ALASKA DEPARTMENT OF FISH AND GAME
DIVISION OF WILDLIFE CONSERVATION
PO Box 115526
Juneau, AK 99811-5526

Alaska Department of Fish and Game
Wildlife Restoration Grant

GRANT NUMBER: AKW-22

PROJECT NUMBER: 1.0

PROJECT TITLE: Recovery, residual and emerging threats assessment for the Steller sea lion (*Eumetopias jubatus*) eastern and western Distinct Population Segments in Alaska

PERIOD OF PERFORMANCE: July 1, 2017 through June 30, 2019

PERFORMANCE YEAR: July 1, 2018 - June 30, 2019; year 2 of a 2-year grant

REPORT DUE DATE: Submit to Coordinator September 26, 2018; due to FAC

PRINCIPAL INVESTIGATOR: Mandy Keogh

COOPERATORS:

Authorities: 2 CFR 200.328
2 CFR 200.301
50 CFR 80.90

I. PROGRESS ON PROJECT OBJECTIVES DURING PERFORMANCE YEAR

OBJECTIVE 1: Determine the health of individuals Steller sea lions in the wDPS and eDPS.

a) Job/activity 1a. Collect tissues and archive samples including blood, hair, skin, whiskers, carcasses and fecal samples from both the eDPS and wDPS.
b) Job/activity 1b. Analyze blood samples for markers of health which may include complete blood cell counts, serology for disease, contaminant concentrations, or biochemical markers of fasting or diet and foraging ecology (e.g. stable isotopes and fatty acids).
c) Job/activity 1c. Analyze reproductive and stress-related hormones in serum, hair, and/or whisker.

ACCOMPLISHMENTS: Work was done on Job/activity 1a-1c. Hair and whisker samples were collected during field efforts supported through other funding or from stranded sea lions were archived. We validated the methods to measure testosterone, a reproductive hormone which is also associated with social stress, in whiskers from male and female Steller sea lions. We sectioned one whisker from 4 male and 4 female Steller sea lions.

Steroid hormones were extracted, and testosterone was measured along the length of the whiskers. An example of the testosterone patterns in 2 male Steller sea lions is presented below.

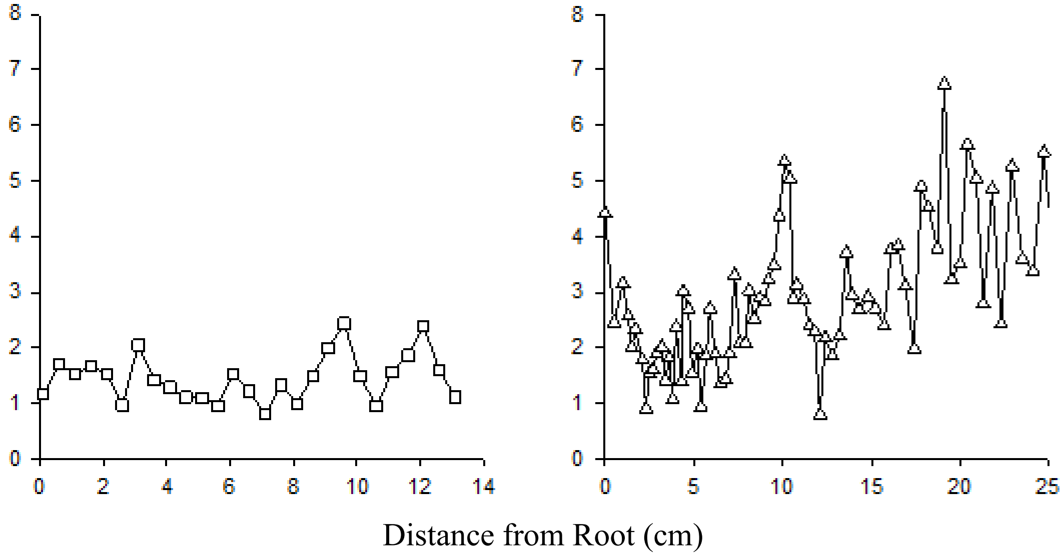

Fig. 1. Testosterone concentrations along the length of a vibrissa from A) an immature male SSL and B) an adult male SSL. Peaks reflect seasonal increases in testosterone associated with the breeding season in sexually mature males.

OBJECTIVE 2: Identify potential residual or emerging threats to Steller sea lions in the wDPS and eDPS.

a) Job/activity 2a. Archive samples including whole blood, serum, viral or bacterial swabs.
b) Job/activity 2b. Compare findings of objective 1 to historical data or results.
c) Job/activity 2c. Analyze archived samples as warranted due to new pathogens or other markers being identified or development of new methods.
d) Job/activity 2d. Publish archived datasets.

ACCOMPLISHMENTS: Work was done on Job/activity 2a. Samples collected during research efforts and from stranded sea lions were archived. Work was done on Job/activity 2d and archived datasets were analyzed, and 2 manuscripts were written and published in peer reviewed journals.

II. SUMMARY OF WORK COMPLETED ON PROJECT TO DATE.

This project is in Year 2 of a 2-year and is the final report for this part of the project. As part of this project we archived samples opportunistically collected as part of field efforts or during bio sampling of carcasses. We expanded the methods available to measure reproductive and stress-related hormones in whiskers to include male Steller sea lions and to include an additional reproductive hormone, testosterone. Our findings suggest that testosterone in whiskers is a useful tool to determine maturity (mature or immature) in male Steller sea lions and could be used to identify seasonality in mature Steller sea lions.

III. SIGNIFICANT DEVELOPMENT REPORTS AND/OR AMENDMENTS.

No Significant Development Reports (SDR) or amendments were submitted during this performance year.

IV. PUBLICATIONS

The following papers were published during this reporting period (pdfs included).

Keogh, M. J., Taras, B. D., Eischens, C., Kennish, J. M., Fadely, B. S., & Rea, L. D. (2019). Variation in milk, serum, and blubber fatty acids in young, free-ranging Steller sea lions. Marine Mammal Science, 35(3), 909-933.

Keogh, Mandy J., Brian Taras, Kimberlee B. Beckmen, Kathleen A. Burek-Huntington, Gina M. Ylitalo, Brian S. Fadely, Lorrie D. Rea, and Kenneth W. Pitcher. "Organochlorine contaminant concentrations in blubber of young Steller sea lion (*Eumetopias jubatus*) are influenced by region, age, sex, and lipid stores." Science of The Total Environment (2019): 134183.

V. RECOMMENDATIONS FOR THIS PROJECT

Project is complete.

Prepared by: PI

Date: 09/26/2019

Marine Mammal Science

MARINE MAMMAL SCIENCE, 9999(9999): 1–25 (2018)

DOI: 10.1111/mms.12574

Variation in milk, serum, and blubber fatty acids in young, free-ranging Steller sea lions

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Abstract

We determined the fatty acid signatures in milk, serum, and blubber samples collected from young free-ranging Steller sea lions (*Eumetopias jubatus*) and investigated the partitioning of fatty acids among these matrices. We assessed the relationship of fatty acids in each matrix with region, age, season, sex, and body condition to gain information needed to determine the most appropriate type of samples to collect and analyze to address future research questions. The variability of fatty acid composition was almost entirely explained by sample type, highlighting the importance of selecting a matrix consistent with the study objectives. Regional differences in fatty acid composition were found in all sample types and these differences varied among matrices. The proportion of fatty acids in milk and serum were influenced by season of capture whereas blubber samples were more affected by age of the pup. The influence of season on the fatty acid signatures in milk and serum highlights the use of these samples over blubber in studies investigating seasonal changes in diet. Further, our findings suggest that the use of milk samples collected from the stomachs of pups may be a viable alternative to directly collecting milk from adult females.

Key words: fatty acid, milk, serum, blubber, Steller sea lion, *Eumetopias jubatus*, age, region.

Understanding the diet of Steller sea lions as well as how their diet is metabolized and incorporated into blubber is important for assessing whether diet composition may be contributing to the lack of recovery of

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the endangered western distinct population segment of Steller sea lions (Pitcher *et al.* 2007, NMFS 2013). Analysis of scat samples indicate that the diet and foraging ecology of female Steller sea lions varies both geographically and seasonally (Sinclair and Zepplin 2002; Waite *et al.* 2012*a*, *b*; Sinclair *et al.* 2013) likely reflecting changes in prey distribution and foraging strategies. However, direct methods to investigate diet such as analysis of scat and stomach contents as well as direct observations, have biases including narrow time frame and underrepresentation of prey with few hard parts (Merrick *et al.* 1997, Sinclair and Zeppelin 2002, Sinclair *et al.* 2013). Increasingly, indirect methods, including stable isotope ratio analysis (Scherer *et al.* 2015, Doll *et al.* 2018) and fatty acid signature analysis (Beck *et al.* 2007; Meynier *et al.* 2008, 2014; Fowler *et al.* 2014), are being used to investigate the diet and foraging ecology of marine predators.

Fatty acids have been analyzed in blubber, blood, and milk from various marine mammals; however, there has been no published data on fatty acids simultaneously analyzed in all three tissues from the same animals. This type of coordinated analysis would provide a clearer understanding of which tissues are best for addressing specific types of research questions and which (if any) tissues may substitute for others to address such questions. Blubber has been widely used for quantifying fatty acids in otariids (Arnould *et al.* 2005, Beck *et al.* 2007, Meynier *et al.* 2008, Lambert *et al.* 2013); however, collecting blubber can be difficult particularly in young animals with small or developing blubber layers. Depending on the research question being addressed, other tissues might be logistically easier to collect and possibly be more informative. For example, fatty acids in otariid milk samples offer insight into the diet of the pup as well as of the mother, fatty acids in serum may offer insight into metabolic use, and those in blubber provide information on energy storage. Collecting milk from adult females can be challenging; however, ingested milk samples can be collected from the stomachs of pups as an alternative source. Steller sea lion pups are routinely handled for population and health studies, allowing an opportunity to collect tissues from dependent pups, which may provide information on the foraging ecology of adult females. There is a great potential for fatty acid profiles in various tissues (*e.g.*, milk, serum, and blubber) to provide information on the seasonal and regional differences in the diet of Steller sea lions; however, a basic understanding of how the fatty acid compositions of these tissues compare and how, within each tissue, they depend on other factors, such as geographic location, age, and sex, is needed.

In the current study, fatty acids were measured in milk, serum, and blubber samples collected from the same young, free-ranging Steller sea lions. The goals of this study were to (1) understand partitioning of fatty acids between diet (milk samples), metabolic use (serum samples), and storage (blubber samples); and (2) assess the relationship of fatty acid signatures in each tissue with region, age, sex, and body condition in young Steller sea lions. Exploring the relationship among these variables within each tissue will provide information needed to determine the most appropriate type of samples to collect and analyze to address future research questions.

MATERIAL AND METHODS

Animals and Sample Collection

We captured, sampled, and released free-ranging Steller sea lions between 2000 and 2004 (Table 1) from 19 locations on or near rookeries and haul outs from Lowrie Island (54.51°N, 133.31°W) in the southeastern panhandle to Adak Island (51.65°N, 176.98°W) in the central Aleutian Islands (Fig. 1). Sea lions were either captured and handled on land with hoop-nets or captured underwater using divers and transferred to a research vessel for measurements and sampling as described by Fadely *et al.* (2005) and Raum-Suryan *et al.* (2004). We measured body mass with the animal held in a capture box or plastic canine kennel suspended from a commercial-grade hanging scale (±0.1 kg) (Rice Lake Weighing Systems, Rice Lake, WI). Physical examinations and sample collection occurred while the sea lion was anesthetized with inhalable isoflurane (Heath *et al.* 1997, Lian *et al.* 2018). We measured standard length as a straight line from tip-of-nose to tip-of-tail while the sea lion was lying on a flat surface with ventral surface down, and axillary girth was measured using a tape measure, both measures to ±0.5 cm. We also calculated a body condition index (BCI) as (axillary girth/standard length) × 100 for each sea lion (Pitcher 1986, Ryg *et al.* 1990, Hastings *et al.* 2011, Rea *et al.* 2016). Ages were estimated based on time of year, tooth eruption, and tooth measurements using the method of King *et al.* (2007).

Table 1. Mean (SD) age, body mass, standard length, axillary girth, and Body Condition Index (BCI) for young Steller sea lions by region and sex.

	n	Age, month	Body mass, kg	Standard length, cm	Axillary girth, cm	BCI
All						
All	68	7.0 (5.9)	71.6 (36.6)	141.3 (18.8)	97.7 (18.8)	69.13 (3.4)
Female	35	7.0 (6.1)	63.4 (30.2)	134.4 (22.4)	94.4 (18.1)	68.6 (3.6)
Male	33	7.0 (5.8)	80.2 (40.9)	145.7 (28.2)	101.2 (19.1)	69.6 (3.1)
Southeast						
All	42	6.5 (5.6)	65.1 (35.7)	136.3 (26.4)	93.2 (18.3)	68.4 (2.9)
Female	19	6.1 (5.0)	53.2 (23.1)	130.0 (20.9)	87.5 (15.0)	67.3 (2.6)
Male	23	6.9 (6.20)	74.9 (41.5)	141.5 (29.7)	97.9 (19.8)	69.3 (2.9)
Prince William Sound						
All	16	9.5 (7.2)	85.6 (40.6)	151.8 (25.5)	104.3 (19.7)	68.4 (3.2)
Female	10	9.9 (8.4)	77.7 (40.2)	137.4 (28.8)	101.5 (21.9)	68.5 (3.5)
Male	6	8.8 (5.5)	98.6 (41.4)	159.2 (25.5)	108.8 (16.2)	68.5 (2.8)
Aleutian Islands						
All	10	5.1 (3.2)	76.5 (28.1)	145.7 (15.5)	106.3 (14.0)	72.8 (3.3)
Female	6	5.3 (3.4)	71.9 (22.2)	143.2 (12.9)	104.5 (12.5)	71.9 (3.6)
Male	4	4.8 (3.3)	83.4 (38.1)	149.5 (20.4)	109.0 (17.6)	72.8 (3.5)

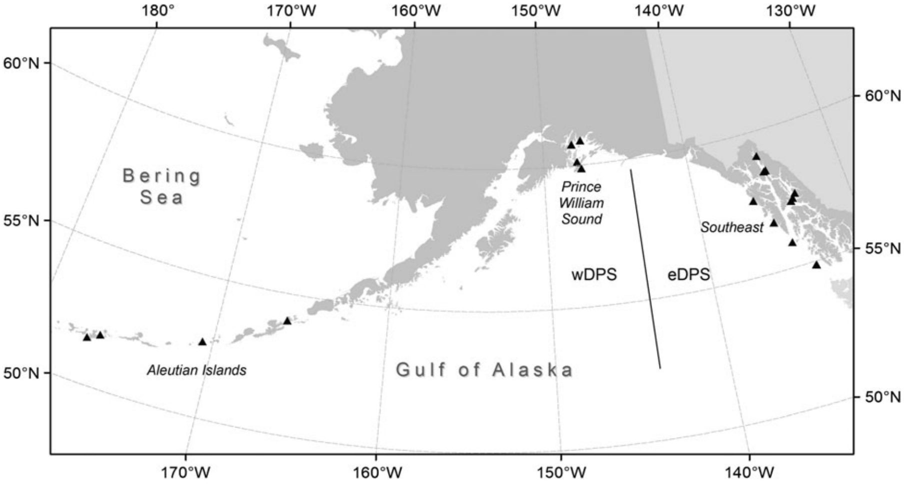

Figure 1. Capture sites (triangles) of Steller sea lions sampled between 2000 and 2004. The line denotes the boundary between the western and eastern distinct population segments. Capture sites include: Sunset Island, Forrester-Lowrie Island, Forrester North Rocks, Sail Island, Brothers Island, Hazy Island, Benjamin Island, Little, Gran Point, Biali Rocks, White Sisters, Glacier Island, The Needle, Fish Island, Perry Island, Cape Morgan Akutan Island, Yunaska Island, Oglala Point Kagalaska Island, Adak Island.

We collected blood samples using standard aseptic techniques *via* venipuncture of a hind flipper vein (21 G butterfly catheter/winged infusion set) or caudal gluteal plexus (18–20 G 1.5–2.5-inch needle) directly into serum separator blood tubes. Serum separator tubes were kept upright and chilled until processing in the laboratory. Serum was stored at −80°C until analyzed.

We collected blubber samples from a sterile surgical biopsy site 3–4 cm cranial and lateral to the femoral joint as described in Beck *et al.* (2007). We wrapped blubber samples in acetone-rinsed aluminum foil, placed them in a polyethylene sample bag (Whirl-Pak), and stored them on dry ice while in the field. Once in the laboratory, we placed samples in a solution of 2:1 chloroform/methanol containing 0.01% 2, 6,-di-tert-butyl-4-methyl-phenol (BHT) and stored them frozen (−80°C) until processed. We collected milk samples by orogastric lavage using a foal stomach tube and drenching syringe to create suction sufficient to aspirate no more than 100 mL of fluid stomach contents. If the fluid had the color, odor, and consistency of milk, a subsample was transferred to a glass vial and capped with a Teflon-lined lid or to a polypropylene cryogenic vial stored at −80°C until analyzed.

Fatty Acid Analysis

We extracted lipids from full-depth blubber and milk samples using a modified Folch method (Folch *et al.* 1957, Iverson *et al.* 2001, Budge *et al.* 2006, Beck *et al.* 2007). Lipids were extracted from tissue samples and fatty acid methyl esters (FAME) were prepared as described in Iverson *et al.* (1997). FAMEs were analyzed at the Applied Science, Engineering and Technology Laboratory at the University of Alaska Anchorage as previously described (Dodds *et al.* 2004, Beck *et al.* 2007). Specific fatty acids were identified using known standard mixtures (Sigma, Supelco, Matreya and/or Nu-check Prep), silver nitrate (argentation) chromatography, and gas chromatography/mass spectrometry. All three tissues were screened for the same 81 fatty acids ranging in chain length from 12 to 24 carbons, though not all fatty acids were detected (discussed further below). Individual fatty acids are reported as percent weight of total fatty acids and are designated using the shorthand nomenclature of carbon chain length: number of double bonds and location (n–x) of the double bond nearest the terminal methyl group. Fatty acids were classified by structural class and the total reported for saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA). For each sample, the ratio of PUFA (n-6) to PUFA (n-3) was calculated. The fatty acid data from blubber samples in the current study were also included in Beck *et al.* (2007).

Statistical Analysis

We compared the fatty acid composition among tissues to better understand if one may substitute for another depending on the research question (*e.g.*, milk for blubber) and to learn more about how pups metabolize milk. In addition, we performed within tissue analysis because each tissue provides insight into a different aspect of Steller sea lion biology. In income breeders, ingested milk reflects the recent diet of the dam, while the fatty acid composition of serum and blubber samples are influenced by the physiology and metabolism of the young animal (Cooper *et al.* 2005). Additionally, serum provides information about the recent past diet while blubber provides a cumulative view of diet over a longer period.

When analyzing the composition of fatty acids, we restricted the data to the 22 fatty acids with mean percentages >0.5; fatty acids below the detection limit of 0.01% were given a value of 0.005% (Iverson *et al.* 2002). The 0.5% cutoff resulted in the same 19 fatty acids for blubber and milk, and 16 fatty acids for serum. Six of the fatty acids in milk and blubber were absent at these levels in serum and three fatty acids not in milk and blubber were present at these levels in serum (Table 2). All 22 fatty acids were included in analyses that compared all tissue types. When analyzing the composition of the classes of fatty acids (SFA, MUFA, and PUFA) we summed across all 81 fatty acids. Of the 81 screened fatty acids, results were not obtained in all tissues for 16 fatty acids. In blubber 15 were not detected, in milk 14 were absent (although one was different than that missing from blubber), and 2 were not detected in serum (both of which were successfully analyzed for in one of the other tissues). All 16 fatty acids had mean percentages less than 0.5%. Thus, summing across all 81 fatty acids did not bias results when comparing tissue types.

Table 2. Descriptive statistics for fatty acid composition of paired milk, serum, and blubber samples from young Steller sea lions. Values are mean (SD) percent of total fatty acid by weight for all 22 fatty acids included in modeling. Bolded means occurred in at <0.5% in the unrestricted data set and were not included in analysis within each tissue.

	Milk				Serum				Blubber			
	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max
Saturated												
14:00	5.57	1.62	3.24	10.71	2.75	0.61	1.70	4.52	5.42	0.89	4.17	7.69
16:00	17.38	1.47	13.60	20.87	27.96	3.98	24.31	37.84	14.13	1.66	9.85	18.96
7Mel16:0	**0.28**	0.08	0.16	0.54	0.97	0.30	0.57	2.00	**0.28**	0.04	0.20	0.42
18:00	2.33	0.49	1.55	3.66	10.50	1.71	6.64	14.48	1.94	0.44	0.96	3.10
Monounsaturated												
16:1n-7	6.63	1.43	4.07	9.54	4.31	0.78	2.85	6.54	9.21	1.66	5.18	14.08
16:1n-11	**0.52**	0.14	0.27	0.90	1.15	0.24	0.74	1.91	**0.49**	0.14	0.26	0.75
18:1n-11	1.97	0.78	0.59	4.47	**0.07**	0.06	0.01	0.20	3.02	1.05	0.62	6.22
18:1n-5	0.57	0.18	0.31	1.04	**0.01**	0.00	0.01	0.01	0.55	0.09	0.36	0.85
18:1n-7	4.47	1.37	2.32	8.11	7.46	0.84	5.78	9.37	4.80	0.82	3.06	7.20
18:1n-9	20.46	3.29	11.78	29.28	30.08	3.95	21.40	37.59	26.79	3.08	15.41	32.07
20:1n-9	3.17	1.42	0.94	6.58	1.51	0.60	0.01	3.34	3.09	0.95	1.49	6.43
20:1n-11	3.94	1.76	1.23	10.02	1.47	0.78	0.01	4.81	5.58	2.21	2.75	12.55
22:1n-9	**0.45**	0.17	0.11	0.81	1.98	1.35	0.56	6.65	**0.32**	0.11	0.17	0.88
22:1n-11	3.10	1.54	0.78	7.37	1.41	1.03	0.01	4.11	1.74	0.81	0.58	4.23
Polyunsaturated												
18:2n-6	1.27	0.39	0.57	2.18	3.02	0.60	1.75	4.45	1.65	0.25	1.06	2.38
18:3n-3	0.81	0.39	0.20	1.62	**0.27**	0.17	0.01	0.54	0.73	0.19	0.37	1.24
18:4n-3	1.34	0.58	0.24	2.85	**0.23**	0.35	0.01	1.66	0.82	0.27	0.30	1.54
20:4n-3	1.27	0.69	0.30	2.89	**0.06**	0.08	0.01	0.64	1.23	0.29	0.69	2.14
20:4n-6	0.72	0.22	0.34	1.11	1.61	0.44	0.54	2.41	0.74	0.18	0.49	1.43
20:5n-3	9.55	2.03	5.00	14.74	1.52	0.35	0.49	2.17	4.79	0.95	2.78	8.33
22:5n-3	3.47	1.30	1.34	6.51	0.25	0.09	0.01	0.65	3.73	0.67	2.36	5.53
22:6n-3	10.72	3.09	5.61	17.05	1.38	0.34	0.74	2.29	8.96	1.62	5.99	13.94
SFA	25.57	2.00	21.77	30.20	42.19	4.44	35.56	53.40	21.77	1.92	16.37	26.81
MUFA	45.28	4.94	36.28	58.36	49.46	4.48	39.33	56.38	55.59	3.60	47.59	63.35
PUFA	29.15	5.79	17.87	39.63	8.36	1.15	6.00	10.74	22.64	2.93	17.16	29.30
PUFA (n-3)	27.16	5.42	16.02	36.80	3.72	0.51	2.65	5.11	20.25	2.76	14.92	26.52
PUFA (n-6)	1.99	0.50	1.12	3.11	4.63	0.88	2.84	6.82	2.39	0.37	1.58	3.28

We used multivariate linear models (MLM), which includes MANOVAs, MANCOVAs, and multivariate regression models, to aid our understanding of how the composition of fatty acids varied among Steller sea lions. We tested for differences in the composition of fatty acids within each tissue type and among tissue types using the Pillai test statistic. Prior to analysis, we transformed these compositional data into Euclidean space using the additive log-ratio (*alr*) transformation (Aitchison 1982). For data summed into the three classes of fatty acids, we divided the proportions of both the SFAs and the MUFAs for each animal by its proportion of PUFAs, an arbitrary choice that does not affect model selection results or significance tests. For the fatty acid analyses, we divided the proportion of each fatty acid for each animal by its proportion of 18:0 following Budge *et al.* (2006). Explanatory variables included tissue type, sex, age, BCI, and geographic region (Aleutian Islands, Prince William Sound, and Southeast Alaska; Fig. 1).

