

Distribution and Abundance of Harbor Seals in Cook Inlet, Alaska. Task III: Movements, Marine Habitat Use, Diving Behavior, and Population Structure, 2004-2006

FINAL REPORT



By

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ABSTRACT

Between 2004 and 2006 we conducted four harbor seal tagging trips in Cook Inlet during the months of October and May. In total, we captured and released 93 harbor seals, 77 of which were tagged with satellite transmitters. Each transmitter was glued to the hair on the back of the seal using durable epoxy. Fourteen of the seals were also equipped with specially developed transmitters that were attached to one of the rear flippers. Transmissions from the 91 tags resulted in 178,536 location estimates and 310,593 dive and haul-out behavior records. These data formed the basis for the development of novel analysis techniques. Johnson et al. (2008) described a novel continuous-time correlated random walk (CTCRW) method for predicting animal locations from satellite tags. Higgs and Ver Hoef (2011) described a new statistical method for analyzing dive behavior based on dive histogram recordings obtained from satellite tags. This report demonstrates the application of the CTCRW model to provide estimates of seasonal changes in behavior and use patterns for harbor seals in Cook Inlet and Gulf of Alaska. Harbor seals tagged in central and southern Cook Inlet ranged as far southwest as the Semidi Islands and some seals used habitats around the north and northeast side of Kodiak Island. There was a strong seasonal pattern of more coastal and restricted spatial use during the spring and summer (breeding, pupping, molting) and more wide-ranging movements within and outside of Cook Inlet during the winter months. Three trip statistics (trip length, trip duration and net trip distance) were examined as response variables in a mixed linear model. All age and sex classes showed a drop in trip duration and length during the pupping and breeding season. Adult female trips were the shortest in length and duration during the month of June (when many are likely tending a pup). Analysis comparing seasonal dive behavior of a single adult female (AF) harbor seal and a juvenile male (JM) harbor seal showed similar results. Dive activity for the adult female was reduced in the month of June. Haul-out behavior also varied seasonally with the proportion of seals hauled out peaking at 0.43 in June compared to 0.32 in October. Combining the previously reported survey counts with these haul-out proportions resulted in a peak seasonal abundance of 23,954 (SE: 1576) seals in August compared to 10,820 (SE: 1240) in April. These insights into seasonal harbor seal movements, behavior and abundance are critical for understanding foraging ecology, marine habitat use, and risk of impacts from industrial accidents such as oil spills.

KEY WORDS: harbor seal, *Phoca vitulina*, movements, seasonal use, Cook Inlet, Alaska

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INTRODUCTION

Harbor seals (*Phoca vitulina*) are important marine predators that occupy a broad range in Alaska from approximately 130°W to 172°E (over 3,500 km east to west) and from 61°N to 51°N (over 1,000 km north to south) (Frost et al. 1982). In recent decades, their abundance has declined at several Alaska locations. For example, in the Aleutian Islands, counts declined 67% between the early 1980s and 1999 (Small *et al.* 2008), with declines of 85% in the western islands. Counts of harbor seals at Tugidak Island declined 85% between 1976 and 1988 (Pitcher 1990). Counts in Prince William Sound declined approximately 63% between 1984 and 1997 (Frost et al. 1999), and 3.3%/yr during 1988-1999 (Ver Hoef and Frost 2003). More recent evidence indicates that harbor seals near Kodiak Island, including those at Tugidak Island, have increased 6.6%/yr during 1993-2001 (Small et al. 2003). Because Cook Inlet is located between regions of contrasting trends, it is important to assess the current status of the population and the potential impacts of oil and gas activities on harbor seal populations within Cook Inlet.

As harbor seals either recover from population declines in the 1970s and 1980s or continue to decline, they are particularly vulnerable to acute and chronic environmental impacts, such as those that could result from industrial accidents such as oil spills (Frost and Lowry 1994, Frost et al. 1994, Spraker et al. 1994, Hoover-Miller et al. 2001). If an oil spill were to occur, harbor seals would be at risk for direct exposure to oil both at sea and ashore when they haul out (Lowry et al. 1994) and secondary exposure caused by ingestion of prey that have been exposed to oil.

Satellite-linked dive recorders (SDRs) deployed on seals are a common tool used by researchers to investigate the marine habitat use, behavior and ecology of marine mammals. It is only recently that statistically advanced techniques such as state-spaced modeling and state-switching models have been available and used to investigate movement and space utilization, while appropriately accounting for location error that is inherent with Argos locations from SDRs deployed on marine mammals. Similarly, advanced methods for analyzing behavior data recorded and transmitted from the SDRs has been lacking. These time-series datasets have a large number of repeated behavior measurements and appropriately dealing with the auto-correlation has been a challenge methodologically and computationally. Dive behavior data are summarized by the SDRs into discrete, unequal bins programmed by the researcher. This categorization and aggregation create data that are a time series of

ordered multi-category counts, which present challenges in terms of statistical modeling and practical interpretation. Similar issues exist with respect to the interpretation of haul-out timeline behavior data. These timeline data are critical not only for understanding harbor seal behavior, but also calculating proper estimates of abundance that account for seals in the water during the time of the aerial surveys. Here, we present statistically sound methodological advancements that overcome many of these issues. We have applied these methods to data obtained from SDRs deployed on harbor seals in the Cook Inlet region of Alaska between 2004 and 2006 in support of our original project objectives and hypotheses.

OBJECTIVES

Objective 1: To assess the seasonal patterns of abundance, distribution, and behavior of harbor seals in Cook Inlet, including an assessment of the seals' diving behavior.

Objective 2: To identify and determine the priority of importance specific marine habitats used by harbor seals in Cook Inlet associated with key life history events such breeding, pup rearing, and foraging.

Objective 3: To enhance estimates of harbor seal abundance in Cook Inlet by determining and applying a haul-out correction factor that adjusts aerial survey counts for the proportion of seals that are at-sea and not observed during aerial surveys.

Objective 4: To evaluate whether or not geographically distinct subpopulations of seals exist within Cook Inlet.

The project's objectives will be addressed by testing the following hypotheses:

Hypothesis 1: Harbor seal diving behavior changes seasonally, presumably reflecting changes in the amount of time spent in social behaviors (e.g., courtship and mating) vs. foraging behaviors, and the depth distribution of local prey aggregations.

Hypothesis 2: Marine habitat use, as measured by at-sea home range sizes and locations, changes seasonally as seals move to access aggregations of migrating prey. Consequently, the risk of exposure to oil at sea changes as seals spend more, or less, time near potential oil hazards.

Hypothesis 3: The proportion of time spent hauled out changes as an estimable function of environmental and temporal covariates, allowing aerial survey counts to be corrected to

absolute abundances.

Hypothesis 4: Two or more geographically distinct subpopulations of harbor seals are present in Cook Inlet, as demonstrated by genetic analyses and movement patterns of tracked animals.

METHODS

Seal Captures and Satellite Tag Deployment

All four hypotheses developed in support of the project objectives relied on the capture and release of harbor seals within Cook Inlet. To accomplish this, four research cruises were conducted aboard charter vessels from the port of Homer, Alaska. The cruises took place in the fall of 2004, spring of 2005, fall of 2005 and spring of 2006. Ninety-three harbor seals were captured and released in Cook Inlet from 2004 to 2006. Seals were captured at a variety of sites widely distributed around central Cook Inlet, including Aurora Rock, Bradley River (both within Kachemak Bay), Yukon Island, Kamishak Bay, Iniskin Bay, Chinitna Bay, Tuxedni Bay, and Kalgin Island. Seals were captured using large-mesh nylon nets deployed from small boats near harbor seal haul-out sites. Seals were carefully removed from the nets and transported to the charter vessel where they were weighed, measured and sedated prior to tag attachment. Genetic tissue samples were collected from each seal.

During the four cruises, 77 seals of all age and sex categories (see Table 1) were captured and released with at least one Argos satellite-linked dive recorder (SDR) manufactured by Wildlife Computers (Redmond, Washington, USA). A total of 91 satellite tags were deployed; 77 tags were of either the ST16 or SPLASH tag type, glued to a seals' pelage, and 14 were SPOT4/5 tags, specially designed for attachment through the webbing of a seal's hind-flipper. The ST16 and SPLASH tags were programmed to collect and transmit information on each seal's location, haul-out time, and diving behavior. They were not duty cycled. The SPOT tags provided only location and haul-out timelines (no dive behavior) and had much smaller batteries. By attaching the SPOT tags to the rear flipper, rather than gluing them to the pelage, deployment lengths through and beyond the molt period was possible. The SPOT tags were duty cycled to transmit data only from the final half of July through the first half of September.

Table 1 Distribution of age and sex categories for harbor seals captured in Cook Inlet, Alaska and released with at least one SDR.

Sex	Adult	Sub-Adult (SUB)	Young of the Year (YOY)
Female	16	15	6
Male	23	13	4
Total	39	28	10

Hypothesis 1: Harbor seal diving behavior changes seasonally, presumably reflecting changes in the amount of time spent in social behaviors (e.g., courtship and mating) vs. foraging behaviors and the depth distribution of local prey aggregations.

Ordered categorical data are pervasive in environmental and ecological data, frequently arising from practical constraints imposed at the time of data collection. Often, the constraints require discretizing a continuous variable into ordered categories. A great deal of data have been collected toward the study of marine mammal dive behavior using dive recorders, which often discretize a continuous variable such as depth. Additionally, data storage or satellite-link transmission constraints may also necessitate the aggregation of data over time intervals of a specified length. Due to the bandwidth limitations of the Argos platform, dive behavior data in this study were transmitted as summarized histograms for ‘maximum dive depth’, ‘dive duration’ and ‘time at depth’. This categorization and aggregation create data that are a time series of ordered multi-category counts, which present challenges in terms of statistical modeling and practical interpretation.

As part of this study, we developed and introduced (Higgs and Ver Hoef 2011) an intuitive strategy for modeling such aggregated, ordered categorical data that is both statistically appropriate and practically useful for the scientists collecting the data. This technique allows for inference regarding the category probabilities and a measure of central tendency on the original scale of the data (e.g. meters), along with incorporation of temporal correlation and over-dispersion. The primary goal of the analysis was to

assess the relationship of covariates, such as time of day, with number of dives and maximum depth of dives.

The computational requirements are currently too high to complete this analysis for the entire dataset of deployments from this study. Here we present summary graphs showing dive behavior of an adult female (AF) and juvenile male (JM) harbor seal across multiple seasons. Higgs and Ver Hoef (2011) is included as an appendix to this report and provides complete details describing the statistical theory and implementation of this novel strategy. Additional studies to further examine the dive behavior of Cook Inlet harbor seals are on-going and we will continue to publish findings from these endeavors in the peer-reviewed literature.

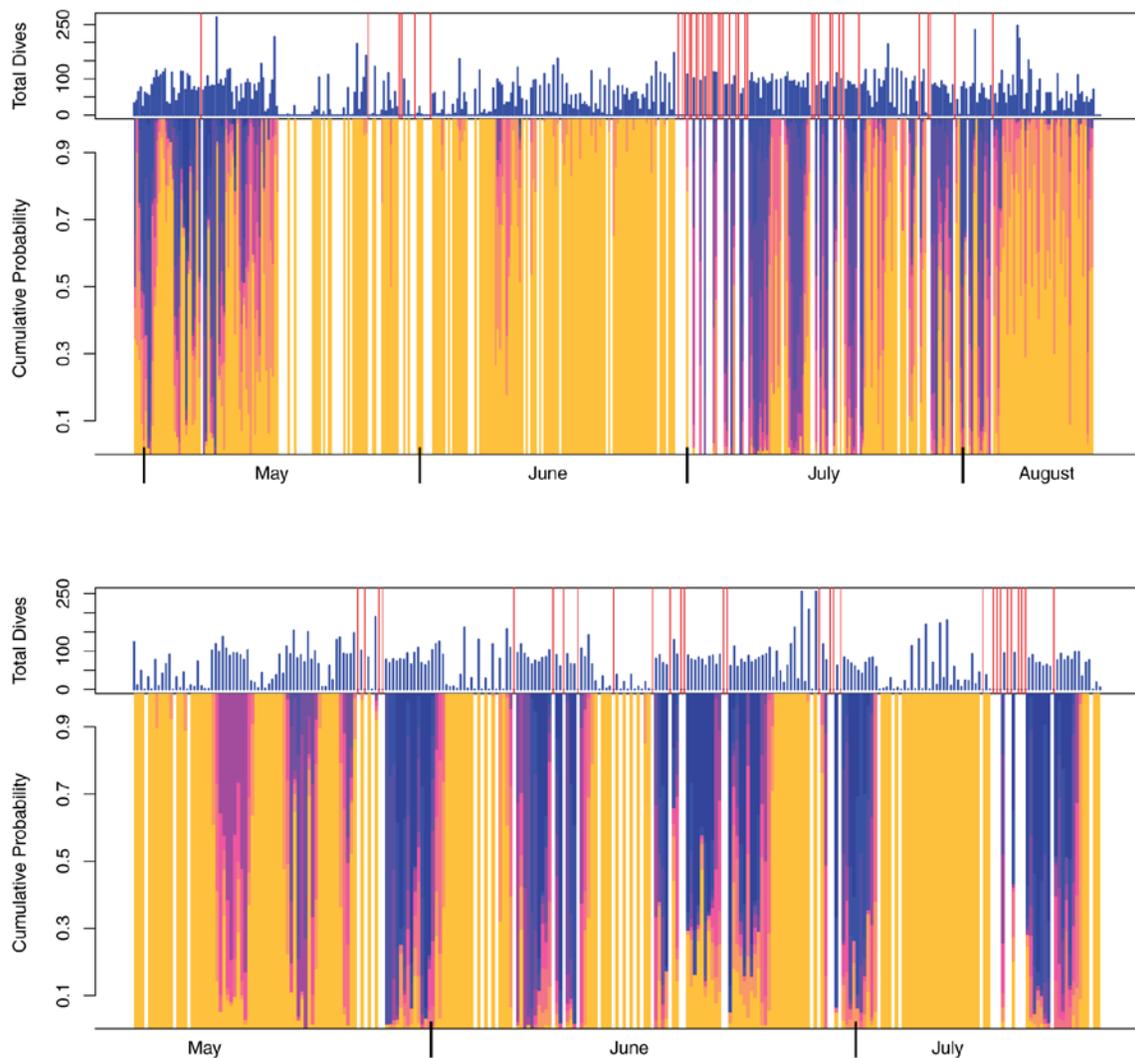


Figure 1 Raw dive data from two seals: (top) an adult female (AF) from April 30 to August 19, 2006 and (bottom) a juvenile male (JM) from May 10 to July 18, 2005. Each plot displays a time series of bars showing the relative proportions of dives from each time period falling in eight (top) or nine (bottom) defined depth classes. Lighter shades correspond to shallower depths, with white indicating either zero dives during that time period or a missing data. The total number of dives per time period is plotted along the top, and the tall red vertical lines indicate missing time periods (reproduced from Higgs and Ver Hoef 2011).

Hypothesis 2: Marine habitat use, as measured by at-sea home range sizes and locations, changes seasonally as seals move to access aggregations of migrating prey. Consequently, the risk of exposure to oil at sea changes as seals spend more, or less, time near potential oil hazards.

As with the dive behavior hypothesis, much of our effort related to this hypothesis focused on development of new statistical methodologies. A novel method using a continuous-time correlated random walk (CTCRW) was developed and published by Johnson et al. (2008) based, in large part, on data collected from this study. Additionally, Johnson maintains an R package, 'crawl', with a high download rate and diverse use-base across multiple disciplines. Here we present two research efforts in support of our second hypothesis, which rely heavily on the CTCRW model and 'crawl' package. First, we examined seasonal and age-related differences in movement metrics and trip characteristics. Second, we documented changes in seasonal use and distribution of harbor seals while at haul-out locations and while at sea.

Argos locations from 77 harbor seal deployments were first pre-filtered with the procedure of Freitas et al. (2008) using the 'argosfilter' package within R. Additionally, for the trip analysis, the focus was on at-sea trip behaviors and, therefore, all locations greater than 1500 meters inland (due to Argos location error) were removed from the dataset. Inland locations were not filtered from the dataset for the space-use analysis as we were interested in both haul-out (which occurs on land) and at-sea use patterns. Following the pre-filtering, animal paths were modeled using the CTCRW model within the 'crawl' R package. We accounted for haul-out time through a dynamic auto-regression parameter that is a function of the collected dry-time data. The dynamic model does not allow for movement while the animal is hauled out. We used the 'dry-stop' function of the crawl package to incorporate harbor seal haul-out data into the movement model. These data were collected and summarized on the tag at hourly intervals, with some missing data due to Argos transmission errors or suboptimal timing of transmissions. These time gaps and uncertainty in the haul-out behavior can lead to additional uncertainty in the use and movement of harbor seals. For the use analysis, a seal was considered hauled out only if the tag reported 100% dry for the hour. CTCRW parameter estimation is accomplished by forming it as a state-space model on which the Kalman filter is used to obtain maximum likelihood

estimates, and to provide hourly location estimates. Six of the deployments did not provide enough data to appropriately model and were removed from final analysis.

