



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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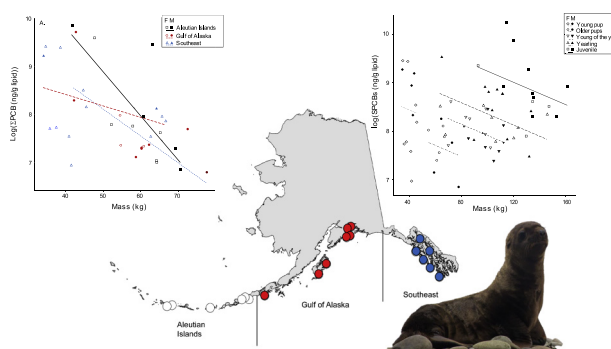
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HIGHLIGHTS

- ΣPCBs were highest in the Aleutian Islands and were higher in males in all regions.
- ΣPCBs and ΣDDTs 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



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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

Abbreviations: (SSL), Steller sea lion.

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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.

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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; Kajiwaru 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 (Ofteidal 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; Ofteidal 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 $-20^\circ C$ 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 (\pm SD) percent recovery of the surrogate standard (1,2,3,4-TCDD) was $81 \pm 6\%$ (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; Kajiwarra 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 \sum PCB 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)			
∑ PCBs	4291 ± 4184	(644–26,107)	120
∑ PCB TEQs	62 ± 64.9	(4.76–405)	120
∑ 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
∑ DDTs	5413 ± 6019	(559–35,821)	113
∑ 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 ∑ PCBs and ∑ PCB load.
^d Denotes DDT isomers in ∑ DDTs and ∑ 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 girth²)] × 10⁶ (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 ∑ DDTs and ∑ PCBs, as well as ∑ DDT loads and ∑ 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 D₂O 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, ∑ PCBs, ∑ 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∑[PCBs], log(PCB load), log∑[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 (ME×ME). Reported terms include the Akaike information criterion with a correction for finite sample sizes (AICc), ΔAICc, R²_{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	ME×ME
log∑[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∑[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 Δ AICc) 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	Δ AICc	Adj R ²	Main effects			Interaction MExME
				ME1	ME2	ME3	
$\log \sum$ [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 \times axial girth²)] $\times 10^6$.

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 μ g; 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 μ g (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 μ g ($37,519 \pm 6706$ μ g). 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 Δ AICc < 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

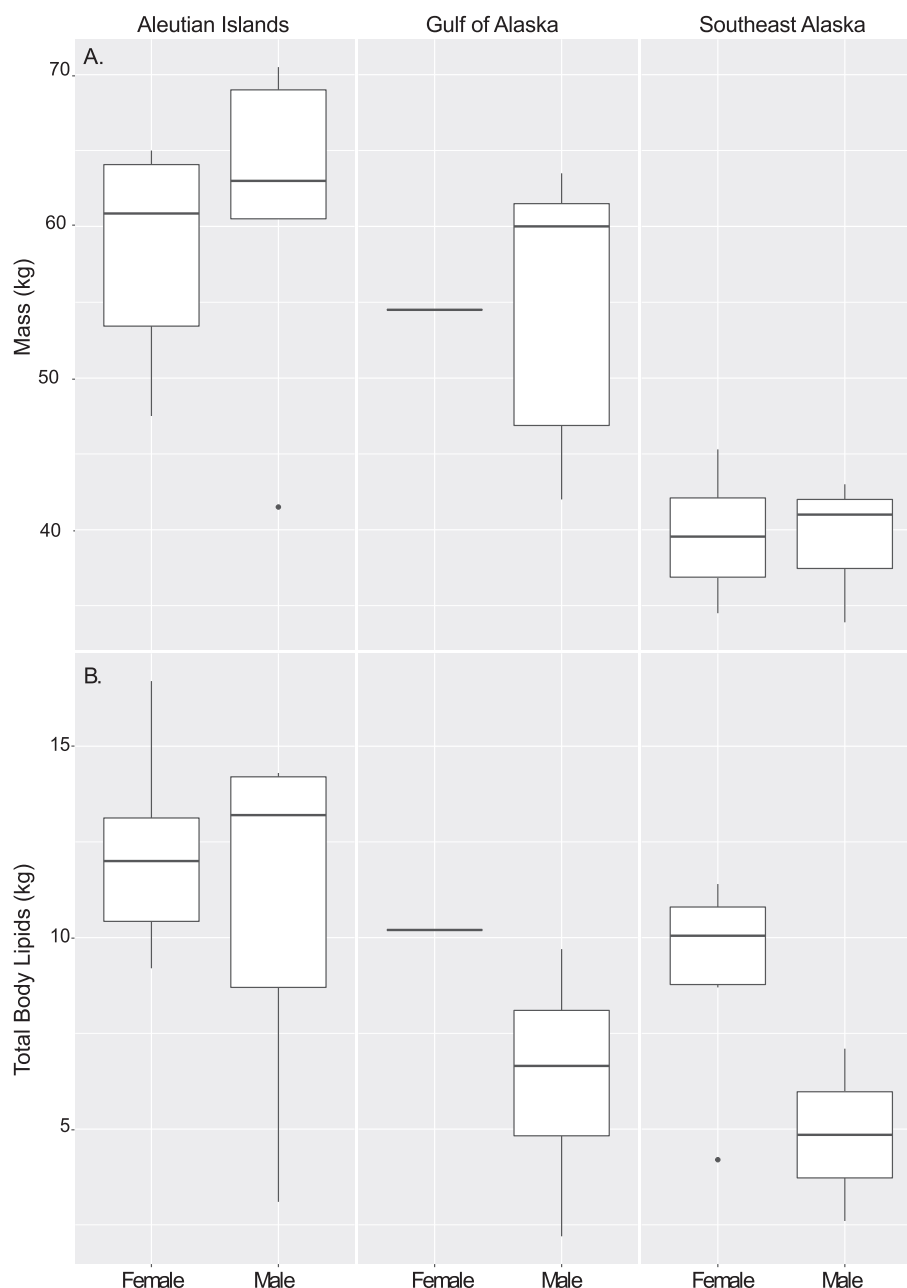


Fig. 2. Boxplots of total body lipid (kg) and mass (kg) for young pups by regions (Southeast Alaska, Gulf of Alaska, and the Aleutian Islands) and sex.

