



Fasting durations of Steller sea lion pups vary among subpopulations—evidence from two plasma metabolites

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Geographic differences in population growth trends are well-documented in Steller sea lions (*Eumetopias jubatus*), a species of North Pacific pinniped listed under the U.S. Endangered Species Act in 1990 following a marked decline in population abundance that began during the 1970s. As population growth is intrinsically linked to pup production and survival, examining factors related to pup physiological condition provides useful information to management authorities regarding potential drivers of regional differences. During dam foraging trips, pups predictably transition among three fasting phases, distinguished by the changes in the predominant metabolic byproduct. We used standardized ranges of two plasma metabolites (blood urea nitrogen and β -hydroxybutyrate) to assign pups to fasting categories ($n = 1528$, 1990–2016, 12 subpopulations): *Recently Fed–Phase I* (digestion/assimilation–expected hepatic/muscle glycogen usage), *Phase II* (expected lipid utilization), transitioning between *Phases II–III* (expected lipid utilization with increased protein reliance), or *Phase III* (expected protein catabolism). As anticipated, the majority of pups were classified as *Recently Fed–Phase I* (overall mean proportion = 0.72) and few pups as *Phase III* (overall mean proportion = 0.04). By further comparing pups in *Short (Recently Fed–Phase II)* and *Long* (all other pups) duration fasts, we identified three subpopulations with significantly ($P < 0.03$) greater proportions of pups dependent upon endogenous sources of energy for extended periods, during a life stage of somatic growth and development: the 1) central (0.27 ± 0.09) and 2) western (0.36 ± 0.13) Aleutian Island (declining population trend) and 3) southern Southeast Alaska (0.32 ± 0.06 ; increasing population trend) subpopulations had greater *Long* fast proportions than the eastern Aleutian Islands (0.10 ± 0.05 ; stabilized population). Due to contrasting population growth trends among these highlighted subpopulations over the past 50+ years, both density-independent and density-dependent factors likely influence the dam foraging trip duration, contributing to longer fasting durations for pups at some rookeries.

Key words: fasting, metabolites, pinnipeds

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Introduction

Background

As a fasting-adapted species, all age and sex classes of Steller sea lions (SSL; *Eumetopias jubatus*) enter fasting states as part of their annual cycle. Following parturition onshore during the late spring, SSL dams fast on the rookery during the perinatal period while provisioning neonatal pups (Pitcher *et al.*, 2001; Maniscalco *et al.*, 2006; Hastings and Jemison, 2016; Kuhn *et al.*, 2017; Maniscalco and Parker, 2018). After 1–2 weeks (Table 1) females depart to forage, leaving the newborn pup onshore to digest and assimilate the latest meal and likely begin the first fast, depending upon the duration of the dam's foraging trip (Milette and Trites, 2003; Maniscalco *et al.*, 2006; Burkanov *et al.*, 2011). Within a day, the dam typically returns to nurse her newborn pup (Table 1). This alternation between foraging and fasting, for both the dam and her pup, continues for approximately 1 year, and sometimes longer, with some observations of dams concurrently nursing a yearling and newborn (Pitcher and Calkins, 1981; Maniscalco *et al.*, 2006). These elements of the maternal attendance pattern—the perinatal nursing period, foraging trip duration and shore visit duration—influence a pup's growth and development, body condition and subsequent fasting capabilities. To successfully raise a pup through weaning, dams must simultaneously balance the metabolic demands of traveling, foraging and producing an energy-rich milk (Schulz and Bowen, 2004) while minimizing consequences to pup health and growth.

Of these maternal attendance metrics, much effort has been dedicated to understanding variability in dam foraging trip duration (Table 1). Differences in dam age, pup age, rookery, year, local bathymetry and local prey availability have demonstrated significant influence on foraging trip duration (Higgins *et al.*, 1988; Hood and Ono, 1997; Maniscalco *et al.*, 2002; Milette and Trites, 2003; Maniscalco *et al.*, 2006; Burkanov *et al.*, 2011). Field studies designed to compute and compare maternal attendance metrics have contributed greatly to the understanding of a species' life history and physiology. These studies are, however, logistically complicated (NRC, 2003), and in order to be comprehensive, often lengthy and cost-prohibitive. Methods have included relying upon on-site observers throughout the early breeding season (Sandegren, 1970; Higgins *et al.*, 1988; Hood and Ono, 1997; Brandon, 2000; Milette and Trites, 2003; Burkanov *et al.*, 2011), installation and maintenance of remote video

surveillance systems (Maniscalco *et al.*, 2006) and deployment of VHF, satellite and/or video-depth recorder transmitters on adult lactating females (Merrick and Loughlin, 1997; Davis *et al.*, 2006; Rehberg *et al.*, 2009; Olivier *et al.*, 2022) to gather maternal attendance data.

Biomarkers of metabolic status

Blood of young pups on rookeries contain markers indicative of their fundamental metabolic state—feeding, fasting, or starvation (Figure 1). As post-prandial pups transition into a fasting state onshore during the dam's foraging trips, they progress through three distinct fasting phases to maintain metabolic homeostasis by systematically catabolizing endogenous energy sources (Castellini and Rea, 1992; Secor and Carey, 2016), resulting in predictable changes in blood metabolite concentrations, as observed by Rea *et al.* (2000) in young SSL pups under experimental fasting conditions. Plasma-derived blood urea nitrogen concentrations ([BUN]) and plasma β -hydroxybutyrate concentrations ([β -HBA]) are two easily-measurable metabolites produced through the catabolism of proteins and lipids, respectively; the concentrations of BUN and β -HBA are expected to reflect the energy source predominantly utilized by the fasting pup (Secor and Carey, 2016).

Distinctions among fasting phases

The primary macronutrient fueling pup metabolic demands is expected to change with fasting duration and characterizes the three phases of fasting (Figure 1). Following the departure of the dam to forage at-sea, pups digest and assimilate their most recent milk meal. In the absence of additional meals, *Phase I* begins, characterized by the catabolism of readily available carbohydrates (stored as glycogen in liver and muscle; Cherel *et al.*, 1988; Lignot and LeMaho, 2012; Secor and Carey, 2016). Pups who recently were fed or had begun *Phase I* fasting were expected to have elevated plasma [BUN], likely a result of the catabolism of both dietary and endogenous proteins (Rea *et al.*, 2000; Rosen, 2009; Secor and Carey, 2016) and very low plasma [β -HBA] (Robin *et al.*, 1988; Lignot and LeMaho, 2012).