Our modeling was exploratory by nature, primarily looking for patterns rather than testing specific hypotheses. More complex models were limited, in part, based on the results from simpler models and to ensure adequate sample sizes within the combinations of explanatory variables. The data set is well-balanced with respect to sex; however, it is unbalanced with respect to location: Southeast Alaska dominates both Prince William Sound and Aleutian Islands (Table 1). Within region there were similar numbers of males and females sampled; however, the age distribution varied somewhat among regions, in that the complete range of ages is not well reflected in all regions. In general, the age distribution was dominated by Steller sea lions ≤5 mo with roughly an equal number ranging up to 17.4 mo. One Steller sea lion exceeded 17.4 mo at 26.6 mo. The lack of balance in some aspects of the sampling led us to only include 2-way interactions involving the independent variable sex in the within tissue type models. We also included 2-way interactions involving tissue type in models that compared the composition of fatty acids among tissue types. We avoided interactions of more than two variables primarily due to a lack of biological rationale.

Three metrics were used to present and interpret the model results: (1) model selection criterion CAIC, (2) R^2 values, and (3) visual representation of the MLM results in the form of canonical discriminant plots. We evaluated parsimony of models in our candidate sets using CAIC, which is a multivariate version of AICc, the small sample size version of AIC (Burnham and Anderson 2003, Fujikoshi and Satoh 1997). We ranked models based on CAIC and generally focused our interpretation to models with ΔCAIC <4. *R*-squared values were calculated from partial eta square values attained using the *etasq* function in R (R Core Team 2016) "heplots" (Fox *et al.* 2016). Partial eta squares are the multivariate version of partial *R*-squares that reflect the proportion of variance explained by each term in the full model.

We used the R package *candisc* (Friendly 2007, Friendly and Fox 2016) to create canonical discriminant plots for the top model (lowest *CAIC*) for each tissue type and among tissue types. These plots depict canonical structure coefficients, which reflect the true relationship among variables in the models. To facilitate interpretation, we transformed the linear discriminant coefficients to the basis of the *clr* coordinate system before calculating the canonical structure coefficients because doing so represents the variation of each fatty acid relative to a mean fatty acid composition rather than a specific fatty acid. Vectors on these figures are proportional to the canonical structure coefficients and the direction and length indicate their relative contribution to discrimination among group means or their relative importance in explaining patterns exhibited by continuous covariates (*e.g.*, trends).

Residuals from the top models were examined to evaluate the normality assumption, to evaluate the mean-variance relationship of the *alr* transformed fatty acids to determine whether it was constant (Warton *et al.* 2012), and to identify outlier data with undue influence. To evaluate the assumption of constant within-group covariance matrices of the *alr* transformed fatty acids compositional data, we examined boxplots, used scatterplots to depict data-concentration ellipses, and performed Box's *M*-test (Rencher and Christensen 2012) using the *boxM* function in the R-package "biotools" (da Silva 2017) and a modified version of the *M*-test (Manley and Navarro Alberto 2017). We performed all analyses using Program R, version 3.3.2 (R Core Team 2016).

Results

Variability in FA Composition among Tissues

Fatty acid classes— Descriptive statistics for mean composition by weight for 22 fatty acids with means >0.5% as well as total SFAs, MUFAs, and PUFAs measured in milk, serum, and blubber samples are reported in Table 2 and depicted in Figures 2 and 3. MUFAs contributed the greatest proportion of fatty acids in milk (range 36.28%–58.36%), serum (range 35.56%–53.40%), and blubber (range 47.59%–63.35%) and the majority of MUFAs in all samples were medium chain (≤18C) (Table 2). In both milk and blubber, PUFAs (range: milk 17.87%–39.63%, blubber 17.16%–29.30%) was the next prominent fatty acid class followed by SFAs (range: milk 21.77%–30.20%, blubber 16.37%–26.81%), whereas PUFAs were underrepresented in serum (range 6.00%–10.74%) and SFAs (range 35.56%–53.40%) contributed more strongly to circulating fatty acids in this matrix. The fatty acid class composition was similar among regions (Fig. 3). Results from models with classes of fatty acid as the response variable were generally much poorer than those with individual fatty acid composition, as the response variable and offered no additional biological insight. The same holds true for models within tissue type (below). As a result, we do not report model results or interpretations for classes of fatty acids; instead, we note any class patterns that arise from the fatty acid analysis.

Descriptive statistics for fatty acids— Myristic acid (14:0) was lower in serum than in paired milk and blubber samples; whereas palmitic acid (16:0), stearic acid (18:0), and 7Mel16:0 were higher in serum than milk and blubber samples (Table 2; Fig. 2). Of the MUFAs, oleic (18:1n-9), vaccenic (18:1n-7), and erucic (22:1n-9) acids were higher in serum than

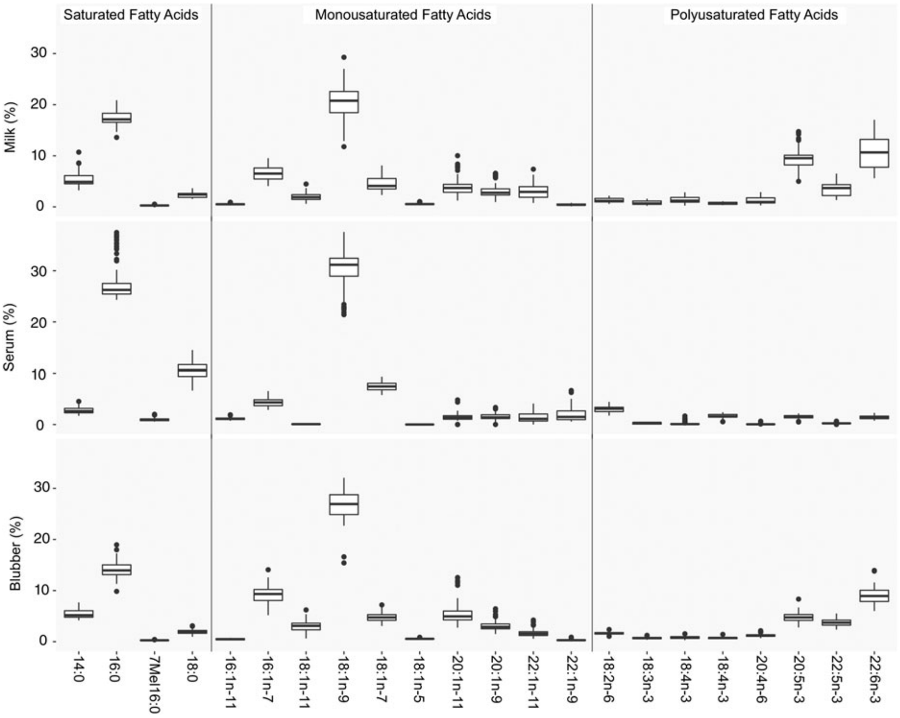

Figure 2. Fatty acid signatures in milk, serum, and blubber samples collected from young Steller sea lions sampled in Aleutian Islands, Prince William Sound, and Southeast Alaska.

milk and blubber whereas 16:1n-7, 18:1n-11, 20:1n-11, 20:1n-9, and 22:1n-11 were lower in serum than milk and blubber samples. Two PUFAs were higher in serum than in milk (18:2n-6, 20:4n-6). Eicosapentaenoic (20:5n-3) and docosahexaenoic (22:6n-3) acids were lower in serum compared to paired milk samples. Docosahexaenoic acid (22:6n-3) was the most prominent PUFA in both milk and blubber while in serum linoleic acid (18:2n-6) was most prominent (Table 2, Fig. 2). The n-3 PUFAs were more prominent in milk and blubber compared to n-6 PUFAs, whereas serum was enriched in n-6 PUFAs (Table 2).

Statistical models for fatty acids—The proportions of fatty acids significantly differed among tissue types when controlling for regional and age effects (approximate $F_{42,\ 350} = 138.2$; $P < 0.0001$; Pillai Trace = 1.89). In the top model (Table 3), tissue type explained 95% of the variability in the composition of fatty acids. Regional and age effects, as well as the tissue type:region and tissue type:age interactions were also significant but collectively explained little of the compositional variability. The effects due to sex and BCI were not statistically significant (Table 3). A canonical discriminant plot depicting these results shows serum to be strongly separated from blubber and milk (Fig. 4). SFAs including

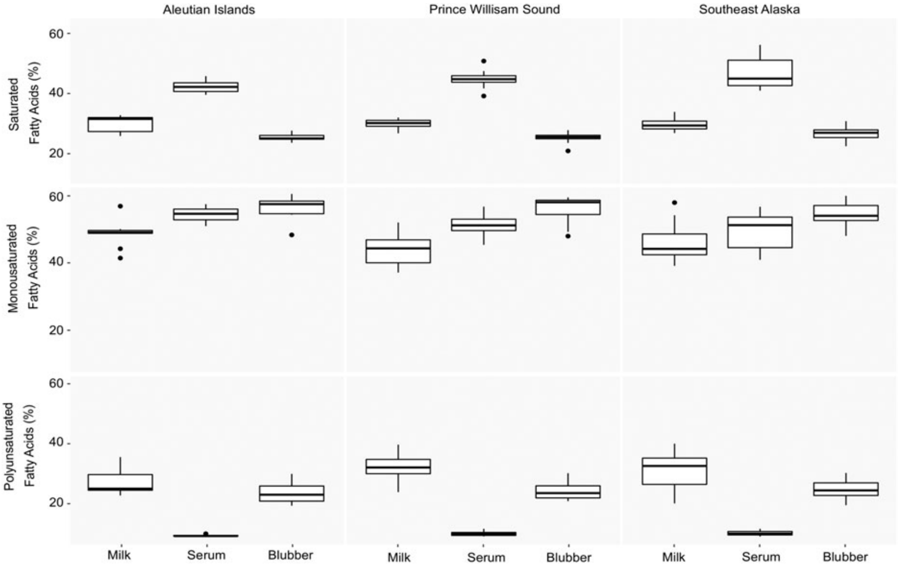

Figure 3. Fatty acid classes (saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids) in milk, serum, and blubber by region (Aleutian Islands, Prince William Sound, and Southeast Alaska).

palmitic acid (16.0) and stearic acid (18.0) were lower in milk and blubber while some MUFAs were higher in blubber compared to milk samples (16:1n-7, 18:1n-11, 20:1n-11). In contrast, several PUFAs including eicosapentaenoic acid (20:5n-3) and eicosatetraenoic acid (20:4n-3) were lower in blubber than in milk (Fig. 4). Linear hypothesis tests for pairwise comparisons show that all tissues are significantly different from each other after accounting for multiple comparisons with $P < 0.0001$.

Variability in the Fatty Acid Composition within Matrices

For milk, the top model with fatty acids as the response variable had region as the only explanatory variable, and the model with both region and age as explanatory variables was second (ΔCAIC = 3.0; Table 4). The region effect was significant in both models ($P < 0.0001$) and allowed for clustering of milk samples by region (Fig. 5A). The second model provides some support for a significant age effect ($P < 0.0001$, Fig. 6A). These models explain 79% and 92% of the variability of individual fatty acids in milk, respectively (Table 4). Milk samples from the Aleutian Islands had a higher proportion of 16:0, 16:1n-7, 18:1n-7, 20:1n-11 and 20:5n-3 and a lower proportion of 18:1n-5 and 20:4n-3 compared to Prince William Sound and Southeast Alaska (Fig. 5A). Southeast Alaska samples had more 18:1n-11, 20:1n-9, and 22:1n-11 and a lower proportion of 18:2n-6 compared to milk samples from Prince William Sound. Milk samples collected from younger Steller sea lions had a greater proportion of several MUFAs including 18:1n-9, 18:1n-11, 20:1n-9, and 22:1n-11 as well as several PUFAs (18:4n-3, 18:2n-6, 18:3n-

Table 3. MLM results for the composition of individual fatty acids with tissue type (TT) as a categorical explanatory variable. The models are listed from most parsimonious (smallest CAIC) to least and include one to four main effects (ME) and relevant interaction terms. Reported terms include Akaike information criterion with a correction for multivariate finite sample sizes (CAIC), ΔCAIC, R^2, P-value for main effects 1–4 (ME1, ME2, ME3, ME4), and interaction terms (*e.g.*, ME1 × ME2).

				Main Effects				Interactions	
Model	CAIC	ΔCAIC	R^2	ME1	ME2	ME3	ME4	ME1 × ME2	ME1 × ME3
TT × Region + TT × Age	9,313.8	0	0.99	<0.0001	<0.0001	<0.0001		<0.0001	<0.0001
TT × Region + Age + BCI	9,396.5	82.7	0.99	<0.0001	<0.0001	<0.0001	0.24	<0.0001	
TT × Region + Age + Sex	9,401.4	87.6	0.99	<0.0001	<0.0001	<0.0001	0.49	<0.0001	
TT × Region + Age	9,419.8	106	0.98	<0.0001	<0.0001	<0.0001		<0.0001	
TT × Region	9,480.6	166.8	0.98	<0.0001	<0.0001				<0.0001
TT + Region + TT × Age	9,503.9	190.1	0.98	<0.0001	<0.0001	<0.0001		<0.0001	
TT + Region + Age	9,611.6	297.8	0.98	<0.0001	<0.0001	<0.0001			
TT + Region + BCI	9,643.1	329.3	0.97	<0.0001	<0.0001	0.18			
TT + Region	9,667.8	354	0.97	<0.0001	<0.0001				
TT × Age	9,755.1	441.3	0.97	<0.0001	<0.0001			<0.0001	
TT + Age	9,855.4	541.6	0.96	<0.0001	<0.0001				
TT + BCI	9,881.1	567.3	0.95	<0.0001	0.009				
TT + Sex	9,899.9	586.1	0.95	<0.0001	0.41				
TT	9,919.1	605.3	0.94	<0.0001					

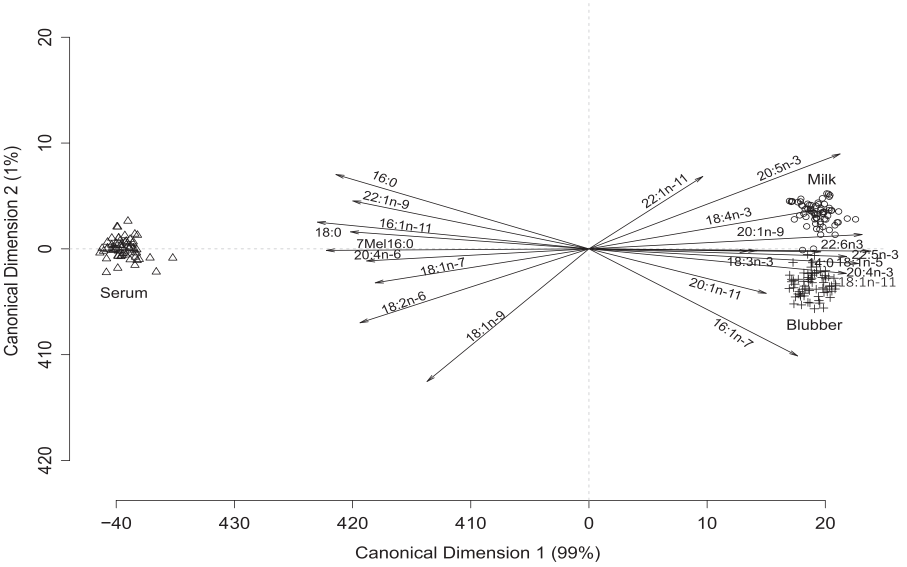

Figure 4. Canonical discriminant plots depicting the MLM results for fatty acid signatures in milk, serum, and blubber samples collected from young Steller sea lions (n = 68). Vector directions and lengths indicate relative contributions of individual fatty acids to the discrimination among tissue types.

3, and 20.4n-3). Various other fatty acids such as 16:1n-7 and 22:5n-3 were more prominent in older Steller sea lions (Fig. 6A; downward pointing vectors in right panel correspond to fatty acids contributing to the lower canonical scores associated with older Stellar sea lions in left panel). BCI and sex were not statistically significant in any model ($P \geq 0.21$, Table 4).

For serum, as with milk, the top model included region as the sole independent variable and the second model included both region and age as independent variables (Table 4). The region effect in the top model was significant ($P < 0.0001$) and the model explained 55% of the compositional variability in the fatty acids. Similar to milk, the fatty acid signatures in serum allowed for the clustering of samples by region (Fig. 5B). Serum samples from the Aleutian Islands had higher proportions of 16:1n-7, 18:1n-7, 18:1n-9 and lower in 16:0, 22:1n-9, and 22:1n-11 compared to sea lions sampled in Southeast Alaska (Fig. 5B). Steller sea lions sampled in Prince William Sound had a higher proportion of 20:5n-3 and lower 20:1n-9 compared to Southeast Alaska. Age, sex, and BCI were not significant in either the single or multiple effect models (Table 4, Fig. 6B), with the exception that BCI was significant ($P = 0.03$) when combined with sex and marginally insignificant as the sole independent variable ($P = 0.09$; Table 4). However, these models had little to no support (ΔCAIC ≥29.1; Table 4).

Table 4. MLM results for the composition of fatty acids in milk, serum, and blubber samples. The models for each tissue are listed from most parsimonious (smallest CAIC) to least and include one to three main effects (ME) and relevant interaction terms. Reported terms include Akaike information criterion with a correction for multivariate finite sample sizes (CAIC), ΔCAIC, R^2, *P*-value for main effect 1–3 (ME1, ME2, and ME3), and interaction term of main effect 1 and 2 (ME1 × ME2).

				Main Effects			Interaction
	CAIC	ΔCAIC	R^2	ME1	ME2	ME3	ME1 × ME2
Milk							
Region	991.8	0.0	0.79	<0.0001			
Region, Age	994.8	3.0	0.92	<0.0001	<0.0001		
Region, Sex	1,033.8	42.0	0.86	<0.0001	0.24		
Region, BCI	1,042.8	51.0	0.84	<0.0001	0.69		
Region, Age, BCI	1,047.5	55.7	0.94	<0.0001	<0.0001	0.63	
Region, Sex, Region × Sex	1,059.1	67.3	0.90	<0.0001	0.21		0.44
Age	1,067.1	75.3	0.64	<0.0001			
Serum							
Region	1,594.3	0.0	0.55	<0.0001			
Region, Age	1,622.3	28.0	0.69	<0.0001	0.15		
BCI	1,623.4	29.1	0.33	0.09			
Region, Sex	1,627.0	32.7	0.67	<0.0001	0.33		
Region, BCI	1,627.8	33.5	0.67	<0.0001	0.37		
Sex	1,631.7	37.4	0.25	0.40			
Age, BCI	1,649.3	55.0	0.54	0.14	0.09		
Sex, BCI	1,649.7	55.4	0.54	0.31	0.03		
Blubber							
Region, Age	548.3	0.0	0.96	<0.0001	<0.0001		
Region, Age, BCI	579.3	31.0	0.98	<0.0001	<0.0001	0.02	
Region	606.1	57.8	0.74	<0.0001			
Age	619.9	71.6	0.83	<0.0001			
Region, BCI	636.8	88.5	0.85	<0.0001	0.03		

For blubber, the top model included region and age as independent variables and the second model included BCI along with region and age effects (Table 4). However, the second model had little support (ΔCAIC = 31) although all the effects were significant ($P \leq 0.020$). The region and age effects in the top model were significant ($P < 0.0001$) and explained 96% of the compositional variability of the individual fatty acids. A canonical discriminant plot depicting the MLM results shows that individual fatty acids could be used to group blubber samples by region (Fig. 5C), as was also the case for both milk and serum samples (Fig. 5A, B). Steller sea lions sampled in the Southeast Alaska region had a greater proportion of 16:0, 18:0, and 20:4n-6 and a lower proportion of 20:1n-11 and 22:1n-11 than samples collected from Prince William Sound. Samples from the Aleutian Islands had a lower proportion of several fatty acids including 18:3n-3, 22:6n-3, and 20:4n-3 while having a greater proportion of 20:5n-3 and 20:1n-9 than blubber samples