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 ($\Delta\text{AICc} < 2$) 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 ($41,979 \pm 54,524$ 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 $\Delta\text{AICc} \leq 2$ and all included mass (Table 2). The only covariate in the top model ($\text{AICc} = 86.43$), 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

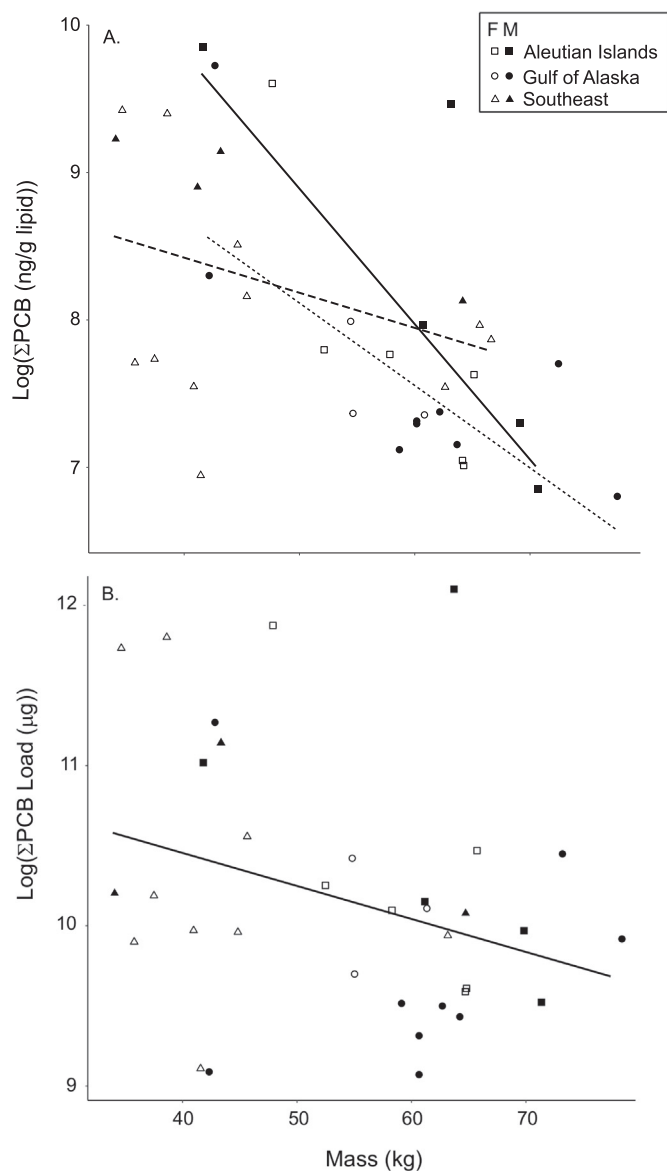


Fig. 3. A. Natural-log transformed Σ PCB concentration (ng/g lipid) and B. log transformed Σ PCB load (μ g) for blubber samples collected from pups (<6 months) by region. We chose to present the second model (Δ AICc = 0.15, Table 2) in order to compare Σ PCBs concentrations and loads in the same figure.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

lipid in the concentration of Σ DDTs. Unlike the concentration of Σ 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 Δ AICc ≤ 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 Σ DDT concentrations being explained by those top models (Table 2). Unlike the top models for the concentration of Σ DDTs, sex was not retained in any model for the Σ 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,

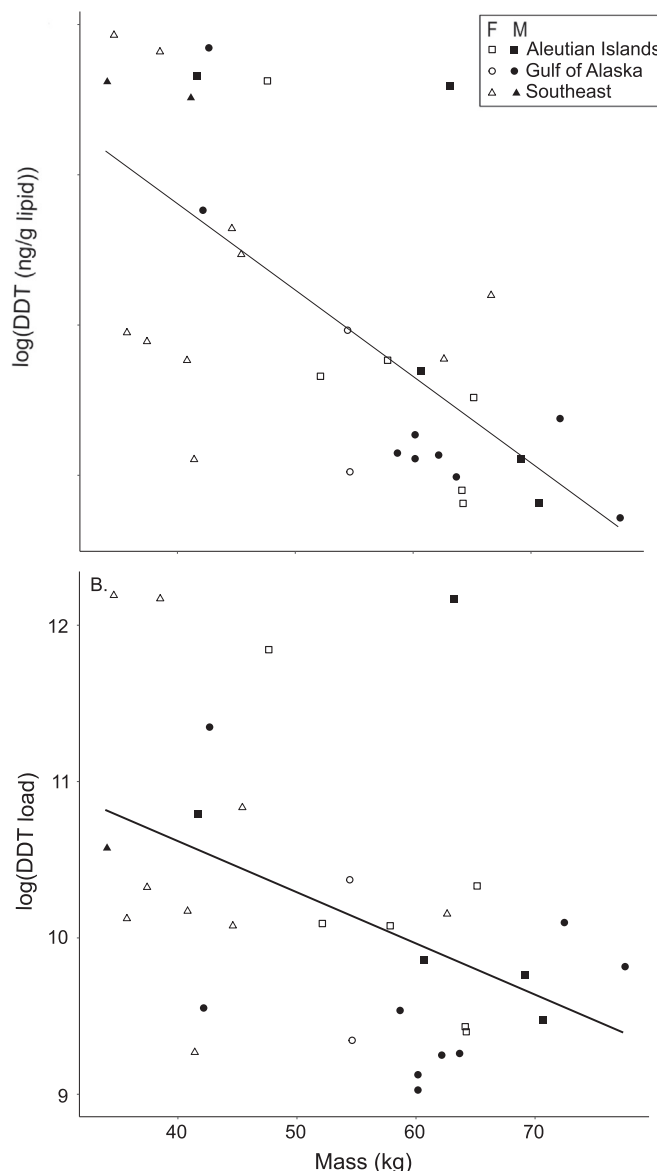


Fig. 4. A. Natural- log transformed Σ DDT concentration (ng/g lipid) and B. log transformed Σ DDT load (μ g) for blubber samples collected from pups (<6 months) by region. Male pups are denoted with filled symbols and female pups by open symbols.

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).

