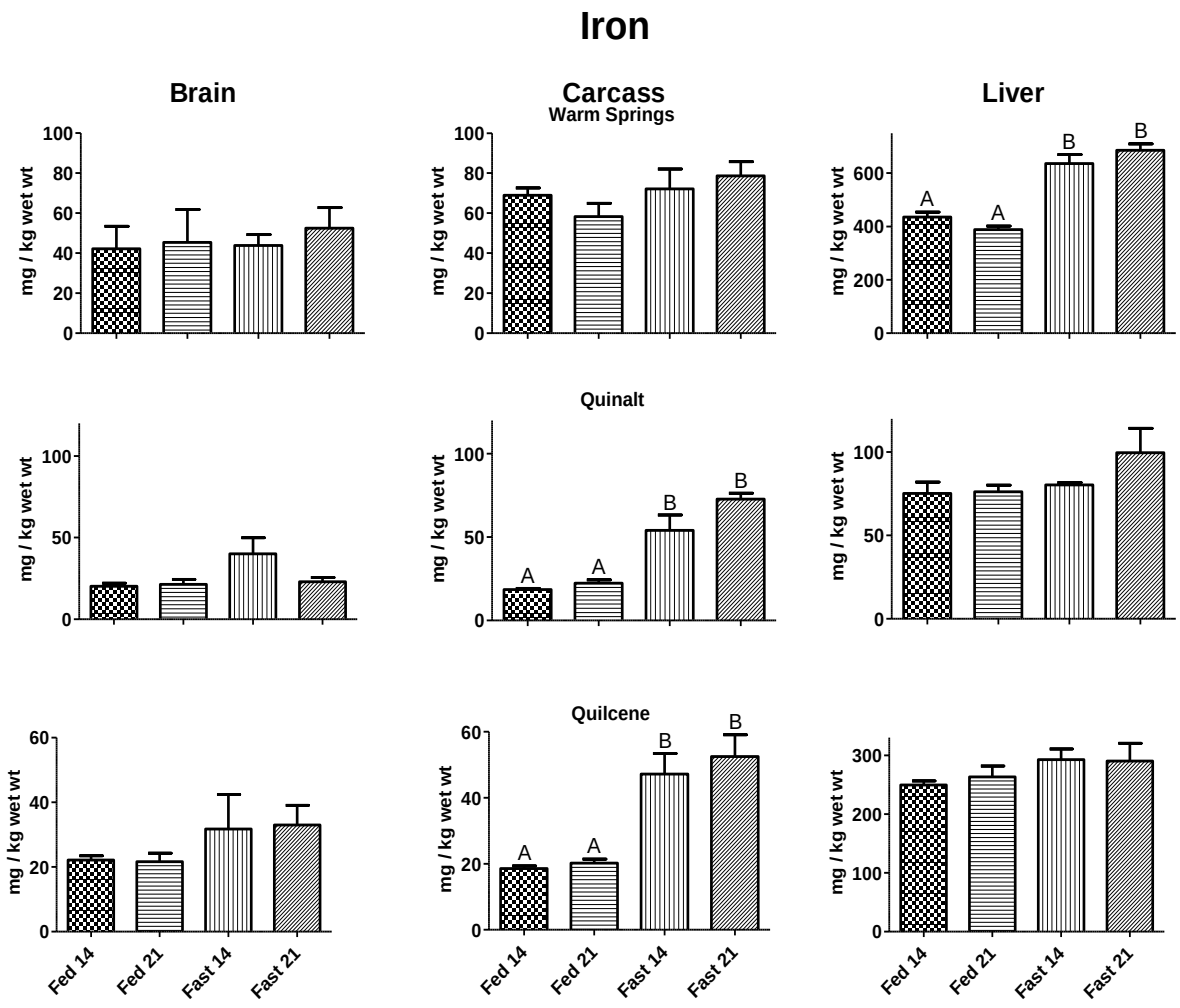
Supplemental Figure 7. Mean (+ 1 SE) cadmium in livers of spring Chinook salmon at Warm Springs National Fish Hatchery after 14 and 21 days of feeding (Fed14, Fed21) or fasting (Fast14, Fast21) beginning at the time the fish would normally have been released to emigrate. Bars within each graph without a letter in common differ ($P < 0.05$) based on one-way general linear models. Sample sizes were three 20-fish pools.