Table 2 Distribution of sex and age class among seals deployed with tags and initially included within the movement analysis procedures

AGE	SEX	NUMBER OF ANIMALS
ADULT	FEMALE	16
SUB-ADULT	FEMALE	12
YOUNG-OF-YEAR	FEMALE	6
YEARLING	FEMALE	3
ADULT	MALE	23
SUB-ADULT	MALE	11
YOUNG-OF-YEAR	MALE	4
YEARLING	MALE	2

Trips were defined based on the haul-out timeline, with the change-point set at 33.3% of an hour in the 'dry' state. Trip distances were estimated with a parametric bootstrap approach. After processing with the Kalman filter, 50 paths were simulated for each animal from the path distribution conditional on the observed Argos data. For each animal and sampled path, total distance, net distance, and total time were calculated. Average travel distance and net distance were used as point estimates, and standard errors were estimated from the distance and net distance variances for each animal. These bootstrap estimates represent approximations to the minimum mean-squared error predictions for travel distance and net distance.

The trip data were modeled using a linear mixed model with sex, age, and month as fixed effects, a random effect for each seal, and temporal autocorrelation for repeated trips by an individual seal. The temporal autocorrelation used a first-order autoregressive model where trips were sequentially indexed. Total trip distance (km), trip duration (days) and net trip distance (km) were examined as response variables. All response variables were transformed to the log scale. For trip distance, two values were removed after checking residuals for outliers; no outliers were apparent for trip duration or net distance. After removing outliers, residuals appeared to be close to normal distributions for all three variables.

This study used a Bayesian approach to estimate the spatial use of tagged seals in the Cook Inlet and Gulf of Alaska regions. By sampling ($n=1000$) from the posterior distributions of the CTCRW predicted tracks, we were able to encapsulate Argos location error and the full range of variability in the movement model in our spatial use analysis. This variability is represented by the overall distribution and the CVs of use for each cell (in the figures, smaller cell size is representative of higher CV values).

We examined the effects of season (fall, early winter, late winter, spring, and summer) on the spatial use patterns of the tagged seals. Use maps were created on a raster grid with cell size of 100 km^2 . Two values were calculated for each cell in each simulation: the number of hours all seals spent in the cell, and the number of seals that spent any hour in the cell. Separate maps were made for each season. Additionally, the use patterns were depicted separately for periods of haul-out and at-sea behavior.

The movement model and use maps did not account for the presence of land. As shown in the space-use figures, the land overlay serves as an assessment of the variability associated with the movement model and Argos location quality.

Hypothesis 3: The proportion of time spent hauled out changes as an estimable function of environmental and temporal covariates, allowing aerial survey counts to be corrected to absolute abundances.

We used a subset 50 seals from our deployment of SDRs to examine haul-out behavior of harbor seals in the Cook Inlet region. SDRs were programmed to record an electrical resistance value of the surrounding medium (water or air). For every hour, the tags recorded the portion of time dry. These dry-time percentages were used to determine haul-out status. Data were transmitted by satellite to Argos (CLS America, Inc., Largo, MD) and downloaded for analysis. The percentage-dry data were converted to a binary response variable with a "1" indicating a deployed tag was dry greater than 50% of the time during the hour. The binary series ranged from 478 to 6,336 hours per seal, for a total of 151,247 observations. There was limited storage on the SDR, and satellites were not always able to upload data before storage capacity was filled, resulting in some periods of missing data.

Our interest centered on factors that affect temporal haul-out patterns. We used restricted maximum likelihood on pseudo-data to fit GLM pseudo-models to the haul-out data. The contains all main effects

and two-way interactions. Additional details regarding details of the GLM pseudo-model can be found in Ver Hoef et al. 2010.

We were primarily interested in using this haul-out model to correct adjusted counts for the proportion of seals that were in the water at the time of the surveys. We encountered two problems with this approach. The count data from aerial surveys do not contain data on sex/age composition of the counts. Thus, we desire an average haul-out proportion, weighted by the population sex/age composition. Although the analysis (Ver Hoef et al. 2010) indicated some sex/age differences, these were minor, and we felt that the distribution of sex and age within our sample (Table 2) was fairly close to that in the population. Thus, we simply fit a pooled model without including sex and age effects.

Table 3 Distribution of age and sex categories for a subset of harbor seal SDR deployments used within the statistical haul-out model.

Sex	Adult	Sub-Adult (SUB)	Young of the Year (YOY)
Female	10	9	6
Male	11	10	4
Total	21	19	10

The second problem was that the satellite transmitters did not remain attached during August, the time of molt and one of our key seasonal survey periods. We addressed this problem by interpolating the fitted models using trigonometric covariates on date and time of day. This also resolved any issues with creating an arbitrary starting day-of-year, and having to use 24 categorical covariates for hour-of-day. We can create an index of “spring-time” by taking $f_s(t_{\text{doy}}) = \sin(2\pi t_{\text{doy}}/365)$, and an index of “summer-time” by taking $f_c(t_{\text{doy}}) = -\cos(2\pi t_{\text{doy}}/365)$, where $t_{\text{doy}} = \text{day-of-year}$, from 1 to 365 beginning with 1 January. Likewise, we can create an index of “6AM-time” by taking $f_s(t_{\text{tod}}) = \sin(2\pi t_{\text{tod}}/24)$, and an index of “noon-time” by taking $f_c(t_{\text{tod}}) = -\cos(2\pi t_{\text{tod}}/24)$, where $t_{\text{tod}} = \text{time-of-day}$, from 1 to 24 beginning with 1 AM. Let Y_i be a random variable for haulout, which is a binary response. The models of Ver Hoef et al. (2010) assume Y_i has expected value $\exp(\mu_i)/(1 + \exp(\mu_i))$, with a temporally auto-correlated error

structure that has a diagonal variance structure like a Bernoulli distribution. Thus, we fit the following model,

$$\begin{aligned} \text{logit}(\mu_i) = & \beta_0 + \beta_1 h_i + \beta_2 h_i^2 + \beta_3 f_s(t_{doy,i}) + \beta_4 f_s^2(t_{doy,i}) + \beta_5 f_c(t_{doy,i}) + \beta_6 f_s(t_{tod,i}) + \\ & \beta_7 f_s^2(t_{tod,i}) + \beta_8 f_c(t_{tod,i}) + \beta_9 f_s(t_{doy,i})f_s(t_{tod,i}) + \beta_{10} f_s(t_{doy,i})f_s^2(t_{tod,i}) + \\ & \beta_{11} f_s(t_{doy,i})f_c(t_{tod,i}) + \beta_{12} f_s^2(t_{doy,i})f_s(t_{tod,i}) + \beta_{13} f_s^2(t_{doy,i})f_s^2(t_{tod,i}) + \\ & \beta_{14} f_s^2(t_{doy,i})f_c(t_{tod,i}) + \beta_{15} f_c(t_{doy,i})f_s(t_{tod,i}) + \beta_{16} f_c(t_{doy,i})f_s^2(t_{tod,i}) + \\ & \beta_{17} f_c(t_{doy,i})f_c(t_{tod,i}), \end{aligned}$$

where h_i is minutes from low tide, $t_{doy,i}$ is day-of-year, $t_{tod,i}$ is time-of-day for the i th observation. Note that we do not include the $f_c^2(t_{doy,i})$ and $f_c^2(t_{tod,i})$ terms because the trigonometric identity $f_s^2(t_{doy,i}) + f_c^2(t_{doy,i}) = 1$ creates a linear dependency with the overall intercept, and hence, non-estimable regression coefficients. In summary, we have 3 main effects for day-of-year and 3 main effects for time-of-day, and all possible interactions between them. This is similar, in a count of the parameters, to having a third-order polynomial for each effect, and their interaction. Here, the virtue is that the covariate values “wrap” on the circular values of day-of-year and time-of-day.

Hypothesis 4: Two or more geographically distinct subpopulations of harbor seals are present in Cook Inlet, as demonstrated by genetic analyses and movement patterns of tracked animals.

The methods used in the genetic analyses of population structure are provided in the contract report attached as Appendix 1.

RESULTS

Hypothesis 1: Harbor seal diving behavior changes seasonally, presumably reflecting changes in the amount of time spent in social behaviors (e.g., courtship and mating) vs. foraging behaviors and the depth distribution of local prey aggregations.

The effort and results related to this hypothesis were focused on the development of analytical methods for the dive data, which present statistical difficulties due to their unusual form. Interesting behavioral patterns, however, were observed in our two example datasets. Higgs and Ver Hoef (2011) presented analyses and results comparing seasonal dive behavior of an adult female (AF) harbor seal and a juvenile male (JM) harbor seal. For the AF harbor seal, the period from approximately the second week in May through June was characterized by relatively low number of dives and a weighted average depth often within the 5 to 10 meter range. The first part of May and the month of July were characterized by increased dive activity and weighted average depth values regularly within the 50 to 100 meter range. The months of May and June correspond with the seasonal peak in pupping, weaning and mating behavior. While we have no capability to confirm whether this AF had a pup, the observed behavior was consistent with a seal tending a pup.

The JM harbor seal maintained a relatively consistent dive behavior pattern throughout the record. There was no evidence of a prolonged period of reduced dive activity as was observed in the AF. The JM dive behavior, however, was more episodic with distinct periods (1-2 weeks) of high dive activity and weighted average depths between 100 and 200 meters followed by periods (1-2 weeks) of low dive activity and weighted average depths between 5 and 10 meters. This behavior is consistent with a non-breeding animal that was focused on the location and exploitation of prey resources. As part of an ongoing effort to publish results from this and other satellite-telemetry studies of harbor seals throughout Alaska, we plan to further develop methods for linking the modeled dive behavior to predicted temporal-spatial locations derived from the Argos data.

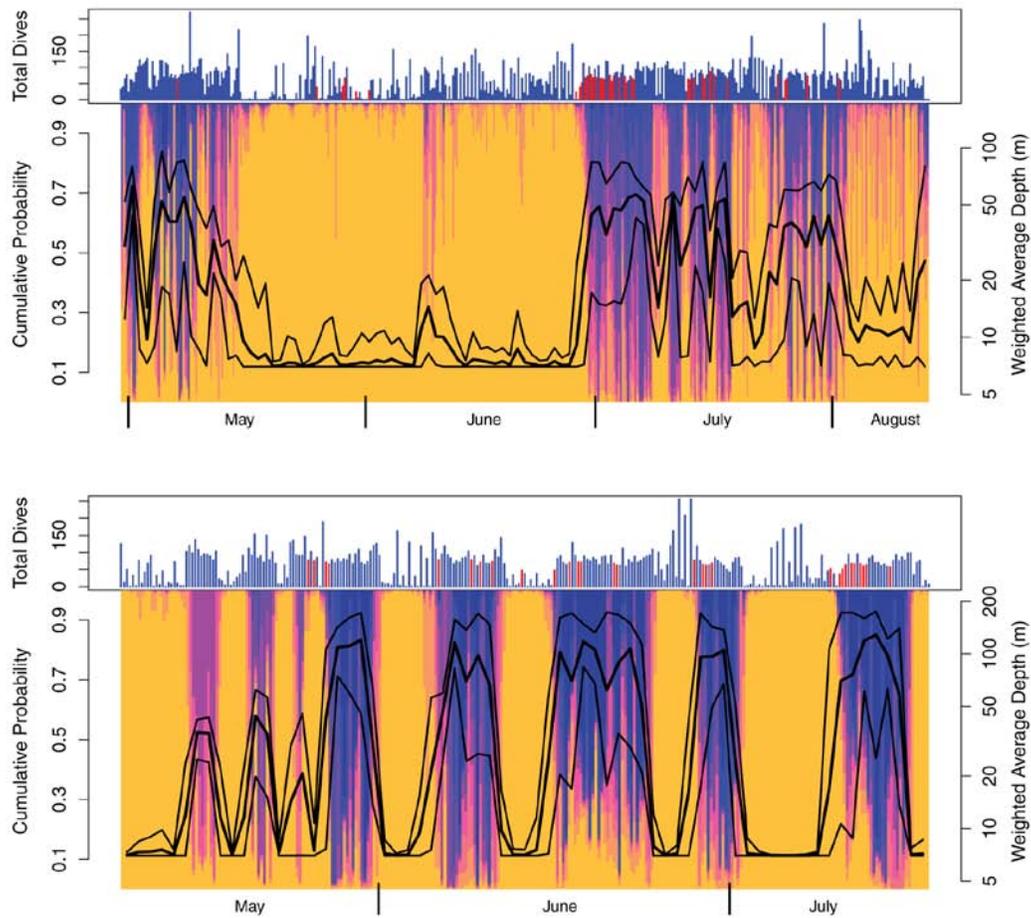


Figure 2 Time Series of barplots showing posterior means of proportion of dives to each depth class for an AF (top) and a JM (bottom). Lighter shades indicate shallower depths. The total number of dives per time period is plotted along the top, where lines in a lighter shade give the predicted number of dives for missing time periods. Posterior means and 95% posterior intervals for daily weighted average depth (averaged over time periods within each day) are plotted in black with respect to the second y-axis (reproduced from Higgs and Ver Hoef 2011).

Hypothesis 2: Marine habitat use, as measured by at-sea home range sizes and locations, changes seasonally as seals move to access aggregations of migrating prey. Consequently, the risk of exposure to oil at sea changes as seals spend more, or less, time near potential oil hazards.

Results from both the analysis of trip characteristics and space-use provided insights suggesting harbor seals in Cook Inlet and the Gulf of Alaska vary in their behavior and distribution across seasons and some of this variability is sex and age related. Figure 3 shows predicted track locations for harbor seals during the months of April, May, June, November, December and January. The tracks are colored with warmer values corresponding to spring and cooler values corresponding to winter. The distribution of movements was much broader in the winter months with some seals ranging as far as the Shumigan Islands. During summer months, movements and distribution was mostly confined to the west side of Cook Inlet and Kachemak Bay.

Trip distances ranged from approximately 10 to 250 kilometers (Figure 4). Shorter trips were evident during the pupping and breeding season. Adult female trips were the shortest in the month of June (when many were likely tending pups). Except for June, adult male trips were shorter than female trips. Trip duration ranged from approximately 4 hours to 4 days (Figure 5). Adult female trips were of shortest duration in June, which corresponds with the period of short trip distances. Lower variability in trip duration was observed among adults when compared to non-adult seals. A similar pattern to trip distance was evident in total net distance traveled during a trip. In general, seals returned to within 10 kilometers of the starting point for the trip and adults were more consistent across months (Figure 6). Higher net distance values were calculated in the spring and fall seasons, which correspond with the seasonal movement of seals in and out of Cook Inlet. Consistent with total trip distance and trip duration, net trip distance was lowest for adult females in June.