Rea *et al.* (2000) subjected four 6-week old captive SSL pups to a $2\frac{1}{2}$ day fast to assess the changes in metabolites over this period. Within 16 hours onset of this experimental fast, Rea *et al.* (2000) observed a transition of SSL pups into the protein-sparing phase, or *Phase II* of fasting, based on

Table 1: Published maternal attendance data are summarized (perinatal period in days, dam foraging trip duration in hours, and dam shore visit duration in hours) for the Steller sea lion breeding range, ordered northwest to northeast Pacific Ocean

SUBPOPULATION	YEAR(S)	PERINATAL PERIOD (d)	FORAGING TRIP (h)	SHORE VISIT (h)	TECHNIQUE
SEA OF OKHOTSK					
Yamsky	2007	10.3	<i>19.9</i>	<i>28.5</i>	visual ¹
KURIL ISLANDS					
Brat Chirpoev	2005	13.6	<i>8.0</i>	<i>42.1</i>	visual ¹
	2006	10.6	<i>9.2</i>	<i>38.3</i>	visual ¹
	2007	11.8	<i>8.6</i>	<i>37.8</i>	visual ¹
Raykoke	2005	12.4	<i>10.6</i>	<i>24.0</i>	visual ¹
	2006	14.2	<i>21.8</i>	<i>28.8</i>	visual ¹
	2007	13.2	<i>20.1</i>	<i>39.6</i>	visual ¹
Lovushki	2005	11.9	<i>6.5</i>	<i>31.7</i>	visual ¹
	2006	12.8	<i>6.5</i>	<i>19.0</i>	visual ¹
	2007	10.7	<i>7.5</i>	<i>18.2</i>	visual ¹
Antiserov	2008–2009		8.7		VDR ⁹
	2005	10.1	<i>13.2</i>	<i>35.5</i>	visual ¹
	2006	12.1	<i>9.6</i>	<i>40.8</i>	visual ¹
2007	14.4	<i>10.5</i>	<i>24</i>	visual ¹	
KAMCHATKA PENINSULA					
COMMANDER ISLANDS					
Medny	2005	11.7	<i>9.7</i>	<i>22.5</i>	visual ¹
	2006	13.7	<i>6.9</i>	<i>42.8</i>	visual ¹
	2007	17.0	<i>15.2</i>	<i>31.6</i>	visual ¹
WESTERN ALEUTIAN ISLANDS					
CENTRAL ALEUTIAN ISLANDS					
Seguam	1997		7.1	23.5	VHF ²
EASTERN ALEUTIAN ISLANDS					
Ugamak	1985	10.6	26.4	55.2	visual ⁶
	1986	9.0	28.8	52.8	visual ⁶
	1990–93		25	18.0	VHF ⁷
WESTERN GULF OF ALASKA					
CENTRAL GULF OF ALASKA					
Marmot	1990–93		21.0	19.0	VHF ⁷
	1993		11.2	20.7	VHF ²
Sugarloaf	1994–95	9.9	19.5	27.0	visual ⁸
EASTERN GULF OF ALASKA					
Lewis	1967–68	9.0	16.7	17.0	visual ¹¹
Fish	1995		19.4	25.2	VHF ²
Chiswell	2001		15.1	22.3	video ⁵
	2002		11.5	19.9	video ⁵
	2003		19.3	27.5	video ⁵
2004		18.1	22.4	video ⁵	
NORTHERN SOUTHEAST ALASKA					
SOUTHERN SOUTHEAST ALASKA					
Hazy & Forrester	1992–93		20.8	20.8	SAT ¹⁰
Lowrie	1993–94, 1996–97		25.6	22.3	VHF ²
	1994–95	7.9	24.9	22.6	visual ⁸
WASHINGTON & OREGON					
CALIFORNIA					
Año Nuevo	1973	3.2	21.8	20.8	visual ^{4, 1}
	1983	6.7	37.0	22.6	visual ³
	1992	5.8	50.2	20.4	visual ^{4, 1}

Mean values are presented in bold text, while median values are distinguished by italics. Data were obtained through five study techniques: visual observations at rookery (visual), through instrumentation of adult female SSL (VHF, SAT, VDR), or through remote video surveillance (video). Subpopulations shaded in light grey have no published quantified observations of maternal attendance metrics. Studies referenced in table: ¹ Burkanov *et al.*, 2011; ² Brandon, 2000; ³ Higgins *et al.*, 1988; ⁴ Hood and Ono, 1997; ⁵ Maniscalco *et al.*, 2006; ⁶ Merrick *et al.*, 1988; ⁷ Merrick and Loughlin, 1997; ⁸ Milette and Trites, 2003; ⁹ Olivier *et al.*, 2022; ¹⁰ Rehberg *et al.*, 2009; ¹¹ Sandegren, 1970.



Figure 1: Illustration of an animal's consumption states (fed, fasting, starvation) and the phases an animal progresses through during each state. With respect to the current study, changes in the plasma concentrations of blood urea nitrogen ([BUN]) and plasma concentrations of β -hydroxybutyrate ([β -HBA]) are indicated by arrow direction (up = increase, down = decrease in concentrations) and arrow magnitude. Homeostasis is maintained throughout both fed and fasting states; when homeostasis is unable to be maintained, a state of starvation ensues. Synthesized from Le Maho *et al.*, 1981; Cherel *et al.*, 1988; Costa, 1991; Castellini and Rea, 1992; Rea *et al.*, 2000; Verrier *et al.*, 2009; Champagne *et al.*, 2012; Lignot and LeMaho, 2012; Secor and Carey, 2016; Crocker and Champagne, 2018; Rosen and Worthy, 2018.

increased [β -HBA] concurrent to a decreased [BUN]. While ketone bodies can serve as a fuel source for much of the nervous system and some other cells, [BUN] did not decrease below detection limits, as gluconeogenesis likely continued, supporting certain portions of the central nervous system (Castellini and Rea, 1992). In the absence of refeeding, pup adiposity is the factor limiting the duration of *Phase II*. The percent total body lipids (%TBL) increases as pups age; an initial 3.4% mean TBL was observed in neonatal pups (1–5 days old; Davis *et al.*, 2006). A study of slightly older pups (1½–3 months old) showed increased %TBL, but also regional differences among the central and eastern Gulf of Alaska (15.1% TBL), Southeast Alaska (17.5% TBL) and central and eastern Aleutian Islands (21.8% TBL; Rea *et al.*, 2016).