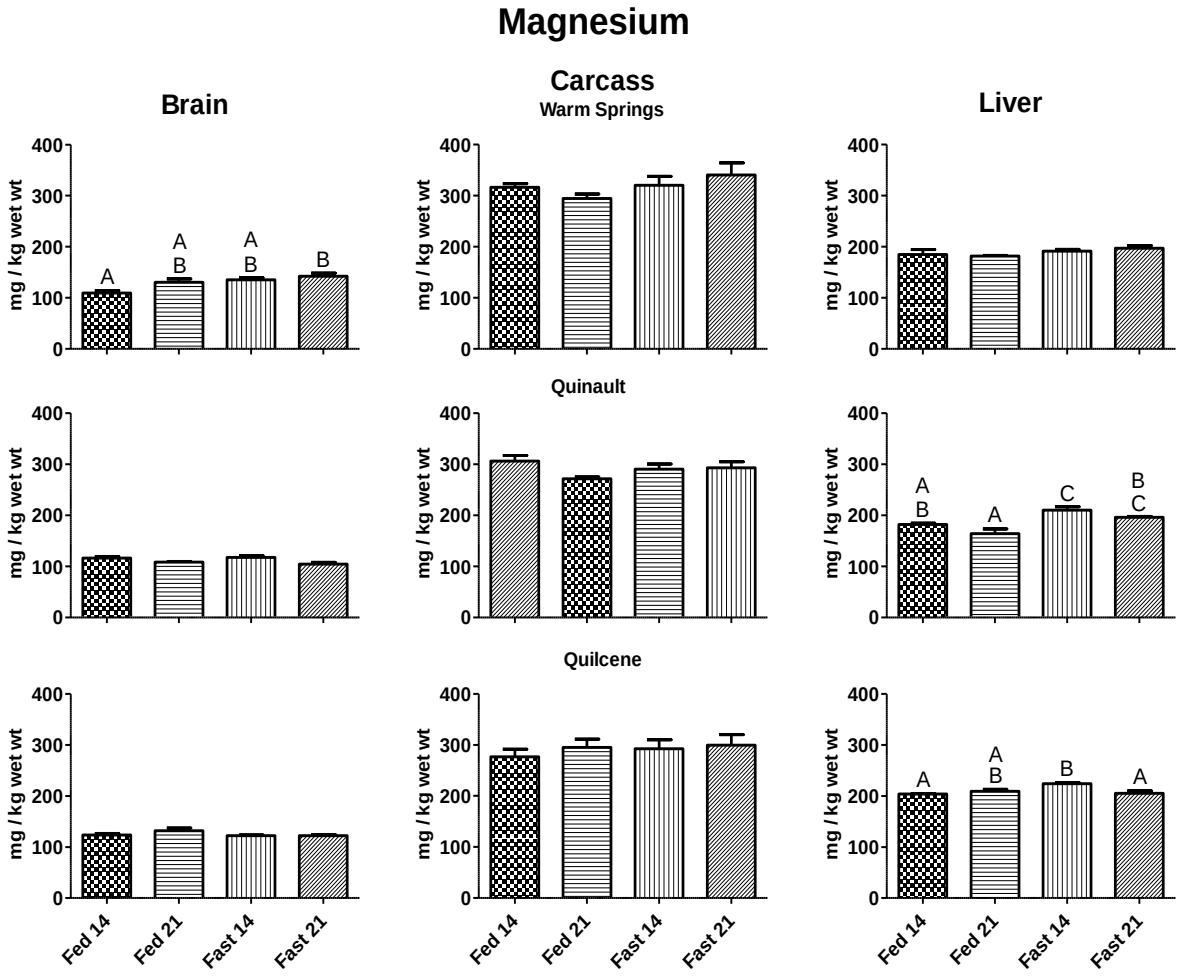
Supplemental Figure 8. Mean (+ 1 SE) tissue-specific chromium in spring Chinook salmon at Warm Springs National Fish Hatchery (NFH), steelhead at Quinault NFH and coho salmon at Quilcene NFH after 14 and 21 days of feeding (Fed14, Fed21) or fasting (Fast14, Fast21) beginning at the time the fish would normally have been released to emigrate. Bars within each graph without a letter in common differ ($P < 0.05$) based on one-way general linear models. Bars within each graph without letters do not differ. Sample sizes were three 20-fish pools.