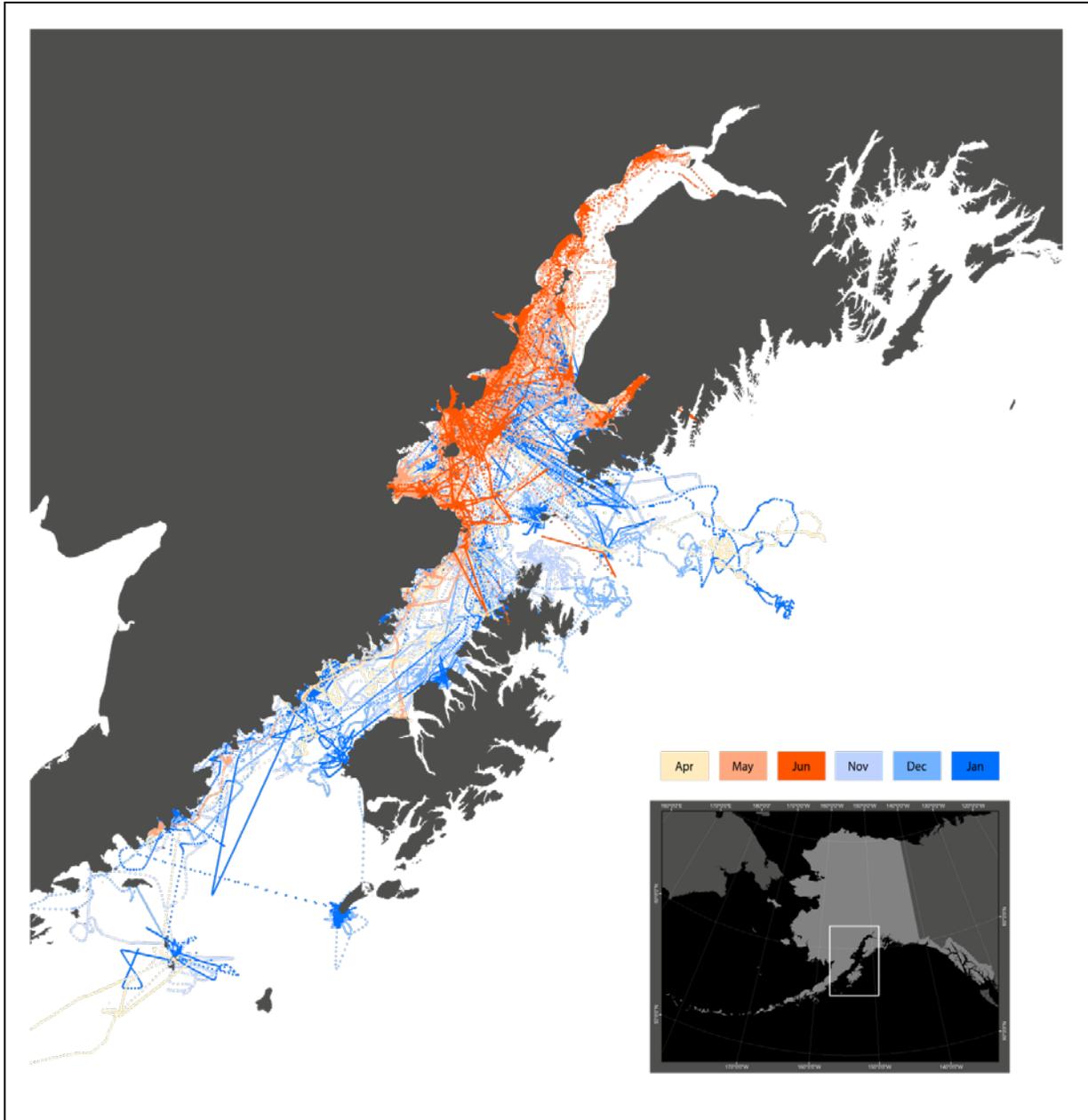


Figure 3 Predicted tracks of harbor seals in Cook Inlet and the Gulf of Alaska. Colors depict seasonal differences in distribution (warmer colors = April to June; cooler colors = November to December)

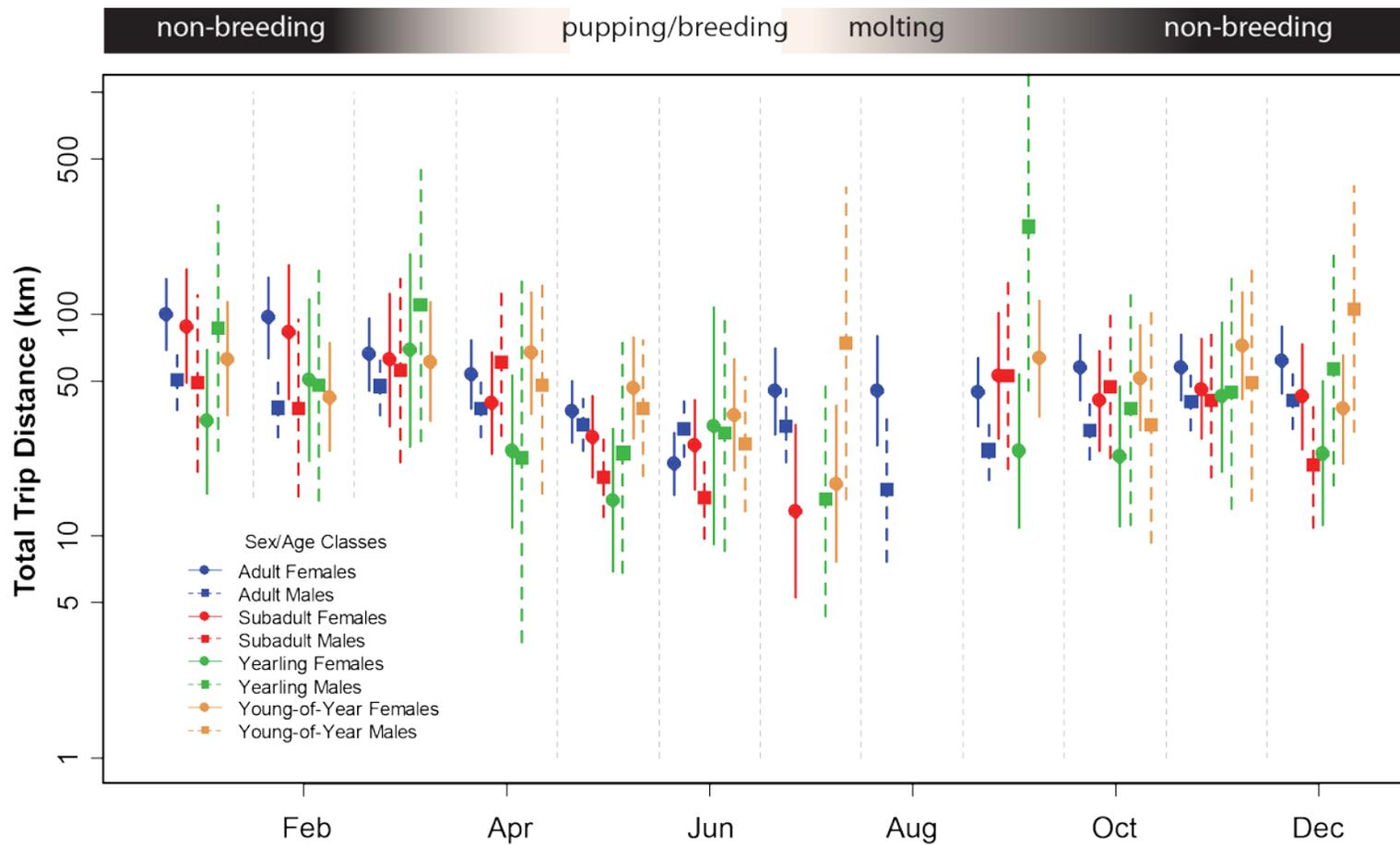


Figure 4 Total trip distance traveled for harbor seals of various age and sex classes in Cook Inlet and the Gulf of Alaska.

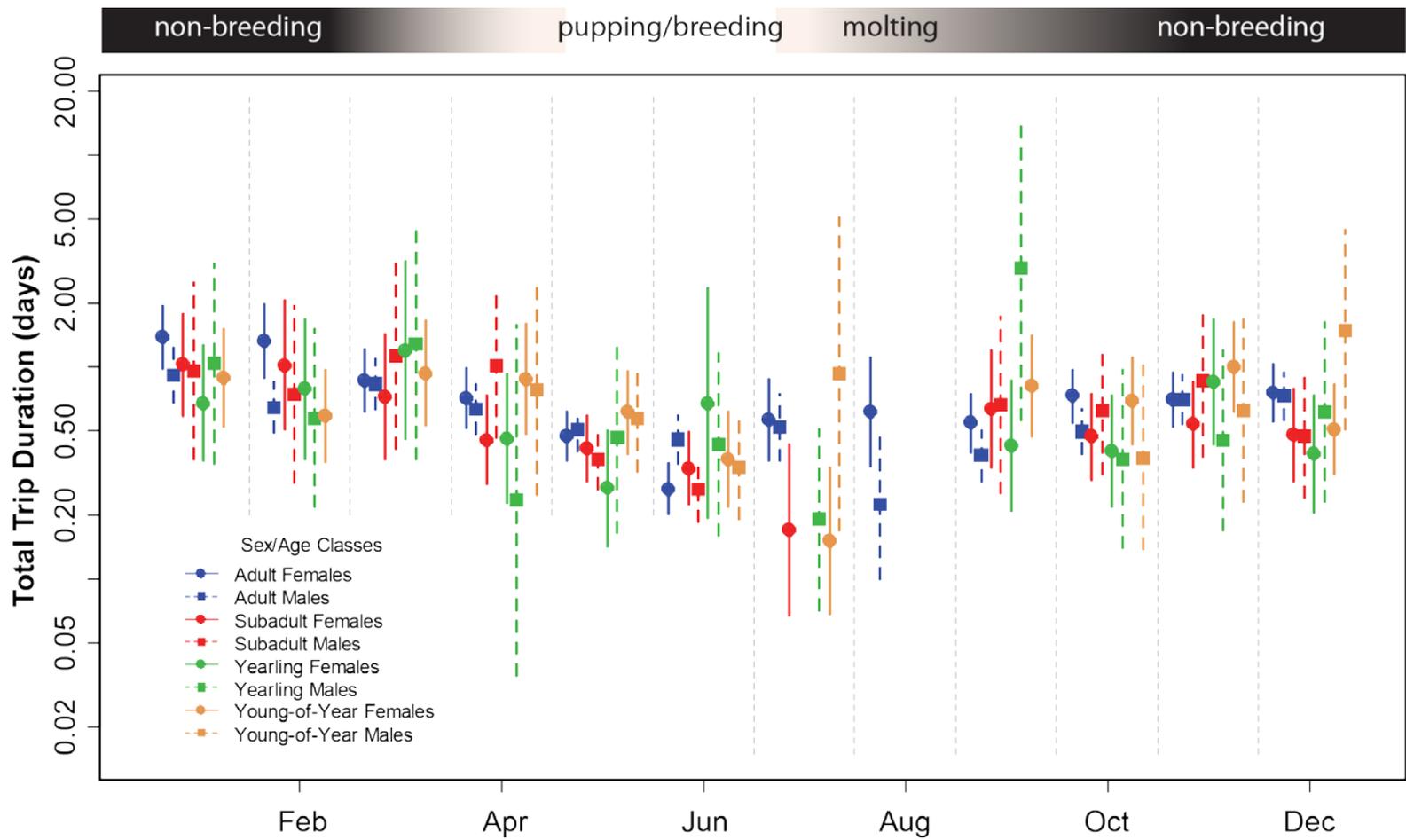


Figure 5 Total duration (in days) of trips for harbor seals of various age and sex classes in Cook Inlet and the Gulf of Alaska.

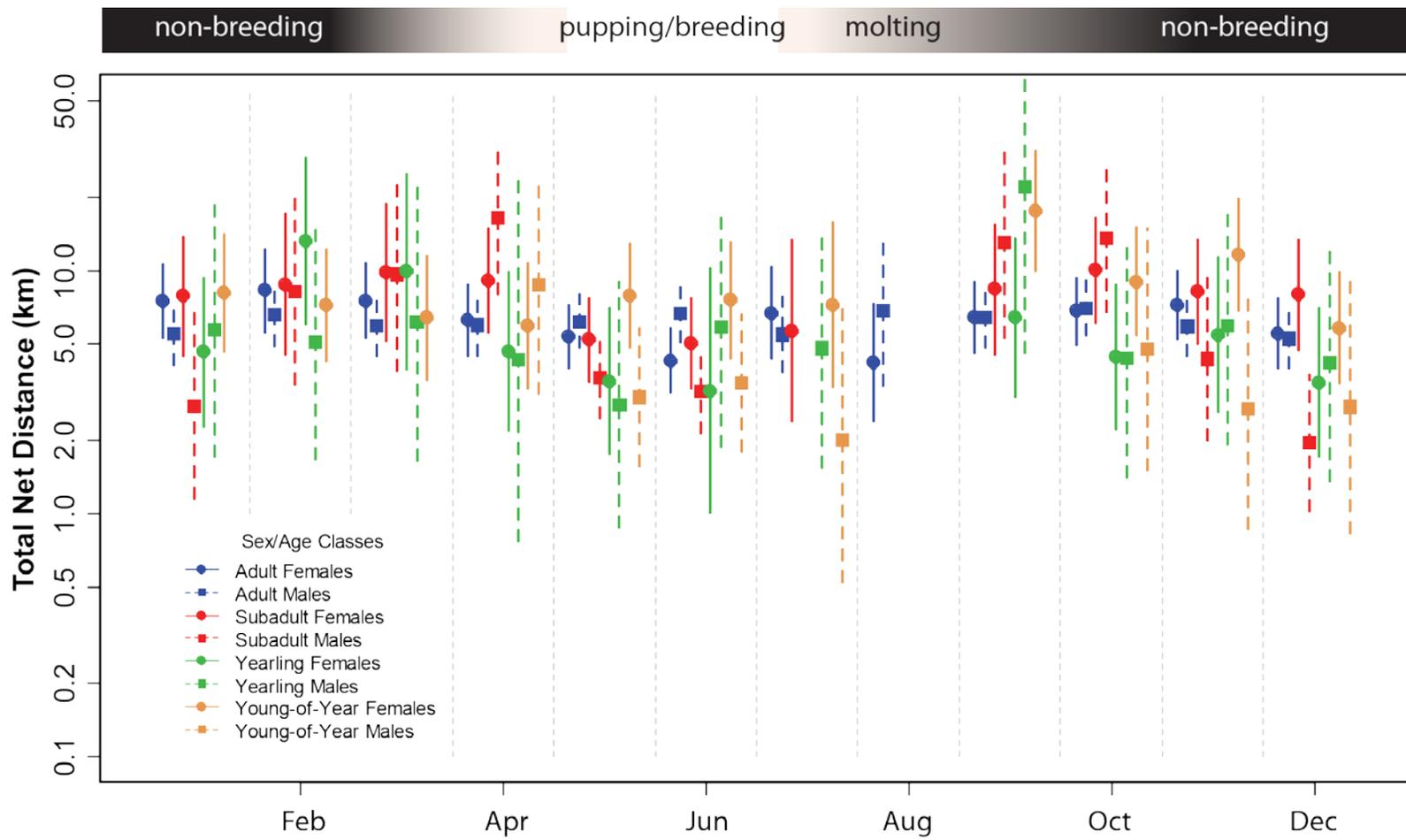


Figure 6 Total net distance of trips for harbor seals of various age and sex classes in Cook Inlet and the Gulf of Alaska

Space use by harbor seals was divided into five seasonal periods: October-November, December-January, February-March, April-May and June-July. August and September were not considered because most of the deployed tags were shed from the seals in August as the seals underwent their annual molt cycle. Within each seasonal period, seal use was calculated and displayed (Figures 7-11) based on 4 distinct categories: average total hours of hauled-out presence in a grid cell, average total hours of in-water presence in a grid cell, average number of seals using a cell while hauled out, and average total number of seals using a cell while in water. Consistent with the findings reported in our analysis of trip durations, seal use was more broadly distributed in the winter months and most confined to the Cook Inlet region during spring and early summer. This pattern was consistent for both in-water behavior and haul-out behavior.

Within each seasonal period, there were distinct geographical regions and habitats where large portions of the seals' time were spent. During the fall months of October and November, seal use was most concentrated in Kachemak Bay, across Cook Inlet toward Iniskin and Iliamna bays, and south through the Kamishak Bay, Cape Douglas and Shelikof Strait regions. Seals also used haul-out and marine habitats along the north side of Kodiak and Afognak islands. One seal traveled farther south and west along the Alaska Peninsula to the Semidi Islands. December and January were characterized by continued use of the core areas within central and southern Cook Inlet as well as expanded use of near-shore habitats along Shelikof Strait, the Alaska Peninsula, north and west sides of Kodiak Island, and Tugidak Island. February and March were similar to December and January. By April and May, harbor seal use contracted significantly and was concentrated mostly within Cook Inlet. Additionally, some seals expanded their use into the northern portion of Cook Inlet (where seals were not captured as part of this study). Our results suggest that most or all harbor seals that are found in our study area of central and lower Cook Inlet during May (our spring tagging period) remain in Cook Inlet through June and July.