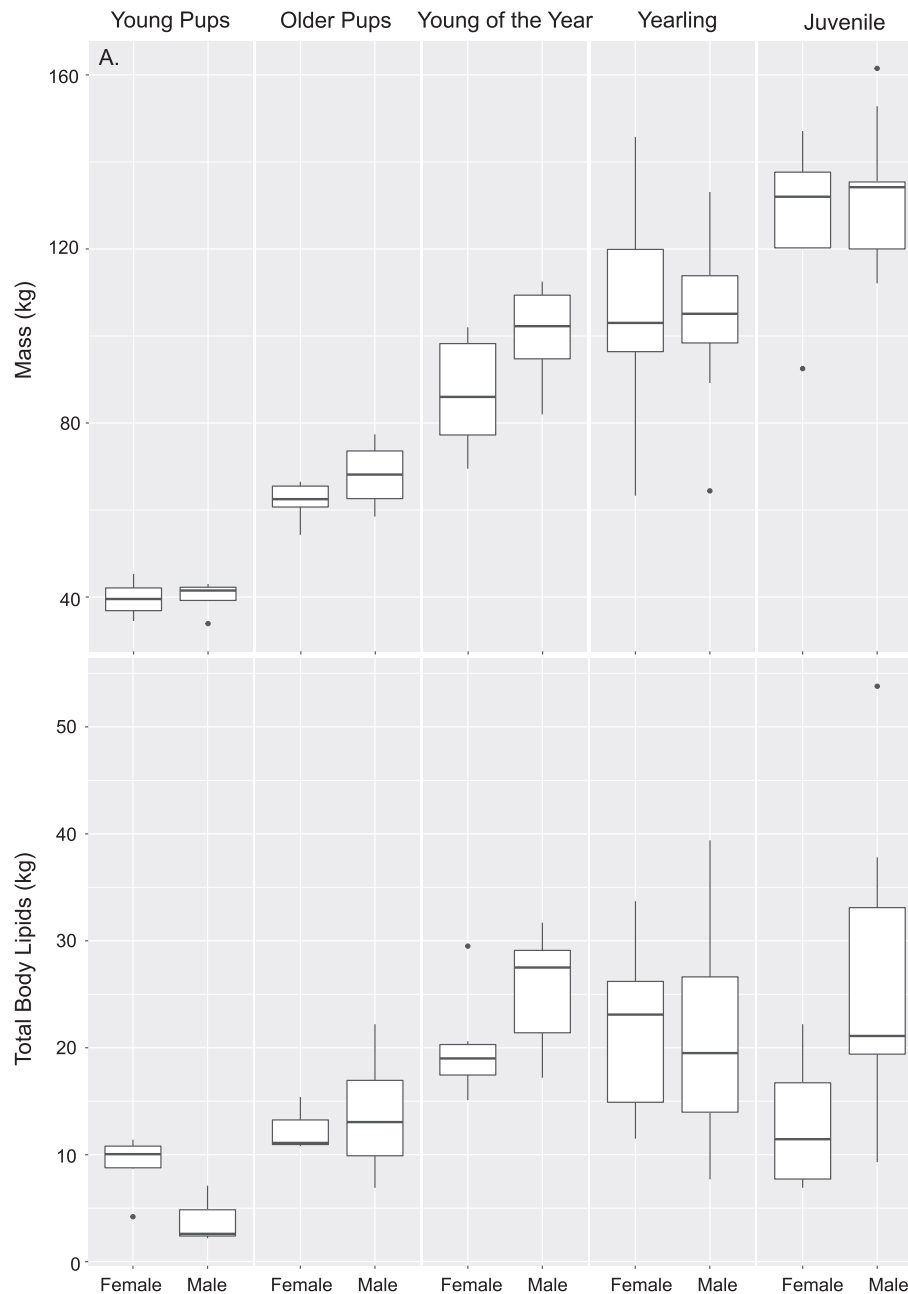


Fig. 5. Boxplots of total body lipid (kg) and mass (kg) by age class (young pups (<2 months), older pups (3–6 months), young of the year (6–11 months), yearling (12–22 months), and juvenile (>23 months)) and sex for Steller sea lions sampled in Southeast Alaska and the eastern portion of the Gulf of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

3.3.2. PCBs in young Steller sea lions

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

Three models examining the variability in \sum PCBs concentration had $\Delta\text{AICc} \leq 2$, 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 \sum 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 \sum PCBs decreasing from young pups to older pups then increasing in the older age classes (Fig. 6A).

The 5 top models ($\Delta\text{AICc} \leq 2$) 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 $\Delta\text{AICc} = 1.03$; 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 \sum 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 \sum PCBs and

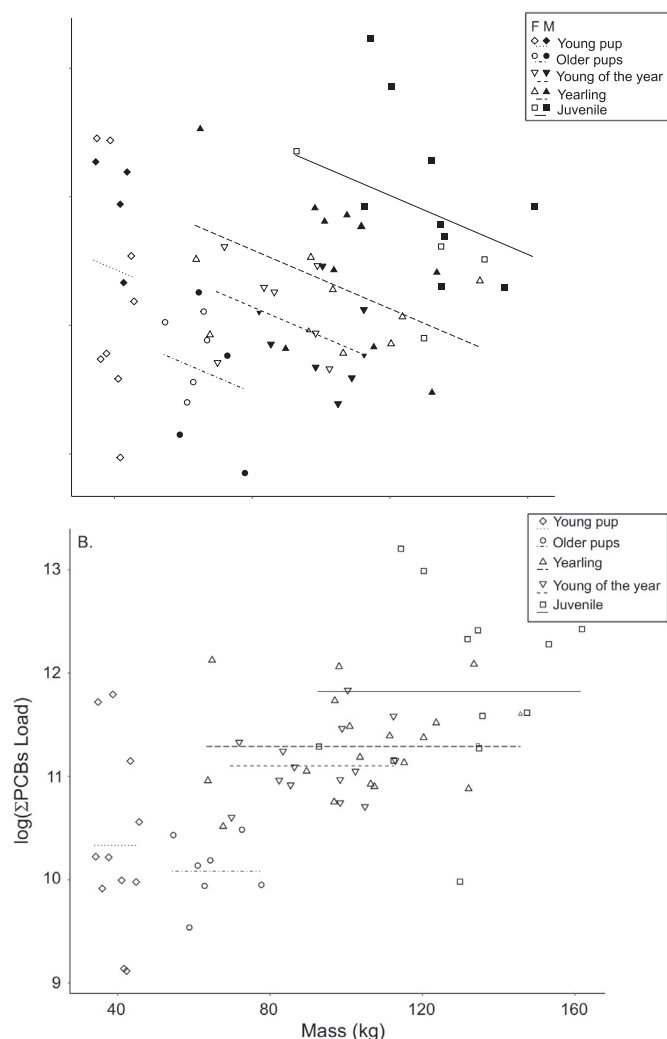


Fig. 6. A. Natural- log transformed Σ PCB concentration (ng/g lipid) and B. log transformed Σ PCB load (μ g) for Steller sea lions by age class including young pups (<2 months), older pups (3–6 months), young of the year (6–11 months), yearling (12–22 months), and juvenile (>23 months). Modeling was 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.

Σ 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 Σ 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 μ g ($114,522 \pm 135,570$ μ g) across the five age classes (Table S3).