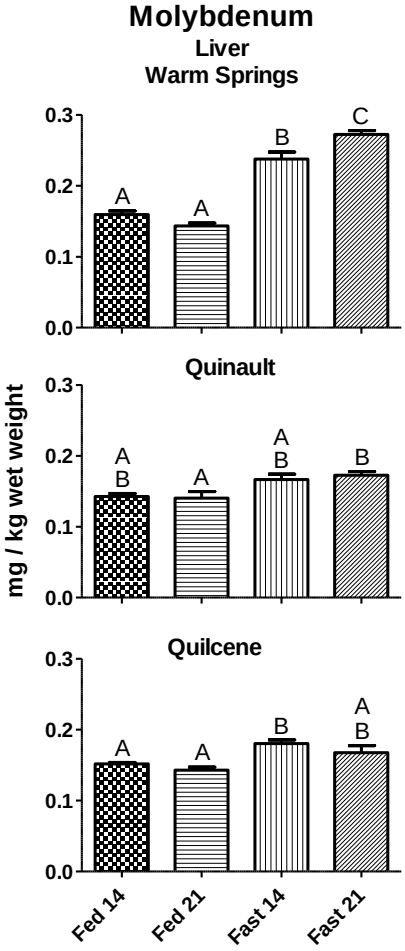
Supplemental Figure 9. Mean (+ 1 SE) tissue-specific copper in spring Chinook salmon at Warm Springs National Fish Hatchery (NFH), steelhead at Quinault NFH and coho salmon at Quilcene NFH after 14 and 21 days of feeding (Fed14, Fed21) or fasting (Fast14, Fast21) beginning at the time the fish would normally have been released to emigrate. Bars within each graph without a letter in common differ ($P < 0.05$) based on one-way general linear models. Bars within each graph without letters do not differ. Sample sizes were three 20-fish pools.



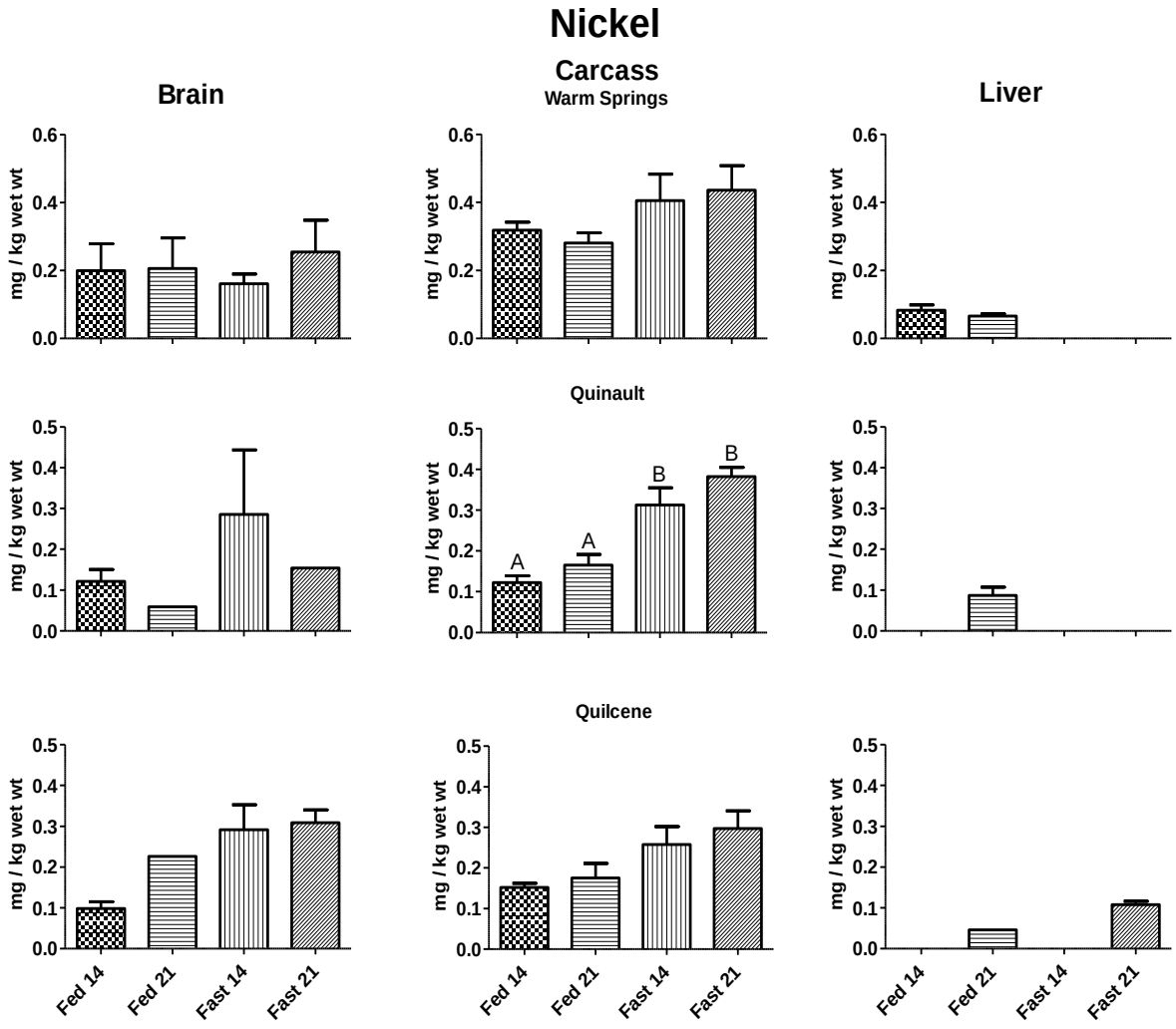
Supplemental Figure 10. Mean (+ 1 SE) tissue-specific iron in spring Chinook salmon at Warm Springs National Fish Hatchery (NFH), steelhead at Quinault NFH and coho salmon at Quilcene NFH after 14 and 21 days of feeding (Fed14, Fed21) or fasting (Fast14, Fast21) beginning at the time the fish would normally have been released to emigrate. Bars within each graph without a letter in common differ ($P < 0.05$) based on one-way general linear models. Bars within each graph without letters do not differ. Sample sizes were three 20-fish pools.