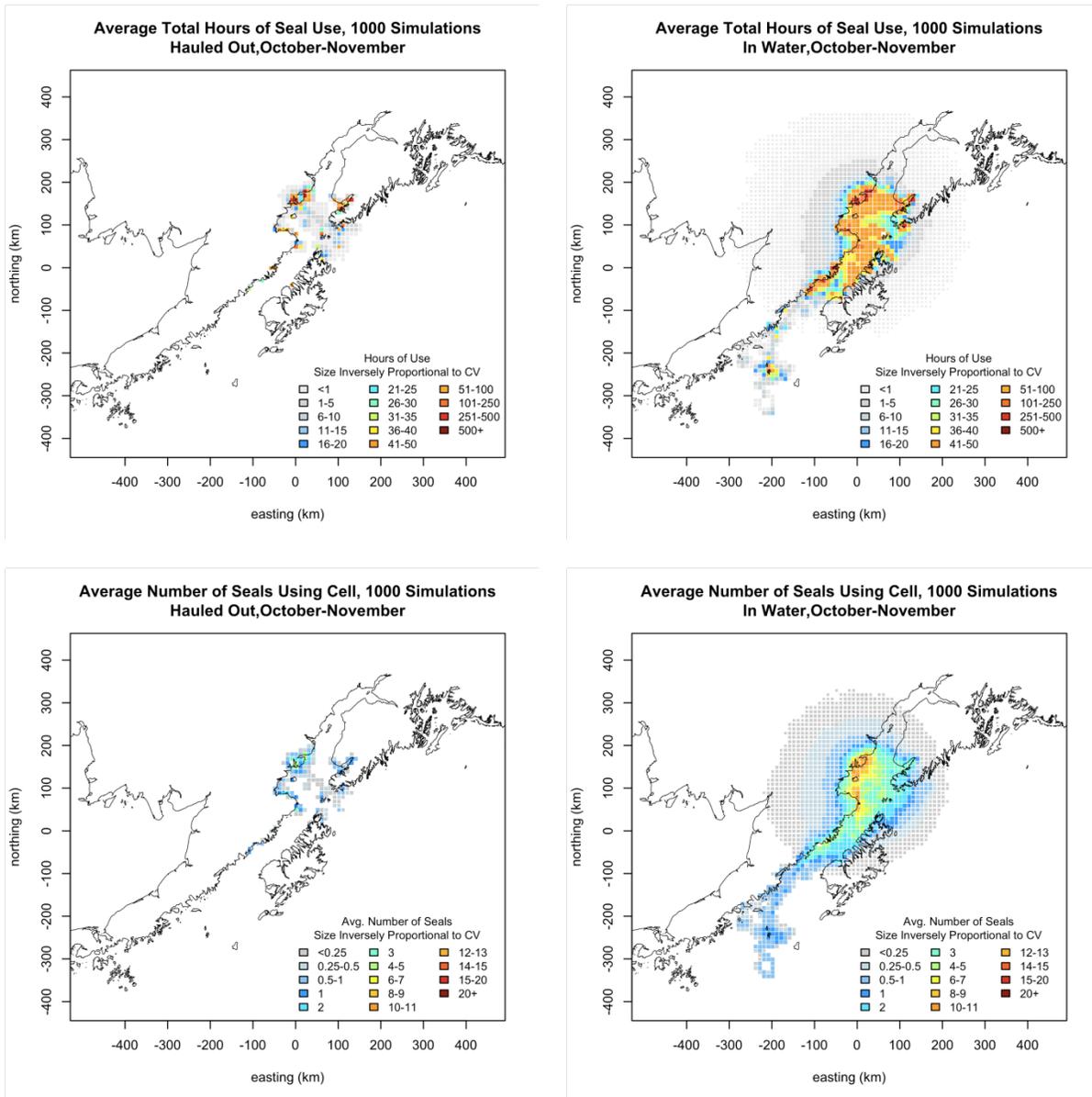


Figure 7 Harbor seal area use maps depicting total hours of seal use and total number of seals using a cell during the months of October and November. Data are combined across years (2004-2006) and the cell size depicted on the maps are inversely proportional to the CV of the estimates.

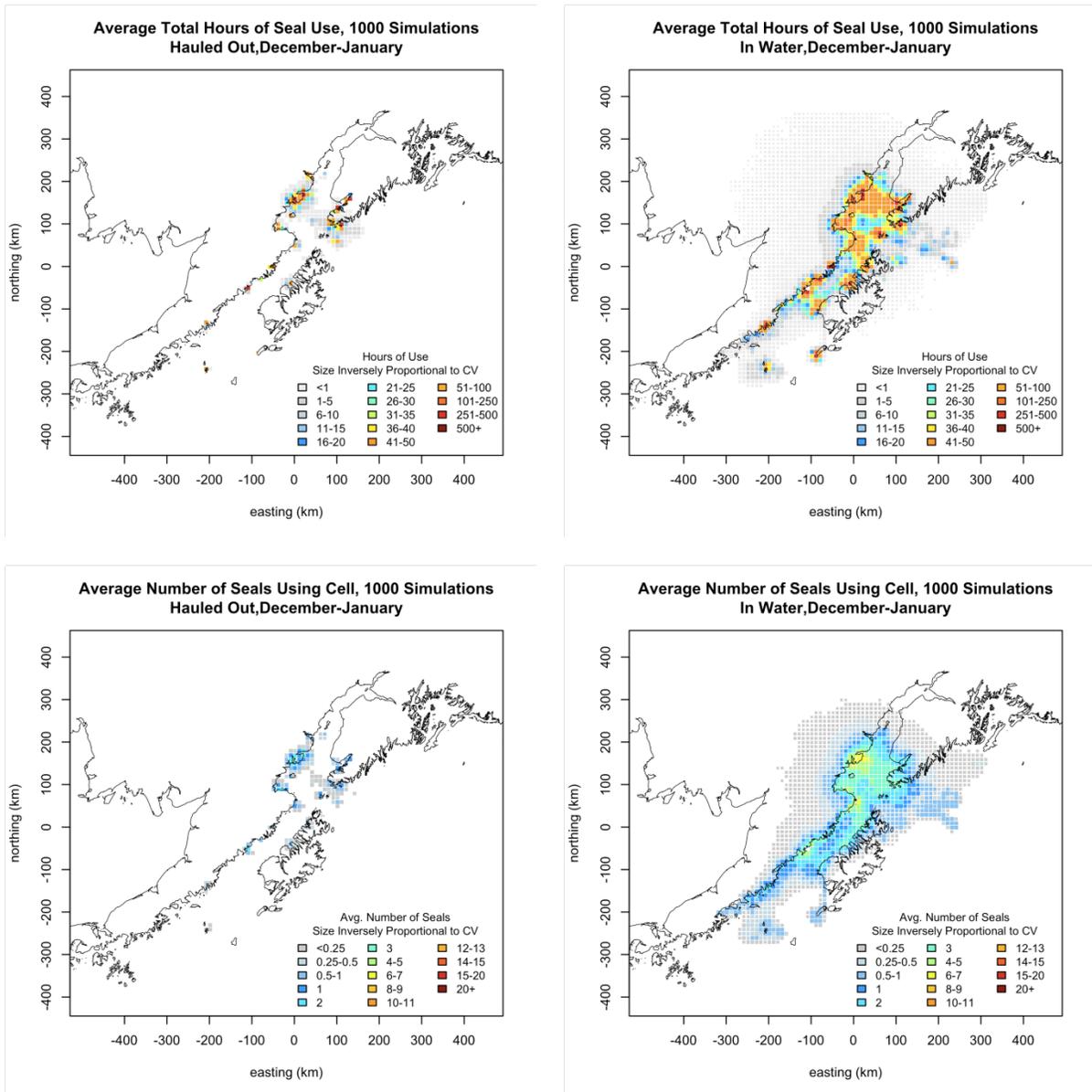


Figure 8 Harbor seal area use maps depicting total hours of seal use and total number of seals using a cell during the months of December and January. Data are combined across years (2004-2006) and the cell size depicted on the maps are inversely proportional to the CV of the estimates.

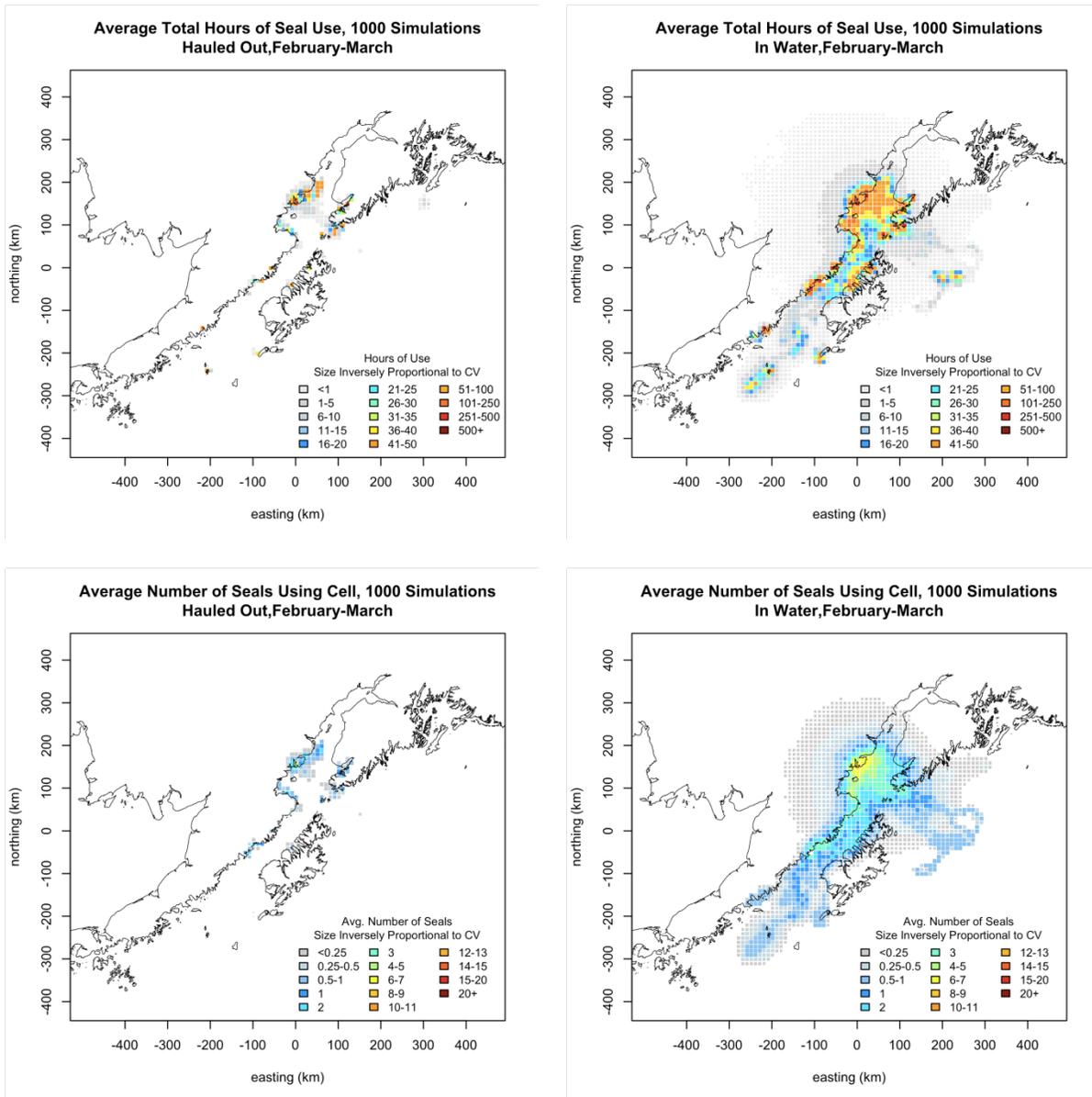


Figure 9 Harbor seal area use maps depicting total hours of seal use and total number of seals using a cell during the months of February and March. Data are combined across years (2004-2006) and the cell size depicted on the maps are inversely proportional to the CV of the estimates.

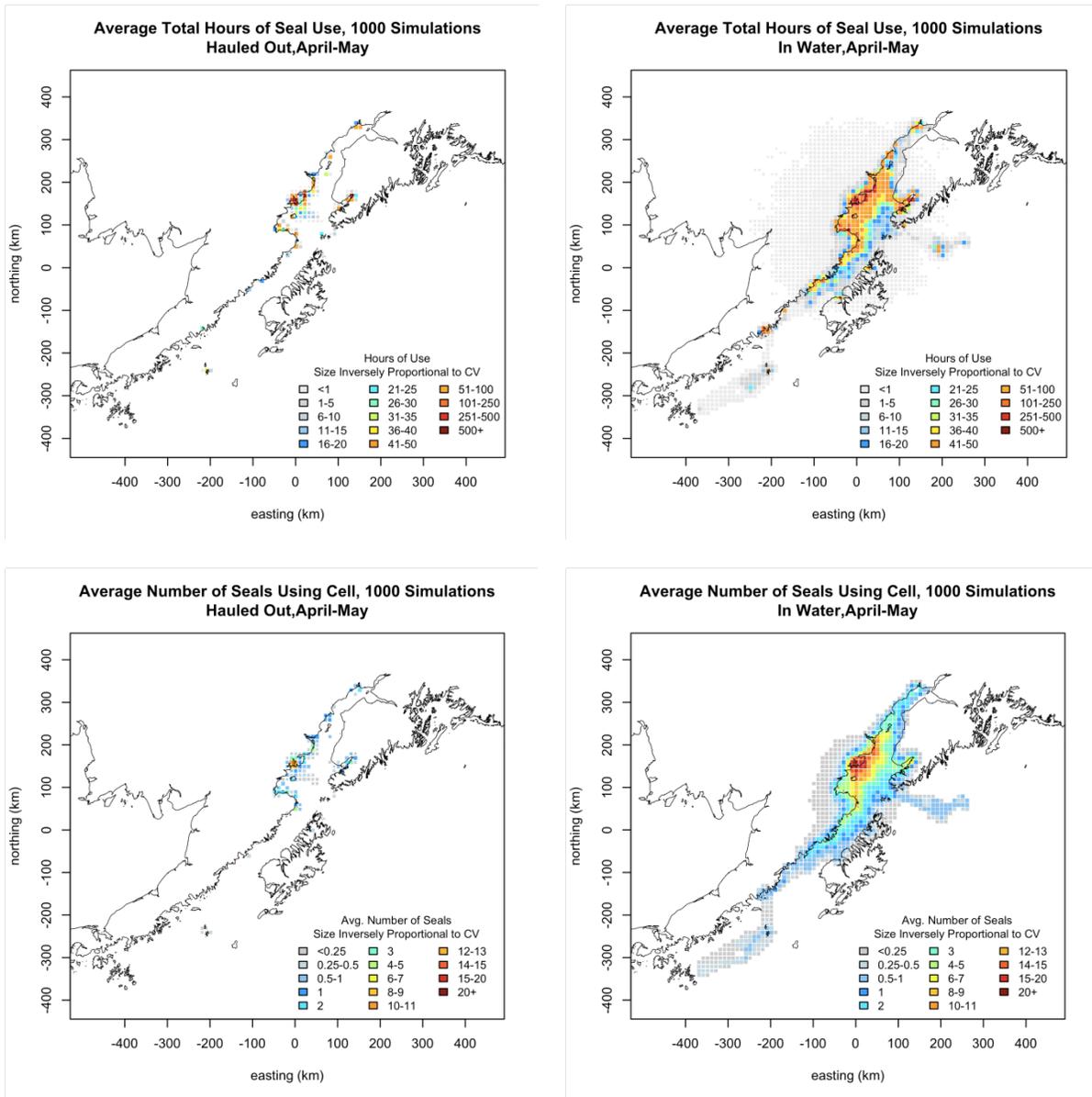


Figure 10 Harbor seal area use maps depicting total hours of seal use and total number of seals using a cell during the months of April and May. Data are combined across years (2004-2006) and the cell size depicted on the maps are inversely proportional to the CV of the estimates.