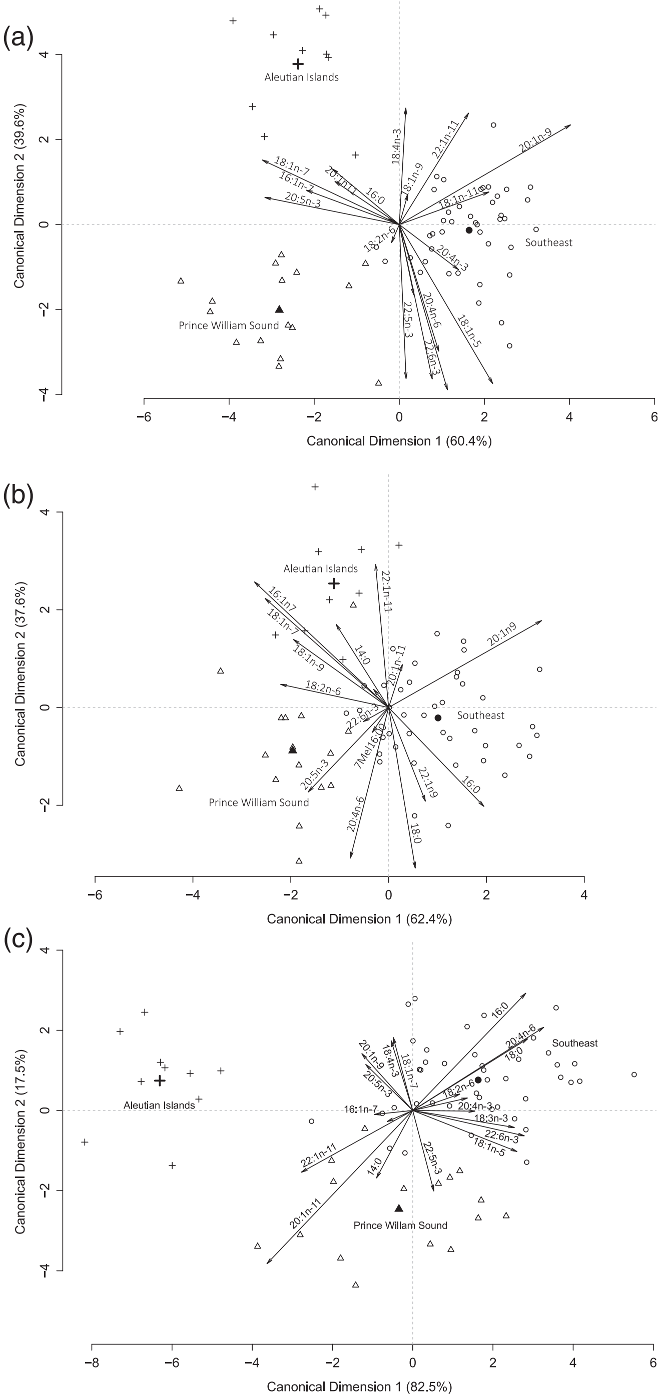
(a)
Aleutian Islands
Southeast
Prince William Sound
Canonical Dimension 2 (39.6%)
Canonical Dimension 1 (60.4%)
18:4n-3
22:1n-11
20:1n-9
18:1n-7
16:1n-7
20:1n11
16:0
20:5n-3
18:1n-9
18:1n-11
18:2n-6
20:4n-3
22:5n-3
20:4n-6
22:6n-3
18:1n-5
(b)
Aleutian Islands
Southeast
Prince William Sound
Canonical Dimension 2 (37.6%)
Canonical Dimension 1 (62.4%)
22:1n-11
16:1n7
18:1n-7
18:1n-9
14:0
20:1n-11
20:1n9
18:2n-6
22:6n-3
7Me16:0
20:5n-3
20:4n-6
18:0
22:1n9
16:0
(c)
Aleutian Islands
Southeast
Prince Willam Sound
Canonical Dimension 2 (17.5%)
Canonical Dimension 1 (82.5%)
16:0
20:4n-6
18:0
20:1n-9
18:4n-3
18:1n-7
20:5n-3
18:2n-6
16:1n-7
20:4n-3
18:3n-3
22:6n-3
18:1n-5
22:1n-11
14:0
22:5n-3
20:1n-11

Figure 5. Canonical discriminant plots depicting the MML results for individual fatty acids by tissue type for (A) milk, (B) serum, and (C) blubber. Vector directions and lengths indicate relative contributions of individual fatty acids to the discrimination among regions.

collected from Prince William Sound and Southeast Alaska. Several fatty acids were proportionally greater in younger Steller sea lions (higher canonical scores) including 16:0, 18:0, 18:2n-6, 20:4n-6, and 22:6n-3 while 14:0, 20:1n-11, and 22:1n-11 were more prominent in older Steller sea lions with lower canonical scores (Fig. 6C). A BCI effect was significant in two multiple explanatory variably models ($P \leq 0.030$) neither of which were supported by model selection (ΔCAIC ≥ 31; Table 3). A sex effect was not significant in any model (Table 4, $P \geq 0.343$).

We conducted a *post hoc* analysis including season of capture as categorical variable with two levels (summer and winter), which revealed that season of capture is a better predictor of fatty acid composition than age for milk and serum but not for blubber. Closely following Sinclair and Zepplin (2002), we defined summer as May through September and winter as November through April (all of our October captures were on 10/30 and were assigned to winter). In our sample, ages 0.5–3.1 mo, 10.8–15.4 mo, and 26.6 mo were captured in the summer and ages 4.6–9.8 mo and 16.7–17.4 mo were captured in the winter. All three regions included Steller sea lions sampled during both seasons. For milk and serum, replacing age with capture season resulted in models that substantially outperformed the previous top models as well as models that included both age and season of capture as independent variables. Models including both season of capture and region had $CAIC = 981.2$ and $R^2 = 0.94$ (milk), $CAIC = 1570.1$ and $R^2 = 0.87$ (serum) and $P < 0.0001$ for both independent variables as compared to previous models (Table 4). For serum, age was not significant in any of the previous models. On the other hand, replacing age with capture season in models for blubber resulted in poorer models. The model with season of capture and region as explanatory variables had $CAIC = 563.2$ *vs.* 548.3, although R^2 remained nearly the same at 0.95 (Table 4). That said, the effect of season of capture was significant ($P < 0.0001$) as was the effect of age in the previous model. Canonical discriminant plots for the effect of season in these models are provided in Figure S1.

Model Diagnostics

In general, model residuals were centered on zero and approximated normality. Furthermore, the mean-variance relationship for the *alr* transformed values was near constant, thus meeting model assumptions. For the MLMs examining fatty acids among tissue types, box plots, scatterplot matrices, Box's *M*-test, and the modified *M*-test indicate departures from the assumption of constant within-group covariance matrices. However, MLM test statistics are generally robust to such departures when group sample sizes are equal as is nearly the case for the independent variable tissue type (n = 66–68) and the Pillai test statistics are

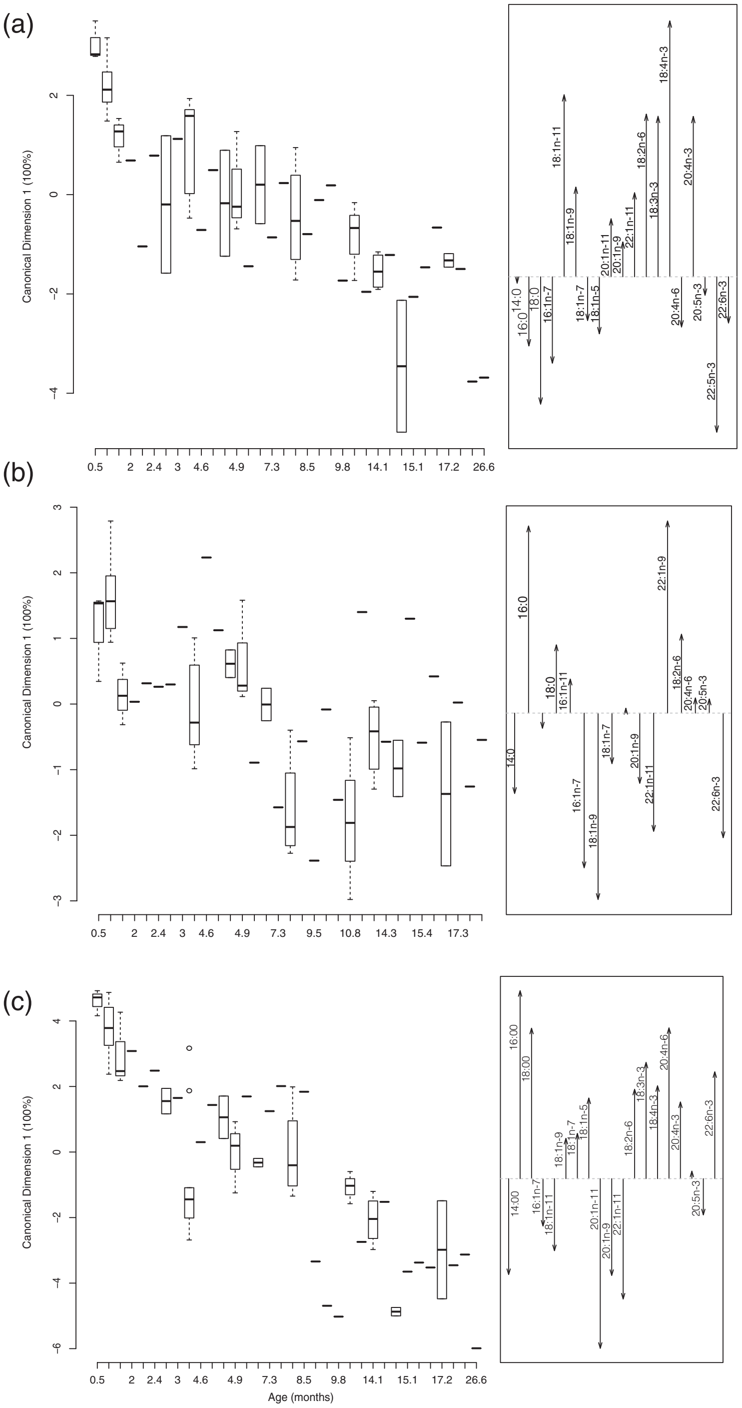
(a)
Canonical Dimension 1 (100%)
2
0
−2
−4
0.5
2
2.4
3
4.6
4.9
7.3
8.5
9.8
14.1
15.1
17.2
26.6
14:0
16:0
18:0
16:1n-7
18:1n-11
18:1n-9
18:1n-7
18:1n-5
20:1n-11
20:1n-9
22:1n-11
18:2n-6
18:3n-3
18:4n-3
20:4n-6
20:4n-3
20:5n-3
22:5n-3
22:6n-3
(b)
Canonical Dimension 1 (100%)
3
2
1
0
−1
−2
−3
0.5
2
2.4
3
4.6
4.9
7.3
9.5
10.8
14.3
15.4
17.3
16:0
14:0
18:0
16:1n-11
16:1n-7
18:1n-9
18:1n-7
20:1n-9
22:1n-11
22:1n-9
18:2n-6
20:4n-6
20:5n-3
22:6n-3
(c)
Canonical Dimension 1 (100%)
4
2
0
−2
−4
−6
0.5
2
2.4
3
4.6
4.9
7.3
8.5
9.8
14.1
15.1
17.2
26.6
Age (months)
16:00
18:00
14:00
16:1n-7
18:1n-11
18:1n-9
18:1n-7
18:1n-5
20:1n-11
20:1n-9
22:1n-11
18:2n-6
18:3n-3
18:4n-3
20:4n-6
20:4n-3
20:5n-3
22:6n-3

Figure 6. Canonical discriminant plots depicting the MML results for fatty acids by tissue type for (A) milk, (B) serum, and (C) Blubber. Vector directions and lengths presented in the right panels indicate the relative contribution of fatty acids to the patterns exhibited by the canonical scores plotted as a function of age (months) in the left panels. For example, downward pointing vectors in the right panels correspond to fatty acids contributing to the lower canonical scores associated with older Steller sea lions as shown in the left panels.

considered the most robust to departures from constant within group covariance matrices (Ito 1980, Rencher and Christensen 2012).

For MLMs testing for differences in composition of fatty acids within tissue type, the covariance matrices for each independent variable grouping are similar only for sex in blubber. Because sample sizes of males and females are nearly equal, the tests for a sex effect in the other tissues are robust to heterogeneity of covariance matrices. For the other independent variables, the larger covariance matrices tended to be associated with the larger sample sizes; therefore, the Pillai test may be somewhat more conservative, meaning that there may be a tendency to fail to reject the null hypothesis when the null is false (Rencher and Christensen 2012). This was not an issue in our study because the effects of region and age were significant in the top models for all tissue types as was the effect of BCI in models for blubber. Diagnostics did not identify any outlier data with undue influence.

Discussion

The observed differences in fatty acid signatures among the three tissues likely reflect differences in dietary time scales and metabolic processing (Tierney *et al.* 2008, Wang *et al.* 2007). The variability of fatty acid composition is almost entirely explained by tissue type, highlighting the importance of considering which tissue is being collected and analyzed in different studies. We discuss the different or similar patterns observed in our study further in relation to region, age (or season), BCI, and sex in more detail below.

Region

Region was in the top model for all three tissues and the fatty acids contributing to the regional differences varied among tissue types. The differences in fatty acid signatures likely reflect the previously reported regional differences in the diet of adult Steller sea lions (Sinclair and Zepplin 2002; Sinclair *et al.* 2005; Sinclair *et al.* 2013, 2015).

As milk is the diet of dependent Steller sea lions, the regional differences in milk would translate into regional differences in the fatty acid composition of both serum and blubber samples, as observed in our study. Milk samples from the Aleutian Islands had proportionally more 18:1n-7, 16:1n-7, and 20:5n-3 compared to samples from Southeast Alaska. Benthic organisms have a greater proportion of n-7 isomers suggesting adult female Steller sea lions within the Aleutian Islands are more dependent on benthic prey than females in Southeast Alaska which had more 22:1n-11 and 20:1n-9, two fatty acids more prominent in pelagic teleost fish (Cooper *et al.* 2009, Iverson *et al.* 2002). However, the Aleutian Island milk samples also had proportionally lower 20:4n-6, another fatty acid prominent in benthic species and in particular sculpin and shrimp (Iverson *et al.* 2002) highlighting the need for obtaining fatty acid signatures from prey across the range of Steller sea lions for accurate fatty acid profile interpretation. As with milk samples, serum samples collected from Steller sea lions within the Aleutian Islands had proportionally more n-7 isomers (16:1n-7, 18:1n-7) compared to Southeast Alaska samples. 20:1n-11, 22:1n-11, and 14:0 also contributed to the regional differences between Prince William Sound and Southeast Alaska in blubber in the current study and was also observed by Beck *et al.* (2007). Similarly, blubber samples from harbor seals in Prince William Sound were higher in 20:1n-11 and 22:1n-11 compared to those sampled in Southeast Alaska. Iverson *et al.* (1997) suggests that 20:1n-11 and 22:1n-11, as well as 20:1n-9 and 22:1n-9 were important for identifying regional differences in fatty acid signatures as these isomers are primarily from diet. The regional differences observed in all three tissues in our study suggest that any one of these tissues collected from young Steller sea lions can be utilized in studies focusing on comparing diets across the range of Steller sea lions and other otariid species.

Age

The proportion of fatty acids in milk varied with age; however, these changes in fatty acid signatures had more to do with season of capture and lactation stage than “age” *per se.* In the current study, all Steller sea lions had recently fed, and the fresh milk samples collected from their stomachs represent the most recent undigested meal and therefore fatty acid composition of milk samples would not be influenced by the condition or metabolism of the pup. Parturition is highly synchronized with Steller sea lions being born between mid-May and mid-July. The narrow pupping window combined with year-round captures and seasonal shifts in prey (Pitcher *et al.* 2001) results in the likelihood that the effect of age on the fatty acid composition of milk (and likely serum) is confounded with the season of capture. The *post hoc* analysis we performed supports that season of capture is a better predictor of fatty acid composition than age for milk and serum. Whereas, models that included age outperformed models with the season of capture for blubber samples.

The influence of season on the fatty acid signature in milk was not surprising. Steller sea lions are income breeders, relying on recent foraging trips to support lactation (Bonner 1984, Oftedal *et al.* 1987, Trillmich 1990, Costa 1991). Milk and serum samples collected in winter had more 22:1n-11 and 20:1n-9 compared to summer samples while 22:6n-3 was more prominent in summer samples. These findings suggest that during winter months adult females are more reliant on pelagic teleost fish (Iverson *et al.* 2002, Cooper *et al.* 2009) than during summer in which benthic species are more prominent in their diet. These findings are in line with previous foraging and seasonal diets of Steller sea lions (Merrick and Loughlin 1997, Merrick *et al.* 1997, Sinclair and Zepplin 2002, Womble and Sigler 2006, Sinclair *et al.* 2013). During early pup-rearing, adult female Steller sea lions forage nearshore and in localized areas while during winter, females with older dependent pups forage up to 400 nmi offshore which may provide more opportunity to feed on pelagic fish. Fatty acid signatures in milk samples have been used to classify the foraging area (continental shelf waters *vs.* oceanic) as well as identifying seasonal shifts in female Australian sea lions and New Zealand fur seals (Baylis and Nichols 2009, Baylis *et al.* 2009). The milk samples from these previous studies were collected directly from lactating females which can be logistically challenging. Our findings support the use of milk samples collected from the stomachs of pups as an alternative source. The fatty acid signatures driving the seasonal difference found in milk were often the same fatty acids contributing to the seasonal difference found in serum. As milk is likely the primary diet of the young Steller sea lions we sampled, the seasonal differences in milk transferred into the serum samples. Both milk and serum represent a short time frame and in our study were useful for comparisons of fatty acid signatures associated with season.

The fatty acid composition in blubber is an accumulated record of diet and the fatty acids in blubber significantly changed with age in our study as well as previously reported in Steller sea lions (Beck *et al.* 2007). At birth, Steller sea lions have an estimated total body lipid stores of 5%–6% of body mass (Brandon *et al.* 2005, Oftedal *et al.* 1987) which on average increases to 28%–36% by 9 mo of age while the sea lions are still nutritionally dependent (Rea *et al.* 2016). In our study, season was not as influential as age on the fatty acid signature in blubber from young Steller sea lions. Beck *et al.* (2007), examined fatty acid signatures of blubber samples, a portion of which are included in our study, and found within regions both seasonal and age-class effects. Although our modeling approach was somewhat different, we also found a significant effect of both age and season; however, the inclusion of season, though significant, did not improve our models suggesting that for blubber, age had a greater influence on the fatty acid signatures. This was not surprising, given that animals of this age are progressively building blubber reserves and so the fatty acid signature at the time of collection would reflect the accumulation of lipids from birth, including those laid down from the milk diet during the previous days to weeks, as is reflected in the ingested milk and serum profiles. Therefore, seasonal or short-term changes in foraging ecology of Steller sea lions are best assessed with serum or milk samples while blubber would be useful for studies without a short temporal component. However, our study was limited to young animals and further studies would be needed to determine if blubber in older Steller sea lions were also influenced by age.

BCI and Sex

Neither BCI nor sex was significant in any of the models for individual fatty acids in milk samples. Lactation is the most energetic part of reproduction (Pond 1977) and the extended period of nutritional dependency found in Steller sea lions requires the adult female to balance her own energetic needs with that of the developing pup, two tasks that may conflict during times of fasting or reduced prey (Champagne *et al.* 2012). The diet and foraging ability of the dam may influence the fatty acid signature of milk which would carry over into the fatty acid signatures of the serum and blubber. Further, as pups are still developing their blubber layer, the fatty acid composition of the blubber layer may influence the BCI of the pup. BCI was significant in some models for fatty acids in serum and blubber; however, BCI in these lower ranking models explained very little of the variability in the fatty acid signatures. Longitudinal sampling, rather than the cross-sectional sampling in our study may find stronger support for the relationships between BCI and fatty acid signatures in serum and blubber. In the current study, Steller sea lions largely relied on a diet of milk which would explain why there was not a difference in individual fatty acids in serum or blubber samples between males and females. Older Steller sea lions that forage for prey may have fatty acid signatures in some tissues that are influenced by sex or BCI as some pinniped species have different foraging abilities or niche selection between males and females (Beck *et al.* 2007, Kernaleguen *et al.* 2015, Baylis *et al.* 2016).

Conclusion

We were able to compare the fatty acid signatures in three matrices simultaneously collected from the same young Steller sea lion. The variability of fatty acid composition was almost entirely explained by sample type, highlighting the importance of selecting a matrix consistent with the study objectives. All three matrices (milk, serum, and blubber) were useful in determining regional differences in fatty acid composition, though the fatty acids driving the regional differences varied among matrices. In addition to the regional effect, the proportion of fatty acids in milk and serum were influenced by season of capture whereas blubber samples were more affected by age of the animal, as expected given the accumulation of lipids during the development of the blubber layer in young Steller sea lions. The influence of season on the fatty acid signatures in milk and serum highlights the use of these samples over blubber in studies investigating seasonal changes in diet. However, blubber may be more appropriate in studies aiming to reduce the influence of season in their sampling design. Further, our findings suggest that the use of milk samples collected from the stomachs of pups may be a viable alternative to directly collecting milk from adult females.

Acknowledgments

We thank the Steller sea lion capture teams at the Alaska Department of Fish and Game (ADF&G) and the Marine Mammal Laboratory, Seattle, for their dedication and assistance in obtaining samples for this project and the captains and crew of the vessels P/V *Woldstad*, P/V *Stimson*, R/V *Tiglax*, and R/V *Medeia*. We also thank B. Applegate, E. Dodds, R. Douglas, A. Geldenhuys, and M. McCoy for their laboratory assistance. This research was funded by a North Pacific Marine Research Institute grant to LDR and JMK (T-2120) and through a cooperative agreement with National Oceanic and Atmospheric Administration (NOAA) and ADF&G (No. NA17FX1079) and FWS AK-22. We thank the efforts of A. Bryan and P. Charapata and the anonymous reviewers whose comments helped improve this manuscript. Captures and field sample collections were conducted under authority

of ESA/MMPA Scientific Collection permits issued by the NOAA Fisheries Office of Protected Resources to the ADF&G and the Marine Mammal Laboratory, #358-1564 and #782-1532, respectively, and within the Alaska Maritime National Wildlife Refuge by Special Use Permit 74500-99004 issued from the U.S. Fish and Wildlife Service. Free-ranging animal handling protocols were reviewed by the ADF&G Division of Wildlife Conservation Animal Care and Use Committee and approved as Assurance of Animal Care #03-0002 and University of Alaska Anchorage Animal Care & Use Committee Permit 2003Beck1. Although the research described has been funded in part by the NOAA, it has not been subjected to NOAA review and therefore does not necessarily reflect the views of the NOAA, and no official endorsement should be inferred. Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA, or the ADF&G. The authors have no conflicts of interests.

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Received: 15 February 2018
Accepted: 24 November 2018

SUPPORTING INFORMATION

The following supporting information is available for this article online at http://onlinelibrary.wiley.com/doi/10.1111/mms.12574/suppinfo.

Figure S1 Canonical discriminant plots for the effect of season of capture (2-level factor) for models with region and season as independent variables for (A) milk, (B) serum, and (C) blubber. Vector directions and lengths presented in the right panels indicate the relative contribution of individual fatty acids to the patterns exhibited by the canonical scores plotted as a function of season in the left panels.