Four models examining the variability in the concentration of Σ DDTs had Δ AICc ≤ 2 and, as with the concentration of Σ 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 Σ 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 Σ 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 Δ AICc ≤ 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 (Δ AICc = 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 Σ 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 Σ DDTs and Σ 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 Σ PCB TEQs concentrations over 209 pg/g lipid and three of these samples also had Σ PCB 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

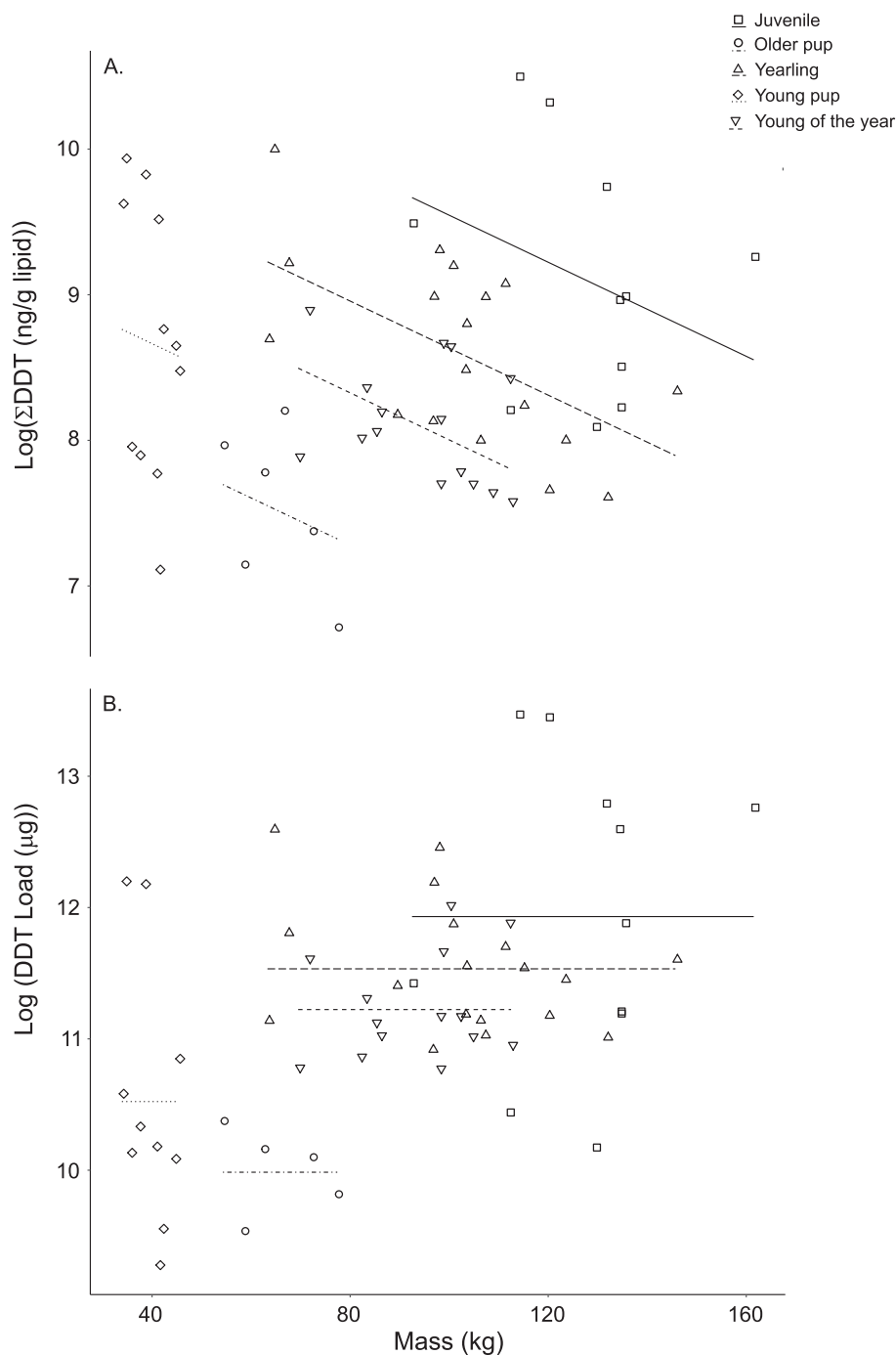


Fig. 7. A. Natural- log transformed Σ DDT concentration (ng/g lipid) and B. log transformed Σ DDT load (μ g) for Steller sea lions by age class including young pups (<2 months), older pups (3–6 months), young of the year (6–11 months), yearling (12–22 months), and juvenile (>23 months). Modeling was 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 laboratory 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 otariid 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 endangered 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 Σ 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 Σ PCBs concentration of 424 ± 388 ng/g lipid (range 96–1400 ng/g lipid), and a mean Σ DDTs concentration of 444 ± 511 ng/g lipid (range 51–1987 ng/g lipid). There was a significant positive relationship between the concentrations of Σ PCBs and Σ DDTs 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 $\delta^{15}\text{N}$ 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 Zeppelin, 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 Σ PCBs for all but the larger pups were highest in pups from the Aleutian Islands. These findings contrast with what we expect if Σ 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 Σ 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 Σ 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 compared 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 Σ PCBs. The decrease in Σ PCBs and Σ 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 Σ 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 Σ 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 Σ PCBs in SSL pups.

The Σ PCB and Σ 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 Σ PCBs and Σ DDTs in SSL pups may be 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 Σ 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.

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References

- Atkinson, S., Demaster, D.P., Calkins, D.G., 2008. Anthropogenic causes of the western Steller sea lion *Eumetopias jubatus* population decline and their threat to recovery. *Mamm. Rev.* 38, 1–18. <https://doi.org/10.1111/j.1365-2907.2008.00128.x>.
- Baker, J.D., Fowler, C.W., 1992. Pup weight and survival of northern fur seals *Callorhinus ursinus*. *J. Zool. Lond.* 227, 231–238. <https://doi.org/10.1111/j.1469-7998.1992.tb04819.x>.