When the captive fasting interval continued for 2½ days, plasma [β -HBA] and plasma [BUN] changed in SSL pups (Rea *et al.*, 2000), suggesting that pups reverted to catabolizing proteins to meet energetic demands prior to exhaustion of available lipid stores—thus entering *Phase III* fasting. During this transition from *Phase II–III*, [BUN] increased and the metabolic byproducts of lipid mobilization began to decrease (Rea *et al.*, 2000). Once pups fully transitioned into *Phase III*, [BUN] increased further and [β -HBA] decreased to levels observed prior to *Phase II* (Rea *et al.*, 2000). During *Phase II*, minimal protein is lost from lean body mass while predominantly lipids are catabolized (Verrier *et al.*, 2009). During *Phase III*, animals can transition from a fasting state into a starvation state that results in the rapid loss of lean body mass (Verrier *et al.*, 2012). While metabolite concentrations

indicating *Phase III* status are rarely observed in free-ranging pinnipeds (Houser and Costa, 2003), an individual pup's departure from *Phase II* and subsequent entry into *Phase III* is expected to have a strong relationship with pre-fasting levels of adiposity (Rea, 1995). Despite the favorable condition of SSL pups in the Rea *et al.* (2000) study (robust and in good body condition prior to the experimental fast; personal communication, Dr L. Rea, University of Alaska Fairbanks, 2020), metabolite concentrations changed over the 60-hour experimental fast suggesting that these pups had transitioned to *Phase III* during this relatively brief period.

There is an important distinction between the state of prolonged fasting and a state of starvation (Figure 1). During all phases of fasting, homeostasis is maintained and essential organ functions are supported (Castellini and Rea, 1992). Fasting, while expected, can only continue for a discrete period, the magnitude of which varies greatly due to the species' adaptive capacity to fasting and an individual's pre-fasting condition (Lignot and LeMaho, 2012). When endogenous energy stores (i.e. muscle and liver glycogen; subcutaneous, visceral and tissue fat; and amino acids from muscle) can no longer maintain homeostasis, starvation commences, potentially resulting in multiple concurrent and cascading organ failures that can progress to irreversible injury and ultimately death. While the state of starvation is unlikely to be reversed in free-ranging pups, *Phase III* can potentially be reversed following refeeding (Lignot and LeMaho, 2012); after significant time in *Phase III*, however, even refeed pups may have a poor prognosis, eventually leading to mortality via starvation (Lignot and LeMaho, 2012;

Rosen and Worthy, 2018). This failure to thrive once fed following a prolonged absence of food is well-documented in human (see reviews in Boateng *et al.*, 2010 and McKnight *et al.*, 2019) and domesticated animal medicine (Chan, 2015; Luethy *et al.*, 2020), as well as in some wildlife (Winn, 2006; Gerlach *et al.*, 2013; Fravel *et al.*, 2016; McRuer and Ingraham, 2019). Indications of refeeding syndrome include a combination of electrolyte imbalances, often characterized by hypophosphatemia, hypomagnesemia and hypokalemia (Crook *et al.*, 2001; Mehanna *et al.*, 2008; Boateng *et al.*, 2010; Crook, 2014). Possible associations with thiamine and other vitamin deficiencies (Crook *et al.*, 2001), as well as trace element deficiencies (e.g. selenium and copper, Boateng *et al.*, 2010) have been suggested as additive and causative agents. Cardiac and renal function are often compromised following a disruption of water balance and associated abnormalities in blood pressure (Crook *et al.*, 2001; Crook, 2014).

Study importance

This study sought to investigate the variability of the fasting status of SSL pups born in different subpopulations, especially within discrete portions of their western range in the U.S., where the population abundance continues to decline (Holmes *et al.*, 2007; Fritz *et al.*, 2014; Altukhov *et al.*, 2015). During the last quarter of the 20th century, the detection of a precipitous decline in SSL population abundance prompted listing the species under the U.S. Endangered Species Act in 1990 (U.S. legal protections well-summarized in NRC, 2003). In 1997, two Distinct Population Segments (DPS) were delineated within the U.S. SSL range: the 1) western (wDPS; listed as endangered) and 2) eastern (eDPS; listed as threatened) (NMFS, 2013). Subsequent research and surveys determined that the eDPS SSL population had been steadily increasing for the past 40+ years at ~3% annually and the threatened status of the eDPS was removed in 2013 (NMFS, 2013). The decline observed throughout most of the wDPS ceased over the 1990s, and most subpopulations within the wDPS have been stable to modestly increasing in abundance for approximately the past two to three decades; there are some locations in the westernmost portion of the wDPS range, however, where the population continues to decline.

This study categorized individual pups into a discrete fasting category using concentrations of plasma BUN and plasma β -HBA, applying thresholds developed from Rea *et al.* (2000). We expected to identify pups along the entire fasting continuum illustrated in Figure 1. Due to variations reported in population trends throughout the SSL breeding range, we tested for differences in pup fasting duration at a finer geographic scale, groupings of rookeries commonly used for comparison by management authorities, hereafter referred to as *subpopulations*. We hypothesized that higher proportions of SSL pups with longer fasting durations would be present in subpopulations where population abundance has failed to recover.

Methods

Study area

This study utilized plasma samples collected during routine capture operations of SSL pups, born at 38 natal rookeries within four broad regions including eastern Russia and the Aleutian Islands, Gulf of Alaska and Southeast Alaska within the United States (Figure 2, Table 2, $n = 1528$, captured 1990–2016). Within these four regions, 12 subpopulations were represented in this study, moving eastward across the northern Pacific Ocean: within eastern Russia, the 1) Sea of Okhotsk, 2) Kuril Islands, 3) Kamchatka Peninsula and 4) Commander Islands and within the United States, the 5) western, 6) central, and 7) eastern Aleutian Islands, the 8) western, 9) central, and 10) eastern Gulf of Alaska, and the 11) northern and 12) southern portions of Southeast Alaska (Figure 2).