Science of the Total Environment 698 (2020) 134183

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Organochlorine contaminant concentrations in blubber of young Steller sea lion (*Eumetopias jubatus*) are influenced by region, age, sex, and lipid stores

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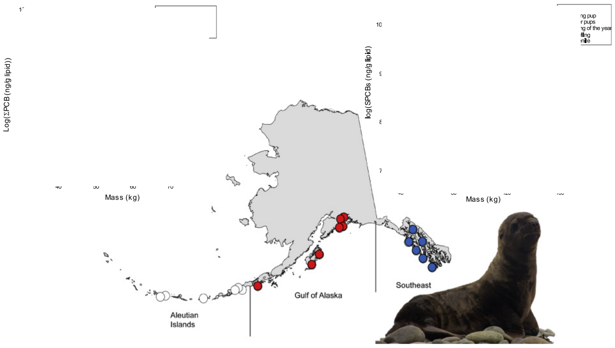
HIGHLIGHTS

- ΣPCBs were highest in the Aleutian Islands and were higher in males in all regions.
- ∑PCBs and ∑DDTs concentrations decreased with increasing mass in pups.
- OC loads in pups decreased with increasing mass with no influence of region or sex.
- OCs increased with age, but decreased with increasing mass within each age class.
- Lipid adjusted OC concentration did not address variability due to energy states.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 13 May 2019
Received in revised form 27 August 2019
Accepted 28 August 2019
Available online 31 August 2019

Editor: Yolanda Picó

Keywords:
Organochlorine contaminants
DDT
PCBs

ABSTRACT

Contaminant exposure is particularly important for species and populations of conservation concern, such as the Steller sea lion (*Eumetopias jubatus*). We used blubber samples ($n = 120$) to determine organochlorine concentrations, including polychlorinated biphenyls (PCBs) and dichloro-diphenyl-trichloroethane (DDTs), and blood samples ($n = 107$) to estimate total body lipids based on the hydrogen isotope dilution method. We assessed the influence of age, sex, condition, and geographic area on contaminant concentrations in blubber and contaminant body load. The concentration of ΣPCBs was highest in pups (<6 months) from the Aleutian Islands, and the concentrations in males were higher than females in all regions. The ΣPCBs and ΣDDTs concentrations and loads decreased with increasing mass in pups, however, there were no regional or sex differences in contaminant load. Within each of the five age classes, the concentrations of ΣPCBs and ΣDDTs decreased with increasing mass, but overall these OCs increased with age. Further, accounting for the lipid content, a potential proxy for energy

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Abbreviations: (SSL), Steller sea lion.
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https://doi.org/10.1016/j.scitotenv.2019.134183

Blubber
Fat
Biopsy
Steller sea lions

balance, in the animal load reduced or removed the regional and sex effects present in age models for contaminants. We propose, that adjusting OCs concentration by the lipid content of the blubber sample alone may not fully account for the variability in OC concentrations associated with differences in condition or energy states between young Steller sea lions.

1. Introduction

Marine mammals are long lived, apex predators with large lipid stores in the form of a blubber layer which is the primary tissue for energy storage as well as providing hydrodynamics and thermal insulation (Liwanag et al., 2012). The blubber layer also acts as a storage tissue for lipophilic contaminants including organochlorines (OCs) which are transferred to pups through the placenta and lactation (Beckmen et al., 1999; Greig et al., 2007; Beckmen et al., 2016). Blubber is widely used for monitoring of OCs including polychlorinated biphenyls (PCBs) and dichloro-diphenyl-trichloroethane (DDTs) in marine mammals (Lee et al., 1996; Kajiwara et al., 2001; Greig et al., 2007; Borrell et al., 2010; Beckmen et al., 2016). Accumulation of certain OCs in marine mammals has been linked to various deleterious biological and physiological effects, including reproductive impairment, immune suppression, and increased risk of cancer and infectious disease (Reijnders, 1986, Ross et al., 1996, Beckmen et al., 2003, Ylitalo et al., 2005, Wang et al., 2010; Murphy et al., 2015, Randhawa et al., 2015). The impacts of contaminant exposure are particularly important for species and populations of conservation concern, such as the Steller sea lion (*Eumetopias jubatus*; SSL). SSLs are managed as two distinct population segments (DPS) with the western DPS listed as endangered under the U.S. Endangered Species Act in 1990 while the eastern DPS was previously listed as threatened until its delisting in 2013 (U.S. Federal Register 62:30772–30773; Fig. 1) (NMFS, 2013). The Alaskan portion of the western DPS continued to decline until 2000 but has since shown regional variability in trends with declines continuing in the western-central Aleutian Islands (Fritz et al., 2014). The causes of the population decline, and slow recovery remain unknown but environmental contaminants including OCs have been hypothesized to be a contributing factor (Barron et al., 2003; Atkinson et al., 2008; NMFS, 2008; Rea et al., 2013).

Otariid (sea lion and fur seal) pups have a limited blubber layer with an estimated total body lipid stores of 5–6% of body mass at birth (Oftedal et al., 1987b; Brandon et al., 2005) which increases to 28–36% by 9 months of age (Rea et al., 2016). Further, as otariids are income breeders, their pups undergo alternating periods of nursing and fasting while the mothers forage (Bonner, 1984; Oftedal et al., 1987a; Trillmich, 1990; Costa, 1991). This strategy leaves pups at risk of entering periods of negative energy balance (Springer et al., 2003; DeMaster et al., 2006; Guénette et al., 2006; Atkinson et al., 2008; Hui et al., 2015). Mass loss has been shown to increase the concentration of PCBs and DDTs (ng/g lipid weight) in blubber sampled from California sea lions (*Zalophus californianus*) while a dilution of PCB and DDT concentrations was

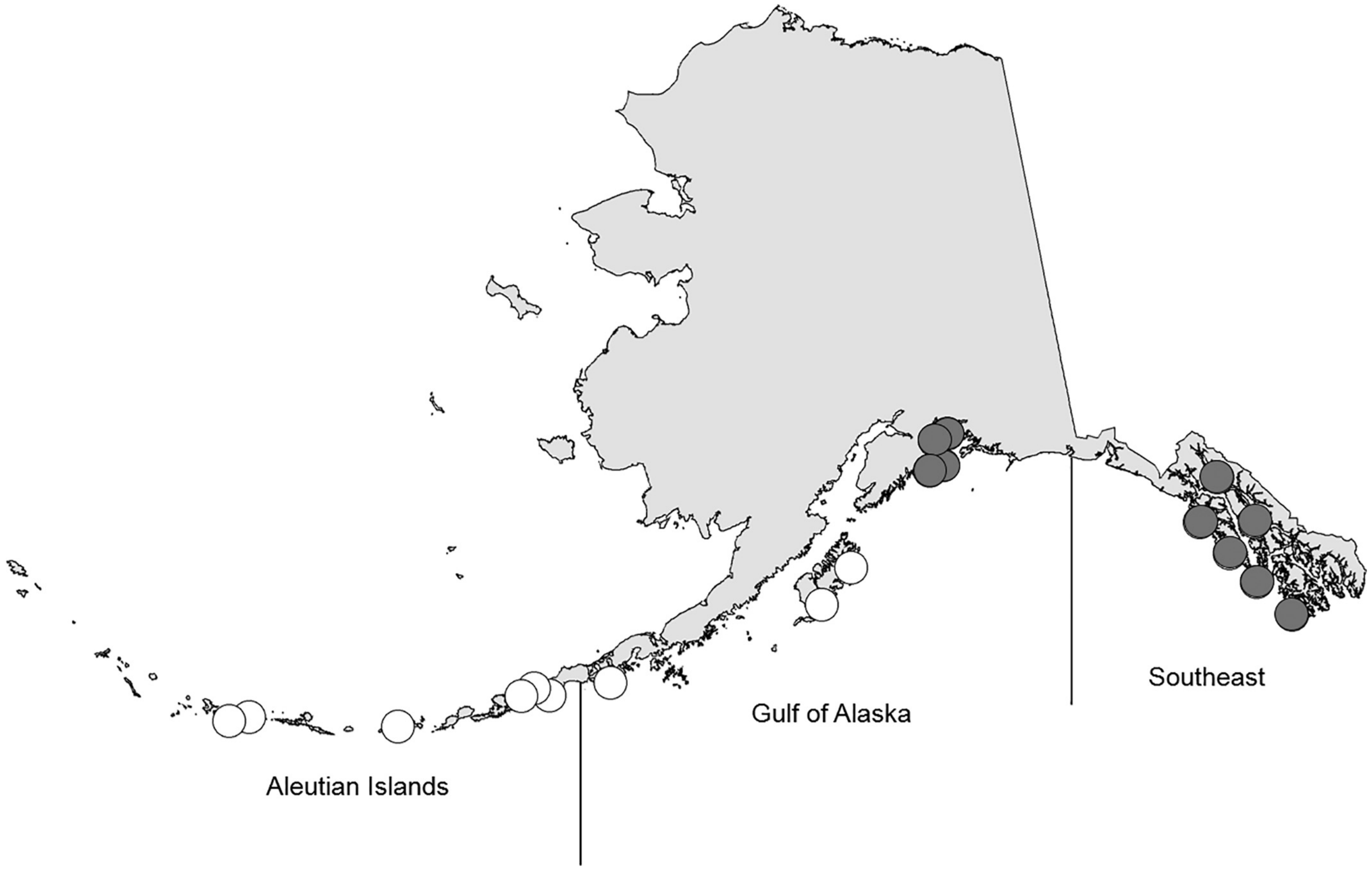

Fig. 1. Capture sites (circle) of Steller sea lions sampled between 1998 and 2003. The lines denote the boundary between the three regions (Aleutian Islands, Gulf of Alaska, and Southeast Alaska). The grey circles denote the samples sampled in Southeast Alaska and the Eastern Gulf of Alaska that were used to assess age effects.

observed when mass was re-gained during rehabilitation (Hall et al., 2008). SSL pups undergo an overall increase in mass and lipid stores during development (Rea et al., 2016) which may lead to a dilution of OCs in their blubber. However, during this period of growth individuals may experience short term periods of mass or lipid loss leading to temporary increases in concentrations of some OCs in blubber and circulating in the blood.

Beckmen et al. (2016) found large variation in PCBs and DDTs in blubber, milk, and feces from young SSLs ($n = 53$) with preferential absorption and storage of some PCB congeners and DDTs leading to biomagnification of these analytes in blubber. However, the sample size did not support assessing factors which may have contributed to the variation in contaminant concentrations, nor was the influence of body condition or energy balance of the pup considered. Further, while environmental contaminants are potential contributors to the regional trends found in reproductive rates and survival, there is limited data available to evaluate whether OC concentrations are a concern and whether studies are needed on the potential impact of contaminants on the health and survival of young SSLs. To help address this data gap, concentrations of OCs, including selected PCB congeners, DDTs and DDT metabolites, and hexachlorobenzene (HCB), were measured in blubber samples collected from 120 free-ranging young SSLs. We assessed the influence of age, sex, condition, and geographic location on contaminant concentrations in blubber samples and contaminant body load.

2. Materials and methods

2.1. Animal captures and sample collection

We captured, sampled, and released 120 free-ranging SSLs in Alaskan waters between 1998 and 2003. We also collected blubber samples from 4 freshly dead SSL pups and 1 fetus during the same period. Capture locations ($n = 19$) were on or near rookeries and haul outs from Lowrie Island (54.51 N 133.31 W) in the southeastern panhandle to Adak Island (51.6459 N 176.9841 W) in the central Aleutian Islands (Fig. 1). Sea lions were caught on land with hoop-nets or underwater by divers and transferred to a research vessel as previously described (Raum-Suryan et al., 2004; Fadely et al., 2005). We estimated ages based on time of year, tooth eruption, and tooth measurements using the method of King et al. (2007), which is accurate through 2 years of age. The ages of four SSLs (35–38.5 months) were estimated using the seasonal stable isotope signature in a whisker (Rea et al., 2015).

We conducted physical examinations and sample collection under isoflurane anesthesia (Heath et al., 1997; Lian et al., 2018). Total body lipids were estimated in 107 SSLs based on hydrogen isotope dilution method utilizing blood samples (Costa, 1987; Reilly and Fedak, 1990; Bowen and Iverson, 1998; Rea et al., 2016) and these values were published as part of a larger dataset (Rea et al., 2016). We included the body lipid content data in our study in order to estimate PCB and DDT loads of the SSLs we sampled. We collected blood samples using standard aseptic techniques via venipuncture of a hind flipper vein (21 G butterfly catheter) or caudal gluteal plexus (18 to 20 G 1.5–2.5-inch needle) directly into polypropylene evacuated serum separator blood tubes. Following an initial blood sample collection, each animal was injected intramuscularly with sterile deuterium oxide (D_2O, 99.99% ^2H_2O) at a dose of 0.3 to 0.5 g/kg body weight, removed from anesthesia, and left to rest during the 2 h equilibration period. Two post D_2O blood samples were collected after a minimum of 2 h of equilibration and approximately 30 min later to measure the concentration of D_2O in serum. Serum separator tubes were kept upright and chilled until processed in the laboratory (1 h) after which serum samples were frozen at −20 °C until analysis as described by Rea et al. (2016). We collected blubber samples ($n = 120$) from a sterile surgical biopsy site 3–4 cm cranial and lateral to the femoral joint as previously described (Beckmen et al., 2016; Keogh et al., 2018). Blubber samples did not include any skin or muscle and were placed on a solvent-rinsed (acetone) Teflon® sheet. After folding in the edges of the Teflon®, the wrapped sample was then sealed in a polyethylene bag (Whirl-pak®). Blubber samples from fresh carcasses were collected on the ventral surface near the xiphoid process and were handled similarly to the biopsies after excision at the beginning of necropsy.

2.2. Blubber analysis for organochlorines

Blubber samples were extracted and analyzed for selected OCs using a high-performance liquid chromatography/photodiode array (HPLC/PDA) method as previously described (Krahn et al., 1994, Beckmen et al., 2016; Supplemental methods).

When interferences resulted in no value for the concentration of an OC, NA (data not available) was assigned. The lower limit of quantitation (LOQ) for the PCB congeners was 0.88 ng/g wet weight (w.w.) whereas for DDT isomers, the LOQ value was 0.6 ng/g w.w. and the LOQ for HCB was 0.26 ng/g w.w. Individual PCB congeners and DDTs isomers below the LOQ were assigned a value of one-half the LOQ value for that analyte. The mean (±SD) percent recovery of the surrogate standard (1,2,3,4-TCDD) was 81 ± 6% (range 66–98%). The OC concentrations determined in blubber of a subset of these SSLs ($n = 48$) were included in Beckmen et al. (2016). OC concentrations from the 5 carcasses are reported in Supplemental Table 1 but not included in the summary statistics or statistical modeling.

2.3. Blubber analysis for lipid content

Blubber samples were analyzed for lipid content by thin layer chromatography with flame ionization detection (TLC/FID) using an Iatroscan Mark 5 (Iatron Laboratories, Tokyo, Japan) as previously described in detail (Krahn et al., 2001; Ylitalo et al., 2005; Beckmen et al., 2016). Total lipid concentrations were calculated by adding the concentrations of the five lipid classes (i.e., sterol esters/wax esters, triglycerides, free fatty acids, cholesterol, and polar lipids) for each sample and each individual lipid class was reported as a percentage of the total lipid. OC concentrations in lipid were calculated by dividing the OC concentrations in the blubber by the proportion of the sample comprised of lipid (Kim et al., 1996; Kajiwara et al., 2001; Loughlin et al., 2002; Myers and Atkinson, 2012). We multiplied the sum of the concentrations (ng/g lipid) of PCB congeners ($\sum$PCBs) or DDT isomers ($\sum$DDTs) by the total body lipid (kg) of each sea lion to calculate the $\sum$PCB and $\sum$DDT loads (μg) for live SSLs (Table 1).

We calculated the PCB toxic equivalent (TEQ) values by multiplying the molar concentration of each dioxin-like PCB congener by the appropriate mammalian toxic equivalent factor (TEF) value for that compound listed in Van den Berg et al. (2006) and then used an additive model of toxicity (Safe, 1990). The ΣPCB TEQs are reported as lipid weight (pg/g lipid). The TEQ values determined by HPLC/PDA in our study are conservative as they were based solely on measurable concentrations of eight (i.e., PCBs 77, 105, 118, 126, 156, 157, 169, 189) of a potential of 12 dioxin-like PCBs and because the PDA LOQ values are higher than those of either low- or high-resolution gas chromatography/mass spectrometry.

2.4. Contaminant modeling

The age distribution of young SSLs was not well represented in all areas sampled; thus, statistical modeling was performed on two subsets of the data that were reasonably well balanced: 1) pups (<6 months) across all locations and 2) all ages of young SSL in Southeast Alaska, or the eastern portion of Gulf of Alaska. With the first subset of data, we used general linear models to assess geographical differences in the concentrations of $\sum$DDTs and $\sum$PCBs, as well as $\sum$DDT loads and $\sum$PCB loads, in pups, while controlling for sex, condition, and body size. Sample locations were described categorically as residing in 1 of

Table 1
Arithmetic mean and standard deviation (SD), range and samples size for age, morphometrics, and organochlorine concentrations for young Steller sea lions (data from the sampled carcasses are not included).

	Mean ± SD	Range	n[a]
Age (months)	10.6 ± 7.7	(2.0–38.5)	120
Body mass (kg)	89.8 ± 32.9	(33.0–161.5)	120
Standard length (cm)	156.1 ± 21.0	(104.0–201.5)	120
Axillary girth (cm)	106.1 ± 11.5	(73.0–136.5)	120
Blubber lipid content (%)	36.6 ± 12.3	(4.0–87.0)	120
Total body lipid (kg)	20.0 ± 11.5	(2.2–53.8)	107
Total body lipid (%)	25.1 ± 8.9	(6.3–41.0)	107
Analyte(s)			
$\sum$PCBs	4291 ± 4184	(644–26,107)	120
$\sum$PCB TEQs	62 ± 64.9	(4.76–405)	120
$\sum$PCBs load	81,105 ± 84,676	(8598–509,089)	107
101/99/149/196[b,c]	701 ± 646	(126.67–4750.0)	120
105[c]	124 ± 120	(126.6–4750)	109
118	510 ± 521	(73.3–3000)	119
128/123[b,c]	81 ± 119	(2.1–1000)	119
138	532 ± 548	(98.2–3571.4)	84
153/87[b,c]	1081 ± 1093	(158.7–7500)	120
170/194[b,c]	71 ± 94	(0.150–607.14)	97
180[c]	168 ± 190	(19.8–1392.86)	103
200[c]	32 ± 47	(0.240–216.0)	73
$\sum$DDTs	5413 ± 6019	(559–35,821)	113
$\sum$DDT load	102,098 ± 124,355	(5616–698,518)	102
p,p′-DDE[d]	4832 ± 5602	(467–33,929)	120
p,p′-DDD[d]	393 ± 398	(83–3500)	120
p,p′-DDT[d]	214 ± 242	(3–1725)	113
o,p′-DDD	23 ± 47	(0.7–244)	110
o,p′-DDT	99 ± 152.7	(0.78–975)	69
HCB	<LOQ		

<LOQ = below the lower level of quantitation.
[a] Indicates the number of samples in which the analyte was detected.
[b] Co-elution.
[c] Denotes PCB congeners in $\sum$PCBs and $\sum$PCB load.
[d] Denotes DDT isomers in $\sum$DDTs and $\sum$DDT load.

3 regions: Southeast Alaska, Gulf of Alaska, and the Aleutian Islands rather than by latitude and longitude because the sampling was discrete and clumped (Fig. 1). Body size metrics were mass (kg), axial girth (cm), and standard length (cm). Condition metrics were body condition index [(axillary girth / standard length) × 100] (Pitcher, 1986; Ryg et al., 1990; Jemison et al., 2011), body-volume-index [mass / (−63.88 + 0.8966 × standard length)] (Trites and Jonker, 2000), and density index [mass / (standard length × axial girth^2)] × 10^6 (Castellini and Calkins, 1993). The body condition index and density index of the sea lions in this study were previously reported in Rea et al. (2016). We also used general linear models to evaluate regional and sex effects on mass and total body lipid in young pups.

With the second subset of data, we used general linear models to evaluate the effect of age on the concentrations of $\sum$DDTs and $\sum$PCBs, as well as $\sum$DDT loads and $\sum$PCB loads, in SSLs sampled in the eastern portion of Gulf of Alaska and Southeastern Alaska while controlling for sex, condition, and body size. Age was included in the models as either a continuous variable, age in months (mo), or as the categorical variable, age class. The five age classes were young pups (<3 months), older pups (4–6 months), young of the year (7–-11 months), yearling (12–22 months), and juvenile (≥23 months). We also used general linear models to explore the effect of age and sex on the mass and total body lipids of SSLs sampled in the Eastern Gulf of Alaska and Southeast Alaska. Models in the candidate sets included only one metric from each of the condition and body size categories. Although there is a relatively strong correlation between the age and size covariates, we included models with all combinations of age class and one size covariate to assess the size effect within age classes. We included first order interactions. The concentration data were natural log transformed to mitigate violations of the model assumptions concerning normality and constant variance. We evaluated parsimony of models in our candidate set using Akaike's information criterion (AIC) for small sample sizes (AICc; Sugiura, 1978, Hurvich and Tsai, 1989) and likelihood ratio tests. We focused our interpretation on models within 2 units of the model with the smallest AICc value (i.e., the top model; Burnham and Anderson, 2003). For top models with significant effects, we specified contrasts to quantify these differences and used tests for multiple comparisons to assess their significance using the general linear hypotheses function "glht" in the R-package "multcomp" (Hothorn et al., 2008). Type III sums of squares were used to test hypotheses regarding effects and *p*-values for these tests are reported in Tables 2 and 3. In the presence of interactions, tests for significance of main effects rarely yield a useful interpretation; therefore, we assumed significant main effects for variables involved in significant interaction terms, even if the main effect p-values exceed 0.05 and describe effect sizes when helpful. Residuals from the top models were examined to determine if model assumptions were met. All analyses were conducted using Program R (R Core Team, 2016).

3. Results

3.1. Descriptive statistics for age, morphometrics, and organochlorine concentrations

Sea lion ages ranged from 2 to 38.5 months (mean age (±SD) was 10.7 ± 7.7 months). Lipid content of blubber samples was highly variable among individuals ranging between 4 and 87% (Table 1). Similarly, the total body lipid, based upon D_2O dilution, varied among individuals ranging between 6% and 41% of body mass, and between 2.2 kg and 53.8 kg lipid (Table 1). Lipid-normalized concentrations of selected PCB congeners, DDT isomers, $\sum$PCBs, $\sum$DDTs, PCB loads, and DDT loads from live sampled sea lions are reported in Table 1. Across age class and region, the most dominant PCB congeners were PCB 153 followed by PCB101, PCB138, and PCB118 (Table 1). Both PCB153 and 101 were detected in all samples analyzed. Concentrations of PCBs 126, 169, and 189 were below the LOQ in all samples and PCBs 77, 157, 190 were detected in only 1–3 samples each. PCB congeners 138 and 180 were unique in that they both contributed significantly to the

Table 2
The models for log$\sum$[PCBs], log(PCB load), log$\sum$[DDTs], and log(DDTs load) for pups (<6 months) across three regions (SE, GOA, AI) in order of most parsimonious (smaller ΔAICc) to least. Models include one to three main effects (ME) and an interaction term (MExME). Reported terms include the Akaike information criterion with a correction for finite sample sizes (AICc), ΔAICc, R^2_{adj}, and *p*-values from hypotheses tests using type 3 sums of squares. Only models with ΔAICc ≤2 are reported.