- Barron, M.G., Heintz, R., Krahn, M.M., 2003. Contaminant exposure and effects in pinnipeds: implications for Steller sea lion declines in Alaska. *Sci. Total Environ.* 311, 111–133. [https://doi.org/10.1016/S0048-9697\(03\)00140-2](https://doi.org/10.1016/S0048-9697(03)00140-2).
- Beckmen, K.B., Ylitalo, G.M., Towell, R.G., Krahn, M.M., O'Hara, T.M., Blake, J.E., 1999. Factors affecting organochlorine contaminant concentrations in milk and blood of northern fur seal (*Callorhinus ursinus*) dams and pups from St. George Island, Alaska. *Sci. Total Environ.* 231, 183–200. [https://doi.org/10.1016/S0048-9697\(99\)00094-7](https://doi.org/10.1016/S0048-9697(99)00094-7).
- Beckmen, K.B., Blake, J.E., Ylitalo, G.M., Stott, J.L., O'Hara, T.M., 2003. Organochlorine contaminant exposure and associations with hematological and humoral immune functional assays with dam age as a factor in free-ranging northern fur seal pups (*Callorhinus ursinus*). *Mar. Poll. Bull.* 46, 594–606. [https://doi.org/10.1016/S0025-326X\(03\)00039-0](https://doi.org/10.1016/S0025-326X(03)00039-0).
- Beckmen, K.B., Keogh, M.J., Burek-Huntington, K.A., Ylitalo, G.M., Fadely, B.S., Pitcher, K.W., 2016. Organochlorine contaminant concentrations in multiple tissues of free-ranging Steller sea lions (*Eumetopias jubatus*) in Alaska. *Sci. Total Environ.* 542, 441–452. <https://doi.org/10.1016/j.scitotenv.2015.10.119>.
- Blasius, M.E., Goodmanlowe, G.D., 2008. Contaminants still high in top-level carnivores in the Southern California Bight: levels of DDT and PCBs in resident and transient pinnipeds. *Mar. Poll. Bull.* 56, 1973–1982. <https://doi.org/10.1016/j.marpolbul.2008.08.011>.
- Bonner, W.N., 1984. Lactation strategies in pinnipeds: problems for a marine mammalian group. *Symp. Zool. Soc. Lond.* 51, 253–272.
- Borrell, A., Garcia-Solà, A., Aguilar, A., García, N., Crespo, E., 2010. Organochlorine residues in South American sea lions, *Otaria flavescens* (Shaw, 1800): bioaccumulation and time trends. *Bull. Environ. Contam. Toxicol.* 84, 731–737. <https://doi.org/10.1007/s00128-010-0025-x>.
- Bowen, W.D., Iverson, S.J., 1998. Estimation of total body water in pinnipeds using hydrogen-isotope dilution. *Physiol. Zool.* 71, 329–332. <https://doi.org/10.1086/515921>.
- Brandon, E.A.A., Calkins, D.G., Davis, R.W., 2005. Neonatal growth of Steller sea lion (*Eumetopias jubatus*) pups in Alaska. *Fish. Bull.* 103, 246–257.
- Burnham, K.P., Anderson, D., 2003. Model Selection and Multi-model Inference. Springer-Verlag, New York <https://doi.org/10.1007/b97636>.
- Castellini, M.A., Calkins, D.G., 1993. Mass estimates using body morphology in Steller sea lions. *Mar. Mamm. Sci.* 9, 48–54. <https://doi.org/10.1111/j.1748-7692.1993.tb00425.x>.
- Champagne, C.D., Crocker, D.E., Fowler, M., Houser, D.S., 2012. Fasting physiology of the pinnipeds: the challenges of fasting while maintaining high energy expenditure and nutrient delivery for lactation. In: McCue, M. (Ed.), *Comparative Physiology of Fasting, Starvation, and Food Limitation*. Springer, Berlin, Germany, pp. 309–336. https://doi.org/10.1007/978-3-642-29056-5_19.
- Cipro, C.V., Bustamante, P., Taniguchi, S., Montone, R.C., 2012. Persistent organic pollutants and stable isotopes in pinnipeds from King George Island, Antarctica. *Mar. Poll. Bull.* 64, 2650–2655. <https://doi.org/10.1016/j.marpolbul.2012.10.012>.
- Costa, D., 1987. Isotopic methods for quantifying material and energy intake of free-ranging marine mammals. *Approaches to Marine Mammal Energetics*. vol. 1, pp. 43–66.
- Costa, D.P., 1991. Reproductive and foraging energetic of pinnipeds: implications for life-history patterns. In: Renouf, D. (Ed.), *Behavior of Pinnipeds*. Chapman & Hall, London, pp. 300–344. https://doi.org/10.1007/978-94-011-3100-1_6.
- deBrito, A.P., Ueno, D., Takahashi, S., Tanabe, S., 2002. Organochlorine and butyltin residues in walleye pollock (*Theragra chalcogramma*) from Bering Sea, Gulf of Alaska and Japan Sea. *Chemosphere* 46, 401–411.
- DeMaster, D., Trites, A.W., Clapham, P., Mizroch, S.A., Wade, P., Small, R.J., Hoef, J.V., 2006. The sequential megafaunal collapse hypothesis: testing with existing data. *Prog. Oceanogr.* 68, 329–342. <https://doi.org/10.1016/j.pcean.2006.02.007>.
- Fadely, B.S., Robson, B.W., Sterling, J.T., Greig, A., Call, K.A., 2005. Immature Steller sea lion (*Eumetopias jubatus*) dive activity in relation to habitat features of the eastern Aleutian Islands. *Fish. Oceanogr.* 14, 243–258. <https://doi.org/10.1111/j.1365-2419.2005.00379.x>.
- Fritz, L.W., Towell, R., Gelatt, T.S., Johnson, D.S., Loughlin, T.R., 2014. Recent increases in survival of western Steller sea lions in Alaska and implications for recovery. *Endang. Spec. Res.* 26, 13–24. <https://doi.org/10.3354/esr00634>.
- Greig, D.J., Ylitalo, G.M., Hall, A.J., Fauquier, D.A., Gulland, F.M.D., 2007. Transplacental transfer of organochlorines in California sea lions (*Zalophus californianus*). *Environ. Toxicol. Chem.* 26, 37–44. <https://doi.org/10.1897/05-609r.1>.
- Guénette, S., Heymans, S.J., Christensen, V., Trites, A.W., 2006. Ecosystem models show combined effects of fishing, predation, competition, and ocean productivity on Steller sea lions (*Eumetopias jubatus*) in Alaska. *Can. J. Fish. Aquatic Sci.* 63, 2495–2517. <https://doi.org/10.1139/f06-136>.
- Hall, A., Gulland, F., Ylitalo, G., Greig, D., Lowenstine, L., 2008. Changes in blubber contaminant concentrations in California sea lions (*Zalophus californianus*) associated with weight loss and gain during rehabilitation. *Environ. Sci. Technol.* 42, 4181–4187. <https://doi.org/10.1021/es702685p>.
- Hardell, S., Tilander, H., Welfinger-Smith, G., Burger, J., Carpenter, D.O., 2010. Levels of polychlorinated biphenyls (PCBs) and three organochlorine pesticides in fish from the Aleutian Islands of Alaska. *PLoS One* 5, e12396.
- Hastings, K., Jemison, L., Gelatt, T., Laake, J., Pendleton, G., King, J., Trites, A., Pitcher, K., 2011. Cohort effects and spatial variation in age-specific survival of Steller sea lions from southeastern Alaska. *Ecosphere* 2, art111. <https://doi.org/10.1890/es11-00215.1>.
- Heath, R.B., DeLong, R., Jameson, V., Bradley, D., Spraker, T., 1997. Isoflurane anesthesia in free ranging sea lion pups. *J. Wildl. Dis.* 33, 206–210. <https://doi.org/10.7589/0090-3558-33.2.206>.
- Hickie, B.E., Ross, P.S., Macdonald, R.W., Ford, J.K., 2007. Killer whales (*Orcinus orca*) face protracted health risks associated with lifetime exposure to PCBs. *Environ. Sci. Technol.* 41, 6613–6619.
- Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous inference in general parametric models. *Biom. J.* 50, 346–363.
- Hui, T.C., Gryba, R., Greg, E.J., Trites, A.W., 2015. Assessment of competition between fisheries and Steller sea lions in Alaska based on estimated prey biomass, fisheries removals and predator foraging behaviour. *PLoS One* 10. <https://doi.org/10.1371/journal.pone.0123786>.
- Hurvich, C.M., Tsai, C.-L., 1989. Regression and time series model selection in small samples. *Biometrika* 76, 297–307. <https://doi.org/10.1093/biomet/76.2.297>.
- Iwata, H., Watanabe, M., Okajima, Y., Tanabe, S., Amano, M., Miyazaki, N., Petrov, E.A., 2004. Toxicokinetics of PCDD, PCDF, and coplanar PCB congeners in Baikal seals, *Pusa sibirica*: age-related accumulation, maternal transfer, and hepatic sequestration. *Environ. Sci. Technol.* 38, 3505–3513. <https://doi.org/10.1021/es035461+>.
- Kajiwara, N., Kannan, K., Muraoka, M., Watanabe, M., Takahashi, S., Gulland, F., Olsen, H., Blankenship, A.L., Jones, P.D., Tanabe, S., 2001. Organochlorine pesticides, polychlorinated biphenyls, and butyltin compounds in blubber and livers of stranded California sea lions, elephant seals, and harbor seals from coastal California, USA. *Archives of Environ. Contamin. Toxicol.* 41, 90–99. <https://doi.org/10.1007/s002440010224>.
- Kannan, K., Blankenship, A.L., Jones, P.D., Giesy, J.P., 2000. Toxicity reference values for the toxic effects of polychlorinated biphenyls to aquatic mammals. *Human Ecol. Risk Assess.* 6, 181–201. <https://doi.org/10.1080/1080703009124491>.
- Keogh, M.J., Atkinson, S., Maniscalco, J.M., 2013. Body condition and endocrine profiles of Steller sea lion (*Eumetopias jubatus*) pups during the early postnatal period. *Gen. Comp. Endocrinol.* 184, 42–50. <https://doi.org/10.1016/j.ygcen.2012.12.016>.
- Keogh, M.J., Taras, B.D., Eischens, C., Kennish, J.M., Fadely, B.S., Rea, L.D., 2018. Variation in milk, serum, and blubber fatty acids in young, free-ranging Steller sea lions. *Mar. Mamm. Sci.* <https://doi.org/10.1111/mms.12574>.
- Kim, G.B., Lee, J.S., Tanabe, S., Iwata, H., Tatsukawa, R., Shimazaki, K., 1996. Specific accumulation and distribution of butyltin compounds in various organs and tissues of the Steller sea lion (*Eumetopias jubatus*): comparison with organochlorine accumulation pattern. *Mar. Poll. Bull.* 32, 558–563. [https://doi.org/10.1016/0025-326X\(96\)84576-0](https://doi.org/10.1016/0025-326X(96)84576-0).
- King, J.C., Gelatt, T.S., Pitcher, K., Pendleton, G.W., 2007. A field-based method for estimating age in free-ranging Steller sea lions (*Eumetopias jubatus*) less than twenty-four months of age. *Mar. Mamm. Sci.* 23, 262–271. <https://doi.org/10.1111/j.1748-7692.2007.00108.x>.
- Krahn, M.M., Ylitalo, G.M., Buzitis, J., Sloan, C.A., Boyd, D.T., Chan, S.-L., Varanasi, U., 1994. Screening for planar chlorobiphenyl congeners in tissues of marine biota by high-performance liquid chromatography with photodiode array detection. *Chemosphere* 29, 117–139. [https://doi.org/10.1016/0045-6535\(94\)90095-7](https://doi.org/10.1016/0045-6535(94)90095-7).
- Krahn, M.M., Ylitalo, G.M., Burrows, D.G., Calambokidis, J., Moore, S.E., Goshio, M., Gearin, P., Plesha, P.D., Brownell Jr., R.L., Tilbury, K.L., Rowles, T., Stein, J.E., 2001. Organochlorine contaminant concentrations and lipid profiles in eastern North Pacific gray whales (*Eschrichtius robustus*). *J. Ceta. Res. Manag.* 3, 19–30.
- Krahn, M.M., Hanson, M.B., Schorr, G.S., Emmons, C.K., Burrows, D.G., Bolton, J.L., Baird, R.W., Ylitalo, G.M., 2009. Effects of age, sex and reproductive status on persistent organic pollutant concentrations in “southern resident” killer whales. *Mar. Poll. Bull.* 58, 1522–1529.
- Kubo, K., Yamaguchi, K., Ishinazaka, T., Yamada, W., Hattori, K., Tanaka, S., 2014. Maternal-to-fetal transfer and concentration profiles of PCB congeners for Steller sea lions (*Eumetopias jubatus*) from Hokkaido, Japan. *Mar. Poll. Bull.* 78, 165–172. <https://doi.org/10.1016/j.marpolbul.2013.10.047>.
- Lee, J.S., Tanabe, S., Umino, H., Tatsukawa, R., Loughlin, T.R., Calkins, D.C., 1996. Persistent organochlorines in Steller sea lion (*Eumetopias jubatus*) from the bulk of Alaska and the Bering Sea, 1976–1981. *Mar. Poll. Bull.*, 535–544. [https://doi.org/10.1016/0025-326X\(96\)84572-3](https://doi.org/10.1016/0025-326X(96)84572-3).
- Levin, M., Morsey, B., DeGuise, S., 2007. Modulation of the respiratory burst by organochlorine mixtures in marine mammals, humans, and mice. *J. Toxic. Environ. Health A* 70, 73–83.
- Lian, M., Johnson, S., Gelatt, T., O'Hara, T.M., Beckmen, K., Rea, L.D., 2018. Field anesthesia of juvenile Steller sea lions (*Eumetopias jubatus*) captured using an underwater capture technique. *Mar. Mamm. Sci.* 34, 125–135. <https://doi.org/10.1111/mms.12445>.
- Liwanag, H.E., Berta, A., Costa, D.P., Budge, S.M., Williams, T.M., 2012. Morphological and thermal properties of mammalian insulation: the evolutionary transition to blubber in pinnipeds. *Biolog. J. Linn. Soc.* 107, 774–787. <https://doi.org/10.1111/j.1095-8312.2012.01992.x>.
- Loughlin, T.R., Castellini, M.A., Ylitalo, G., 2002. Spatial aspects of organochlorine contamination in northern fur seal tissues. *Mar. Pollut. Bull.* 44, 1024–1034. [https://doi.org/10.1016/S0025-326X\(02\)00149-2](https://doi.org/10.1016/S0025-326X(02)00149-2).
- Maniscalco, J.M., 2014. The effects of birth weight and maternal care on survival of juvenile Steller sea lions (*Eumetopias jubatus*). *PLoS One* 9, e96328. <https://doi.org/10.1371/journal.pone.0096328>.
- Maniscalco, J.M., Parker, P., Atkinson, S., 2006. Interseasonal and interannual measures of maternal care among individual Steller sea lions (*Eumetopias jubatus*). *J. Mammal.* 87, 304–311. <https://doi.org/10.1644/05-mamm-a-163r2.1>.
- Merrick, R.L., Loughlin, T.R., 1997. Foraging behavior of adult female and young-of-the-year Steller sea lions in Alaskan waters. *Can. J. Zool.* 75, 776. <https://doi.org/10.1139/z97-099>.
- Mori, C., Morsey, B., Levin, M., Nambiar, P.R., Guise, S.D., 2006. Immunomodulatory effects of in vitro exposure to organochlorines on T-cell proliferation in marine mammals and mice. *J. Toxic. Environ. Health A* 69, 283–302.
- Mos, L., Cameron, M., Jeffries, S.J., Koop, B.F., Ross, P.S., 2010. Risk-based analysis of polychlorinated biphenyl toxicity in harbor seals. *Integr. Environ. Assess. Manag.* 6, 631–640.