Sample collection

Free-ranging SSL pups were physically (Brown *et al.*, 1995; Castellini *et al.*, 2012; Keogh *et al.*, 2013) or chemically restrained via gas anesthesia (Heath *et al.*, 1997; Haulena, 2014) at their natal rookeries (see Supplemental data for comparison of handling techniques). Captures within the United States were conducted by the Alaska Department of Fish and Game (ADF&G), National Atmospheric and Oceanic Administration (NOAA) National Marine Mammal Laboratory (NMML), and the Alaska SeaLife Center (ASLC) under authority granted through permits issued by the NOAA National Marine Fisheries Service (Marine Mammal Protection Act Permit #s: 809, 965, 14 325, 14 326, 18 528-00, 15 837-00, 358-1564, 358-1769, 358-1888, 782-1447, 782-1532, 782-1889, 782-1523, 881-1668, 881-18 900-02) and following Institutional Animal Care and Use protocol standards (ADF&G # 03-002, 06-07, 09-28, 2010-1412; ASLC # 07-01; Marine Mammal Laboratory # A/NW2010-4, A/NW 2013-2, A/NW 2016-3). Rookeries in eastern Russia were sampled in 2003–2007 and in 2009 with approval of Russian permitting agencies (Burkanov *et al.*, 2011). Blood samples obtained from the vein of the caudal gluteal plexus were drawn into blood collection tubes containing ethylenediaminetetraacetic acid or sodium heparin anticoagulants. Blood samples were kept chilled in the field until centrifuging (3000–3500 rpm for ten minutes), typically within four hours of collection (Rea *et al.*, 1998; Keogh *et al.*, 2013). Efforts were made to minimize disturbance to SSL on the rookery. Typically, individual pups were directly handled for 10–20 minutes for measurements, sample collection and/or tagging. Total rookery disturbance intervals (researchers' arrival to departure) were typically < 8 hours. The time of blood collection with respect to the onset of rookery disturbance was not available for evaluation for many pups in this study. Plasma aliquots were stored at -20°C during the remainder of the field research trips (3–10 days) and at -80°C thereafter (months to years, maximal interval of 8 years) following return to the laboratory. Plasma samples were loaned to the

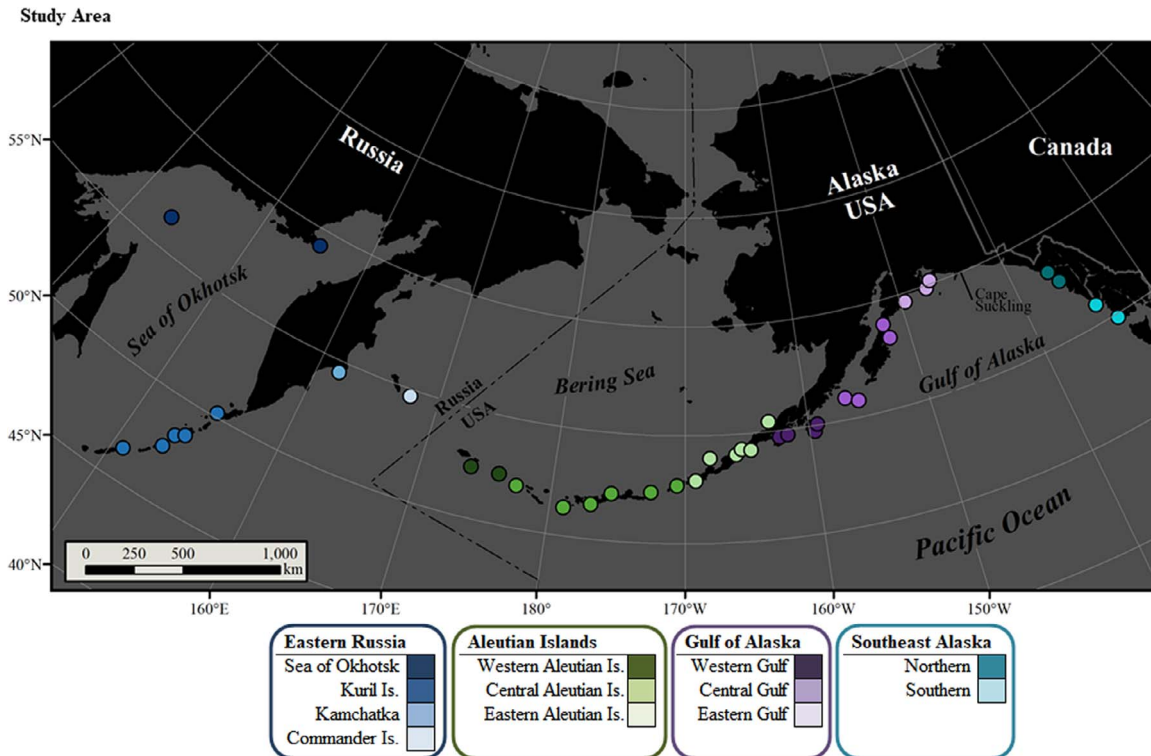


Figure 2: Steller sea lion natal rookeries (dots) sampled in this study. Pups from four regions were included: eastern Russia (blues), Aleutian Islands (greens), Gulf of Alaska (purples), and Southeast Alaska (aquas). The 12 subpopulations represented are color coded in different shades of the regional color. Within the USA, the designation at Cape Suckling separates the western and eastern Distinct Population Segments at 144° longitude.

University of Alaska Fairbanks (UAF) by the permitted agency for use in these analyses. The UAF Office of Research Integrity indicated that an additional Institutional Animal Care and Use protocol was not necessary as this laboratory project utilized archived tissue samples and did not directly involve handling live animals. During field physical examinations, morphometrics (mass, standard length, axillary girth) and sex were recorded for most pups, as well as any observed external abnormalities. Sampling dates ranged between May 26–August 26. Due to the semi-synchronous nature and geographic variation of parturition in SSL, pups on a rookery could have been within six weeks of age of each another (Pitcher *et al.*, 2001; Maniscalco and Parker, 2018).