	AICc	ΔAICc	Adj R^2	Main effects			Interaction
				ME1	ME2	ME3	MExME
log$\sum$[PCBs]							
Sex, mass, region, mass ∗ region	86.58		0.50	0.01	<0.001	0.045	0.025
Sex, mass, sex ∗ mass	88.09	1.51	0.40	0.06	0.03		0.12
Sex, mass	88.11	1.53	0.37	0.08	<0.001		
log(PCB load)							
Standard length	85.14		0.08	0.06			
Mass	85.29	0.15	0.07	0.06			
Axial girth	86.30	1.16	0.05	0.11			
Mass, region	86.43	1.29	0.12	0.09	0.16		
Axillary girth, region	86.57	1.43	0.12	0.10	0.11		
Mean, no covariate	86.57	1.43	0.00				
Standard length, region	86.86	1.72	0.11	0.12	0.20		
log$\sum$[DDTs]							
Mass	86.43		0.43	<0.001			
Mass, sex	86.78	0.35	0.45	<0.001	0.16		
Mass, sex, mass ∗ sex	87.48	1.05	0.47	0.01	0.11		0.18
log(DDT load)							
Standard length	82.55		0.20	0.006			
Mass	83.68	1.12	0.17	0.01			

Table 3
The models for log$\sum$[PCBs], log(PCB load), log$\sum$[DDTs], and log(DDTs load) in order of most parsimonious (smaller ΔAICc) to least. Models include one to four main effects (ME) and an interaction term (MExME). Reported terms include the Akaike information criterion with a correction for finite sample sizes (AICc), delta AICc, R^2_{adj}, and p-values from hypotheses tests using type 3 sums of squares. Only models with delta AICc ≤2 are reported. Age (class) is age-class and Age (mo) is age in months.

All SSL $\sum$PCBs (SE/EGOA) age effects	AICc	ΔAICc	Adj R2	Main effects			Interaction
				ME1	ME2	ME3	MExME
log$\sum$[PCBs]							
Age class, mass, sex	119.20		0.38	<0.001	0.003	0.050	
Age class, mass	120.96	1.76	0.35	<0.001	0.01		
Age class, mass, sex, mass ∗ sex	121.19	1.99	0.37	<0.001	0.003	0.050	0.44
log(PCB load)							
Age class, BCI	130.02		0.46	<0.001	0.07		
Age (mo), sex, age (mo) ∗ sex	130.19	0.17	0.43	<0.001	0.03		0.001
Age class, BCI, sex	130.48	0.46	0.47	<0.001	0.10	0.17	
Age class, sex	130.95	0.93	0.45	<0.001	0.12		
Age class	131.05	1.03	0.44	<0.001			
log$\sum$[DDTs]							
Age class, mass, sex	129.45		0.38	<0.001	0.002	0.10	
Age class, mass	129.88	0.43	0.36	<0.001	0.004		
Age class, mass, sex, age class ∗ sex	130.85	1.40	0.44	0.062	0.003	0.070	0.07
Age class, standard length	131.32	1.87	0.35	<0.001	0.008		
log(DDT load)							
Age (mo), sex, age (mo) ∗ sex	136.56		0.36	0.29	0.03		0.001
Age class	137.02	0.46	0.37	<0.001			
Age class, DSL	137.61	1.05	0.38	0.001	0.18		
Age class, mass, sex, mass ∗ sex	138.14	1.58	0.40	0.006	0.140	0.087	0.031
Age class, BCI	138.36	1.80	0.37	<0.001	0.29		
Age class, sex	138.40	1.84	0.37	<0.001	0.30		
Age class, DI	138.56	2.00	0.37	<0.001	0.333		

Density Index (DI): [body mass / (standard length × axial girth^2)] × 10^6.

total $\sum$PCBs and they also were undetected in numerous samples due to analytical interferences occurring in the HPLC/PDA system and therefore assigned "NA". To avoid the possibility of model results being biased by the distribution of NAs (e.g., one region having more NAs than another) these congeners were not included when calculating the $\sum$PCBs and PCB load values used in the models and reported in Tables 1 and S1. That said, models run with and without these 2 congeners included in $\sum$PCBs yielded no differences in interpretation. There was a large variation among sea lions in the concentration of $\sum$PCBs (644–26,107 ng/g lipid) and $\sum$PCB load (8598–509,089 μg; Table 1). The $\sum$PCB TEQ level was 62 ± 65 pg/g lipid (range 5 to 410 pg/g, lipid), with PCBs 105 and 118 being the predominant dioxin-like congeners contributing to these summed values (Table 1). Further, the concentrations of $\sum$PCB TEQs and $\sum$PCBs (both natural log transformed) were highly correlated ($r = 0.95$) with nearly a 1:1 correspondence between PCB118 and TEQ (slope = 1.04; SE = 0.01; adjusted $R^2 = 0.99$) as PCB118 is the primary dioxin-like PCB contributing substantially to $\sum$PCB TEQs. Given this strong relationship, we focused on $\sum$PCBs and did not model $\sum$PCB TEQs results. The highest concentration of $\sum$PCBs (44,615 ng/g lipid), $\sum$PCB TEQs (731 pg/g lipid) and $\sum$DDTs (65,385 ng/g lipid) were all from one deceased newborn sea lion sampled in Southeast Alaska in 2002 (Table S1).

Concentrations of $\sum$DDTs also varied greatly among SSLs (559–35,821 ng/g lipid; Table 1), with *p,p′*-DDE accounting for 83% of the sum on average (range 34–100%). The DDT load of each sea lion ranged between 5613 and 698,518 μg (Table 1). $\sum$DDT concentrations were calculated by adding the concentrations of three of the DDT isomers (*p,p′*-DDD, *p,p′*-DDE, *p,p′*-DDT). The two remaining DDT isomers (*o,p′*-DDD, *o,p′*-DDT) were not included as interferences resulted in NA values <LOQ in 88% and 79% of the samples, respectively, and values >LOQ contributed a negligible amount to $\sum$DDTs. HCB concentrations were <LOQ for all samples except one; therefore, no further analysis was done with these data.

3.2. Regional differences in Steller sea lion pups (<6 mo)

3.2.1. Differences in mass and total body lipid of young pups

We restricted the analysis for the effects of region and sex on mass and total body lipid stores to young pups because older pups were, on average, 14 kg larger than young pups ($p = 0.001$) and sampling was not balanced across regions (i.e., no older pups were sampled in the Aleutian Islands). Models examining the effects of region and sex on mass of young pups indicate a regional effect ($p < 0.001$) and no sex effect ($p = 0.73$; Fig. 2A). The top model had region as the only covariate and explained 58% of the variability in mass. Mean mass of young pups was 59.5 kg (SE = 2.3) in the Aleutian Islands, 54.9 kg (SE = 3.6) in Gulf of Alaska, and 39.6 kg (SE = 3.2) in Southeast Alaska. The mass of pups in both the Aleutian Islands and Gulf of Alaska was significantly higher than pups in Southeast Alaska ($p < 0.001$) while there was no difference between masses of pups in the Aleutian Islands and the Gulf of Alaska ($p = 0.43$).

Models examining the effects of region and sex on the total body lipid stores of young pups indicate a significant regional effect ($p \leq 0.02$) and a marginally significant sex effect ($p = 0.045$), but no significant region:sex interaction ($p = 0.60$; Fig. 2B). The top model with region and sex as covariates explained 31% of the variability in total body lipid. Mean total body lipids of female pups was 2.8 kg (SE = 1.34) greater than that for male pups but not significantly so when accounting for multiple comparisons ($p = 0.14$). Mean total body lipids of pups from the Aleutian Islands was 3.8 kg (SE = 1.4) greater than that of pups from Southeast Alaska ($p = 0.04$) and 3.5 kg (SE = 1.6) greater than pups from Gulf of Alaska but this difference was not statistically significant ($p = 0.11$). The difference between young pups from Gulf of Alaska and Southeast Alaska was not significant (0.28 kg, SE = 1.75; $p = 0.99$).

3.2.2. PCBs in pups (<6 months)

The concentration of $\sum$PCBs in pups ($n = 38$) ranged from 889 to 18,750 ng/g lipid (4591 ± 4908 ng/g lipid) with the $\sum$PCB loads ranging from 8598 to 168,240 μg (37,519 ± 6706 μg). Summary statistics for regional differences in the concentration of $\sum$PCBs and $\sum$PCB loads in pups are provided in Table S2.

The three models examining the variability in the concentration of $\sum$PCBs in SSL pups with ΔAICc <2 include mass and sex (Table 2). Hypothesis tests indicate the effect of mass was significant in all models ($p \leq 0.03$). The top model also included a significant sex effect ($p = 0.01$), a marginally significant region effect ($p = 0.045$) and a significant interaction between mass and region ($p = 0.025$). The addition of age class to the top model results in an insignificant age effect ($p = 0.15$; AICc = 2.2, not presented in Table 2), which supports grouping the young and older pups for inference (Section 3.3).

The top model explains a moderate amount of the variation in $\sum$PCBs concentration (adjusted $R^2 = 0.50$, Table 2). At the mean mass of 54 kg, female SSLs from the Aleutian Islands had the largest mean $\sum$PCBs concentration at 3885 ng/g lipid followed by those from Southeast Alaska with a mean value 2787 ng/g lipid, and then Gulf of Alaska with a mean value of 1721 ng/g lipid. Whereas, the expected $\sum$PCBs concentrations for males at a mass of 54 kg were, 3219 ng/g lipid greater than that in females for each region (Aleutian Islands: 7104 ng/g lipid; Southeast Alaska 6006 ng/g lipid; Gulf of Alaska: 4940 ng/g lipid). Multiple comparison test shows the sex effect to be significant ($p = 0.04$) as well as the difference between the Aleutian Islands and Gulf of Alaska ($p = 0.050$). In addition, concentration of $\sum$PCBs decreased with increasing mass in sea lion pups from all three regions; however, the rate of decrease differed by region as indicated

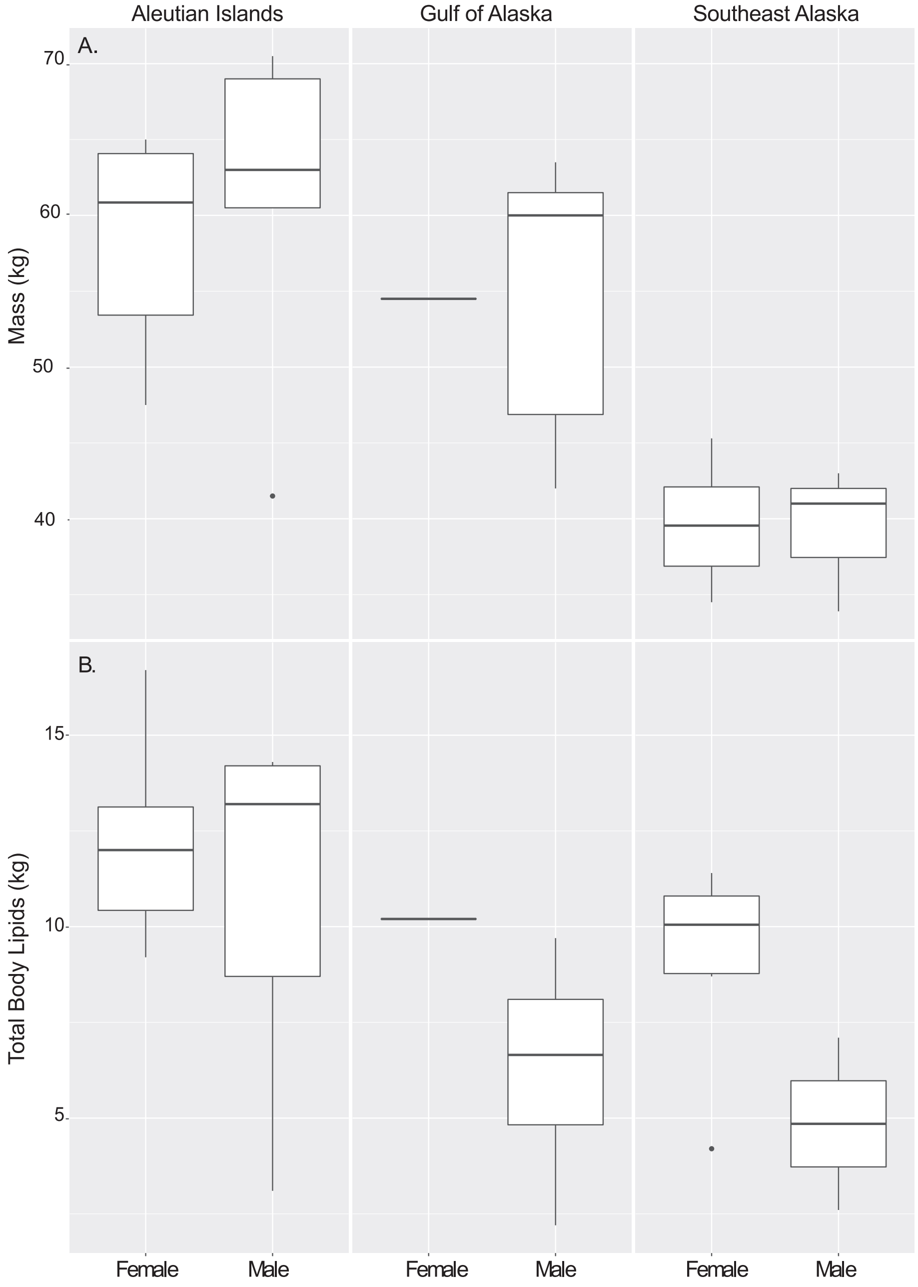

Fig. 2. Boxplots of total body lipid (kg) and mass (kg) for young pups by regions (Southeast Alaska, Gulf of Alaska, and the Aleutian Islands) and sex.

by the different slopes of each line (Fig. 3A). For an increase in 1 kg over the mean weight of 54 kg the concentration of $\sum$PCBs are expected to be 360 ng/g lipid less for pups from the Aleutian Islands, 100 ng/g lipid less for pups in the Gulf of Alaska, and 63 ng/g lipid less for pups in Southeast Alaska.

The seven top models (ΔAICc <2) explained little of the variability of $\sum$PCB load in pups (adjusted $R^2 \leq 0.12$; Table 2). The three top models each included one size metric (standard length, mass, or axial girth); however, none of these effects were significant ($p \geq 0.06$, Table 2). Further, none of the top models for $\sum$PCB loads contained the variable sex and for three models that included region, its effect was not significant ($p \geq 0.11$; Table 2; Fig. 3B). Generally, there was less variability between sexes and among regions with $\sum$PCB loads as the response variable compared with the concentration of $\sum$PCBs (ng/g lipid).

3.2.3. DDTs in pups

The concentration of $\sum$DDTs in pups ranged from 813 to 20,403 ng/g lipid (5266 ± 1036 ng/g lipid) and the $\sum$DDT loads ranged between 5613 and 195,867 μg (41,979 ± 54,524 μg). Summary statistics for regional differences in the concentration of $\sum$DDTs and $\sum$DDT loads in pups are provided in Table S2.

Three of the models examining the variability in the concentration of $\sum$DDTs had ΔAICc ≤2 and all included mass (Table 2). The only covariate in the top model (AICc = 86.43), mass, was significant ($p < 0.001$) but this relationship explains only a moderate amount of the variability (adjusted $R^2 = 0.43$, Table 2). The only other variables in the top models were sex and its interaction with mass, which were not significant in the second and third models ($p \geq 0.11$; Table 2). As with concentration of $\sum$PCBs, the concentration of $\sum$DDTs decreased with increasing mass of pups (Fig. 4A). The top model indicates an increase in 1 kg over the mean weight of 54 kg is expected to result in a decrease of 170 ng/g

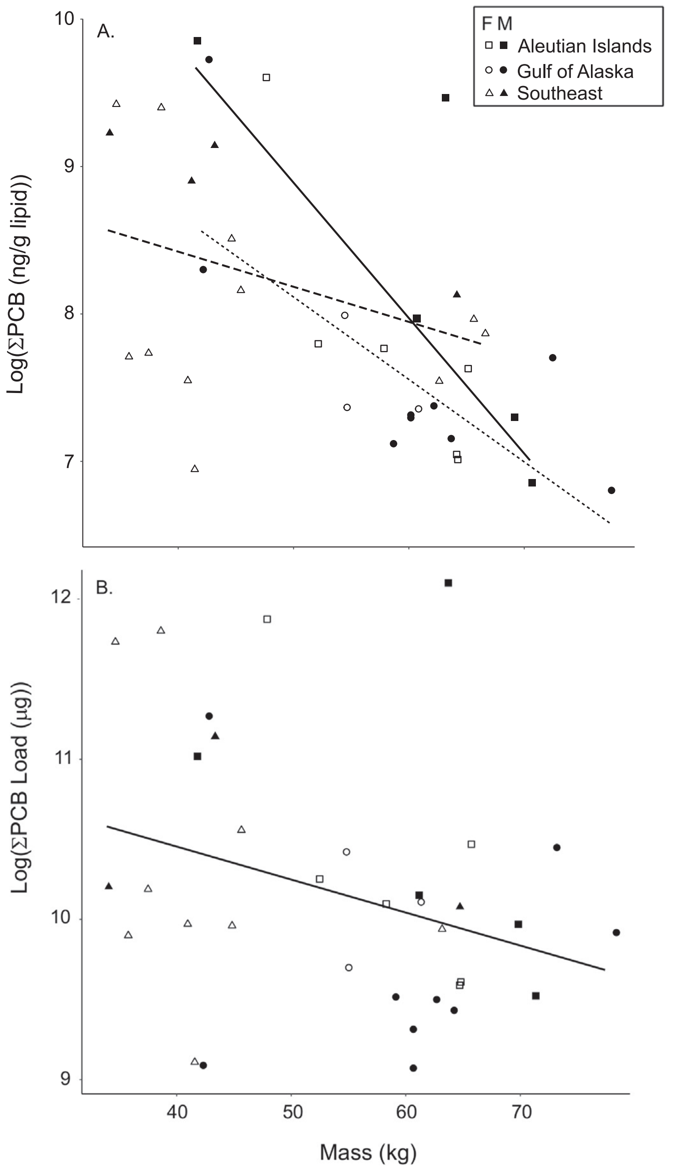

Fig. 3. A. Natural-log transformed ∑PCB concentration (ng/g lipid) and B. log transformed ∑PCB load (μg) for blubber samples collected from pups (<6 months) by region. We chose to present the second model (ΔAICc = 0.15, Table 2) in order to compare ∑PCBs concentrations and loads in the same figure. Male pups are denoted with filled symbols and female pups by open symbols. Note the figure does not show parallel lines for each sex.

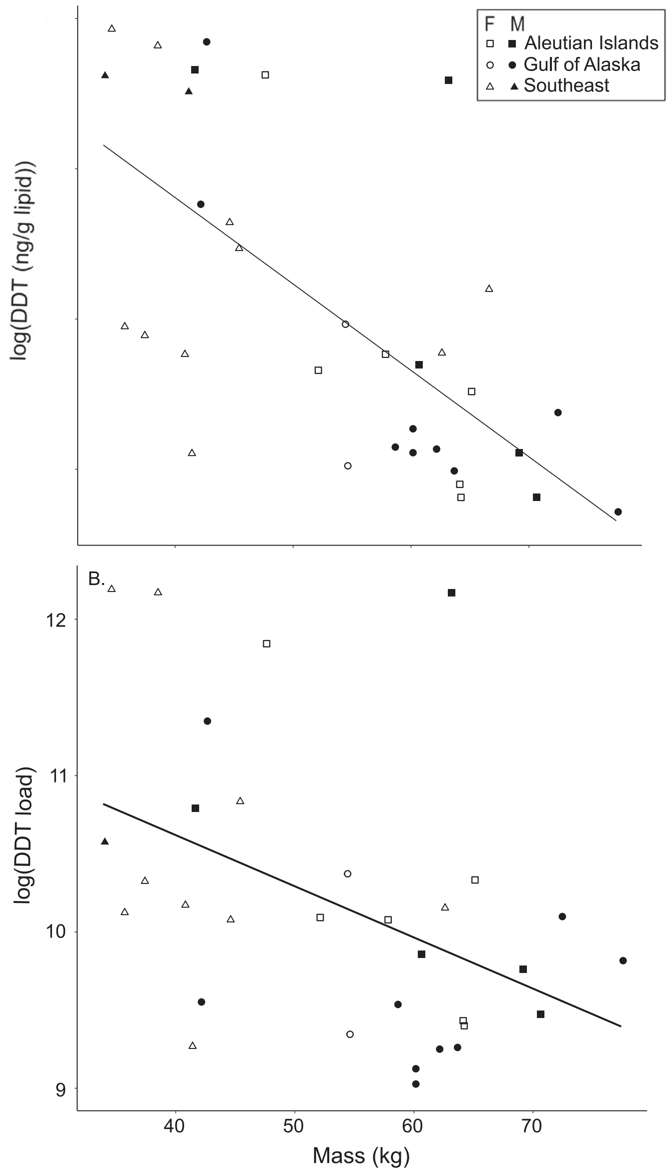

Fig. 4. A. Natural- log transformed ∑DDT concentration (ng/g lipid) and B. log transformed ∑DDT load (μg) for blubber samples collected from pups (<6 months) by region. Male pups are denoted with filled symbols and female pups by open symbols.

lipid in the concentration of ∑DDTs. Unlike the concentration of ∑PCBs, however, region was not in any of the top models (Table 2, Fig. 4A).

The two models explaining the variability in DDT loads in SSL pups with ΔAICc ≤2 included one size metric each (Table 2). However, these models only explained up to 20% of the variability in DDT loads compared to 43–47% of the variability in ∑DDT concentrations being explained by those top models (Table 2). Unlike the top models for the concentration of ∑DDTs, sex was not retained in any model for the ∑DDT load (Table 2).

3.3. Age differences in young Steller sea lions

3.3.1. Differences in mass and total body lipid of young Steller sea lions

Relationships among age, mass, and total body lipids were explored using SSLs sampled in the Eastern Gulf of Alaska ($n = 38$) and Southeast regions ($n = 30$; Table S3). Across all individuals in this subset of data, mass ranged between 33.9 kg and 161.5 kg, lipid ranged between 3.2 kg and 40.3 kg, and percent lipid ranged between 6.3 and 40.3%.

Models examining the effects of age class and sex on mass of SSLs indicate an age effect ($p \leq 0.001$) and no significant sex effect ($p = 0.13$) or age class and sex interaction ($p = 0.86$). The top model had age class as the only covariate and explained 78% of the variability in mass. With age class as an ordered categorical variable, the linear term in the top model was significant but no higher terms (e.g., quadratic) were significant consistent with the linear increase shown in Fig. 5A.

Models examining the effects of age class and sex on the total body lipid of young SSLs indicate a significant age class effect ($p < 0.001$) without a sex effect ($p = 0.18$) or an effect of sex within age class ($p = 0.10$). The top model had age class as the only covariate and explained 28% of the variability in the total body lipid stores. With age class as an ordered categorical variable, the top model has a significant negative quadratic term consistent with a leveling off and slight decrease in total body lipid at older age classes (Fig. 5B).