- Muir, D., Savinova, T., Savinov, V., Alexeeva, L., Potelov, V., Svetoch, V., 2003. Bioaccumulation of PCBs and chlorinated pesticides in seals, fishes and invertebrates from the White Sea, Russia. *Sci. Total Environ.* 306, 111–131. [https://doi.org/10.1016/S0048-9697\(02\)00488-6](https://doi.org/10.1016/S0048-9697(02)00488-6).
- Muir, D.C.G., Norstrom, R.J., Simon, M., 1988. Organochlorine contaminants in arctic marine food chains: accumulation of specific polychlorinated biphenyls and chlordanes-related compounds. *Environ. Sci. Technol.* 22, 1071–1079. <https://doi.org/10.1021/es00174a012>.
- Murphy, S., Barber, J.L., Learmonth, J.A., Read, F.L., Deaville, R., Perkins, M.W., Brownlow, A., Davison, N., Penrose, R., Pierce, G.J., 2015. Reproductive failure in UK harbour porpoises *Phocoena phocoena*: legacy of pollutant exposure? *PLoS One* 10, e0131085. <https://doi.org/10.1371/journal.pone.0131085>.
- Myers, M.J., Atkinson, S., 2012. Temporal variability in organochlorine contamination in blood and blubber of captive Steller sea lions (*Eumetopias jubatus*). *Mar. Mamm. Sci.* 28, 105–123. <https://doi.org/10.1111/j.1748-7692.2011.00466.x>.
- Myers, M.J., Litz, B., Atkinson, S., 2010. The effects of age, sex, season and geographic region on circulating serum cortisol concentrations in threatened and endangered Steller sea lions (*Eumetopias jubatus*). *Gen. Comp. Endocrinol.* 165, 72–77.
- National Marine Fisheries Service (NMFS), 2008. Recovery Plan for the Steller Sea Lion (*Eumetopias jubatus*). Revision. National Marine Fisheries Service, Silver Spring, MD, p. 325.
- National Marine Fisheries Service (NMFS), 2013. Status Review of the Eastern Distinct Population Segment of Steller Sea Lion (*Eumetopias jubatus*). 144pp + Appendices. Protected Resources Division, Alaska Region, National Marine Fisheries Service, 709 West 9th St, Juneau, Alaska 99802.
- Oftedal, O.T., Boness, D.J., Tedman, R.A., 1987a. The behavior, physiology, and anatomy of lactation in the pinnipedia. In: Genoways, H.H. (Ed.), *Current Mammalogy* https://doi.org/10.1007/978-1-4757-9909-5_6.
- Oftedal, O.T., Iverson, S.J., Boness, D.J., 1987b. Milk and energy intakes of suckling California sea lion *Zalophus californianus* pups in relation to sex, growth, and predicted maintenance requirements. *Physiol. Zool.* 60, 560–575. <https://doi.org/10.1086/physzool.60.5.30156130>.
- Pendleton, G.W., Hastings, K.K., Rea, L.D., Jemison, L.A., O'Corry-Crowe, G.M., Beckmen, K.B., 2016. Short-term survival of Steller sea lion (*Eumetopias jubatus*) pups: investigating the effect of health status on survival. *Mar. Mamm. Sci.* 32, 931–944. <https://doi.org/10.1111/mms.12308>.
- Peñín, I., Levin, M., Acevedo-Whitehouse, K., Jasperse, L., Gebhard, E., Gulland, F., De Guise, S., 2018. Effects of polychlorinated biphenyls (PCB) on California sea lion (*Zalophus californianus*) lymphocyte functions upon in vitro exposure. *Environ. Res.* 167, 708–717.
- Pitcher, K.W., 1986. Variation in blubber thickness of harbor seals in Southern Alaska. *The J. Wildl. Manag.* 50, 463–466. <https://doi.org/10.2307/3801106>.
- Pond, C.M., 1977. The significance of lactation in the evolution of mammals. *Evolution*, 177–199 <https://doi.org/10.1111/j.1558-5646.1977.tb00995.x>.
- R Core Team, 2016. R: A Language and Environment for Statistical Computing. Vienna, Austria. <https://www.R-project.org/>.
- Randhawa, N., Gulland, F., Ylitalo, G.M., DeLong, R., Mazet, J.A., 2015. Sentinel California sea lions provide insight into legacy organochlorine exposure trends and their association with cancer and infectious disease. *One Health* 1, 37–43. <https://doi.org/10.1016/j.onehlt.2015.08.003>.
- Raum-Suryan, K.L., Rehberg, M.J., Pendleton, G.W., Pitcher, K.W., Gelatt, T.S., 2004. Development of dispersal, movement patterns, and haul-out use by pup and juvenile Steller sea lions (*Eumetopias jubatus*) in Alaska. *Marine Mammal Science* 20, 823–850. <https://doi.org/10.1111/j.1748-7692.2004.tb01195.x>.
- Rea, L.D., Castellini, J.M., Correa, L., Fadely, B.S., O'Hara, T.M., 2013. Maternal Steller sea lion diets elevate fetal mercury concentrations in an area of population decline. *Sci. Total Environ.* 454, 277–282. <https://doi.org/10.1016/j.scitotenv.2013.02.095>.
- Rea, L.D., Christ, A.M., Hayden, A.B., Stegall, V.K., Farley, S.D., Stricker, C.A., Mellish, J.-A.E., Maniscalco, J.M., Waite, J.N., Burkanov, V.N., Pitcher, K.W., 2015. Age-specific vibrissae growth rates: a tool for determining the timing of ecologically important events in Steller sea lions. *Mar. Mamm. Sci.* 31, 1213–1233. <https://doi.org/10.1111/mms.12221>.
- Rea, L.D., Fadely, B.S., Farley, S.D., Avery, J.P., Dunlap-Harding, W.S., Stegall, V.K., Eischens, C.A., Gelatt, T.S., Pitcher, K.W., 2016. Comparing total body lipid content of young-of-the-year Steller sea lions among regions of contrasting population trends. *Mar. Mamm. Sci.* 32, 1200. <https://doi.org/10.1111/mms.12327>.