Metabolite assays

Plasma-derived [BUN] and [β -HBA] were measured via spectrophotometer (SpectraMax 340PC384, Molecular Devices, San Jose, CA) using commercially-available endpoint assay kits: [BUN] via StanBio Kit #2050 and Sigma Aldrich Kit #66–20 and #MAK006; [β -HBA] via StanBio Kit #2440 and Sigma Aldrich Kits #310 and #MAK001 (StanBio, EKF Diagnostics USA, Boerne, TX; Sigma Aldrich, now Millipore Sigma, St. Louis, MO) (Castellini and Costa, 1990; Castellini *et al.*, 1993). Plasma samples with moderate to

severe hemolysis were not included in this study. Technical replicates were $\leq 10\%$ coefficient of variation; assays were repeated for samples exceeding this threshold. Calculations of metabolite concentrations were made using Softmax Pro (v. 4.8) software or the open-access interface *MyAssays.com*, applying a four-parameter logistic curve. Beginning in 2016, a handheld ketometer (Precision Xtra™, Abbott Laboratories, Abbott Park, IL, ketometer precision = 0.1 mmol/L [β -HBA]), was used to identify plasma samples with [β -HBA] above and below a 0.3 mmol/L threshold needed to categorize fasting phase (Crawford *et al.*, In Review). Samples measuring between 0.2–0.4 mmol/L via ketometer were further analysed via the biochemical assay to improve precision around that threshold (assay precision ≈ 0.01 mmol/L [β -HBA]). Earlier investigations in SSL pup fasting did not find predictable variation in concentrations of non-esterified fatty acids (Rea *et al.*, 1998) or glucose over the fasting period (Rea *et al.*, 2000). No other biomarkers were measured in the scope of this study.

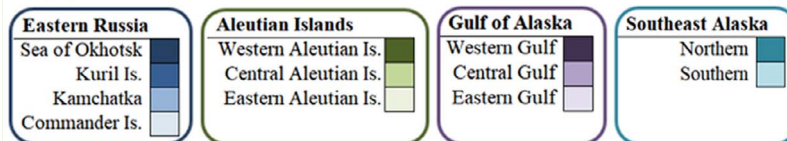
Fasting phase categorization

Thresholds defined for each fasting state were based upon the changes observed in plasma [BUN] and plasma [β -HBA] during an experimental fast imposed upon four captive

Table 2: Annual numbers (n) of plasma samples from individual Steller sea lion pups. Sampling breaks in the sequence of years are indicated by a thick grey line

Birth Year	Region				Annual Subtotal								
	EASTERN RUSSIA		ALEUTIAN ISLANDS			GULF OF ALASKA		SOUTH-EAST					
1990					9				9				
1991					15	9	7			31			
1992					11	7	10		13	41			
1993					17	10	33		19	79			
1994							10		13	23			
1995						19				19			
1996					10	10	12			32			
1997				20	14	11				45			
1998									13	13			
1999								11		11			
2000									12	12			
2001								8		98			
2002				22	36			55		29			
2003		99				22		11	24	64			
2004	61		11	9		25	16	10	40	27			
2005		50				46		8	21	43			
2006	60		29	28						32			
2007		147											
2009		19											
2013					11	9							
2016									12	11			
Subpopulation Subtotal	121	315	40	37	53	84	187	54	132	69	128	308	Study n=1528
Region Total	513		324		255		436						

Subpopulation Legend



The striped area in the northern Southeast Alaska subpopulation represents years (1990–1998) that no samples could exist, as rookeries had not yet been established to create this subpopulation.

six-week old SSL pups (Rea et al., 2000; Table 3). Using these thresholds, one of four fasting categories was assigned to each individual pup: 1) Recently Fed–Phase I fasting (*Fed-I*), 2) Phase II fasting (*II*), 3) transitioning between Phases II and III fasting (*II-III*), 4) Phase III fasting (*III*). Using additional information available for 87 SSL pups, *a priori* expectations of fasting classifications were compared to the classification assigned using metabolite thresholds. This additional information was obtained via field records identifying individuals as neonates, emaciated/starveling/orphaned, skinny/thin, or having milk in their stomachs (sampled via gastric lavage during another study, Beckmen et al., 2016).

Data selection

Quantitative analyses were conducted using open-source software R (R Core Team, 2019). Criteria for inclusion in this

study required a minimum of five pups sampled on each location/date combination to assess the proportion of pups in different fasting phases (Table 2). Previously published results from 16 pups sampled during 1990 and 1991 in the Gulf of Alaska (Castellini et al., 1993) and an additional 220 pups sampled in Southeast Alaska, the Gulf of Alaska and the Aleutian Islands between 1991–1996 (Rea et al., 1998) were included in this study for comparison.

Maniscalco et al. (2006) identified an inflection point in the maternal attendance cycle when dam foraging trip durations significantly increased, occurring around July 19th at the Chiswell rookery (eastern Gulf of Alaska). Before including pups sampled after July 19th of any year into the current study, a Kruskal–Wallis nonparametric one-way ANOVA on ranks (stats R package) was used to compare the proportion of pups categorized as *Fed-I* phase before and after July 19th of

Table 3: Thresholds used to assign fasting phase of Steller sea lion pups, based on changes in concentrations of blood urea nitrogen (BUN) and β -hydroxybutyrate (β -HBA) measured in plasma during a fast imposed upon 6-week old captive Steller sea lions (Rea *et al.*, 2000)

Fasting Phase Category	[BUN] (mmol/L)	[β -HBA] (mmol/L)	Description	Duration Category (binomial model)
Recently Fed / Phase I	<10	<0.3	Digestion and assimilation of milk Carbohydrate stores of glycogen depleted	Short Fast
Phase II	<4.5	≥ 0.3	Transitioned to mobilizing stored lipids (protein sparing phase)	
Transitioning Phase II–III	4.5–9.9	≥ 0.3	Lipid stores largely utilized Protein catabolism recommences	Long Fast
Phase III	≥ 10	<0.3	Lipid stores depleted Protein greatest remaining energy source	
Unclassifiable	≥ 10	≥ 0.3	[β -HBA] elevated indicative of fasting; elevated [BUN] attributed to refeeding or transition to Phase III	

any year for subpopulations where such late summer samples were collected.