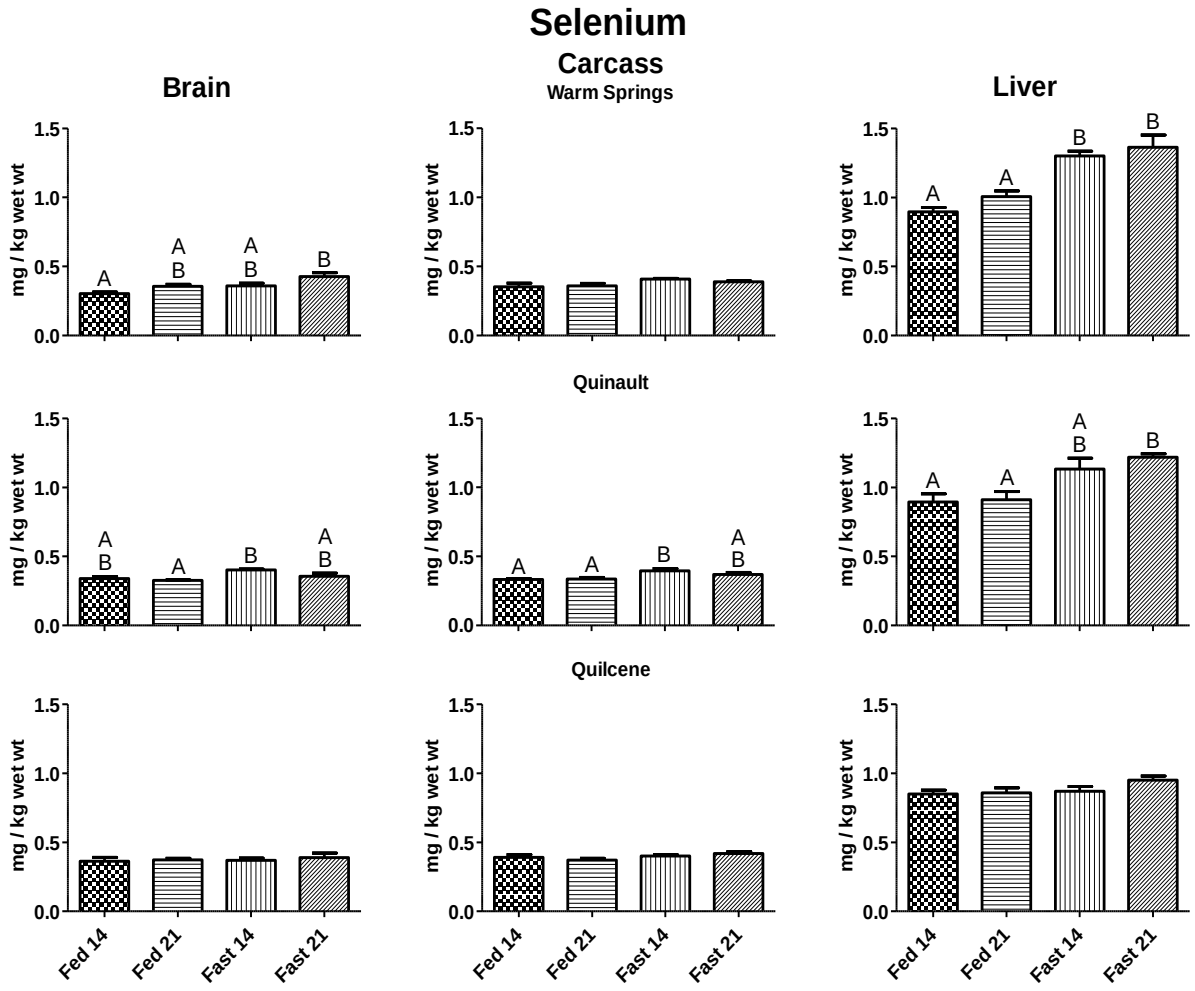
Supplemental Figure 11. Mean (+ 1 SE) tissue-specific magnesium in spring Chinook salmon at Warm Springs National Fish Hatchery (NFH), steelhead at Quinault NFH and coho salmon at Quilcene NFH after 14 and 21 days of feeding (Fed14, Fed21) or fasting (Fast14, Fast21) beginning at the time the fish would normally have been released to emigrate. Bars within each graph without a letter in common differ ($P < 0.05$) based on one-way general linear models. Bars within each graph without letters do not differ. Sample sizes were three 20-fish pools.