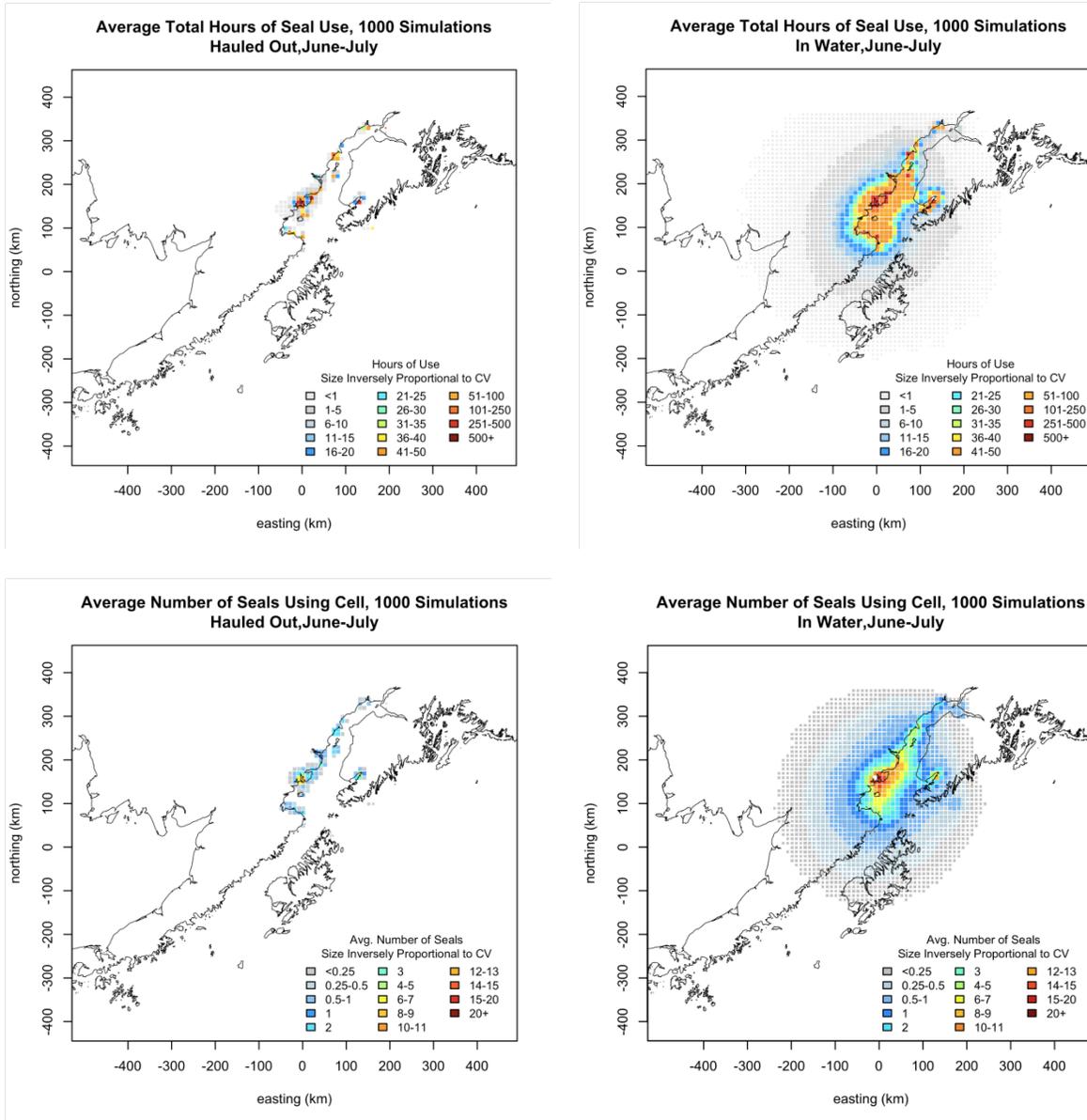


Figure 11 Harbor seal area use maps depicting total hours of seal use and total number of seals using a cell during the months of June and July. Data are combined across years (2004-2006) and the cell size depicted on the maps are inversely proportional to the CV of the estimates.

Hypothesis 3: The proportion of time spent hauled out changes as an estimable function of environmental and temporal covariates, allowing aerial survey counts to be corrected to absolute abundances.

In the final model for the proportion of harbor seals ashore (hauled out), there were no significant year effects, but with such large amounts of data, most other effects were significant. We were especially interested in the probability of hauling out throughout hours of the day and dates of the year, and the interaction of hour and date. The significance of sex and age implied a separate fit of the date/hour-of-day interaction for each sex/age interaction. We held the tide effect constant at low tide, which corresponded to the maximum probability of hauling out while holding all other factors constant. The fitted model for date and time of day for adult females is shown in Figure 12, which shows that haul-out probabilities, in general, were highest in the summer and around mid-day.

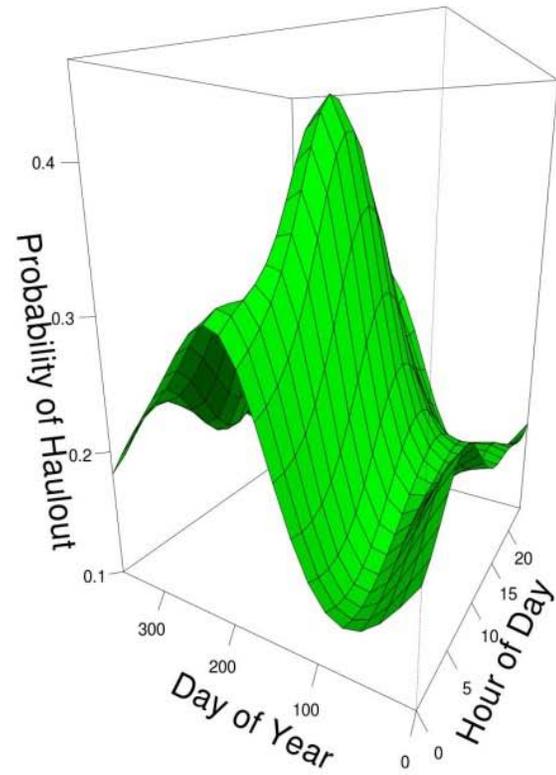
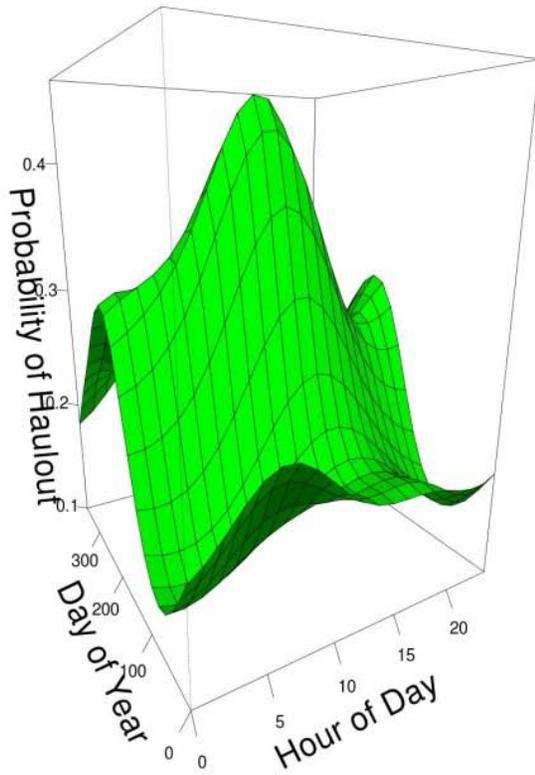


Figure 12 Fitted haul-out probability model, from two perspectives. Time from low-tide is set to 0 minutes.

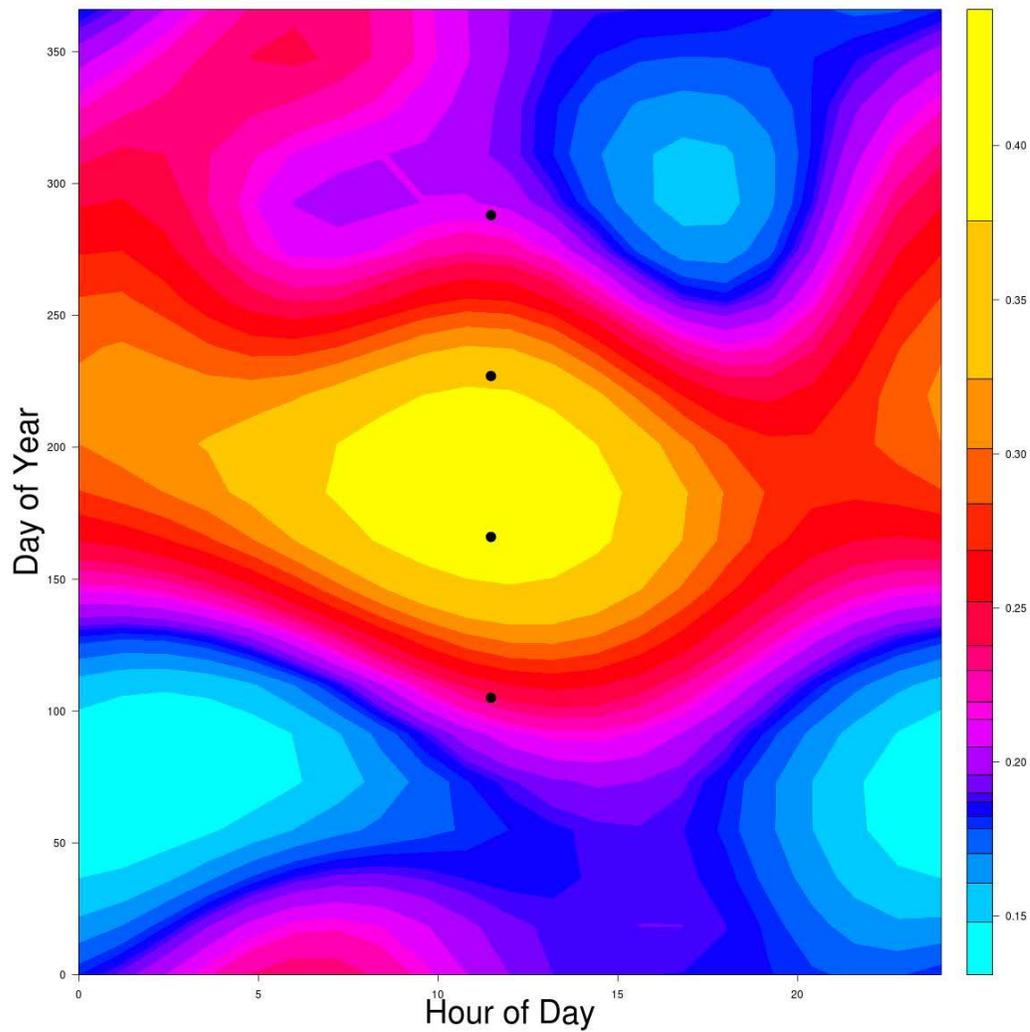


Figure 13 Contour plot depicting the seasonal and hourly changes in the proportion of harbor seals ashore. The black dots represent our survey dates (15 Apr, 15 June, 15 Aug and 15 October) at noon. The haul-out proportion is adjusted to 0 minutes from low tide.

The black dots in the filled contour plot (Figure 13) show the dates (15 Apr, 15 June, 15 Aug, and 15 Oct) at noon, and the curve is adjusted for 0 minutes from low tide, so the black dots are at the same set of covariates as the adjusted counts. From this model, we made a correction factor for each of the adjusted counts. The estimated proportions that are hauled out, and the correction factors with their estimated standard errors, are shown for each date in (Table 3).

Table 4 Estimates of the proportion of seals hauled out (Prop. HO) and the corresponding survey correction factors (CF) for each of the survey months.

		Estimate		
		Prop. HO	CF	CF (SE)
Month	April	0.244	4.106	0.249
	June	0.429	2.333	0.104
	Aug	0.347	2.886	0.160
	Oct	0.201	4.967	0.362

The correction factor is the inverse of the fitted proportion of seals that were hauled-out. The variance of the correction factor was obtained using the delta method (Dorfman 1938). By multiplying the correction factors in the table above by the adjusted count totals, we arrived at corrected total estimates for each season. The standard errors of the corrected total estimates were obtained using Goodman's (1960) variance of a product. The estimates are given Table 4.

Table 5 Covariate adjusted counts and estimates of total harbor seal abundance for each of the survey months

		Estimate				Total	Total (SE)
		Count	Count (SE)	CF	CF (SE)		
Month	April	2635.258	257.15	4.106	0.249	10820.51	1240.885
	June	7111.024	313.44	2.333	0.104	16586.73	1038.409
	Aug	8301.03	292.39	2.886	0.160	23954.43	1576.032
	Oct	2406.84	454.11	4.967	0.362	11954.31	2412.58

Hypothesis 4: Two or more geographically distinct subpopulations of harbor seals are present in Cook Inlet, as demonstrated by genetic analyses and movement patterns of tracked animals.

The results of the genetic analyses of population structure are provided in the contract report attached as Appendix 1.

DISCUSSION

Hypothesis 1: Harbor seal diving behavior changes seasonally, presumably reflecting changes in the amount of time spent in social behaviors (e.g., courtship and mating) vs. foraging behaviors and the depth distribution of local prey aggregations.

The focus of our work to date has been on the development of new, statistically sound methods for interpreting dive behavior. We expect the efforts here to provide a key foundation for future analysis of the unique dive records obtained from satellite tags. To fully explore population level behavior patterns will require more time and more advances in computing power. However, the results from a comparison of one exemplar adult female and juvenile male suggest differing seasonal patterns that coincide with the timing of key social behaviors. The adult female had a relatively low number of dives during May and June that corresponded with the known timing of peak pupping. Reduced dive activity is expected for a harbor seal tending a newly born pup. The juvenile male, however, maintained a relatively consistent dive behavior with episodic, distinct periods of high dive activity. The weighted average depths for these likely foraging periods were between 100 and 200 meters. This was followed by periods of low dive activity and weighted average depths between 5 and 10 meters. This behavior is consistent with a non-breeding animal that is focused on the location and exploitation of prey resources.

Hypothesis 2: Marine habitat use, as measured by at-sea home range sizes and locations, changes seasonally as seals move to access aggregations of migrating prey. Consequently, the risk of exposure to oil at sea changes as seals spend more, or less, time near potential oil hazards.

We addressed this hypothesis in a two-stage approach. The first task involved the development of a novel method that relies on the estimate locations from satellite tags to inform a state-space movement model. This state-space model incorporates the both the error associated with the Argos location estimates and any error related to uncertainty within the underlying movement model. The second approach focused on novel applications of the movement model to examine and describe the seasonal movement and use patterns of harbor seals captured in Cook Inlet. This analysis provides an unprecedented and detailed examination of seasonal changes in harbor seal movement.

The large-scale patterns indicate a portion of harbor seals captured in Cook Inlet move out of the area in the fall and into habitats within Shelikof Strait, Northern Kodiak Island, and coastal habitats of the Alaska Peninsula. Many of these seals tend to be younger individuals and these larger movements are reflected in the estimates of trip distance and duration for younger age classes in the fall months. Animals begin moving back into Cook Inlet during the spring months and nearly all tagged animals are back within Cook Inlet by May and June, which coincides with peak breeding and pupping. Small-scale patterns of movement within Cook Inlet were also evident. Seals captured in Kachemak Bay were more likely to remain within Kachemak Bay and were rarely recorded leaving for other parts of Cook Inlet. Use of the western habitats of Cook Inlet was higher than the eastern coastline. Seals captured at various sites in Cook Inlet were observed in the Kalgin Island region on more than one occasion. The nearby sand bars may serve as a key hub location for seals as they move throughout Cook Inlet. Some seals moved into the northern portion of Cook Inlet, but, in general, seals captured in the southern portion of Cook Inlet tended to stay south of the Forelands.

Hypothesis 3: The proportion of time spent hauled out changes as an estimable function of environmental and temporal covariates, allowing aerial survey counts to be corrected to absolute abundances.

The haul-out behavior analysis and survey correction factors are the first attempt at a comprehensive, statistically sound model of harbor seal haul-out behavior and abundance ashore across multiple seasons. The seasonal haul-out pattern corresponds with the information from the dive behavior and movement analysis. Seals are more likely to be hauled out during the pupping and breeding period and haul-out less frequently during late fall and winter. The importance of sex and age in the model also confirms our results from the dive and movement analysis that suggest different seasonal behaviors across age and sex. The haul-out model allows us to estimate abundance for October, April and June. The lowest seasonal abundance is estimated for April at 10,821 (SE: 1240.885) seals. The movement model indicates much of the seals' spatial use is still outside of Cook Inlet in February and March. The dive behavior for both the adult female and the juvenile male indicate relatively intense diving in early May that could be associated with either travel or foraging.

The estimates for proportion of seals ashore in August are much lower than previous estimates for Alaska harbor seals. Therefore, the survey correction factor results in a higher than expected abundance estimate. August corresponds with the annual molt cycle and, therefore, most of our tags fall off during this period. For this reason, we have a very limited number of data records that correspond with the timing of our August survey. To compensate for this, we rely on trigonometric functions to create better covariates for the circular data (time-of-day and day-of-year) and then interpolate across August. While this approach allows us to generate an estimate for the survey correction factor in August, it likely underestimates the proportion of seals ashore during the survey. Other studies have shown the proportion of seals ashore to peak during the molt. These values are often equal or in excess of the proportion estimated for pupping period. Without additional data from August and by interpolating the model across this period, we are unable to detect an expected increase in haul-out availability during the August surveys. Given this limitation, the estimate of total abundance of harbor seals in Cook Inlet for the month of August is likely between 16,587 (June estimate) and 23,954 (interpolated August estimate).

As part of this study, we tested the deployment of a few prototype flipper-mounted satellite transmitters that are designed to stay on the animal through the molt period. We have continued to deploy these transmitters in other regions of Alaska and hope additional analyses will improve our haul-out model and allow a more precise estimation of abundance in August.

Additional assumptions were made regarding the distribution of age and sex within the population compared to the subset of animals tagged and used in our analysis. Our analysis of the haul-out model shows significant effects for sex, age, and the interactions with date. Unfortunately, we are unable to determine sex or age from our survey photographs and must assume the distribution of age and sex with our tagging study is representative of the population surveyed.

Hypothesis 4: Two or more geographically distinct subpopulations of harbor seals are present in Cook Inlet, as demonstrated by genetic analyses and movement patterns of tracked animals.