Body condition indices

Body condition indices have proven more useful for assessing Otariid condition than individual morphometrics, as SSL are a highly sexually dimorphic species, even distinguishable as young pups (Higgins *et al.*, 1988; Brown *et al.*, 1995; Ono and Boness, 1996). Though there are multiple published calculations of body condition applied to SSL pups, this study used an index detailed by Trites and Jonker (2000):

Body condition index

$$= \left(\frac{\text{body mass (kg)}}{-63.88 + 0.8966 \times \text{dorsal standard length (cm)}} \right).$$

Data analysis

Initial within fasting category comparisons were made at the subpopulation level using Kruskal–Wallis nonparametric tests, followed by a Dunn test for multiple comparisons and using a Bonferroni correction (FSA R package). To identify differences in pup fasting duration among subpopulations, we further classified pups into *Short* or *Long* fast categories. Pups initially characterized as *Fed–I* or *II* were combined into the *Short* category; all other pups were combined into the *Long* category (Table 3). This allowed us to fit a binomial regression to assess differences attributed to pups in *Short* or *Long* fasts with respect to body condition index, sex and/or subpopulation (Warton and Hui, 2011; *stats* R package). Using the categorical response variable of fasting duration (*Short* or *Long*), the global model included the fixed-effects of the body condition index, subpopulation, sex, timing of sampling (before or after July 19) and interactions between variables. The top model was selected using a backward selection process. Statistical significance was identified using $\alpha < 0.05$. Parameter estimates were calculated for the marginal means (95% confidence interval). Tukey contrasts were used to assess differences between each combination of

the 12 subpopulations. Post-hoc Chi-square tests were used to test for differences in sex distribution within fasting phases with respect to subpopulation (*stats* R package).

Statistical comparisons for interannual variability in fasting state across the breeding range, within subpopulation, or within rookery were not made, due to few rookeries with robust, multi-annual sampling regimes (Table 2). Unfortunately, the resulting dataset was discontinuous and unbalanced and not appropriate for statistical analysis as a time series.

Results

Following review of the capture records for pups included in this study, we concluded that the vast majority of pups appeared to be clinically normal as determined by external physical examination. Exceptions are described below in our evaluation of *a priori* expectations. Our comparison of metabolite concentrations in chemically- and physically-restrained SSL pups identified no significant differences attributable to either anesthesia or manual restraint (see Supplemental data).

Our assignment of concentration thresholds of plasma metabolites among categories of fasting were in most cases validated, using a subset of pups with documented conditions (poor body condition, presence of milk in stomach at time of capture; Table 4). Pups noted as emaciated/starveling/orphaned ($n = 10$) were all classified as either *Phase II–III* or *III*, fully meeting our expectations, while three pups described as skinny/thin were categorized as *Fed–I*. Pups identified as neonates were expected to be classified as *Fed–I* ($n = 33$), but only 59% of cases matched expectations. Similarly, only 58% of the pups identified with stomachs containing milk were categorized as *Fed–I* ($n = 27$). Pups observed with dam via video surveillance within 8.2 hours prior to blood sampling met our expectations of *Fed–I* (Figure 4). However, our expectations-based upon the pup metabolite concentrations supporting an entrance into *Phase II* within 16 hours of fasting onset (as

Table 4: Comparison of *a priori* expected fasting phase classifications to outcomes following application of the thresholds (Table 3) for a subset of pups with field records indicating a certain status (n = 87)

Outcomes for <i>a priori</i> Expectations							
Field Note Documentation or Known Status	n	<i>a priori</i> Expectation	Proportions by Fasting Category				Unclassified
			Fed-I	II	II-III	III	
Neonate	33	Fed-I	0.59	0.03	0.25	0.13	n=1
Orphan	5	II-III+			0.40	0.60	
Starving / emaciated	5	II-III+			0.60	0.40	
Skinny / thin	3	II+	1.00				
Milk collected via gastric tube	27	Fed-I	0.58		0.31	0.12	n=1
Minimum # hours since last observed with dam prior to blood sampling (via remote video)							
< 16 hours	9	Fed-I	0.78	0.11	0.11		
16-48 hours	5	II+	0.60	0.40			

observed by Rea *et al.*, 2000)–were not met for the interval 8.2–16 hours. Pups with a last known observation with the dam ranging from 16–48 hours only met expectations in two of five cases (Table 4).

Before applying the binomial model, fasting phase distributions in the three subpopulations with pups sampled post-July 19th of any year were examined. No significant differences in the proportion of pups categorized as *Fed-I* were observed between pups sampled post-July 19th in northern Southeast Alaska (*Fed-I* proportion = 0.70, n = 19) or in the eastern Gulf of Alaska (*Fed-I* proportion = 0.83, n = 12) when compared to all other pre-July 19th samples (*Fed-I* proportion = 0.72, n = 1468). The southern Southeast Alaska subpopulation, however, exhibited a significantly lower proportion of pups in *Fed-I* in this same comparison (*Fed-I* proportion = 0.38, n = 29; Kruskal-Wallis: H = 10.72, P < 0.01). Further comparisons between pups from two southern Southeast Alaska rookeries, Forrester Island Complex and Hazy Island, sampled between June–August indicated that the post-July 19th proportions of *Fed-I* were not significantly different than those observed pre-July 19th within this subpopulation (Kruskal-Wallis: H = 1.62, P = 0.445, Figure 3). Finding no evidence of longer dam foraging in these areas, data for all young pups were included in subsequent analyses.

Of 1528 pups included in this study, only 21 (1.4%) were unable to be categorized into one of the four discrete fasting categories. Each unclassifiable pup had elevated [β -HBA] (indicative of lipid oxidation) and [BUN] (indicative of protein catabolism); we therefore categorized these 21 pups into the *Long* fast category for inclusion in the binomial model only. We observed a mean plasma [BUN] of 5.7 ± 0.07 mmol/L and a mean plasma [β -HBA] of 0.23 ± 0.004 mmol/L for all SSL pups in this study combined (Figure 5).