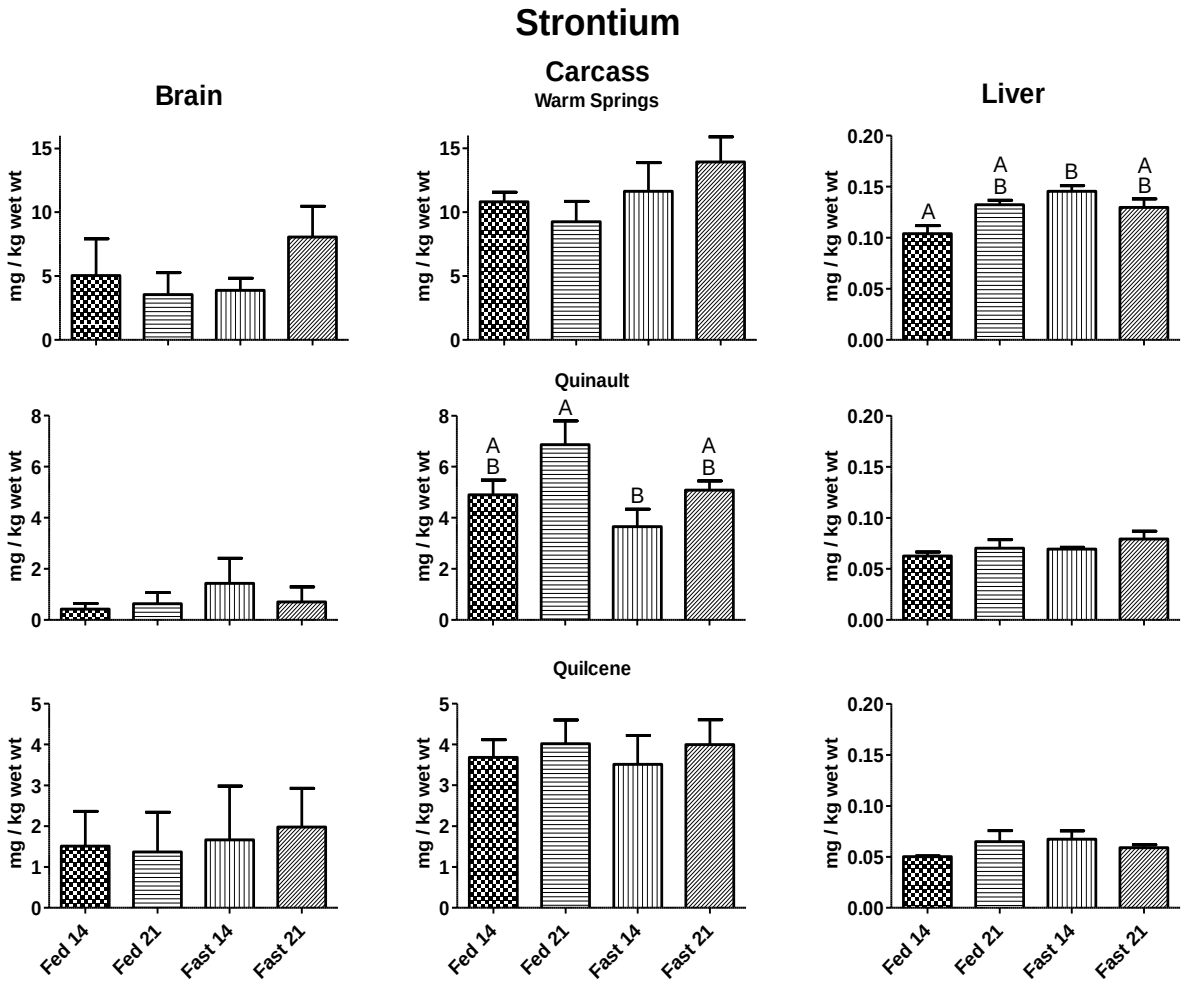
Supplemental Figure 12. Mean (+ 1 SE) molybdenum in livers of spring Chinook salmon at Warm Springs National Fish Hatchery after 14 and 21 days of feeding (Fed14, Fed21) or fasting (Fast14, Fast21) beginning at the time the fish would normally have been released to emigrate. Bars within each graph without a letter in common differ ($P < 0.05$) based on one-way general linear models. Sample sizes were three 20-fish pools.