- Reijnders, P.J.H., 1986. Reproductive failure in common seals feeding on fish from polluted coastal waters. *Nature (London)* 324, 456–457. <https://doi.org/10.1038/324456a0>.
- Reilly, J.J., Fedak, M.A., 1990. Measurement of the body composition of living gray seals by hydrogen isotope dilution. *J. Appl. Physiol.* 69, 885–891. <https://doi.org/10.1152/jappl.1990.69.3.885>.
- Ross, P., Swart, R.D., Addison, R., Loveren, H.V., Vos, J., Osterhaus, A., 1996. Contaminant-induced immunotoxicity in harbour seals: wildlife at risk? *Toxicol.* 112, 157–169. [https://doi.org/10.1016/0300-483x\(96\)03396-3](https://doi.org/10.1016/0300-483x(96)03396-3).
- Ross, P.S., De Swart, R.L., Reijnders, P., Van Loveren, H., Vos, J.G., Osterhaus, A., 1995. Contaminant-related suppression of delayed-type hypersensitivity and antibody responses in harbor seals fed herring from the Baltic Sea. *Environ. Health Perspect.* 103, 162.
- Ryg, M., Lydersen, C., Markussen, N.H., 1990. Estimating the blubber content of phocid seals. *Can. J. Fish. Aquat. Sci.* 47, 1223–1227. <https://doi.org/10.1139/f90-142>.
- Safe, S., 1990. Polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and related compounds: environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). *CRC Crit. Rev. Toxicol.* 21, 51–88. <https://doi.org/10.3109/10408449009089873>.
- Scherer, R.D., Doll, A.C., Rea, L.D., Christ, A.M., Stricker, C.A., Witteveen, B., Kline, T.C., Kurle, C.M., Wunder, M.B., 2015. Stable isotope values in pup vibrissae reveal geographic variation in diets of gestating Steller sea lions (*Eumetopias jubatus*). *Mar. Ecol. Prog. Ser.* 527, 261–274. <https://doi.org/10.3354/meps11255>.
- Schulz, T.M., Bowen, W.D., 2005. The evolution of lactation strategies in pinnipeds: a phylogenetic analysis. *Ecol. Monogr.* 75, 159–177. <https://doi.org/10.1890/04-0319>.
- Sinclair, E., Zeppelin, T., 2002. Seasonal and spatial differences in the diet in the western stock of Steller sea lions (*Eumetopias jubatus*). *J. Mammal.* 83, 973–990. [https://doi.org/10.1644/1545-1542\(2002\)083<0973:sasdid>2.0.co;2](https://doi.org/10.1644/1545-1542(2002)083<0973:sasdid>2.0.co;2).
- Sinclair, E., Johnson, D., Zeppelin, T., Gelatt, T., 2013. Decadal variation in the diet of Western stock Steller sea lions. U.S. Dep. Commer., NOAA Tech. Memo. NMFS AFSC-248 (67 p.).
- Springer, A.M., Estes, J.A., van Vliet, G.B., Williams, T.M., Doak, D.F., Danner, E.M., Forney, K.A., Pfister, B., 2003. Sequential megafaunal collapse in the North Pacific Ocean: an ongoing legacy of industrial whaling? *PNAS* 100, 12223–12228. <https://doi.org/10.1073/pnas.1635156100>.
- Sugiura, N., 1978. Further analysis of the data by akaike's information criterion and the finite corrections: further analysis of the data by akaike's. *Communications in Statistics-Theory and Methods* 7, 13–26. <https://doi.org/10.1080/03610927808827599>.
- Tollit, D., Fritz, L., Joy, R., Miller, K., Schulze, A., Thomason, J., Walker, W., Zeppelin, T., Gelatt, T., 2017. Diet of endangered Steller sea lions (*Eumetopias jubatus*) in the Aleutian Islands: new insights from DNA detections and bioenergetic reconstructions. *Can. J. Zool.* 95, 853–868. <https://doi.org/10.1139/cjz-2016-0253>.
- Trillmich, F., 1990. The behavioral ecology of maternal effort in fur seals and sea lions. *Behav.* 114, 3–20. <https://doi.org/10.1163/156853990x00022>.
- Trites, A.W., Jonker, R.A.H., 2000. Morphometric measurements and body condition of healthy and starveling Steller sea lion pups (*Eumetopias jubatus*). *Aquat. Mamm.* 26, 151–157.
- Van den Berg, M., Birnbaum, L.S., Denison, M., De Vito, M., Farland, W., Feeley, M., Fiedler, H., Hakansson, H., Hanberg, A., Haws, L., 2006. The 2005 World Health Organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. *Toxicol. Sci.* 93, 223–241.
- Wang, D., Atkinson, S., Hoover-Miller, A., Lee, S.-E., Li, Q.X., 2007. Organochlorines in harbor seal (*Phoca vitulina*) tissues from the northern Gulf of Alaska. *Environ. Poll.* 146, 268–280. <https://doi.org/10.1016/j.envpol.2006.01.050>.
- Wang, D., Shelver, W.L., Atkinson, S., Mellish, J.-A., Li, Q.X., 2010. Tissue distribution of polychlorinated biphenyls and organochlorine pesticides and potential toxicity to Alaskan northern fur seals assessed using PCBs congener specific mode of action schemes. *Arch. Environ. Contam. Toxicol.* 58, 478–488.
- Ylitalo, G.M., Stein, J.E., Hom, T., Johnson, L.L., Tilbury, K.L., Hall, A.J., Rowles, T., Greig, D., Lowenstine, L.J., Gulland, F.M.D., 2005. The role of organochlorines in cancer-associated mortality in California sea lions (*Zalophus californianus*). *Mar. Poll. Bull.* 50, 30–39. <https://doi.org/10.1016/j.marpolbul.2004.08.005>.