Within-fasting category comparisons

The majority of pups in each subpopulation were classified as *Fed-I* (Figure 6A); a grand mean proportion of 0.72 within *Fed-I* was observed (n = 1077). The greatest

Recently Fed-Phase I Pups in Southern Southeast Alaska

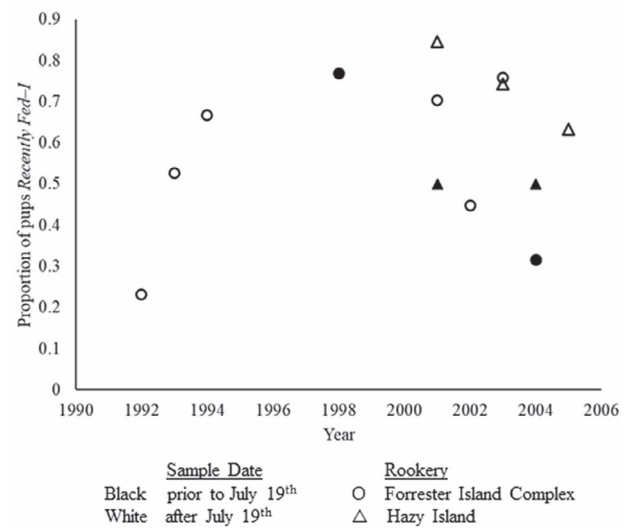


Figure 3: Proportion of Steller sea lion pups categorized as *Recently Fed-Phase I* fasting for plasma samples collected before (open symbols) and after (closed symbols) July 19th at two rookeries in the southern Southeast Alaska subpopulation: Forrester Island complex (circles) and Hazy Island (triangles). In the Gulf of Alaska, July 19th was found to be an inflection point at which adult females began extending the duration of their foraging trips (Maniscalco *et al.*, 2006).

proportion of pups in *Fed-I* was observed in both the Kamchatka Peninsula and western Gulf of Alaska subpopulations (proportion = 0.83), while the lowest proportion of pups observed in *Fed-I* was in the western Aleutian Islands (proportion = 0.57). A significant difference among subpopulations was detected within the *Fed-I* category (Kruskal-Wallis $\chi^2 = 29.502$, df = 11, P = 0.0019); post-hoc tests identified no specific significant differences. No significant differences were identified among subpopulations within *Phase II* of fasting (Kruskal-Wallis $\chi^2 = 16.935$, df = 11, P = 0.1098); the range of proportions was 0.02–0.12 among subpopulations, representing 133 individual

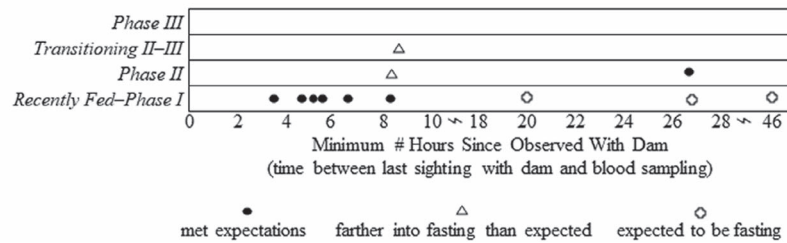
Dam Sightings and *a priori* Expectations

Figure 4: Fasting phase classification of Steller sea lion pups by number of hours since observed with adult female via video camera surveillance at Chiswell Island rookery, eastern Gulf of Alaska in 2016. Black dots indicate classifications for individual pups that met *a priori* predictions (Table 4). Triangles indicate pups classified farther into the fasting process, and those not fasting when a fasting state was expected are indicated by open circles.

SSL pups (Figure 6B). Notably, all subpopulations had higher proportions of pups categorized as transitioning *II-III* ($n = 242$ individual SSL pups) than *Phase II*. Significant differences were detected for pups transitioning between *II-III* (Kruskal-Wallis $\chi^2 = 44.903$, $df = 11$, $P < 0.0001$). While three subpopulations had more than 20% of pups in the transition phase (the central and western Aleutian Islands and southern Southeast Alaska), post-hoc tests revealed that the western Aleutian Islands (Dunn test with Bonferroni adjustments $Z = -3.8538$, $P = 0.0077$) and southern Southeast Alaska (Dunn test with Bonferroni adjustments $Z = -5.2350$, $P < 0.0001$) pups were significantly more prevalent in this transition phase than the eastern Aleutian Islands (Figure 6C). Southern Southeast Alaska also had a higher proportion of pups in *II-III*, when compared to the Kuril Islands (Dunn test with Bonferroni adjustments $Z = -3.7774$, $P = 0.0105$). No significant differences were identified among subpopulations within *Phase III* of fasting (Kruskal-Wallis $\chi^2 = 14.800$, $df = 11$, $P = 0.1919$; Figure 6D). *Phase III* had the lowest proportions of pups observed (4% of total observations, $n = 55$), and two subpopulations in Russia were unrepresented within *Phase III* (Kamchatka Peninsula and Commander Islands).

Binomial model of fasting duration

The most parsimonious model identified both sex ($Z = 2.8987$, $P = 0.0038$, $df = 1524$) and subpopulation ($Z = 3.0409$, $P = 0.0024$, $df = 1524$) as highly significant explanatory variables for fasting duration (Top model, Table 5). The parameter estimates for the proportion of *Long* fasts with 95% confidence intervals for each subpopulation, corrected for sex are reported in Table 6. The parameter estimates within the three Aleutian Island subpopulations included both the highest (western) and lowest (eastern) proportions of *Long* fasting pups, within a relatively small portion of the overall SSL breeding range. Only four of 66 contrasts were highly significant (Tukey, $P < 0.03$ for all four; Figure 7): the eastern Aleutian Island subpopulation had a lower proportion of *Long* fasting pups than the 1) western Aleutian Islands, 2) central Aleutian Islands and 3) southern Southeast Alaska

subpopulations; the Kuril Islands also had a significantly lower proportion of *Long* fasting pups in contrast to the 4) southern Southeast Alaska subpopulation.

While a significantly greater proportion of female pups were categorized into a *Long* fasting durations, no post-hoc comparisons were significant when comparing distribution into fasting phases by subpopulation with respect to the observed sex ratios (Table 7). The overall observed sex ratio was 54♂:46♀. Observed sex ratios within each subpopulation followed the same skew towards more males in all but two cases: a 50:50 sex ratio was observed in the Sea of Okhotsk, and the western Aleutian Islands had more female pups than males (45♂:55♀, Table 7). Though no statistical differences between sexes for *Long* fasting pups by subpopulation were identified, Figure 8 illustrates the magnitude of the variability between the proportions of all males/subpopulation and the proportions of all females/subpopulation categorized into the four fasting categories.