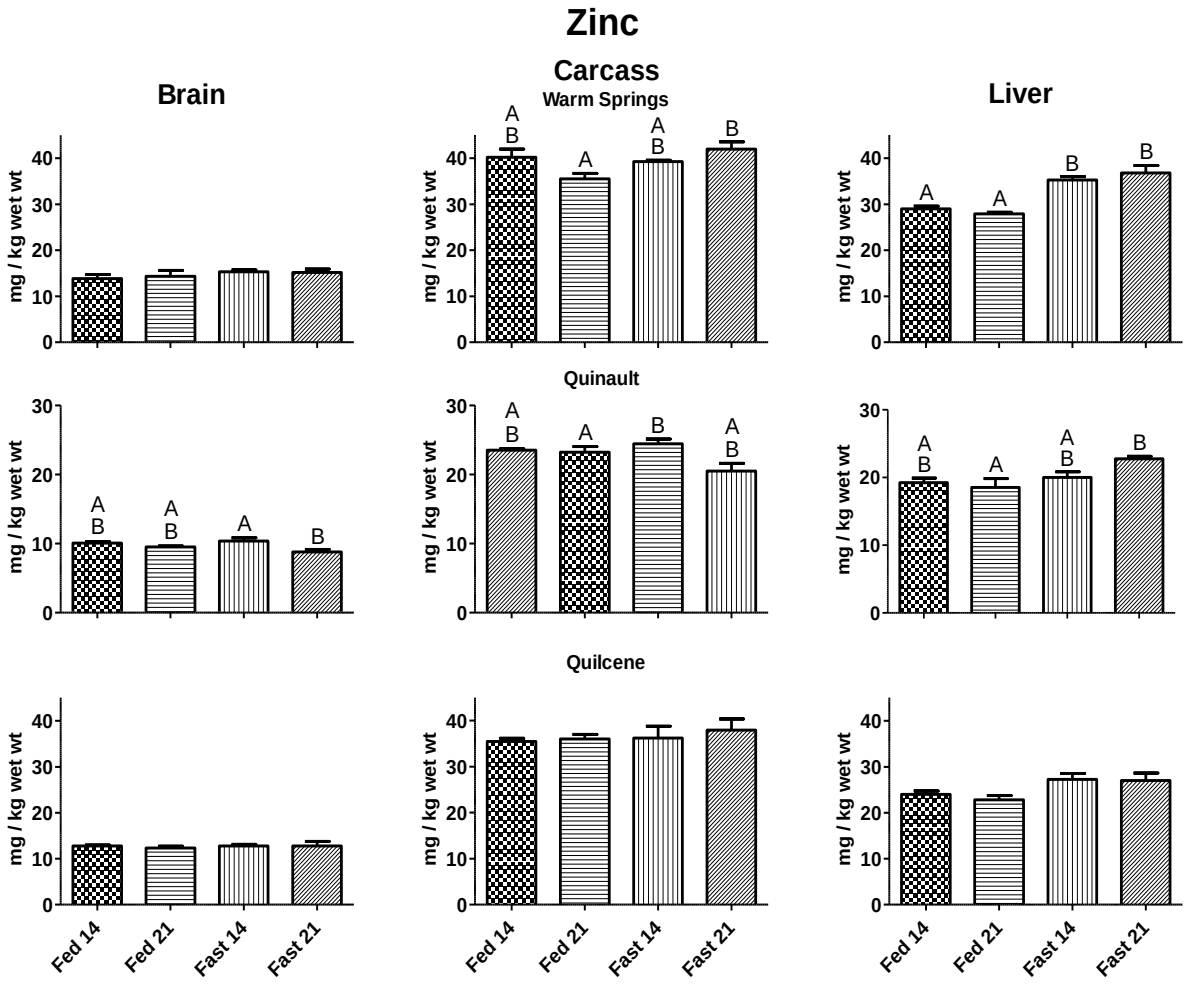
Supplemental Figure 13. Mean (+ 1 SE) tissue-specific nickel in spring Chinook salmon at Warm Springs National Fish Hatchery (NFH), steelhead at Quinault NFH and coho salmon at Quilcene NFH after 14 and 21 days of feeding (Fed14, Fed21) or fasting (Fast14, Fast21) beginning at the time the fish would normally have been released to emigrate. Bars within each graph without a letter in common differ ($P < 0.05$) based on one-way general linear models. Bars within each graph without letters do not differ. Sample sizes were three 20-fish pools.