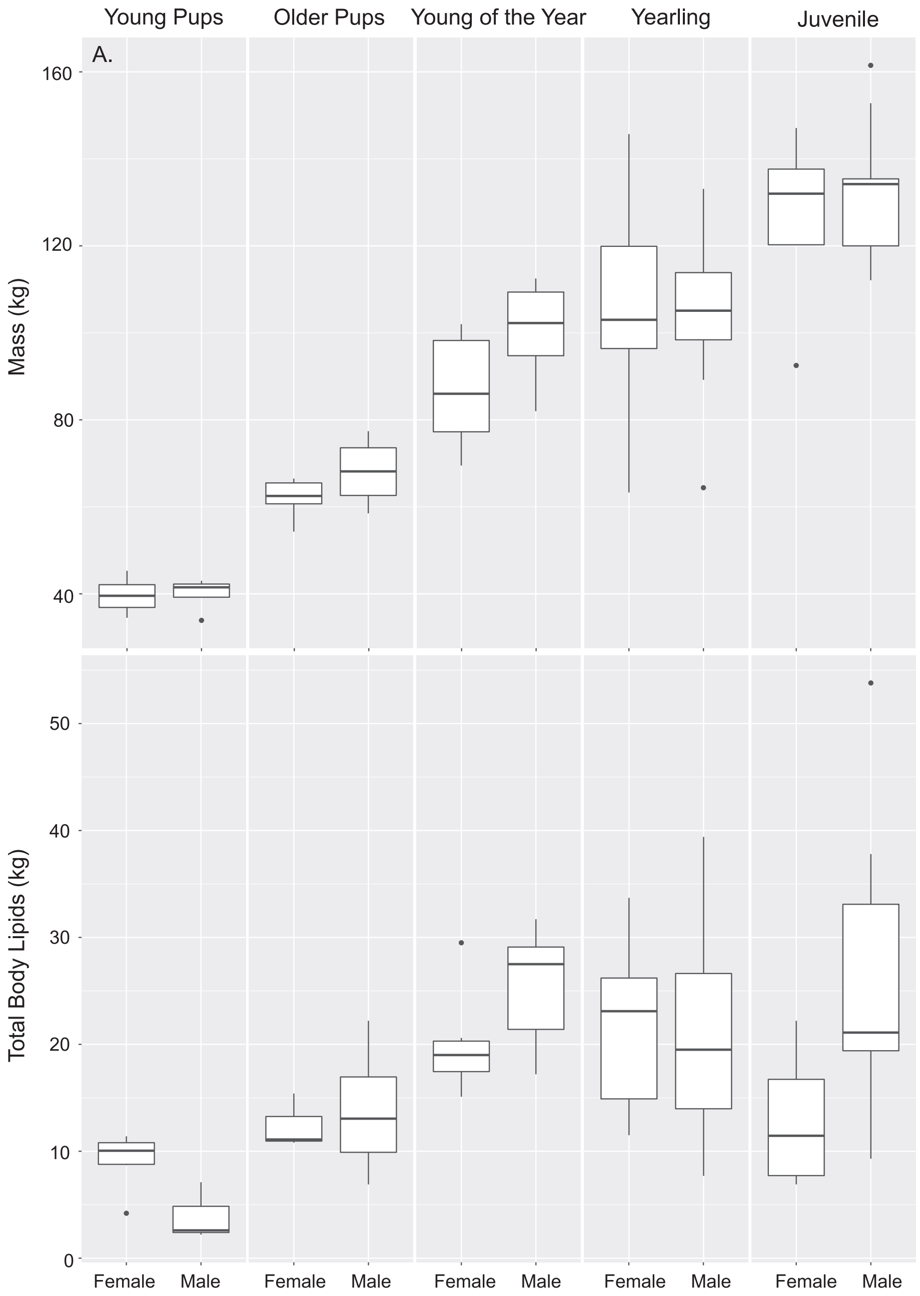

Fig. 5. Boxplots of total body lipid (kg) and mass (kg) by age class (young pups (<2 months), older pups (3–6 months), young of the year (6–11 months), yearling (12–22 months), and juvenile (>23 months)) and sex for Steller sea lions sampled in Southeast Alaska and the eastern portion of the Gulf of Alaska (see Fig. 1).

3.3.2. PCBs in young Steller sea lions

The concentration of ∑PCBs for this subset ranged between 889 and 26,107 ng/g lipid (4885 ± 4202 ng/g lipid) and the ∑PCB loads ranged between 8745 and 509,089 μg (86,823 ± 87,031 μg) across the five age classes (Table S3).

Three models examining the variability in ∑PCBs concentration had ΔAICc ≤2, all of which included the age class ($p < 0.001$) and mass ($p \leq 0.01$; Table 3). The top model also included a marginally significant sex effect ($p = 0.050$) while the second model had no additional covariates. Each of the top models explains a relatively small proportion of the variance in the concentration of ∑PCBs with adjusted R^2 ranging from 0.35 to 0.38 (Table 3). The top model, with age class treated as an ordered categorical variable has significant quadratic ($p < 0.0001$) and linear ($p = 0.0002$) terms, which is consistent with log concentration of ∑PCBs decreasing from young pups to older pups then increasing in the older age classes (Fig. 6A).

The 5 top models (ΔAICc ≤2) explaining the variability in PCB loads in SSLs included age (mo) or age class (Table 3). Sex, mass, and condition effects were insignificant and accounted for little of the variation in PCB loads. In fact, the model with age class as the only independent variable (ranked fifth with ΔAICc = 1.03; Table 3) explained the vast majority of the variance with adjusted $R^2 = 0.44$ vs. 0.46 for the top model. That said, the second model contains a significant age (mo) and sex interaction, indicating some support for variation in age effect by sex. No such interaction is supported for age class and sex. For the top model with age class, the linear term in this model was significant but, unlike for concentration of ∑PCBs no higher order terms (e.g., quadratic; $p = 0.10$) were significant (Fig. 6B).

Additional models that included geographic location as an independent variable indicated that both the concentration of ∑PCBs and

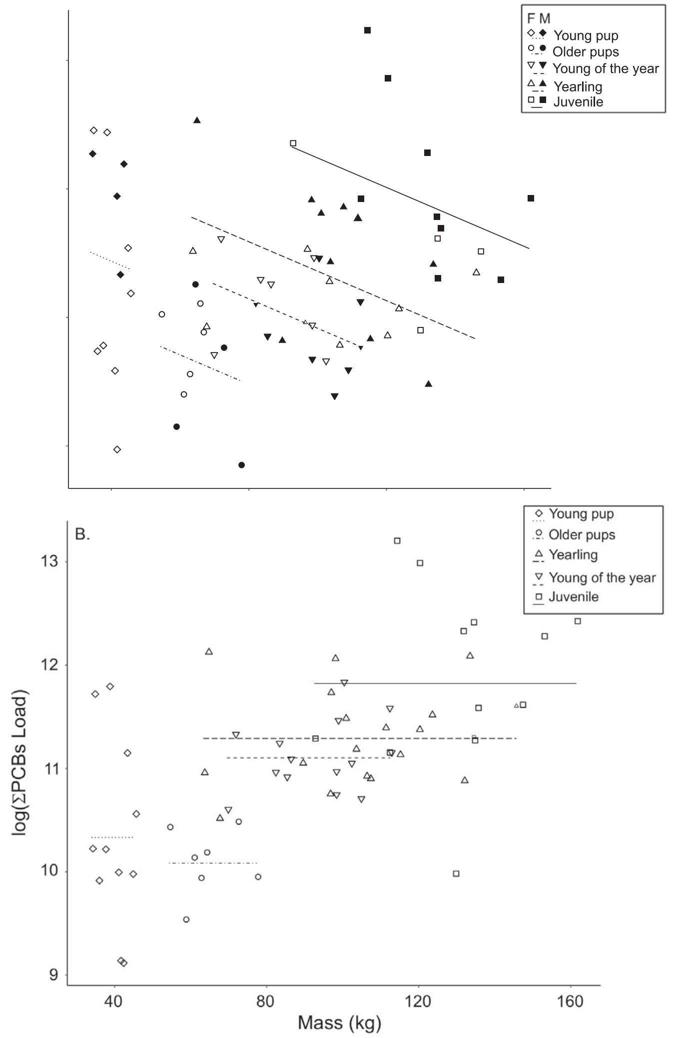

Fig. 6. A. Natural- log transformed $\sum$PCB concentration (ng/g lipid) and B. log transformed $\sum$PCB load (μg) for Steller sea lions by age class including young pups (<2 months), older pups (3–6 months), young of the year (6–11 months), yearling (12–22 months), and juvenile (>23 months). Modeling was restricted to sea lions sampled in Southeast Alaska and the eastern portion of Gulf of Alaska. Note the figure does not include separate parallel lines for each sex.

$\sum$PCB loads for Eastern Gulf of Alaska and Southeast Alaska were not significantly different thus justifying pooling these data to obtain a dataset relatively balanced with respect to age class distribution.

3.3.3. DDTs in young Steller sea lions

Concentrations of $\sum$DDTs ranged between 813 and 35,821 ng/g lipid (6627 ± 6627 ng/g lipid) and the DDT loads ranged between 5612 and 698,518 μg (114,522 ± 135,570 μg) across the five age classes (Table S3).

Four models examining the variability in the concentration of $\sum$DDTs had ΔAICc ≤2 and, as with the concentration of $\sum$PCBs, all these models included the age class and one size metric (mass or in one model standard length) as covariates (Table 3). For models without a significant interaction term involving age class, age class was highly significant ($p < 0.001$; Table 3) as was mass in the top three models ($p \leq 0.004$) and standard length in the fourth model ($p = 0.008$). Neither sex ($p = 0.10$) nor its interaction with age class ($p = 0.07$) were significant in these models. The top model with covariates age class ($p < 0.001$), mass ($p = 0.002$), and sex ($p = 0.10$) explains only a moderate amount of the variability in the data (adjusted $R^2 = 0.38$). A likelihood ratio test indicates no significant difference in the top two models ($p = 0.10$) and points to sex as a relatively poor predictor as does a comparison of their adjusted R^2 values (0.38 vs. 0.36). As with concentration of $\sum$PCBs, when age class is treated as an ordered categorical variable, the model has significant quadratic ($p = 0.0002$) and linear terms ($p < 0.0001$) consistent with the log concentration of $\sum$DDTs decreasing from young pups to older pups then increasing in the older age classes (Fig. 7A).

Analysis of DDT load resulted in seven models with ΔAICc ≤2, all of which include age as either a continuous (top model) or categorical (remaining models) variable and exhibited a narrow range in adjusted R^2 values (0.36 to 0.40; Table 3). The main effect of age was significant ($p \leq 0.006$; for models without a significant interaction term involving age). In addition to age, most models included one or more of the following variables sex, a size metric, and a body condition index. For additive models (i.e., no interaction term), the effects of these variables were not significant ($p \leq 0.18$). Age (mo) appears in only the top model; otherwise, the categorical age class is preferred. In addition, the second model (ΔAICc = 0.46) with age class as the only covariate has a slightly larger adjusted R^2 value (0.37 vs. 0.36) than the top model. We prefer interpreting the second model because: it performs as well as the top model, there is a general preference for age class over age (mo) in the top models, and because model 2 is simpler. With age class as an ordered categorical variable, the linear term in model 2 was significant but, unlike for the concentration of $\sum$DDTs, the quadratic was not significant ($p = 0.27$; Fig. 7A, B).

As for PCBs, additional models that included geographic location as an independent variable indicated that the concentration of $\sum$DDTs and $\sum$DDT loads for Eastern Gulf of Alaska and Southeast were not significantly different thus justifying pooling these data to obtain a dataset that was relatively balanced with respect to age class distribution.

4. Discussion

4.1. General distribution and patterns

Monitoring PCB and DDT concentrations in the blubber of marine mammals is an effective means of assessing their exposure to toxic OCs, as well as exploring the influence of age, sex, body condition, and geographic location on the levels of these contaminants. The samples in our study were collected between 1998 and 2003 with concentrations spanning the reported ranges for SSLs sampled in Alaska in 1976 (Lee et al., 1996), highlighting the retention of these compounds in the marine environment and food web for decades.

Studies have attempted to establish thresholds for contaminant exposure above which sublethal effects were observed or expected based on data and modeling from phocid seals, European otters, and mink (Ross et al., 1995; Kannan et al., 2000; Iwata et al., 2004; Mos et al., 2010). In our study, 6 of the 120 samples from live animals (5%) had ΣPCB TEQs concentrations over 209 pg/g lipid and three of these samples also had ΣPCB concentrations above 17,000 ng/g lipid, both concentrations above which immune suppression (natural killer cell activity, T-cell proliferation) was observed in harbor seals (Ross et al., 1995). However, if we use the tissue residue dose (TRD) of 1300 ng/g lipid proposed by Mos et al. (2010), we find that 104 of the 120 samples (87% of the samples) fall above the TRD, a concentration below which no unacceptable adverse health effects are expected. The dramatic difference between these thresholds highlights the difficulty in categorizing samples based on values from a single sample, particularly for young growing pinnipeds that undergo dilution of OCs. Application of thresholds based on phocid or other fish-eating mammals to otariid species is further complicated by differences in life history, breeding strategies, fasting durations and energy status. Recent studies have found that OC concentrations alter immune function in otariid species, demonstrating the potential for sub-lethal impacts of contaminant exposure while highlighting the difficulty in predicting the effect of mixtures of OCs (Mori et al., 2006; Levin et al., 2007). Peñín et al. (2018) found that in vitro exposure of T cells and natural killer cells to PCB

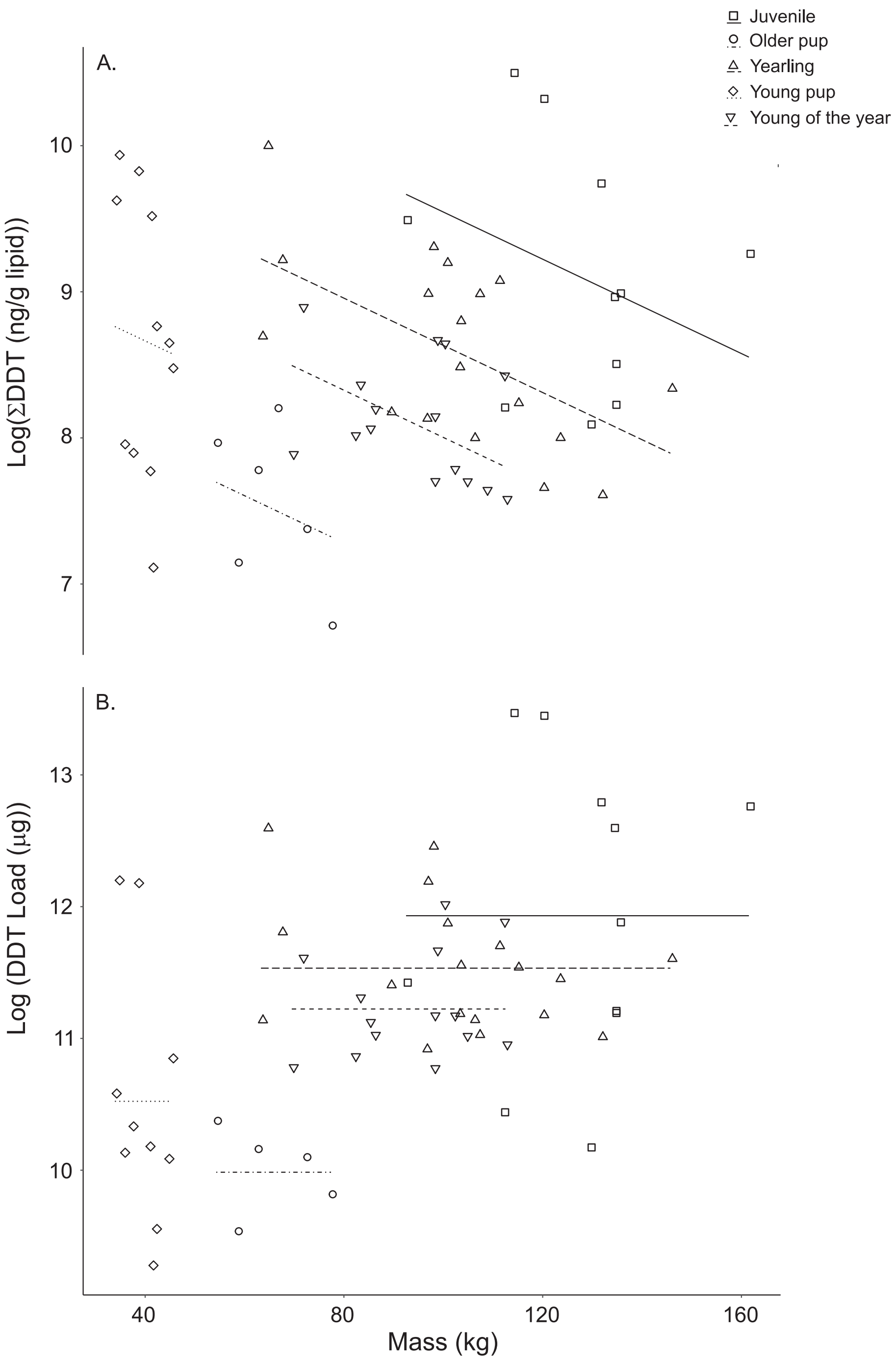

Fig. 7. A. Natural- log transformed ∑DDT concentration (ng/g lipid) and B. log transformed ∑DDT load (μg) for Steller sea lions by age class including young pups (<2 months), older pups (3–6 months), young of the year (6–11 months), yearling (12–22 months), and juvenile (>23 months). Modeling was restricted to sea lions sampled in Southeast Alaska and the eastern portion of Gulf of Alaska.

congeners (individually and mixed) altered immune function, including non-dioxin-like congeners and that the effect of mixture PCB congeners are not simply additive. These laborary based studies demonstrate the potential sub-lethal effects of PCBs on the immune system of otariids; however, more studies are needed to better understand how OC concentrations in blubber samples may be associated with sublethal effects in Steller sea lions and other ottariid species.

4.2. Regional effects

4.2.1. Mass and lipid content in young pups

It is important to assess the influence of sex and body mass or condition when considering other parameters such as OC concentrations as there is strong evidence that these factors are predictive of survival during the early postnatal period (Baker and Fowler, 1992; Jemison et al., 2011; Keogh et al., 2013; Maniscalco, 2014; Pendleton et al., 2016). Further, we found young pups (<3 months) from Southeast Alaska were significantly smaller than pups from both the Gulf of Alaska and the Aleutian Islands. Similarly to mass, the lipid stores of young pups from the Aleutian Islands and the Gulf of Alaska did not differ, whereas young pups from Southeast Alaska had significantly lower lipid stores compared to the other regions, similar to patterns reported in Rea et al. (2016). Interestingly, the eastern DPS of SSLs which includes Southeast Alaska has increased in population leading to its removal from the list of endnagered and threatened wildlife under the Endangered Species Act in 2013. While regional differences in size of SSLs are well documented, the factors contributing to these differences remain unclear and may be related to genetics, diet, fasting durations, and maternal investment, all factors that may also influence the deposition and mobilization of OCs in blubber.

4.2.2. PCBs and DDTs in pups

Foraging differences across the range of adult females likely contribute to the regional differences in the concentrations of $\sum$PCBs in SSL pups, as diet is the primary exposure route of these lipophilic contaminants. SSLs under 6 months of age are nutritionally dependent and while they disperse from the natal rookery with their mothers, they remain within the same region (Bonner, 1984; Raum-Suryan et al., 2004; Schulz and Bowen, 2005). The blubber OC concentrations for pups in our study represent the OCs accumulated through transplacental transfer and through their diet of milk (Kubo et al., 2014; Beckmen et al., 2016). Beckmen et al. (2016) found that SSL milk samples ranged between 9.9 and 36% lipid, had a mean ΣPCBs concentration of 424 ± 388 ng/g lipid (range 96–1400 ng/g lipid), and a mean ΣDDTs concentration of 444 ± 511 ng/g lipid (range 51–1987 ng/g lipid). There was a significant positive relationship between the concentrations of ΣPCBs and ΣDDTs in milk and blubber samples, though as expected milk had lower concentrations compared to blubber samples.

As income breeders, the milk produced by SSLs reflect the contaminants largely gained from recent foraging trips with contributions from the females blubber stores (Lee et al., 1996; Kubo et al., 2014). However, the contribution of OCs from blubber stores would be greater during the perinatal period immediately following birth when females remain on shore fasting between 1.7 and 17.1 days (Merrick and Loughlin, 1997; Maniscalco et al., 2006). Lactation is energetically expensive (Pond, 1977) and adult females must balance their own energetic needs with that of the developing pup, two tasks that may conflict during times of fasting or reduced prey (Champagne et al., 2012). Females with larger blubber stores, whether due to age or regional differences in size (Rea et al., 2016), would be expected to support longer perinatal periods, leading to a greater transfer of lipids and OCs from her blubber layer. Whether the length of the perinatal period or foraging trips vary between regions and how differences may influence the transfer of OCs to young pups is not known.

The trophic level at which adult females are feeding could influence the OC content of the milk because OCs biomagnify within the marine food web (Muir et al., 1988; Muir et al., 2003). Overall, female SSLs in the central and eastern Gulf of Alaska had higher δ^{15}N values compared to females in the central and western Aleutian Islands and Southeastern Alaska (Scherer et al., 2015). Diet modeling and scat analysis found that females in the Gulf of Alaska relied more on salmon and pollock whereas females in the central and western Aleutian Islands rely on lower trophic prey including squid and Atka mackerel, and Southeast Alaska female sea lions relied heavily on forage fish and pollock (Sinclair and Zepplin, 2002; Sinclair et al., 2013; Scherer et al., 2015; Tollit et al., 2017). The greater the proportion of squid, forage fish, and other lower trophic prey in the diet of SSLs would be expected to be associated with lower exposure of OCs; whereas sea lions consuming a greater proportion of predator fish, such as cod and pollock, would be expected to have higher concentrations of dietary derived contaminants. However, in our study, the concentration of $\sum$PCBs for all but the larger pups were highest in pups from the Aleutian Islands. These findings contrast with what we expect if $\sum$PCBs was solely due to adult female trophic level and suggests that other factors such as atmospheric transport and local input of contaminants may be contributing to the concentration of $\sum$PCBs found in SSL pups in our study. Hardell et al. (2010) found substantial levels of PCBs in all fish species sampled in the Aleutian Islands, including many known prey of SSLs and Walleye pollock sampled in the Sea of Japan had higher *p,p′*-DDT compared to pollock from the Bering Sea and Gulf of Alaska (deBrito et al., 2002). Besides region, the top models explaining the variation in $\sum$PCBs concentrations included mass and sex while none of the condition indexes were retained. When reported as a non-lipid adjusted value, the concentration of OCs in blood were higher in female SSL pups complared to males and for both sexes, OC concentrations were higher in the rookeries in the Russian Far East compared to the Western Aleutian Islands (Myers et al., 2010).