The results presented in the report attached as Appendix 1 were clear with regard to dispersal patterns and genetic exchange among broadly defined major concentration areas of harbor seals in Alaska, that female dispersal and male-mediated gene flow in and out of Cook Inlet is restricted. Within Cook Inlet, however, there were contrasting patterns of differentiation between mtDNA and nDNA markers. Low but significant differentiation between eastern and western Cook Inlet seals was found in mtDNA, but not in nDNA, possibly reflecting greater female philopatry to breeding location and greater male-mediated gene flow throughout the Inlet. Also, a failure to detect clear mtDNA differences between western Cook Inlet and parts of the Kodiak archipelago may indicate female dispersal between these areas. Overall, the differentiation between Cook Inlet harbor seals and other major groups of harbor seals in the Gulf of Alaska implies that the Cook Inlet population may be slow to recover from a large perturbation such as an oil spill or a serious disease outbreak; dispersal rates from nearby populations are likely to be low.

ACKNOWLEDGMENTS

The Kachemak Bay Estuarine Research Reserve (KBERR) staff, especially Ori Badajos, Steve Baird, Kim Donahue, and Scott Pegau were extremely helpful throughout the project. We also relied upon the skillful assistance of Michael Cameron, Bob Caruso, Shawn Dahle, Dave Withrow, John Jansen, and Robert Montgomery of the NOAA National Marine Mammal Laboratory (NMML); Steve Jeffries and Bryan Murphie of the Washington Department of Fish and Wildlife, Shawn Johnson of Johnson Veterinary Services and Julie Richmond, currently of the University of North Florida during the logistics and field portions of this project. Mike Simpkins (NOAA) was crucial in developing the proposal, and mobilizing the initial tagging cruises. Capt. Jeff Johnson, the M/V Peregrine, Glenn Seaman, L.A. Holmes and many other kind people and merchants of Homer, Alaska, were also very helpful. We are extremely grateful to the Minerals Management Service (MMS), now called the Bureau of Ocean Energy Management (BOEM), and especially Chuck Monnett for the support that enabled us to conduct this work.

PRESENTATIONS

- Boveng, P. A summary of harbor seal research conducted in Alaska by NOAA Fisheries. Oral presentation, Eighteenth Working Group Meeting, Project 02.05-61, "Marine Mammals," under Area V of the U.S.-Russia Agreement on Cooperation in the Field of Environmental Protection Seattle, Washington, September 23-26, 2004.
- Boveng, P.L., M.A. Simpkins, and J.L. Bengtson. Seasonal dynamics of harbor seals in Cook Inlet. Poster presentation, Alaska Marine Science Symposium, 24-26 January 2005, Anchorage, Alaska.
- Boveng, P.L., J.L. Bengtson, and M.A. Simpkins. Distribution and abundance of harbor seals in Cook Inlet: Seasonal variability in relation to key life history events. Oral presentation, Minerals Management Service, Alaska OCS Region, Tenth Information Transfer Meeting, March 14-16, 2005, Anchorage, AK. OCS Study MMS 2005-036.
- Boveng, P.L. A summary of harbor seal research conducted by NOAA Fisheries in Alaska. Oral presentation to the Alaska Harbor Seal Research Planning meeting (NOAA NMFS Alaska Fisheries Science Center, NOAA NMFS Alaska Region, Alaska Department of Fish and Game, Alaska Native Harbor Seal Commission, and Alaska Sea Life Center) in Juneau, 29 March 2005.
- Boveng, P. A summary of harbor seal research conducted in Alaska by NOAA Fisheries. Oral presentation, Working Group Meeting, Project 02.05-61, "Marine Mammals," under Area V of the U.S.-Russia Agreement on Cooperation in the Field of Environmental Protection, St. Petersburg, Russia, September 2006.
- Boveng, P., J. London, and R. Montgomery. Strong seasonal dynamics of harbor seals, an upper-trophic predator in Cook Inlet. Oral presentation, Alaska Marine Science Symposium, January 2007, Anchorage, Alaska.
- Boveng, P. and J. London. Harbor seals in Cook Inlet. Oral presentation, Port Graham Wisdom Keepers' Meeting, Port Graham, Alaska, September 2007.
- London, J., D. Johnson, P. Boveng, J. VerHoef. Seasonal Movements of Harbor Seals Related to Sex and Age. 2007 Biennial Conference on the Biology of Marine Mammals, Capetown, South Africa (Poster); 2008 Alaska Marine Science Symposium, Anchorage, Alaska (Oral).
- London, J., D. Johnson, and P. Boveng. Using a Continuous Time Correlated Random Walk and Bayesian Inference to Examine Spatial Use Patterns of Harbor Seals in Cook Inlet, Alaska, USA. 2009 Biennial Conference on the Biology of Marine Mammals, Quebec, Canada (Poster); 2010 Alaska Marine Science Symposium, Anchorage, Alaska (Poster).

LITERATURE CITED

- Dorfman, R. 1938. A note on the delta-method for finding variance formulae. *The Biometric Bulletin* **1**:129-137.
- Freitas, C., C. Lydersen, M. A. Fedak, and K. M. Kovacs. 2008. A simple new algorithm to filter marine mammal Argos locations. *Marine Mammal Science* **24**:315-325.
- Frost, K. J. and L. F. Lowry. 1994. Assessment injury to harbor seals in Prince William Sound, Alaska, and adjacent areas following the *Exxon Valdez* oil spill. Alaska Department of Fish and Game, Wildlife Conservation Division, Fairbanks, AK.
- Frost, K. J., L. F. Lowry, and J. J. Burns. 1982. Distribution of marine mammals in the coastal zone of the Bering Sea during summer and autumn. Pages 365-561 *Environmental Assessment of the Alaskan Continental Shelf. Final Reports of Principal Investigators, Volume 20, December 1983.* U.S. Department of Commerce, NOAA and U.S. Department of the Interior, Mineral Management Service, Juneau, AK.
- Frost, K. J., L. F. Lowry, and J. M. Ver Hoef. 1999. Monitoring the trend of harbor seals in Prince William Sound, Alaska, after the Exxon Valdez oil spill. *Marine Mammal Science* **15**:494-506.
- Frost, K. J., C. A. Manen, and T. L. Wade. 1994. Petroleum hydrocarbons in tissues of harbor seals from Prince William Sound and the Gulf of Alaska. Pages 331-358 *in* T. R. Loughlin, editor. *Marine Mammals and the Exxon Valdez.* Academic Press, Inc., San Diego, CA.
- Goodman, L. A. 1960. On the exact variance of products. *Journal of the American Statistical Association* **55**:708-713.
- Higgs, M. D. and J. M. Ver Hoef. 2011. Discretized and aggregated: modeling dive depth of harbor seals from ordered categorical data with temporal autocorrelation. *Biometrics*.
- Hoover-Miller, A., K. R. Parker, and J. J. Burns. 2001. A reassessment of the impact of the Exxon Valdez oil spill on harbor seals (*Phoca vitulina richardsi*) in Prince William Sound, Alaska. *Marine Mammal Science* **17**:111-135.
- Johnson, D. S., J. M. London, M.-A. Lea, and J. Durban. 2008. Continuous-Time Correlated Random Walk Model for Animal Telemetry Data. *Ecology* **89**:1208-1215.
- Lowry, L. F., K. J. Frost, and K. W. Pitcher. 1994. Observations of oiling of harbor seals in Prince William Sound. Pages 209-225 *in* T. R. Loughlin, editor. *Marine Mammals and the Exxon Valdez.* Academic Press, Inc., San Diego, CA.
- Pitcher, K. W. 1990. Major decline in number of harbor seals, *Phoca vitulina richardsi*, on Tugidak Island, Gulf of Alaska. *Marine Mammal Science* **6**:121-134.

- Small, R. J., G. W. Pendleton, and K. W. Pitcher. 2003. Trends in abundance of Alaska harbor seals, 1983-2001. *Marine Mammal Science* **19**:344-362.
- Spraker, T. R., L. F. Lowry, and K. J. Frost. 1994. Gross necropsy and histopathological lesions found in harbor seals. Pages 281-311 *in* T. R. Loughlin, editor. *Marine Mammals and the Exxon Valdez*. Academic Press, Inc., San Diego, CA.
- Ver Hoef, J. M. and K. J. Frost. 2003. A Bayesian hierarchical model for monitoring harbor seal changes in Prince William Sound, Alaska. *Environmental and Ecological Statistics* **10**:201-219.
- Ver Hoef, J. M., J. M. London, and P. L. Boveng. 2010. Fast computing of some generalized linear mixed pseudo-models with temporal autocorrelation. *Computational Statistics* **25**:39-55.

APPENDIX 1- Population structure of Cook Inlet harbor seals revealed by mitochondrial DNA and microsatellite analysis

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Introduction

Harbor seals (*Phoca vitulina*) occupy a near-continuous distribution in the coastal and continental shelf waters of Alaska south of 61°N. This important marine predator utilizes a diverse range of habitats, hauls out at thousands of discrete coastal sites and represents a significant marine resource to a range of users. While the near-continuous distribution and recorded capacity of this pinniped to undertake long distance movements (Lowry et al 2001, Small et al. 2005) might suggest limited population subdivision in Alaska, the non-uniform abundance and differing population trends of harbor seals across their Alaskan range (Boveng et al. 2003, Small et al. 2003, 2008) as well as the behavioral philopatry to discrete breeding populations and foraging grounds observed elsewhere in this species (Stanley et al. 1996, Goodman 1998), identifies the potential for substantial population structure in Alaskan harbor seals. Resolving patterns of dispersal and population structure in such a species, however, is challenging.

The limited temporal scale (i.e., months) over which telemetry can record movements means that such an approach will rarely confirm dispersal in long-lived species including harbor seals, and thus cannot provide detailed insight into dispersal patterns or population structure. Genetic analysis, specifically the examination of spatial patterns of variation at genetic loci, can reveal much about dispersal behavior and breeding patterns over time that facilitate inference about contemporary and historical demographic and reproductive relationships among groups of seals. Markers of choice in these endeavors include the mitochondrial genome (mtDNA), a maternally inherited genome that traces female dispersal patterns over time. As such, this marker is ideal for resolving the demographic relationships among groups of organisms. Highly polymorphic nuclear markers (nDNA), including microsatellites, are widely used to infer breeding patterns of both males and females and to estimate rates of genetic exchange. Incorporating these kinds of genetic analysis with direct assessment of movement patterns and habitat use via telemetry provide a comprehensive assessment of population structure in harbor seals that will assist in the assessment of population status and the risk of anthropogenic impacts and in the identification of management units. This report summarizes the findings of a genetic study of harbor seals in Cook Inlet, Alaska that primarily comprise seals sampled during satellite telemetry studies between 2004 and 2006 (Boveng et al. 2012).

Tissue samples have been collected from harbor seals across their Alaskan range, comprising samples from subsistence hunts and field studies including the current study. Prior to the present investigation, an analysis of 881 seal samples from 180 locations across Alaska for mtDNA variation revealed genetic subdivision indicative of restricted female dispersal at multiple spatial scales (Westlake and O’Corry-Crowe 2002, O’Corry-Crowe et al. 2003). Sample size from Cook Inlet and adjacent areas, however, was low limiting inference of population structure in this region. Nonetheless, initial indications suggested restricted female dispersal between Cook Inlet and the Gulf of Alaska. These earlier studies, while informative on larger geographic scales, highlighted the need for larger sample sizes, greater sample coverage and the inclusion of nDNA as well as mtDNA in the analysis of harbor seal population structure in Cook Inlet. Furthermore, the seasonal patterns of seal movements, haulout behavior and habitat use indicated that hypotheses about population genetic structure need to take seal biology into consideration.

In the current study, we analyzed all 93 samples collected during tagging operations between 2004-2006 for patterns of variation in 435 base pairs (bp) of the mtDNA control region and for allelic polymorphism at 10 independent microsatellite loci. These data were compared to existing data from Cook Inlet and to data from across Alaska to assess patterns of gene flow and dispersal within Cook Inlet and between Cook Inlet and the Gulf of Alaska.

Objectives

Objective 1: To evaluate whether or not seals within Cook Inlet are genetically distinct from other seal populations

Objective 2: To evaluate whether or not geographically distinct subpopulations of seals exist within Cook Inlet

The project's objectives were formally tested as the following hypotheses:

Hypotheses

Hypothesis 1: Harbor seals within Cook Inlet are genetically distinct from seals in the Gulf of Alaska for –

1a: mtDNA, indicating limited female dispersal over time

1b: nDNA, indicating limited genetic exchange via male-mediated gene flow

Hypothesis 2: Seals in western Cook Inlet are genetically distinct from seals in eastern Cook Inlet for -

2a: mtDNA and indicating limited female dispersal over time

2b: nDNA, indicating limited genetic exchange via male-mediated gene flow

Methods

DNA extraction

Small biopsies of skin and blubber tissue were collected from each seal during capture and tagging operations and preserved in 95% EtOH. Total DNA was successfully isolated from all samples using standard cell lysis-protein digestion methods followed by silica-based DNA extraction and recovery protocols. Tissue lysis and digestion steps were automated using the FastDNA™ kit and the FastPrep™ instrument (BIO 101, Carlsbad, California, USA), and DNA was recovered using the DNeasy™ Tissue Kit (QIAGEN, Valencia, California, USA). The concentration and quality of the purified DNA from all samples were estimated by spectrophotometry.

mtDNA analysis

A 588 base-pair (bp) region of the mitochondrial genome was amplified by the polymerase chain reaction (PCR) with custom designed primers (Kocher et al, 1989, Rosel et al., 1994; Westlake

and O’Corry-Crowe, 2002). The amplified PCR products were purified by membrane-based filtration using Microcon® (Millipore, Bedford, Massachusetts) or QIAquick® (Qiagen, Valencia, California) columns. A total of 435 bp of the mtDNA control region and adjacent proline tRNA gene were sequenced by direct dideoxy sequencing using 4-dye fluorescent technology of Applied Biosystems (ABI, 1992). Excess dye-labeled terminators were removed from sequencing reactions using Centri-Sep™ spin columns (empBiotech GmbH, Berlin, Germany) or by ethanol precipitation. Sequences were electrophoresed on an ABI 373A or ABI 377 automated sequencer, and were edited and aligned with the SeqEd™ multiple-sequence editor program (ABI, 1992).

Microsatellite analysis

All samples were also analyzed for variation within ten pinniped microsatellite loci. Forward primers were labeled with a fluorescent tag and PCR amplification of target DNA was performed in 25 or 50µl reactions in either a 9600 thermal cycler (Perkin-Elmer, Norwalk, Connecticut, USA), a GeneAmp 2700 PCR system (Applied Biosystems, Foster City, California, USA) or a PTC-100 Thermal Controller (MJ Research, Inc., Watertown, Massachusetts, USA). Reactions contained approximately 10ng template DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.0 mM MgCl₂, 0.01% gelatin, 150 µM of each dNTP, 0.3 µM of each primer, and 2.5 units of *Taq* DNA polymerase. Following denaturation at 90°C for 2 min., DNA was amplified by 35 cycles consisting of denaturation at 94°C for 45 sec., annealing at 48°C for one min., and extension at 72°C for 1.5 min. A final extension period of 5 min at 72°C was followed by cooling of the PCR product to 4°C. PCR products were run on an ABI 377 gel or 3100/3130xl Genetic Analyzer. Comparisons of observed to expected genotypic frequencies using the Micro-Checker program (version 2.2.3; van Oosterhout et al. 2004) found no evidence of null alleles (i.e., homozygote excess), large allelic dropout or scoring errors due to stuttering.

Data Analysis

To assess patterns of genetic subdivision on a number of spatial scales tissue samples were stratified into regional and sub-regional groupings using information on seal distribution and abundance. At the regional scale all the Cook Inlet samples were compared to harbor seals from other broadly defined regions across Alaska, including the Kodiak Archipelago, Prince William

Sound, Southeast Alaska and Bristol Bay. At the sub-regional level, we used the criteria and groupings defined in O’Corry-Crowe et al. (2003). At this level, Cook Inlet samples were stratified into an eastern Cook Inlet (ECI) group comprising samples from Kachemak Bay and Yukon Island, and a western Cook Inlet (WCI) group comprising the samples from Kamishak Bay, Iniskin Bay, Chinitna Bay, Tuxedni Bay and Kalgin Island. Seal samples from Japan, Russia and California were used as outgroups in all analyses.