Supplemental Figure 14. Mean (+ 1 SE) tissue-specific selenium in spring Chinook salmon at Warm Springs National Fish Hatchery (NFH), steelhead at Quinault NFH and coho salmon at Quilcene NFH after 14 and 21 days of feeding (Fed14, Fed21) or fasting (Fast14, Fast21) beginning at the time the fish would normally have been released to emigrate. Bars within each graph without a letter in common differ ($P < 0.05$) based on one-way general linear models. Bars within each graph without letters do not differ. Sample sizes were three 20-fish pools.



Supplemental Figure 15. Mean (+ 1 SE) tissue-specific strontium in spring Chinook salmon at Warm Springs National Fish Hatchery (NFH), steelhead at Quinault NFH and coho salmon at Quilcene NFH after 14 and 21 days of feeding (Fed14, Fed21) or fasting (Fast14, Fast21) beginning at the time the fish would normally have been released to emigrate. Bars within each graph without a letter in common differ ($P < 0.05$) based on one-way general linear models. Bars within each graph without letters do not differ. Sample sizes were three 20-fish pools.



Supplemental Figure 16. Mean (+ 1 SE) tissue-specific zinc in spring Chinook salmon at Warm Springs National Fish Hatchery (NFH), steelhead at Quinault NFH and coho salmon at Quilcene NFH after 14 and 21 days of feeding (Fed14, Fed21) or fasting (Fast14, Fast21) beginning at the time the fish would normally have been released to emigrate. Bars within each graph without a letter in common differ ($P < 0.05$) based on one-way general linear models. Bars within each graph without letters do not differ. Sample sizes were three 20-fish pools.

III. MANAGEMENT ACTIONS

This investigation identified contaminants in fish and feed at three Pacific Region NFHs with the purpose of determining levels of contaminants and mitigating for them. Based on the results, the following direct management actions could be implemented.

1. The FWS should work with the contracted manufacturers of the fish feed supplied to the Pacific Region NFHs to reduce or eliminate contaminant sources in fish feed. This will reduce or eliminate the risk to threatened and endangered salmonids produced at NFHs. By selecting the least contaminated feeds, NFHs will also reduce the amount of contaminants released into the aquatic environment from sources like excess food, effluent and fish carcasses.

We collected data from three NFHs describing contaminant loads in adult fish returning to the hatcheries, in their progeny during their 18-month residence time within the hatcheries, and in the feeds on which these progeny were reared. We determined, with a few exceptions, that eggs and unfed swim-up fry are more heavily contaminated relative to their size than the adult females from which they were produced. As the fish grew there was an overall dilution effect, i.e., newly synthesized tissues in the juvenile fish contained lower concentrations of contaminants and reduced the overall concentration of those contaminants deposited within the egg by the adult female. Although contaminants were found in all feeds fed to juvenile fish reared within the NFHs, none of the feeds used during the sampling time period contained PCB concentrations above the level found to adversely affect fish health (residual effect threshold, RET, of 2400 ng PCB/g lipid; Meador et al. 2002). Returning adult female fish to Warm Springs and Quilcene NFHs carried levels of total PCBs that were less than the males on a wet weight basis. That being the case, if there is a need to distribute carcasses in the watershed for nutrient enhancement, female fish carcasses without eggs should be used. On a wet weight-basis, males contained higher contaminant loads than females. Madenjian (2011) suggests that the males will have higher PCB loads due to sex differences in gross growth efficiency. Unfed fry at Quilcene and Quinault NFHs carried a total PCB level that exceeded the RET for PCBs. Since these fish have not been on feed and appeared to have obtained their contaminant load from maternal transfer little can be done to mitigate for this source of contamination.

2. The investigation could result in added feed specifications which would specify acceptable levels of contaminants to be included in government feed contracts with manufacturers, ensuring quality feed to the NFH System.