Differences in mass, body lipid stores, and the change in these parameters with age appear to contribute to the regional differences in concentrations of $\sum$PCBs. The decrease in $\sum$PCBs and $\sum$DDTs concentrations with increasing mass in pups (Figs. 3A and 4A) appears to result from increasing lipid stores during the postnatal development of the blubber layer effectively diluting the accumulating OCs in pups under 6 months of age, as found in other pinniped species (Hall et al., 2008). SSL pups under 6 months of age are nutritionally dependent on milk when the apparent dilution of PCBs occurred in blubber, this is in contrast to the growth dilution previously found in cetaceans which occurred after the transition to a fish-based diet (Hickie et al., 2007; Krahn et al., 2009). We found that for every 1 kg above the average mass for a SSL pup the concentration of $\sum$PCBs was 360 ng/g lipid lower in pups from the Aleutian Islands, 100 ng/g lipid lower in pups in the Gulf of Alaska, and 63 ng/g lipid lower for pups in Southeast Alaska. This pattern in the PCB data suggests that the rate at which pups gain mass and lipid stores also varied regionally as reported by Rea et al. (2016) however the DDT data did not vary regionally. We found larger pups (3–6 months) had lower concentrations of $\sum$DDTs than smaller pups similarly across the three regions. These findings are somewhat contradictory as both PCBs and DDTs are lipophilic; however, it may be that multiple factors (e.g. local input, preferential deposition) that we cannot fully address in our study are contributing to the concentrations of $\sum$PCBs in SSL pups.

The $\sum$PCB and $\sum$DDT loads in pups also decreased with increasing mass; however, there were no significant regional or sex influences as there were for the concentration models. Similar to the concentration models, none of the condition indices were found in the load models. The lack of regional and sex influences on contaminant load suggests that the observed regional and sex effects on contaminant concentrations in pups may be an artifact of how the level of contamination was quantified rather than real regional differences in contaminant exposure. It appears that accounting for body lipid stores diminished the apparent regional and sex effects. Indeed, regional and sex influences on the concentrations of $\sum$PCBs and $\sum$DDTs in SSL pups may be a driven by the differences in the total body lipid stores of pups (Rea et al., 2016). In addition, models indicated that total body lipid was marginally influenced by sex and more significantly by region consistent with the regional and sex differences observed in the concentration models being driven by the regional and sex differences in lipid stores in young SSLs.

To explore this possibility, we replaced mass with total body lipid (kg) as a covariate in the top concentration models. We found that including total body lipid in the $\sum$PCBs models for pups led to the elimination of a significant sex effect ($p = 0.81$) but only marginally diminished the regional effect. However, the model including total body lipid had a much larger AICc and an adjusted R^2 reduced by 50% compared to model with mass as a covariate. Our findings suggest that adjusting OCs concentration by the lipid content of the blubber sample alone may not fully account for the variability in OC concentrations associated with differences in condition or energy states between sea lion pups.

4.3. Age effects

4.3.1. Mass and lipid content in young Steller sea lions

Mass and lipid stores increased across the five age categories in the current study. For mass, the age effect was linear while, for total body lipids, the top model also included a negative quadratic term supporting a plateauing of lipid content with little difference between the young of the year, yearling, and juvenile age classes. For both mass and lipid content, there was no influence of sex across all age classes. The lack of sex differences is due to our samples being restricted to young, sexually immature SSLs.

4.3.2. PCBs and DDTs in young Steller sea lions

The concentrations of $\sum$PCBs and $\sum$DDTs decreased from young to older pups followed by an increase in the older age classes. Within each age class the concentrations of $\sum$PCBs and $\sum$DDTs decreased with increasing mass, but overall these OCs increased with age, similar to previous reports (Lee et al., 1996; Blasius and Goodmanlowe, 2008). There was no relationship between mass and either $\sum$PCB or $\sum$DDT loads, though a few of the condition indices were found in these models. $\sum$PCB and $\sum$DDT loads increased linearly with age over all age classes but the difference in the $\sum$PCB and $\sum$DDT loads between young and older pups was not significant. We propose the decline in OC concentrations between young pups (<2 months) and older pups (3–6 months) in our study is due to a rapid increase in deposition of lipids in the blubber layer leading to an initial dilution of the OCs. The model shows young pups averaged 7.9 kg of body lipid store which nearly doubled to 13.2 kg by 6 months of age, while the total body lipid content remained consistent in the older age categories including young of the year (22.6 kg), yearlings (21.3 kg), and juveniles (22.1 kg). Both the concentration and load of $\sum$PCBs and $\sum$DDTs increased in those older age classes suggesting that while the lipid stores in these age classes were consistent, the concentration of these contaminants increased, implying the contaminants in the blubber samples were from the accumulation of OCs received through diet and not as influenced by changes in mass or lipids as observed in young and older pup age classes. In harbor seals, the concentration of $\sum$PCBs was negatively correlated with blubber thickness in females (Wang et al., 2007) and the concentration of PCBs decreased in female California sea lions during rehabilitation as mass and blubber stores increased supporting a dilution effect of increasing lipid stores (Hall et al., 2008), whereas decreases in mass due to extreme fasting or starvation was associated with increasing concentration of $\sum$PCBs (Hall et al., 2008; Cipro et al., 2012). Our findings highlight the importance of nutritional status or energy state and body condition when interpreting and comparing OC concentrations between individuals and populations. As with the pup dataset, we replaced mass with total body lipid as a covariate in the top concentration models. We found that including total body lipid in the $\sum$PCBs models for Steller sea lions did not substantively change the model results. For $\sum$PCBs, sex remained marginally significant, age class was strongly significant, the adjusted R^2 decreased slightly (0.38 to 0.35), and AICc increased by about 1.3. Therefore, total body lipid did not supplant age and was a slightly worse predictor than mass alone. Similarly, for the concentration of $\sum$DDT, sex remained insignificant with age remaining significant and the effect of total body lipid was marginal ($p = 0.06$). The adjusted R^2 decreased (0.39 to 0.31) and the AICc increased by 7. Therefore, a model with mass was better than the models with total body lipid, supporting that mass explains variability in the concentrations of OCs not accounted for by total body lipid.

5. Conclusion

This study adds to the limited data available on the concentrations of OCs in an apex predator of conservation concerns, the Steller sea lion. PCBs and DDTs were found at significant levels in SSL tissues collected between 1998 and 2003 through their range in Alaska highlight the retention of these contaminants in the marine environment and food web. In both the analysis of pups across regions, and the analysis of age classes of SSL within the SE/EGOA region the blubber concentrations of $\sum$PCBs and $\sum$DDTs were more variable compared to $\sum$PCB and $\sum$DDT loads. Further, accounting for the lipid content (a potential proxy for energy balance) by using contaminant loads as the response variable, reduced or removed the regional and sex effects present in models for contaminants concentrations in SSL pups. We also found that the concentrations of contaminants decreased with increasing mass within each age class but an overall increase in contaminants was found with increasing age. In pups <6 months of age, we found that contaminant loads also decreased with increasing mass. However, we found no influence of mass on contaminant loads within age classes. Taken together, our findings suggest that adjusting OCs concentration by the lipid content of the blubber sample alone may not fully account for the variability in OC concentrations associated with differences in condition or energy states between sea lion pups. Further, given the OC concentrations found in blubber samples (1998–2003) in our study, monitoring of OC concentrations in SSLs and other marine mammal species in these areas should be continued and future studies are needed to understand the mechanisms that increase/decrease lipid stores in SSL pups among regions.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.134183.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We gratefully acknowledge the contributions of M.M. Krahn, M. Castellini, D. Schaeffer, R. Towell, R. Ream, T. Loughlin, P. Tuomi, D. Boyd, J. Buzitis, G. Yanagida, K. Tilbury, T. Gelatt, K. Raum-Suryan, V. Stegall, S. Harper, and J. Sterling as well as the many Alaska Department of Fish & Game crewmembers, NMFS Marine Mammal Laboratory personnel who assisted in the collection of samples. We thank the efforts of the anonymous reviewers. We also thank the captains and crew of the vessels P/V Woldstad, P/V Stimson, M/V Tiglax, and R/V Medeia. Captures and field sample collections were conducted under authority of ESA/MMPA Scientific Collection permits issued by the NOAA Fisheries Office of Protected Resources to the Alaska Department of Fish & Game and the Marine Mammal Laboratory, #358-1564 and #782-1532, respectively, and within the Alaska Maritime National Wildlife Refuge by Special Use Permit 74500-99004 issued from the U.S. Fish and Wildlife Service. Free-ranging animal handing protocols were reviewed by the ADF&G Division of Wildlife Conservation Animal Care and Use Committee and approved as Assurance of Animal Care #03-0002. This research was funded through the National Oceanic and Atmospheric Administration (NOAA) awards to ADF&G (Nos. NA17FX1079; NA15NMF4390170) and the US Fish and Wildlife Service (grant number AK-22). The findings and conclusions in this paper are those of the authors and do not necessarily represent the views of the National Marine Fisheries Service. The authors have no conflicts of interests.

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ALASKA DEPARTMENT OF FISH AND GAME
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Alaska Department of Fish and Game
Wildlife Restoration Grant

GRANT NUMBER: AKW-22

PROJECT NUMBER: P1.0

PROJECT TITLE: Steller sea lion recovery

PERIOD OF PERFORMANCE: July 1, 2017 – June 30, 2020 (Year 3 by no-cost extension)

PERFORMANCE YEAR: July 1, 2018 – June 30, 2019; year 2 of a 3-year grant

REPORT DUE DATE:

PRINCIPAL INVESTIGATOR: Michael Rehberg

COOPERATORS:

Authorities: 2 CFR 200.328
2 CFR 200.301
50 CFR 80.90

I. PROGRESS ON PROJECT OBJECTIVES DURING PERFORMANCE YEAR

OBJECTIVE 1: Contribute to estimates of population abundance and vital rates of Steller sea lions.

ACCOMPLISHMENTS:

A set of three aerial surveys of the ephemeral, spring Steller sea lion aggregations at the Alsek/Doame/Akwe rivers on the outer coast south of Yakutat were conducted during 3 days in March – April 2019 using flights from Juneau. This aggregation of mammals and birds coincides with a large eulachon run along the coastline, and has grown in size over the past 10 years. Sea lions are drawn to this location from throughout the Gulf of Alaska: one sea lion satellite tagged at Sitka by NOAA Fisheries visited the location in early March, and brand resights indicate sea lions drawn from southern Southeast Alaska through the Alaska Peninsula.

Analysis of these aerial photos is partially complete; funding limitations in Year 3 caused us to lose the temporary employee assigned this work. This work will be resumed as funding issues are addressed or the work is re-assigned. Thus far, 33 confirmed brands (21 female, 12 male) were identified: 27 sea lions branded on their natal rookery included 3 sea lions from the endangered western population and 2 sea lions from Oregon. Six brands had been previously captured by us as non-pups. Daily counts included 4,416 and 2,481 at Alsek River,104 and 120 at Inian Islands, 123 at Cannery Creek, and 4 at the new Libby Island haulout identified recently during these surveys. Two days of aerial photos remain to be counted.

For the second year, we used our Spring 2017 SDR to expand our reproductive rate surveys westward to Kodiak Island, a region otherwise un-surveyed. This year, we prioritized surveys at two large rookeries: the Barren Islands south of Cook Inlet, and Marmot Island off Kodiak. This provided coverage of an area that has seen recent (December 2017) 17% drop in pup production. During these surveys, we confirmed 125 brand-resights. At Marmot Island rookery, 50 females and 12 males; at Sugarloaf Island rookery, 43 femalse and 7 males. Latax Rocks, Ushagat Island and Sea Otter Island haulouts were also surveyed.

During June 2019, we travelled by skiff to service and download the remote time-lapse cameras installed at Graves Rocks rookery and South Marble Island haulout, Glacier Bay. The seasonal Graves Rocks camera was re-installed for the breeding season (this camera is exposed to high winter surf and removed each fall.) To address concerns from the Park Service regarding the visibility of the South Marble Island camera installation to Park visitors, we re-positioned the camera to a less visible location (below).

We took delivery of an APH-28 hexacopter system (Aerial Imaging Solutions, LLC) purchased under this grant by SDR, and during Year 3 we are preparing to train in its use and integrate it with our ongoing survey work. This system is compatible with the imaging hexacopters in use by our collaborators at the NOAA Marine Mammal

Laboratory, to ensure data are compatible and due to their known success using this system.

OBJECTIVE 2: Determine population structure and habitat use of Steller sea lions.

ACCOMPLISHMENTS:

In order to collect skin samples for the purpose of genetic analysis, initially in support of the DNA methylation method for aging, and ultimately for other population structure and vital rates analysis, this year we developed our ability to collect skin samples using remotely-delivered biopsy darts. During the April 2019 capture cruise, and other training, we trained and qualified two additional personnel under our research permit to collect skin biopsy samples using biopsy darts. Collected samples are being held frozen until analysis, and biopsy darting continues into Year 3.

OBJECTIVE 3: Conduct a pilot project to examine retention time of new telemetry instruments that may provide information on movements, dive behavior, and environmental data as a means of long-term monitoring of adult and subadult Steller sea lions.

ACCOMPLISHMENTS:

During Year 2, we confirmed that the new, flipper-mounted satellite tags are working successfully on adult male Steller sea lions and retention is good. Flipper mounted tags use semi-permanent attachments through the flipper, similar to the numeric ear tags used on terrestrial mammals. These tags permit year-round, and potentially multi-year, tracking of sea lion locations, which is not currently possible with fur-mounted instruments that molt off every autumn.

During this reporting period, we conducted a successful capture and satellite tagging trip during April 2019 in Southeast Alaska, capturing 3 adult males in Frederick and Chatham Straits. Sea lions were tracked (Figure 1) using fur-mounted dive-and GPS-location recording tags as well as semi-permanent, flipper-mounted location-only recording tags.

Prior to these deployments, we worked with the manufacturer to test and alter flipper-tag programming to fix problems we identified in previous deployments. Flipper tags had been rapidly burning through their transmission allotments and failing earlier than predicted. The fixes accomplished during this project appear to have solved this issue for the current deployment. We also worked to confirm whether these tags can be used successfully on Steller sea lions. Because the tags were originally designed for seals that haul out on ice, the manufacturer had warned us that electrical problems would occur when used on land-associated seals. During this deployment, we have confirmed this is not a problem for Steller sea lions.

Finally, we confirmed that flipper tags provided equivalent and accurate indications of where Steller sea lions haul out as the temporary, higher-resolution head-mounted GPS tags (Figure 2). This means flipper tags – which can last years instead of months – will be suitable to track long-term Steller sea lion movement across multiple years and through the breeding season.

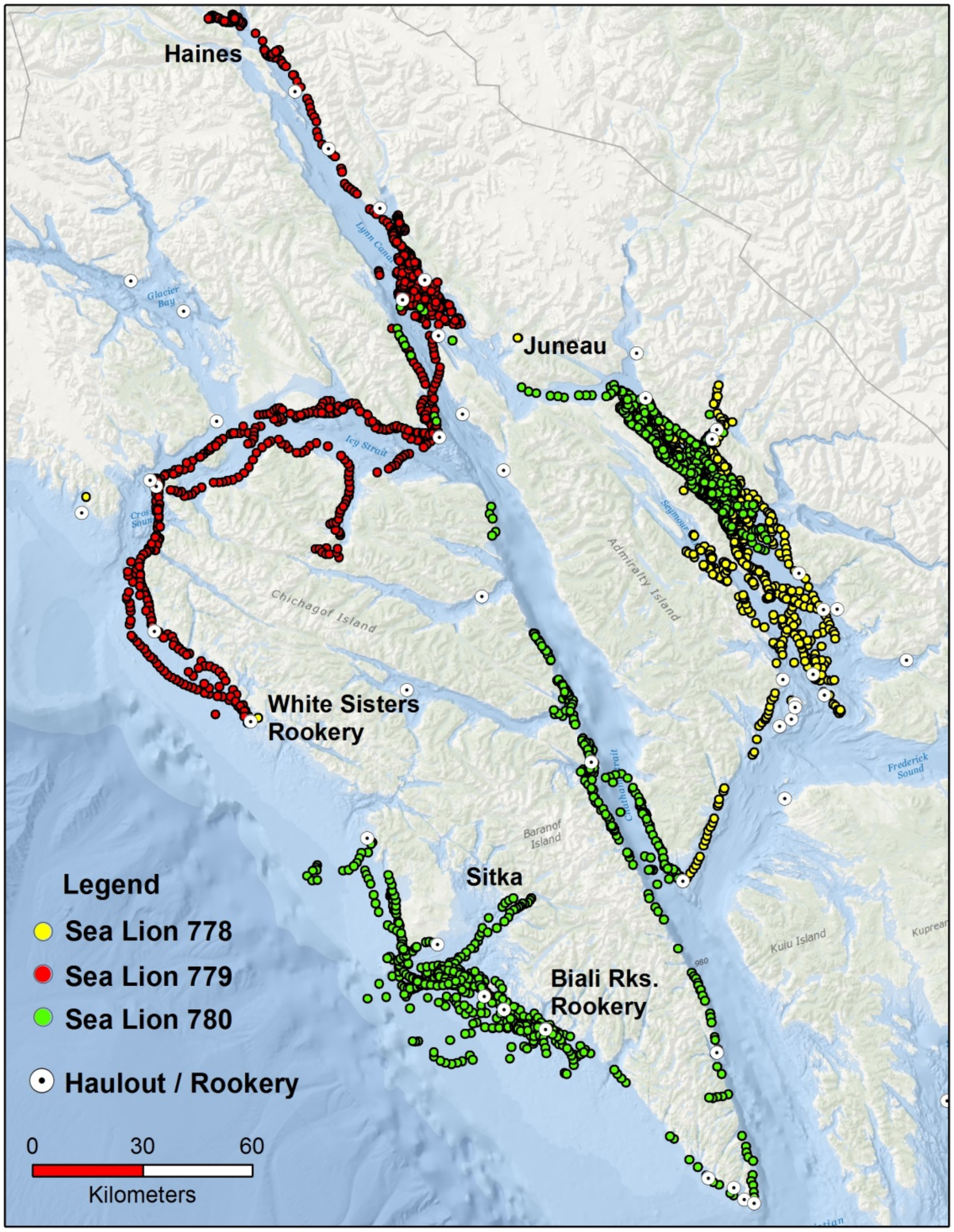

Figure 1. Locations of 3 Steller sea lion adult males captured during April 2019 and tracked through September 2019. Animals were fitted with head-mounted dive- and GPS-location-recording satellite tags. Horizontal resolution is ~25m and temporal resolution is 15 minutes.

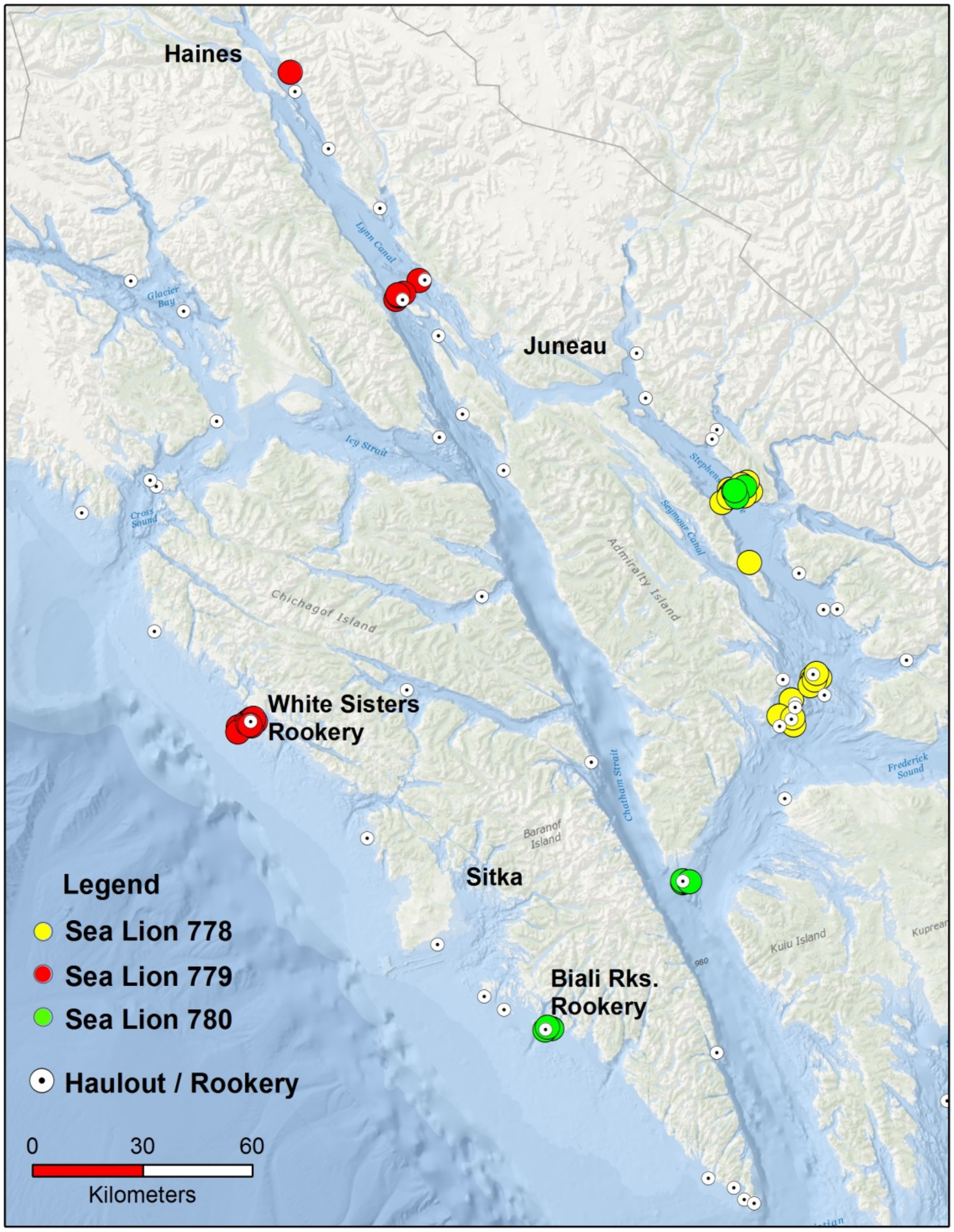

Figure 2. Locations collected by flipper-mounted tags on the 3 adult male Steller sea lions described in Figure 1. These tags are semi-permanent, but lower resolution and transmit during haulouts only. Most haulout behavior captured by temporary, high-resolution GPS tags (Figure 1) was also captured by these smaller, longer-lasting tags. Horizontal resolution is 0.2 - 5 km and temporal resolution is minutes to days.

OBJECTIVE 4: Assess foraging areas and diet of Steller sea lions.

ACCOMPLISHMENTS:

Contracting with the COHO Lab at Alaska SeaLife Center, we began analysis of scats to determine the prey taken by sea lions using DNA techniques. The COHO Lab designed primers, a necessary first step in DNA prey analysis, for the 27 prey items we requested (Table 1). A primer is required for each prey species we wish to identify using this method.