Both equilibrium and non-equilibrium methods were used to investigate patterns of genetic differentiation, dispersal and population structure. Traditional F -statistics, homogeneity tests and likelihood-based methods, as well as *a priori* stratification and landscape genetic approaches were used to infer seal behavior from patterns of genetic variation. Details of all methods used will be available in subsequent manuscripts. Frequency (F_{st} , Chi-Square) and distance-based (R_{st} and Φ_{st}) analysis of genetic heterogeneity with permutation-based estimation of statistical significance were conducted using Arlequin and GENEPOP software. For microsatellite data, the Bayesian model-based clustering method STRUCTURE (v. 2.3.3) was also used to infer population structure and assign individuals to population of origin (Pritchard et al. 2000, 2009). Under the assumption of HW equilibrium within clusters (“populations”) and complete linkage equilibrium between loci STRUCTURE infers the most likely number of population clusters, K , given the data, $Pr(K|X)$. Analyses were run both with and without admixture ancestry models. In most analyses we used the models of Hubisz et al. (2009) that use sampling location as prior information. The authors found that weighting clustering outcomes that correlate with grouped sample location can reveal further underlying structure without the risk of detecting structure that is not present.

Results

Hypothesis 1: Harbor seals within Cook Inlet are genetically distinct from seals in the Gulf of Alaska for - 1a: mtDNA, indicating limited female dispersal over time

A total of 85 seal samples collected during capture operations between 2004 and 2006 yielded high quality sequence data. These data were combined with 50 other samples from Cook Inlet in

a comparison of regional mtDNA differentiation across Alaska comprising 1,308 seals. A further 70 samples from Russia, Japan and California were used as outgroups. *F* statistics and homogeneity tests revealed that Cook Inlet was genetically differentiated from other major regions in Alaska (Table 1). All other analysis gave similar results (not shown).

Table 1. Estimates of mtDNA differentiation (F_{st}) and significance (p) values from 50,175 permutations among major centers of harbor seal abundance in Alaska.

	Southeast AK	Gulf Coast	PWS	Cook Inlet	Kodiak	Bristol Bay	Pribilofs	Russia	Japan	California
n=	371	110	297	135	254	125	16	26	14	30
Southeast AK										
Gulf Coast	0.000									
PWS	0.003	0.001								
Cook Inlet	0.010	0.007	0.010							
Kodiak	0.007	0.004	0.005	0.007						
Bristol Bay	0.040	0.031	0.039	0.042	0.035					
Pribilofs	0.063	0.061	0.066	0.057	0.045	0.081				
Russia	0.098	0.097	0.102	0.103	0.099	0.125	0.154			
Japan	0.094	0.092	0.099	0.101	0.097	0.121	0.147	0.191		
California	0.056	0.054	0.060	0.061	0.057	0.080	0.097	0.140	0.133	

Dark shading indicates statistical significance at $p < 0.05$

Light shading indicates statistical significance at $p < 0.10$

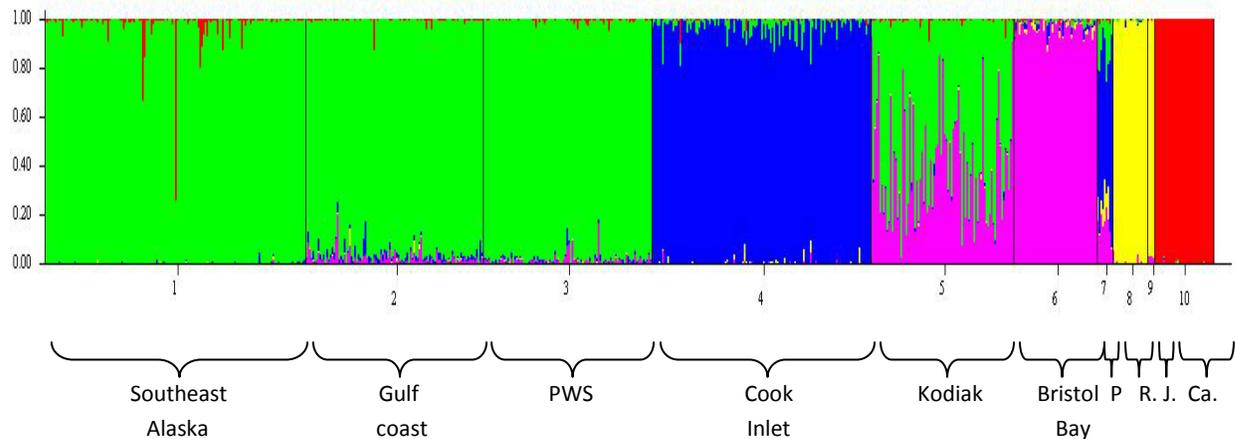
PWS = Prince William Sound

Hypothesis 1: Harbor seals within Cook Inlet are genetically distinct from seals in the Gulf of Alaska for - **1b:** nDNA, indicating limited genetic exchange via male-mediated gene flow

A total of 126 seals from Cook Inlet were successfully screened for allelic polymorphism in 10 independent microsatellite loci. Cook Inlet genotypes were compared to 488 seals genotyped from the other major regions of seal distribution across Alaska and a further 58 from north Pacific outgroups Russia, Japan and California. Both frequency-based and distance based F -

statistics revealed substantial genetic differentiation between Cook Inlet and other regions (not shown). The Bayesian model-based cluster analysis, STRUCTURE, incorporating the LOCPRIOR option, found the most likely number of population clusters to be $K=5$. The same value of K and groupings of individuals into the K clusters was found for analyses that allowed admixture and did not allow admixture. One of the genetically distinct clusters corresponds to all the seals sampled in Cook Inlet (Figure 1).

Figure 1. Model-based cluster analysis of nDNA data from harbor seals in the North Pacific using STRUCTURE 2.3.3. The analysis using prior sample region information (LOCPRIOR) revealed $K=5$ clusters as the most likely. Each of 672 individuals is represented by a vertical line with estimated membership, Q , in each of K clusters denoted by the ratio of the 5 different colors. The analysis was based on ten microsatellite loci, MCMC reps of 50,000 burn-in followed by a run of 1×10^6 reps, and used both the admixture and no admixture (shown) models of ancestry. The Pribilof Islands, Russia, Japan and California samples are denoted by P., R., J. and Ca.



Hypothesis 2: Seals in western Cook Inlet are genetically distinct from seals in eastern Cook Inlet for - 2a: mtDNA and indicating limited female dispersal over time

The 135 Cook Inlet samples were partitioned into two geographic sub-regions, eastern ($n=56$) and western ($n=79$) Cook Inlet, respectively, based on the location of coastal sampling sites to determine whether there was population subdivision on smaller spatial scales. Low, but

statistically significant differentiation was found between the two Cook Inlet groupings for mtDNA (Table 2). When compared with other similarly scaled sub-regions across Alaska for this marker, both the eastern and western groupings differed from most other areas. A notable exception involved western Cook Inlet where we failed to detect differences between these seals and seals sampled in Northern Kodiak ($F_{st} = -0.001$). Low levels of differentiation were also documented between western Cook Inlet and the outer coast of the Kenai Peninsula, western Kodiak and eastern Kodiak (Table 2). All other analysis methods gave similar results (not shown).

Table 2. Estimates of mtDNA differentiation (F_{st}) and significance (p) values from 50,175 permutations among sub-regions harbor seal abundance from northern Southeast Alaska to the Pribilof Islands.

	SE Alaska 5	Gulf Coast 1	PWS 1	PWS 2	PWS 3	E Kenai	E Cook Inlet	W Cook Inlet	N Kodiak	W Kodiak	E Kodiak	S Kodiak	Bristol Bay 1	Bristol Bay 2	Pribilofs
	96	72	79	52	166	47	56	79	29	37	68	51	77	32	16

SE Alaska 5

Gulf Coast 1	0.001															
PWS 1	0.005	0.001														
PWS 2	0.006	0.002	-0.005													
PWS 3	0.004	0.000	0.000	-0.001												
E Kenai	0.003	-0.003	0.000	-0.003	-0.002											
E Cook	0.043	0.036	0.036	0.038	0.039	0.040										
W Cook	0.010	0.007	0.013	0.009	0.008	0.004	0.050									
N Kodiak	0.004	-0.004	0.003	0.004	0.000	0.000	0.037	-0.001								
W Kodiak	0.015	0.007	0.017	0.015	0.012	0.008	0.046	0.005	-0.006							
E Kodiak	0.012	0.004	0.005	0.005	0.004	0.001	0.036	0.003	-0.005	0.001						
S Kodiak	0.012	0.012	0.008	0.004	0.009	0.008	0.043	0.021	0.013	0.021	0.008					
Bristol Bay	0.047	0.033	0.038	0.036	0.039	0.038	0.063	0.046	0.035	0.043	0.034	0.040				
Bristol Bay	0.043	0.029	0.035	0.036	0.038	0.036	0.057	0.045	0.027	0.036	0.030	0.033	-0.009			
Pribilofs	0.073	0.061	0.062	0.068	0.067	0.070	0.075	0.068	0.045	0.044	0.042	0.060	0.086	0.064		

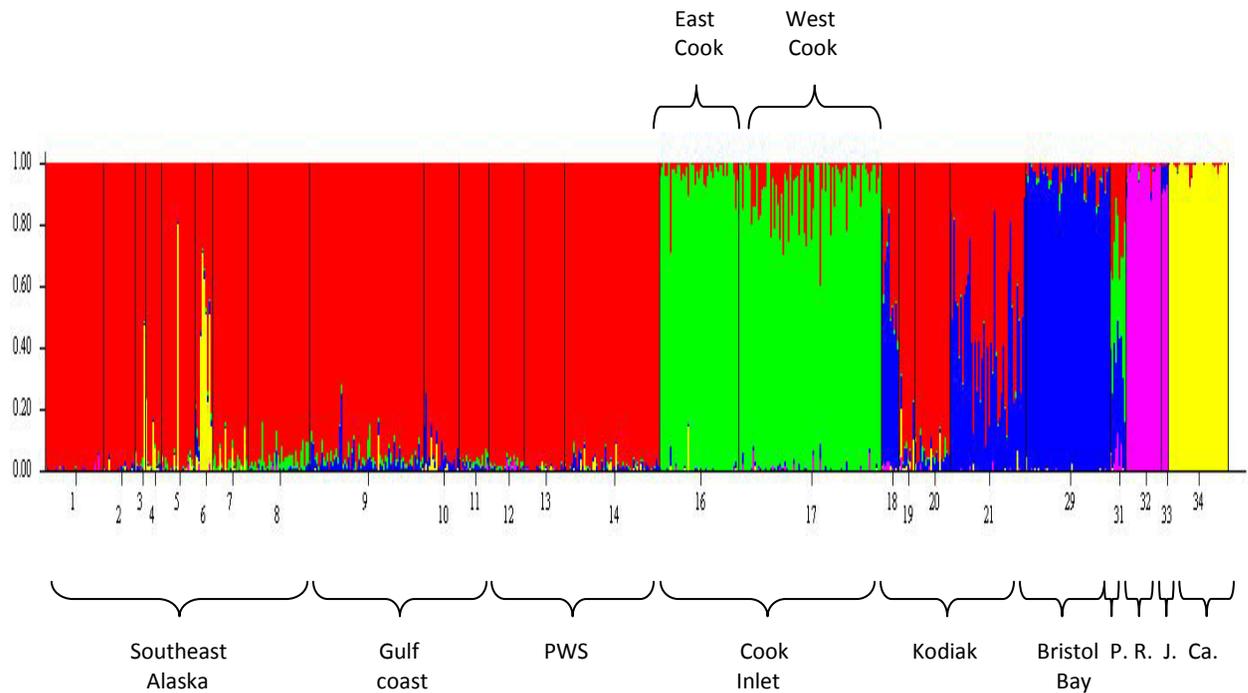
Dark shading indicates statistical significance at $p < 0.05$

Light shading indicates statistical significance at $p < 0.10$

Hypothesis 2: Seals in western Cook Inlet are genetically distinct from seals in eastern Cook Inlet for **-2b:** nDNA, indicating limited genetic exchange via male-mediated gene flow

The 126 genotyped Cook Inlet samples were partitioned into the two geographic sub-regions, eastern (n=45) and western (n=81) Cook Inlet and, as with mtDNA, were compared with seals from sub-regions across Alaska and the North Pacific Ocean. Overall, microsatellite differentiation at these spatial scales was lower than that observed for mtDNA. Unlike the mtDNA analysis we were unable to detect statistically significant differentiation at nDNA between the two Cook Inlet groupings. As with the macrogeographic scale of the regional comparisons the model-based cluster analysis found the most likely number of population clusters to be $K=5$. The same value of K and groupings of individuals into the K clusters was found for analyses that allowed admixture and did not allow admixture. One of the genetically distinct clusters comprises seals from the east and west Cook Inlet sub-regions (Figure 2).

Figure 2. Model-based cluster analysis of nDNA data from harbor seals in the North Pacific using STRUCTURE 2.3.3. The analysis using prior sample sub-region information (black vertical lines demarcate the different sub-regions) revealed $K=5$ clusters as the most likely. Each of 672 individuals is represented by a vertical line with estimated membership, Q , in each of K clusters denoted by the ratio of the 5 different colors. The analysis was based on ten microsatellite loci, MCMC reps of 50,000 burn-in followed by a run of 1×10^6 reps, and used both the admixture and no admixture (shown) models of ancestry. The Pribilof Islands, Russia, Japan and California samples are denoted by P., R., J. and Ca.



Discussion

The results presented here provide new insights into the dispersal patterns, breeding behavior and population structure of harbor seals in Cook Inlet and across Alaska. The findings of significant genetic differentiation between seals in Cook Inlet and other regions and sub-regions indicate limited dispersal and gene flow in and out of the Inlet over contemporary or ecological time frames, and possibly over even longer time scales. The findings from the clustering analysis of the nDNA data were quite surprising revealing that Cook Inlet seals were among the most distinct of any groupings within the Gulf of Alaska. An initial phylogeographic analysis of the

mtDNA data, using genetic distance among mtDNA haplotypes (not shown), also indicates a unique genetic signature for these animals that may date back thousands of years to the early Holocene or even the Pleistocene. More analysis, however, is required, including Bayesian and likelihood-based coalescent methods, to fully ascertain the demographic and evolutionary import of these genetic findings. It is already clear however, with regard to dispersal patterns and genetic exchange among broadly defined major concentration areas of harbor seals in Alaska, that female dispersal and male-mediated gene flow in and out of Cook Inlet is restricted.

The analysis of genetic heterogeneity on smaller spatial scales that are more likely to correspond to dispersal distance in this species revealed a more varied picture that may reflect a more complex pattern of dispersal and reproductive interactions involving Cook Inlet seals. The contrasting patterns of differentiation between mtDNA and nDNA markers within Cook Inlet, for example, may reflect greater female philopatry to breeding location and greater male-mediated gene flow throughout the Inlet. While both the eastern and western Cook Inlet groupings were differentiated from most other sub-regions across Alaska, they differed in the observed degree of mtDNA differentiation with a number of nearby areas. The failure to detect clear mtDNA differences between western Cook Inlet and parts of the Kodiak archipelago may indicate female dispersal between these areas. Furthermore, the nDNA cluster analysis, while clearly placing all Cook Inlet seals into a separate cluster, revealed that several seals from western Cook Inlet may be of mixed origins (Figure 2).

The findings on Cook Inlet harbor seal movements and habitat use presented in this report by Boveng, London and van Hoef are especially interesting in this light. The majority of satellite tracks of Cook Inlet seals moving into the Gulf of Alaska were in waters surrounding the northern and western Kodiak archipelago, including Shelikof Strait (Figure 3, Boveng et al. 2012). That some of these movements may reflect some demographic relationship between areas is intriguing but conjecture at this point. Most of the tagged seals provided tracks spanning just a few seasons and even then were typically observed to return to their capture location. Furthermore many (but not all) of the movements into the Gulf were recorded in the winter outside the breeding season.

Another observation worth noting is that the north Kodiak sub-region was not clearly differentiated from most other areas across Alaska for mtDNA (Table 2). While this may have some biological basis it should be pointed out that north Kodiak had a small sample size relative to most other areas.

Finally, analysis is ongoing into the potential role sampling season and the age and gender of seals may have on our ability to resolve population structure in harbor seals in Cook Inlet. Initial analysis of the data by season, age and sex largely confirm the pattern of genetic structure reported on here.