Examining the contaminant load in the feeds used during the sampling period showed that all the feeds carried a similar amount of all classes of contaminants included in the analyses. Two feed samples had higher levels of total PCBs--1029.41 and 1713.38 ng/g lipid in starters #0 and #1, respectively, but the level dropped to less than half that amount in the next feed sizes (Appendix I). Accumulation of total PCBs did occur in the fish raised at the hatchery but these levels are probably the result of maternal transfer since some of the greatest increases occurred from egg to

fry prior to the fish being fed commercial feeds (Table 1-5, 1-9, 1-13). Three feed companies are represented in this survey. Two feed formulations from one company are also included, making for an easy comparison among these feeds. The majority of the protein and energy in one of these formulations originated from fish meal and fish oil, respectively; and the protein and energy sources in the second formulation originated from a mix of ingredients including blood, feather, poultry and corn gluten meals, and poultry fat in addition to fish meal and fish oil. Since many "alternative ingredients" were included in the second formulation, fish meal and oil inclusion levels were lower in the second formulation compared to the first. Regardless of the inclusion of alternative ingredients, contaminant loads are largely similar among these feeds. Possible variations in source ingredients appear to cause the observed differences. Berntssen et al. (2010) were able to reduce the contaminants in Atlantic salmon by using alternative ingredients, however, their ingredients consisted of all plant-based sources, including wheat and corn gluten, and soybean meal without any fish meal or fish oil. Also, in the Berntssen et al. study, it is not known if either group of fish were fatter than the other due to the ingredients used. Oo et al. (2007) was able to successfully replace fish oil with palm oil without compromising growth to reduce the dietary dioxin level in the feed and fish. Drew et al. (2007) were also able to reduce contaminants in fish fillets by altering the ingredients in the feed. They used a combination of canola protein concentrate and a canola/linseed oil mixture to replace the fish meal and oil. The replacements decreased growth in the fish when both fish meal and oil were reduced, but the contaminants were reduced somewhat as well. The loss of growth would be a trade-off for a reduction in contaminants, however, reductions in contaminants within fish tissues by partial substitution of fish meal and oil with alternative ingredients are not guaranteed, as we see in the current survey comparing the two feed formulations discussed above. Some contaminant data concerning hatchery fish supplied by Johnson et al. (2010) and Arkoosh et al. (2011) indicated that hatcheries were not the major source of contaminants in the fish. However, any reduction in contaminants in the feed would be a benefit. It should be noted that every hatchery is going to have a different situation due to their unique infrastructure. Other possible sources of contaminants in a hatchery setting could be paint or caulking.

3. The investigation will help participating NFHs determine the contaminant load in fish and feeds, if any, possible sources of contamination, and ultimately the feasibility of releasing NFH reared fish for human consumption or "seeding"/carcass placement programs.

In reference to other work that has been done (Johnson et al. 2010, Arkoosh et al. 2011) hatchery raised fish seemed to be more likely to accumulate contaminants after leaving the hatchery. If that is the case, Warm Springs NFH is the farthest from the ocean so their smolts may be the most impacted of the three hatcheries sampled in this study. Concerning the use of carcasses, as discussed in #1, female carcasses without eggs may be the best to use for nutrient enhancement programs.

4. The investigation will provide an initial examination of the ecological impacts of contaminated fish feed by looking at the physiological effects on salmonids during smoltification and after they are released from the NFH.

This investigation confirmed that a relatively short period of fasting—similar to being released from a hatchery—had physiological effects on the three salmon species. These effects included the potential to lose mass and, as a result, the redistribution of contaminants from lipids to organs (brain and liver). The combination of fasting and increased contaminant loads altered the expression of genes in the liver that are important for various metabolic pathways and genes known to result from contaminant exposure and metabolism of toxins. The changes in gene expression point to metabolic costs of fasting and contaminants that may reduce bioenergetic resources available for disease resistance, predator avoidance, and the process of smoltification or other activities necessary for survival. While this investigation did not explore alternative hatchery release strategies, the results suggest that an investigation of alternatives might be prudent. For example, the use of acclimation ponds or in-stream acclimation facilities where natural food items are available might eliminate or reduce the time required for fish to adapt to these new food sources.

5. The investigation will aid in the development and refinement of ***Interim National Guidelines for Hatchery Management Decisions Regarding Contaminants in Catchable-Size Fish Produced by the National Fish Hatchery System.***

Addressing management Action #5, the current work was done in the Pacific Region. In this Region fish are released as smolts and not as catchable-size fish. Data from the current project can provide additional information for Guideline I (Activities for which current contaminant information is available) of the aforementioned strategy/guidance document. For the three facilities addressed in this study, the adults contributed the majority of contaminants to the eggs and unfed fry. Knowledge of the returning adult contaminant load may aid with decisions about distribution of carcasses, i.e., whether they are used for stream enhancement purposes.

IV. SCHEDULE

The above two chapters of this final report are written as such because they are draft manuscripts in preparation and will be submitted to peer reviewed journals some time after the completion and submission of this final report. Upon acceptance, the manuscripts will be uploaded into CID along with the final report for this off-refuge investigation.