The COHO lab extracted and began analysis of the 60 initial sea lion scat samples we provided. These samples represent sea lion foraging off Long Island, near Kodiak, during January and March 2013-2014. This is the pre-sample of sea lion diet collected before the current, reduced Steller sea lion pup counts observed in this area, and before the major "blob" phenomenon – high water temperatures that are affecting potential sea lion prey abundance and diversity. Funding-dependent, we hope to collect additional scats during January or March 2020, which will represent sea lion diet that is happening during the present "blob" event.

Long Island is also a key location in the ongoing survey work during Year 1 and Year 2, and the upcoming adult female capture work in October 2019. We hope to paint an interesting picture of sea lion interactions with their changing ecosystem.

Table 1. Primers for development to identify prey species.

1	Walleye pollock	*Gadus chalcogrammus*
2	Pacific cod	*Gadus macrocephalus*
3	Arrowtooth flounder	*Atheresthes stomias*
4	Salmon spp.	*Oncorhynchus spp.*
5	Pacific sand lance	*Ammodytes hexapterus*
6	Pacific herring	*Clupea pallasi*
7	Sculpin/Irish Lord spp.	Cottidae
8	Snailfishes	Liparidae
9	Rock sole	*Lepidopsetta spp.*
10	Skates	*Raja spp.*
11	Rockfishes	*Sebastes spp.*
12	Pacific sandfish	*Trichodon trichodon*
13	Capelin	*Mallotus villosus*
14	Eulachon	*Thaleichthys pacificus*
15	Smooth Lumpsucker	*Aptocyclus ventricosus*
16	Pacific Tomcod	*Microgadus proximus*
17	Shrimp spp.	Pandalidae
18	Octopus spp.	*Enteroctopus* & *Octopus spp.*
19	Squid spp.	Gonatidae
20	Pacific hake	*Merluccius productus*
21	Smelt spp.	Osmeridae

22	Pacific halibut	*Hippoglossus stenolepis*
23	Unidentified sculpins	Cottoidea
24	Unidentified polychaetes	Polychaeta
25	Spiny dogfish	*Squalus acanthias*
26	Pacific lamprey	*Entosphenus tridentatus*
27	Saffron cod	*Eleginus gracilis*

Sources: Trites et al. 2007, McKenzie and Wynne 2008, Pitcher 1981, Sinclair et al. 2013, Trites and Calkins 2008, Alaska SeaLife Center unpublished data

The results of our satellite tagging work (see Objective 3, above) also provided new information about Steller sea lion foraging. The head-mounted satellite tags we attach to sea lion fur provide data including GPS locations (to ~25 meters), the depth, duration and shape of individual dives, whether a sea lion is hauled out or at-sea, and a temperature profile of the water column. These data, combined with environmental characteristics we plot on maps (such as seafloor depth, seafloor features, currents, and the shoreline), provide inference about locations important for sea lion foraging. This analysis is underway during Year 3 of this grant.

Illustrations are a good way to explain these data. Adult male sea lion #778 spent all of its time within Frederick Sound and Stephens Passage (Figure 3a). Clusters of deep diving occur over seafloor canyons show sea lions targeting benthic prey (e.g., squid, cod) while shallow diving in otherwise deep water indicate pelagic prey (e.g., salmon, herring) are being taken. This male never approached a breeding rookery and likely did not breed this year. Sea lion # 779 did venture to an offshore breeding rookery (Figure 3b), where it completely ceased diving for 6 weeks while presumably holding a territory. Upon return to inside waters, this male resumed targeting prey in earnest. Sea lion #780 also ventured offshore (Figure 3c), but did not pause to breed. Instead, spending its time relatively shallow offshore foraging through the summer. Finally, Figure 3d show how clusters of diving at various depths can indicate potential foraging hot spots.

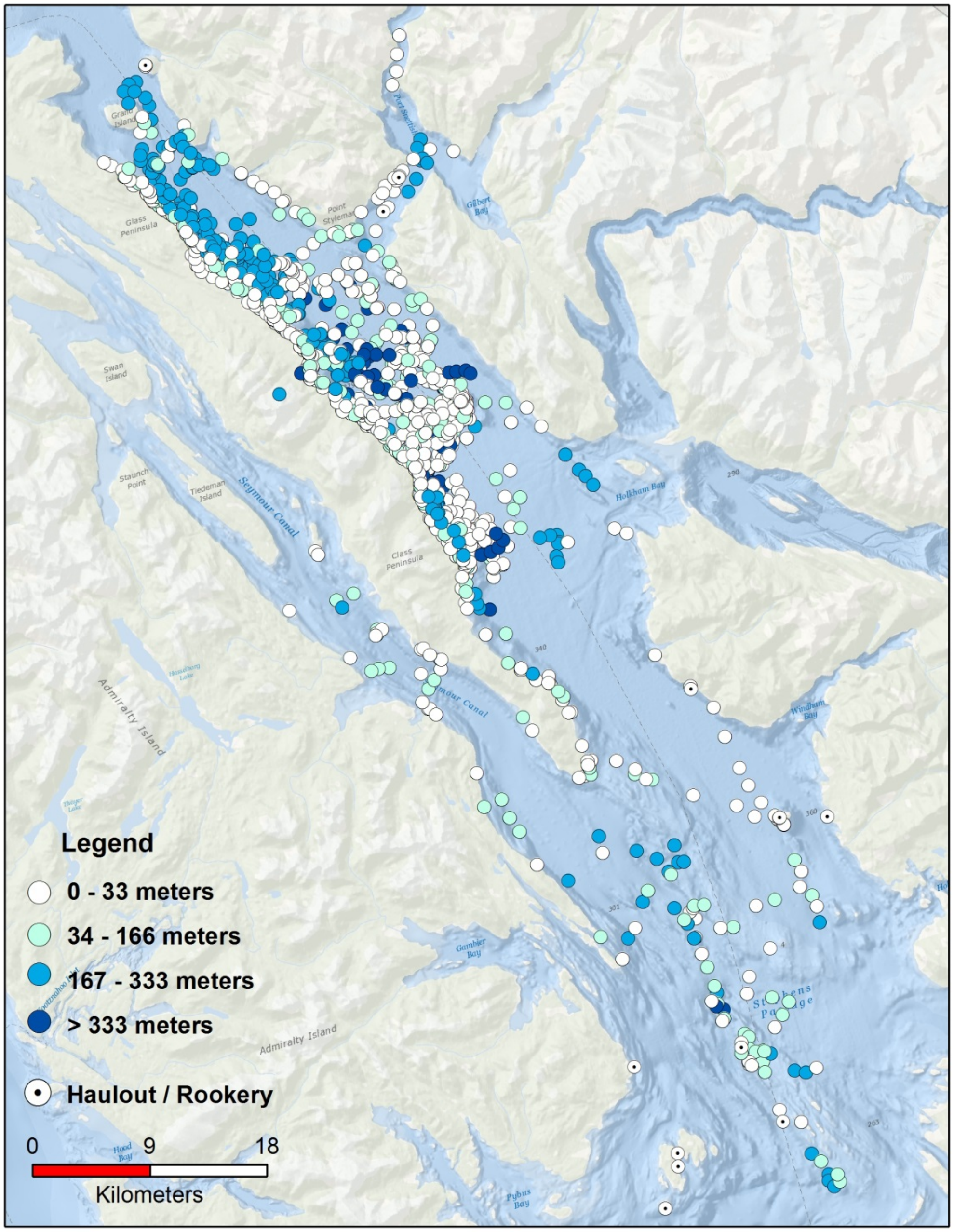

Figure 3a. Diving behavior linked to GPS locations collected during April - September 2019 for adult male Steller sea lion #778. Shaded dots indicate diving depths at each location, and are overlaid onto an image of seafloor depths (color) and undersea features (shading). Horizontal resolution is ~25 meters, temporal resolution is sporadic (locations shown only when a sea lion dives.)

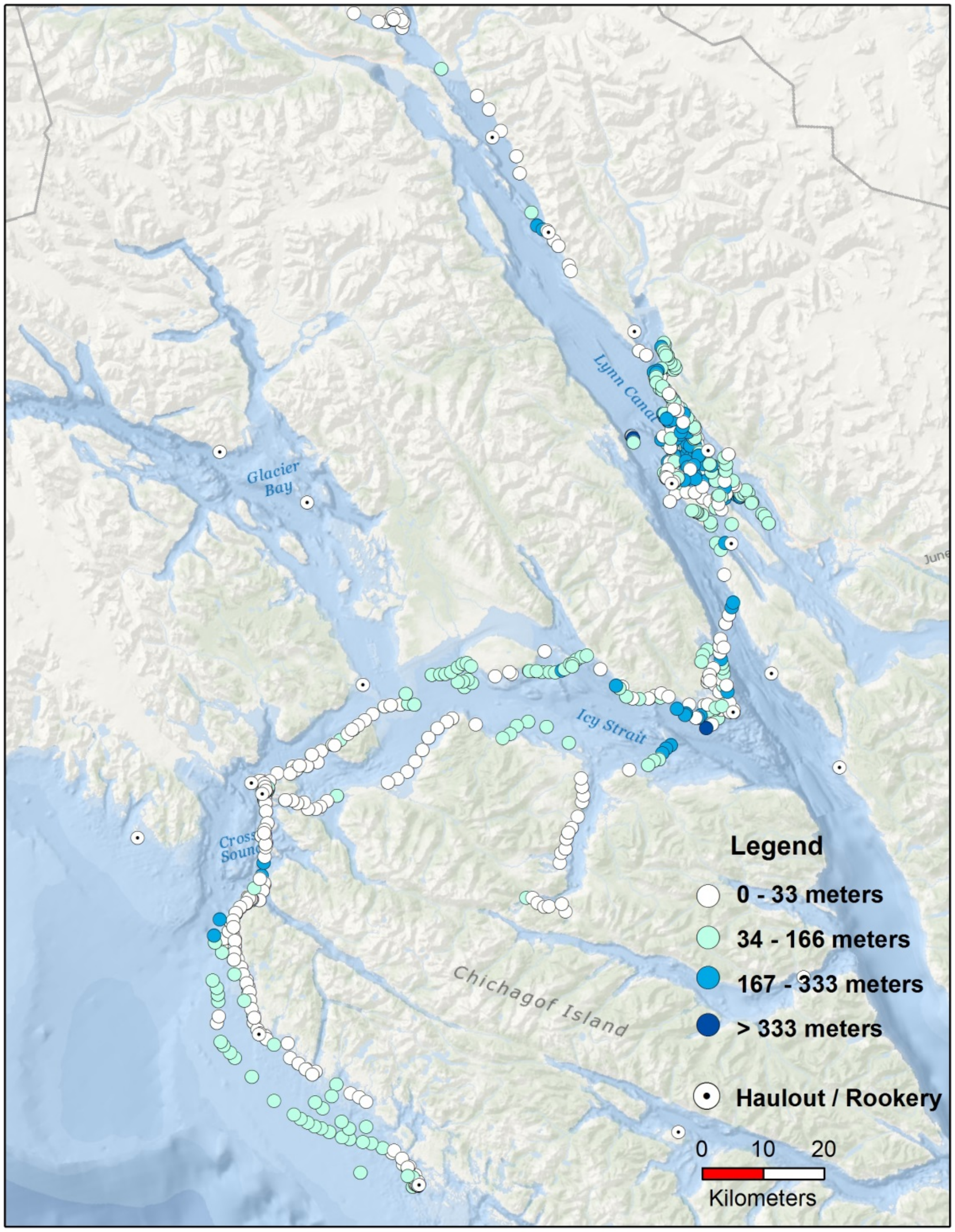

Figure 3b. Diving behavior linked to GPS locations collected during April - September 2019 for adult male Steller sea lion #779. Shaded dots indicate diving depths at each location, and are overlaid onto an image of seafloor depths (color) and undersea features (shading). Horizontal resolution is ~25 meters, temporal resolution is sporadic (locations shown only when a sea lion dives.)

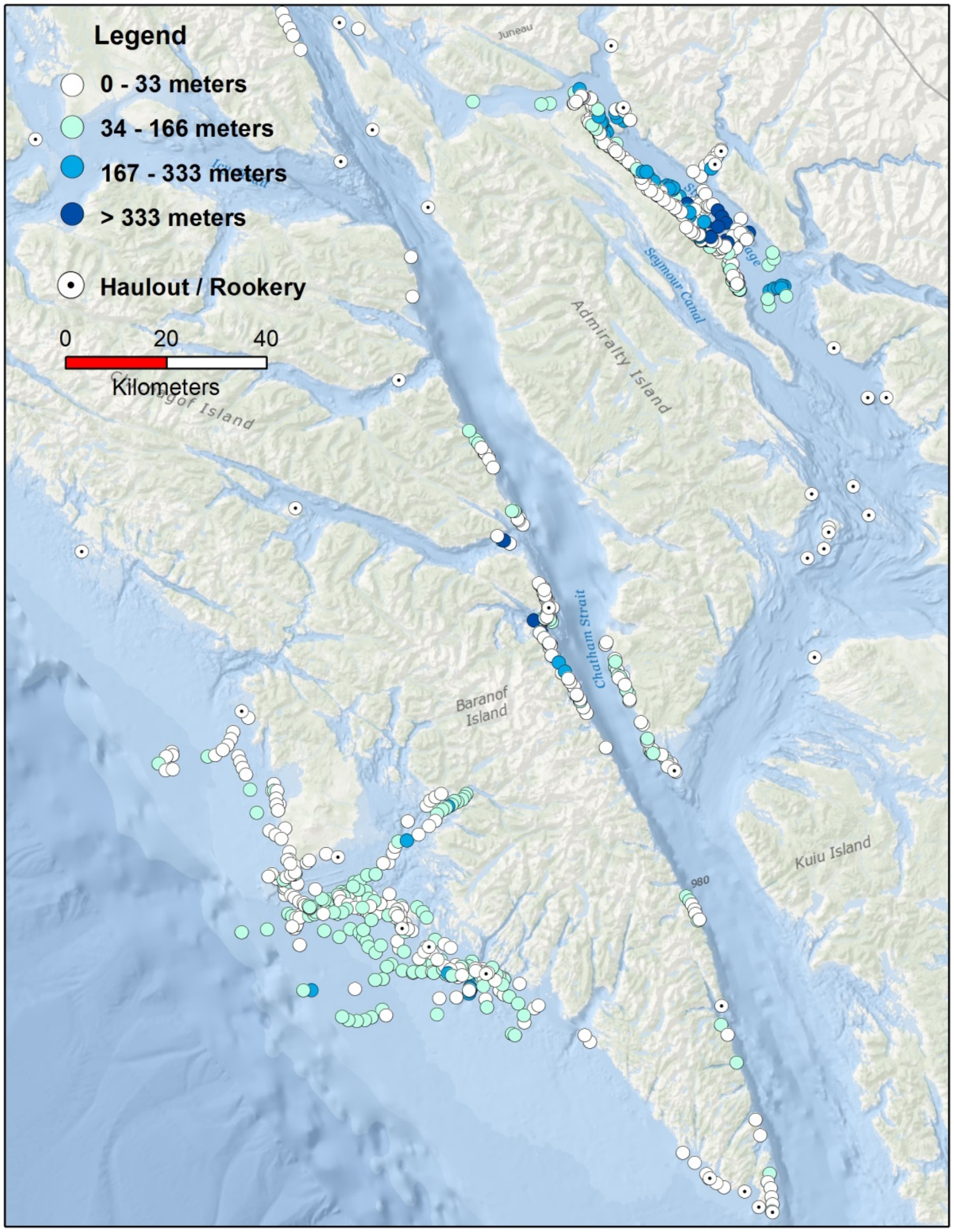

Figure 3c. Diving behavior linked to GPS locations collected during April - September 2019 for adult male Steller sea lion #780. Shaded dots indicate diving depths at each location, and are overlaid onto an image of seafloor depths (color) and undersea features (shading). Horizontal resolution is ~25 meters, temporal resolution is sporadic (locations shown only when a sea lion dives.)

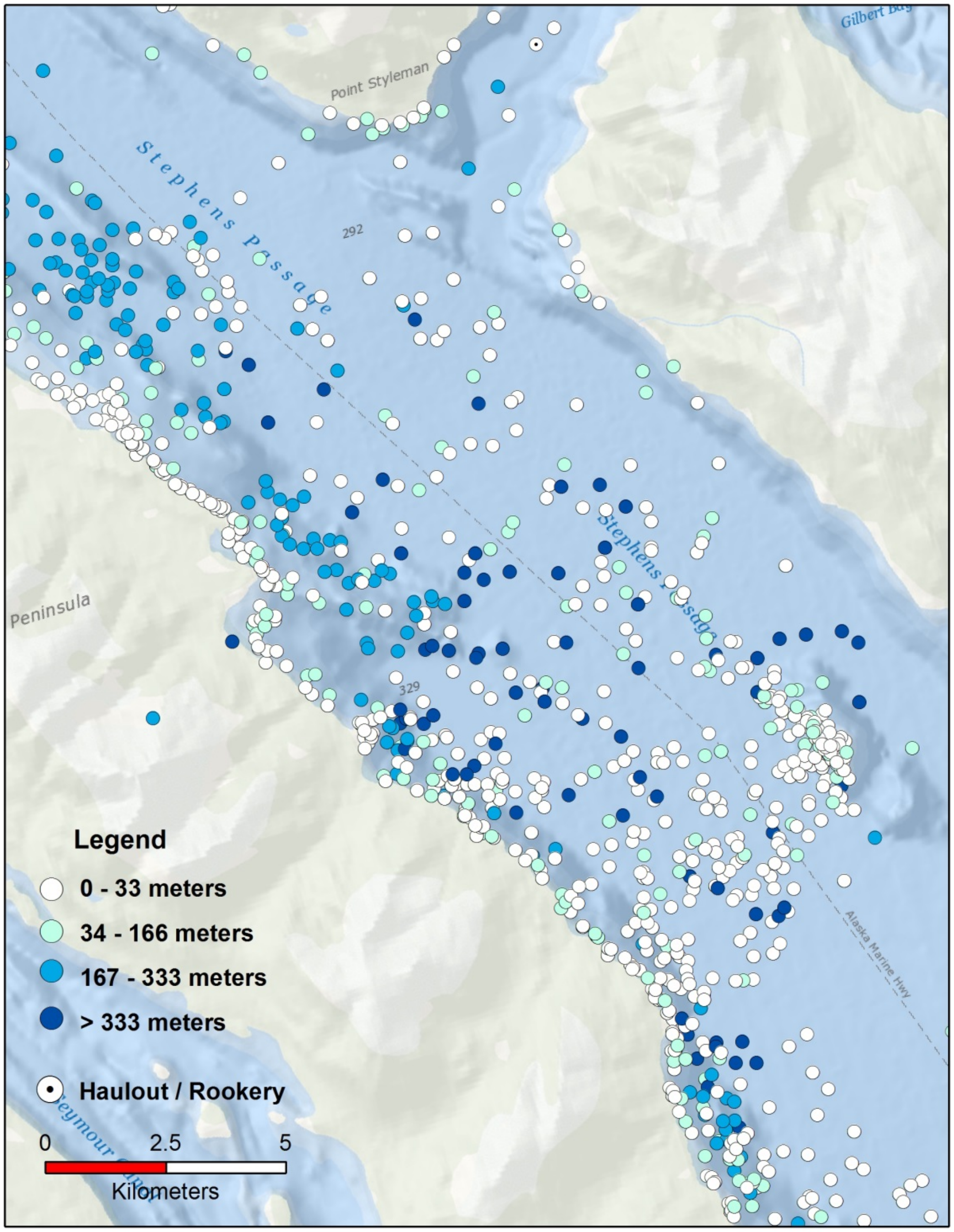

Figure 3d. Detailed view of foraging behavior (dives over seafloor) by adult male Steller sea lions 778 and 780 in Stephens Passage. Shallower foraging over shallow seafloor features is seen center-right, deeper foraging dives scatter throughout this deep fjord, and concentrations of diving behvaior at different locations, suggesting foraging, is evident. Horizontal resolution is ~25 meters, temporal resolution is sporadic.

OBJECTIVE 5: Document marine debris and fisheries interactions with Steller sea lions.

ACCOMPLISHMENTS:

ADF&G attempted but was unsuccessful in performing any disentanglements during calendar year 2019. However, we did invite two experts from the Marine Mammal Center (Sausalito, California) to collaborate and exchange knowledge with us during the April 2019 adult male captures. Their experience in "rescue response" type work on California sea lions provided valuable methods that we are working to adopt in both our research and disentanglement capture work. One such method is the "Incident Command Structure" which clarifies decisionmaking and roles of a team during the fast-paced and unpredictable sea lion capture work we do. In turn, we provided them valuable experience with Steller sea lion capture before their planned work to teach entanglement response techniques in eastern Russia.

OBJECTIVE 6: Determine the health of individuals and identify residual and emerging threats to Steller sea lions.

ACCOMPLISHMENTS:

Health-related work and publication is reported elsewhere in this IPR.

II. SUMMARY OF WORK COMPLETED ON PROJECT TO DATE.

This is Year 2 of a three-year (by no-cost extension) project. Summaries of work completed to date are included in Section I, above. Summarized results and publications emerging from planned or ongoing analysis of data collected (e.g., photo processing, instrument testing) should occur during Year 3.

III. SIGNIFICANT DEVELOPMENT REPORTS AND/OR AMENDMENTS.

Two Significant Development Reports were submitted and approved. These reports included the following no-cost changes:

1. Modifying the approach to Job 1a, *Retrieve data, service and re-deploy time-lapse cameras* and Job 1b, *Analyze images to collect brand resight and count data*, by using unobligated funds to purchase and train on a remote unoccupied aircraft system (UAS, or "drone") for remote aerial population and vital rates data collection.
2. Documenting in-kind match provided by the vessel operator for the adult male Steller sea lion capture in April 2019.

One amendment was approved, providing a no-cost extension to continue this work into a Year 3, presently underway. During this extension, analysis of prey found in scats will continue, adult male Steller sea lion tracking data will be analyzed and submitted, an additional year of expanded surveys in the Kodiak Island region will be possible, and we will work on learning and incorporating the newly-acquired UAS for our survey work.

IV. PUBLICATIONS

No publications were produced during this reporting period.

V. RECOMMENDATIONS FOR THIS PROJECT Because the Gulf of Alaska, Bering and Chukchi Seas are now experiencing a major ecosystem disruption due to two years reduced sea ice and/or high ocean temperatures, using the WSFR as a platform to accomplish broader, system- or multi-species research would be an ideal and timely next step for the Marine Mammal Program. Broader than possible under agency-specific species or management mandates under other grants. The Marine Mammal Program could pursue marine systems- and seascape-scale research initiatives similar to the Division of Wildlife Conservation's progress on terrestrial landscape ecology in recent years.

Should match be secured in advance and objectives well defined, the WSFR program would be a relatively stable funding source to continue key portions of our research that are not funded by NOAA Fisheries. Expansion into other marine mammal work, such as Northern sea otter and harbor seal work, is a possibility.

Prepared by: Michael Rehberg, Wildlife Biologist IV

Date: September 24, 2019