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References

- Boveng P, Bengtson J, Withrow D, Cesarone J, Simpkins M, Frost K and Burns J. 2003. The abundance of harbor seals in the Gulf of Alaska. *Marine Mammal Science* 19(1): 111-127.
- Boveng PL, London JM and Ver Hoef JM (2012) Distribution and abundance of harbor seals in Cook Inlet, Alaska. Task III: Movements, marine habitat use, diving behavior, and population structure, 2004-2006. Final Report. BOEM Report 2012-xxx. Bureau of Ocean Energy Management, Alaska Outer Continental Shelf Region Anchorage, Alaska. 57 pp.
- Goodman SJ. 1998. Patterns of extensive genetic differentiation and variation among European harbor seals (*Phoca vitulina vitulina*) revealed using microsatellite DNA polymorphisms. *Mol Biol Evol* 15(2): 104-118.
- Hubisz M, Falush D, Stephens M and Pritchard J. 2009. Inferring weak population structure with the assistance of sample group information. *Molecular Ecology Resources*.
- Kocher TD et al. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences* 86:6196-6200
- Lowry LF, Frost KJ, Ver Hoef JM and DeLong RA. 2001. Movements of satellite-tagged subadult and adult harbor seals in Prince William Sound, Alaska. *Marine Mammal Science* 17(4): 835-861.
- O’Corry-Crowe GM Martien KK and Taylor BL. 2003. The analysis of population genetic structure in Alaska harbor seals, *Phoca vitulina*, as a framework for the identification of management stocks. Southwest Fisheries Science Center, National Marine Fisheries Service Administrative Report LJ-03-08. 54pp.
- Pritchard JK, Stephens M and Donnelly P. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155:945–959.
- Pritchard JK, Wen X and Falush D. 2010. Documentation for STRUCTURE software: version 2.3.
- Rosel PE, Dizon AE and Heyning JE. 1994. Genetic analysis of sympatric morphotypes of common dolphins (genus *Delphinus*). *Marine Biology* 119:159-167.

Small RJ, Pendleton GW and Pitcher KW. 2003. Trends in abundance of Alaska harbor seals, 1983-2001. *Marine Mammal Science* 19(2): 344-362.

Small RJ, Lowry LF, Ver Hoef JM, Frost KJ, DeLong RA and Rehberg MJ. 2005. Differential movements by harbor seal pups in contrasting Alaska environments. *Marine Mammal Science* 21(4): 671-694.

Small RJ, Boveng PL, Byrd GV and Withrow DE. 2008. Harbor seal population decline in the Aleutian Archipelago. *Marine Mammal Science* 24(4): 845-863.

Stanley HF, Casey S, Carnahan JM, Goodman S, Harwood J and Wayne RK. 1996. Worldwide patterns of mitochondrial DNA differentiation in the harbor seal (*Phoca vitulina*). *Mol Biol Evol* 13(2): 368-382.

Van Oosterhout C, Hutchinson WF, Wills DPM and Shipley PF. 2004. *Micro-Checker User Guide*. The University of Hull. 28pp.

Westlake RL and O'Corry-Crowe GM. 2002. Macrogeographic structure and patterns of genetic diversity in harbor seals (*Phoca vitulina*) from Alaska to Japan. *Journal of Mammalogy* 83(4):1111-1126.

APPENDIX 2 – Tagged Harbor Seals in Cook Inlet, 2004-2006

SPENO	SPECIES	AGE	SEX	TAGTYPE	DEPLOYDATE	ENDDATE
PV2004_0201	Harbor seal	YRLG	F	ST16	2004-08-27 UTC	2005-02-15 UTC
PV2004_0206	Harbor seal	ADULT	M	ST16	2004-08-27 UTC	2005-02-07 UTC
PV2004_0208	Harbor seal	SUB	F	ST16	2004-08-28 UTC	2005-05-02 UTC
PV2004_0216	Harbor seal	ADULT	M	ST16	2004-08-30 UTC	2005-05-25 UTC
PV2004_0217	Harbor seal	ADULT	F	ST16	2004-08-30 UTC	2005-07-16 UTC
PV2004_0219	Harbor seal	ADULT	F	ST16	2004-08-30 UTC	2005-07-09 UTC
PV2004_0215	Harbor seal	SUB	F	ST16	2004-08-30 UTC	2009-01-01 UTC
PV2004_0221	Harbor seal	ADULT	M	ST16	2004-08-30 UTC	2005-02-15 UTC
PV2004_0223	Harbor seal	ADULT	F	ST16	2004-08-30 UTC	2005-01-25 UTC
PV2004_0214	Harbor seal	SUB	F	ST16	2004-08-30 UTC	2005-05-17 UTC
PV2004_0226	Harbor seal	SUB	F	ST16	2004-08-31 UTC	2005-02-11 UTC
PV2004_0233	Harbor seal	YOY	F	SPLASH	2004-08-31 UTC	2005-01-03 UTC
PV2004_0231	Harbor seal	ADULT	F	ST16	2004-08-31 UTC	2005-04-25 UTC
PV2004_0230	Harbor seal	ADULT	M	ST16	2004-08-31 UTC	2005-04-01 UTC
PV2004_0229	Harbor seal	SUB	F	ST16	2004-08-31 UTC	2004-09-03 UTC
PV2004_0227	Harbor seal	SUB	M	ST16	2004-08-31 UTC	2004-10-30 UTC
PV2004_0224	Harbor seal	ADULT	F	ST16	2004-08-31 UTC	2004-10-13 UTC
PV2004_0235	Harbor seal	ADULT	M	ST16	2004-09-01 UTC	2004-09-08 UTC
PV2004_0234	Harbor seal	ADULT	M	ST16	2004-09-01 UTC	2005-04-28 UTC
PV2004_0001	Harbor seal	YOY	F	SPLASH	2004-09-15 UTC	2005-02-28 UTC
PV2005_0236	Harbor seal	ADULT	M	ST16	2005-04-30 UTC	2005-05-11 UTC
PV2005_0238	Harbor seal	ADULT	M	ST16	2005-04-30 UTC	2005-07-11 UTC
PV2005_0239	Harbor seal	ADULT	M	ST16	2005-04-30 UTC	2005-05-16 UTC
PV2005_0237	Harbor seal	SUB	F	SPLASH	2005-04-30 UTC	2005-06-26 UTC
PV2005_0240	Harbor seal	SUB	F	SPLASH	2005-05-05 UTC	2005-06-30 UTC
PV2005_0241	Harbor seal	ADULT	M	ST16	2005-05-08 UTC	2005-07-08 UTC
PV2005_0246	Harbor seal	SUB	F	SPLASH	2005-05-09 UTC	2005-06-25 UTC
PV2005_0245	Harbor seal	SUB	M	ST16	2005-05-09 UTC	2005-06-18 UTC
PV2005_0244	Harbor seal	SUB	M	SPLASH	2005-05-09 UTC	2005-06-18 UTC
PV2005_0243	Harbor seal	YRLG	M	SPLASH	2005-05-09 UTC	2005-07-17 UTC
PV2005_0247	Harbor seal	SUB	F	SPLASH	2005-05-09 UTC	2005-07-18 UTC
PV2005_0249	Harbor seal	ADULT	M	ST16	2005-05-09 UTC	2005-07-01 UTC
PV2005_0248	Harbor seal	ADULT	M	ST16	2005-05-09 UTC	2005-07-09 UTC
PV2005_0250	Harbor seal	SUB	M	SPLASH	2005-05-10 UTC	2005-06-05 UTC
PV2005_0251	Harbor seal	ADULT	M	SPLASH	2005-05-10 UTC	2005-07-04 UTC
PV2005_0252	Harbor seal	ADULT	F	SPLASH	2005-09-13 UTC	2006-05-23 UTC
PV2005_0253	Harbor seal	ADULT	M	SPLASH	2005-09-13 UTC	2006-04-21 UTC
PV2005_0254	Harbor seal	YOY	M	SPLASH	2005-09-13 UTC	2006-01-28 UTC

PV2005_0255	Harbor seal	ADULT	M	SPLASH	2005-09-15 UTC	2006-06-10 UTC
PV2005_0259	Harbor seal	ADULT	F	SPLASH	2005-09-15 UTC	2006-05-21 UTC
PV2005_0256	Harbor seal	ADULT	F	SPLASH	2005-09-15 UTC	2006-08-11 UTC
PV2005_0257	Harbor seal	ADULT	M	SPLASH	2005-09-15 UTC	2006-06-19 UTC
PV2005_0258	Harbor seal	ADULT	M	SPLASH	2005-09-15 UTC	2006-08-04 UTC
PV2005_0260	Harbor seal	YOY	F	SPLASH	2005-09-16 UTC	2006-05-27 UTC
PV2005_0261	Harbor seal	ADULT	M	SPLASH	2005-09-16 UTC	2006-07-25 UTC
PV2005_0262	Harbor seal	YRLG	M	SPLASH	2005-09-18 UTC	2006-04-13 UTC
PV2005_0263	Harbor seal	ADULT	M	SPLASH	2005-09-18 UTC	2006-06-14 UTC
PV2005_0264	Harbor seal	ADULT	M	SPLASH	2005-09-18 UTC	2006-06-14 UTC
PV2005_0270	Harbor seal	YOY	F	SPLASH	2005-09-19 UTC	2006-06-14 UTC
PV2005_0269	Harbor seal	ADULT	M	SPLASH	2005-09-19 UTC	2006-06-19 UTC
PV2005_0268	Harbor seal	YRLG	F	SPLASH	2005-09-19 UTC	2006-05-19 UTC
PV2005_0267	Harbor seal	SUB	M	SPLASH	2005-09-19 UTC	2006-06-06 UTC
PV2005_0266	Harbor seal	SUB	F	SPLASH	2005-09-19 UTC	2006-05-27 UTC
PV2005_0265	Harbor seal	YRLG	F	SPLASH	2005-09-19 UTC	2006-06-11 UTC
PV2005_0275	Harbor seal	ADULT	M	SPLASH	2005-09-21 UTC	2006-08-08 UTC
PV2005_0276	Harbor seal	ADULT	F	SPLASH	2005-09-21 UTC	2006-06-02 UTC
PV2005_0274	Harbor seal	ADULT	M	SPLASH	2005-09-21 UTC	2006-06-14 UTC
PV2005_0273	Harbor seal	YOY	F	SPLASH	2005-09-21 UTC	2006-07-01 UTC
PV2005_0272	Harbor seal	ADULT	F	SPLASH	2005-09-21 UTC	2006-02-03 UTC
PV2005_0277	Harbor seal	ADULT	F	SPLASH	2005-09-21 UTC	2006-06-26 UTC
PV2005_0271	Harbor seal	ADULT	F	SPLASH	2005-09-21 UTC	2006-05-29 UTC
PV2006_0280	Harbor seal	YOY	F	SPLASH	2006-04-26 UTC	2006-07-22 UTC
PV2006_0280	Harbor seal	YOY	F	SPOT5	2006-04-26 UTC	2009-01-01 UTC
PV2006_0278	Harbor seal	YOY	M	SPLASH	2006-04-26 UTC	2006-07-09 UTC
PV2006_0278	Harbor seal	YOY	M	SPOT5	2006-04-26 UTC	2009-01-01 UTC
PV2006_0279	Harbor seal	YOY	M	SPLASH	2006-04-26 UTC	2006-07-05 UTC
PV2006_0279	Harbor seal	YOY	M	SPOT5	2006-04-26 UTC	2009-01-01 UTC
PV2006_0281	Harbor seal	SUB	M	SPLASH	2006-04-29 UTC	2006-06-24 UTC
PV2006_0281	Harbor seal	SUB	M	SPOT5	2006-04-29 UTC	2009-01-01 UTC
PV2006_0287	Harbor seal	SUB	F	SPLASH	2006-04-29 UTC	2006-06-08 UTC
PV2006_0286	Harbor seal	ADULT	F	SPOT5	2006-04-29 UTC	2009-01-01 UTC
PV2006_0286	Harbor seal	ADULT	F	SPLASH	2006-04-29 UTC	2006-08-18 UTC
PV2006_0285	Harbor seal	SUB	M	SPLASH	2006-04-29 UTC	2006-06-18 UTC
PV2006_0284	Harbor seal	SUB	F	SPOT5	2006-04-29 UTC	2009-01-01 UTC
PV2006_0284	Harbor seal	SUB	F	SPLASH	2006-04-29 UTC	2006-06-14 UTC
PV2006_0283	Harbor seal	SUB	M	SPOT5	2006-04-29 UTC	2009-01-01 UTC
PV2006_0283	Harbor seal	SUB	M	SPLASH	2006-04-29 UTC	2009-01-01 UTC
PV2006_0282	Harbor seal	SUB	M	SPOT5	2006-04-29 UTC	2009-01-01 UTC
PV2006_0282	Harbor seal	SUB	M	SPLASH	2006-04-29 UTC	2006-06-24 UTC
PV2006_0288	Harbor seal	ADULT	F	SPOT5	2006-04-30 UTC	2009-01-01 UTC

PV2006_0288	Harbor seal	ADULT	F	SPLASH	2006-04-30 UTC	2006-07-03 UTC
PV2006_0289	Harbor seal	ADULT	F	SPLASH	2006-05-04 UTC	2006-07-27 UTC
PV2006_0289	Harbor seal	ADULT	F	SPOT5	2006-05-04 UTC	2009-01-01 UTC
PV2006_0290	Harbor seal	SUB	M	SPLASH	2006-05-04 UTC	2006-06-20 UTC
PV2006_0290	Harbor seal	SUB	M	SPOT5	2006-05-04 UTC	2009-01-01 UTC
PV2006_0293	Harbor seal	SUB	M	SPOT5	2006-05-05 UTC	2009-01-01 UTC
PV2006_0291	Harbor seal	YOY	M	SPOT5	2006-05-05 UTC	2009-01-01 UTC
PV2006_0292	Harbor seal	ADULT	F	SPLASH	2006-05-05 UTC	2006-06-28 UTC
PV2006_0292	Harbor seal	ADULT	F	SPOT5	2006-05-05 UTC	2009-01-01 UTC
PV2006_0293	Harbor seal	SUB	M	SPLASH	2006-05-05 UTC	2006-06-26 UTC
PV2006_0291	Harbor seal	YOY	M	SPLASH	2006-05-05 UTC	2006-06-14 UTC

APPENDIX 3 – Abstracts of peer-reviewed publications resulting from this project:

Johnson, D. S., J. M. London, M.-A. Lea, and J. Durban. 2008. Continuous-Time Correlated Random Walk Model for Animal Telemetry Data. *Ecology* 89:1208-1215.

ABSTRACT: We propose a continuous-time version of the correlated random walk model for animal telemetry data. The continuous-time formulation allows data that have been nonuniformly collected over time to be modeled without subsampling, interpolation, or aggregation to obtain a set of locations uniformly spaced in time. The model is derived from a continuous-time Ornstein-Uhlenbeck velocity process that is integrated to form a location process. The continuous-time model was placed into a state–space framework to allow parameter estimation and location predictions from observed animal locations. Two previously unpublished marine mammal telemetry data sets were analyzed to illustrate use of the model, by-products available from the analysis, and different modifications which are possible. A harbor seal data set was analyzed with a model that incorporates the proportion of each hour spent on land. Also, a northern fur seal pup data set was analyzed with a random drift component to account for directed travel and ocean currents.

<http://www.esajournals.org/doi/abs/10.1890/07-1032.1>

Ver Hoef, J. M., J. M. London, and P. L. Boveng. 2010. Fast computing of some generalized linear mixed pseudo-models with temporal autocorrelation. *Computational Statistics* 25:39-55.

ABSTRACT: This paper considers ways to increase computational speed in generalized linear mixed pseudo-models for the case of many repeated measurements on subjects. We obtain linearly increasing computing time with number of observations, as opposed to $O(n^3)$ increasing computing time using numerical optimization. We also find a surprising result; that incomplete optimization for covariance parameters within the larger parameter estimation algorithm actually decreases time to convergence. After comparing various computing algorithms and choosing the best one, we fit a generalized linear mixed model to a binary time series data set with over 100 fixed effects, 50 random effects, and approximately 1.5×10^5 observations.

<http://www.springerlink.com/content/w87058283420x1p5/>

Higgs, M. and J. M. Ver Hoef. 2011. Discretized and aggregated: Modeling dive depth of harbor seals from ordered categorical data with temporal autocorrelation. *Biometrics* DOI: 10.1111/j.1541-0420.2011.01710.x

ABSTRACT: Ordered categorical data are pervasive in environmental and ecological data, and often arise from constraints that require discretizing a continuous variable into ordered categories. A great deal of data have been collected toward the study of marine mammal dive behavior using satellite depth recorders (SDRs), which often discretize a continuous variable such as depth. Additionally, data storage or transmission constraints may also necessitate the aggregation of data over time intervals of a specified length. The categorization and aggregation create a time series of ordered multicategory counts for each animal, which present challenges in terms of statistical modeling and practical interpretation. We describe an intuitive strategy for modeling such aggregated, ordered categorical data allowing for inference regarding the category probabilities and a measure of central tendency on the original scale of the data (e.g., meters), along with incorporation of temporal correlation and overdispersion. The strategy extends covariate-specific cutpoint models for ordinal data. We demonstrate the method in an analysis of SDR dive-depth data collected on harbor seals in Alaska. The primary goal of the analysis is to assess the relationship of covariates, such as time of day, with number of dives and maximum depth of dives. We also predict missing values and introduce novel graphical summaries of the data and results.

<http://onlinelibrary.wiley.com/doi/10.1111/j.1541-0420.2011.01710.x/full>