Discussion

Key findings

As expected, the majority of pups in this study and within each subpopulation were categorized as *Fed-I* and having *Short* fasting durations, as the means and medians of the [BUN] and [B-HBA] were relatively low (Figure 5). Consistent with other pinniped fasting studies, very few pups were classified into *Phase III* (Houser and Costa, 2003), as anticipated for a fasting-adapted species that tends to extend *Phase II* (Verrier *et al.*, 2009). Also, as expected, pups were distributed into all categories described across the fasting continuum (Figure 1).

Both the within-fasting category comparisons and the binomial model identified the same three subpopulations with greater proportions of pups in the more prolonged fasting categories: 1) the western and 2) central Aleutian Islands and 3) southern Southeast Alaska subpopulations. These areas of the Aleutian Islands and Southeast Alaska experienced divergent population growth patterns for the past

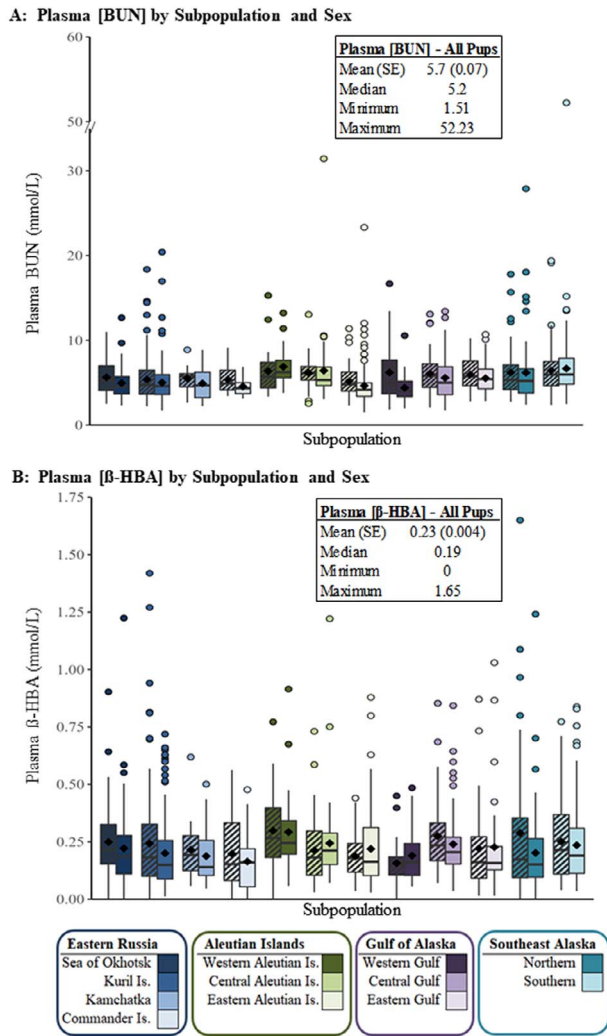


Figure 5: (A) Plasma [BUN] and (B) plasma [β-HBA] for 1528 SSL pups by 12 subpopulations and sex (females striped, male solid bars). The black diamond within each boxplot represents the mean observed concentration for that subpopulation and sex.

50 years: SSL populations in Southeast Alaska (eDPS) steadily increased (Allen and Angliss, 2013), while populations in the western Aleutians and portions of the central Aleutians continued to decline (Holmes *et al.*, 2007), suggesting support for both density-dependent and -independent influences on the duration of fasting periods.

Findings from the increasing population in the eDPS support the potential for density-dependent influences in this area. SSL pups from the eDPS had reduced body masses (Brown *et al.*, 1995; Rea *et al.*, 1998; Brandon *et al.*, 2005; Hastings *et al.*, 2020a), and young-of-the-year eDPS SSL had reduced body mass and percent total body lipids (Rea *et al.*, 2016), as compared to the wDPS. The mean dam foraging trip durations in the eDPS were amongst the longest observed

(Brandon, 2000; Milette and Trites, 2003). Pups from eDPS weaned later than those from the wDPS, with exception of pups from the eastern Gulf of Alaska subpopulation (Hastings *et al.*, 2020a). Three new rookeries were established (northern Southeast Alaska subpopulation in our study; Mathews *et al.*, 2011), and more recent genetic investigations have elucidated more complex dispersive movements within the eDPS and between the eDPS and wDPS than was previously understood (Jemison *et al.*, 2013, 2018; Hastings *et al.*, 2020b).

Addressing potentially confounding factors

There are few studies on fasting blood chemistry of free-ranging Otariid pups upon which to compare our approach and findings (Castellini *et al.*, 1993; Rea *et al.*, 1998; Arnould *et al.*, 2001; Verrier *et al.*, 2011, 2012). The majority of the research measuring metabolite concentrations on young pinniped pups has been conducted on Phocids, which exhibit considerable differences in life history as neonates through weaning, complicating comparisons. Phocid pups have a single intensive nursing period prior to weaning (varying between 4 days to 7 weeks among Phocid species); there is not an alternation between fasting and fed states as in Otariid pups (Crocker and McDonald, 2016). Furthermore, we established our thresholds used to assign fasting category in this study, based upon results from the Rea *et al.* (2000) 2½ day experimental fast imposed on four captive 6-week old SSL pups. Plasma samples collected from free-ranging SSL pups are limited to providing a snapshot view of pup metabolite status. Our interpretation of these data is limited as we lack additional information about pups’ prior fasting histories, pre-fasting adiposity, or specific birthdate.

Variations in metabolic analytes may result from physiological responses to other scenarios than fasting. In a clinical setting, a differential diagnosis for SSL pups with elevated [BUN] should minimally include diagnostics for dehydration, infection of *Leptospira* spp., reduced cardiac function, renal function and glomeruli filtration rate, amyloidosis and bleeding within the gastrointestinal tract (Chinnadurai *et al.*, 2008; Colegrove *et al.*, 2009; Field *et al.*, 2018; Lawler *et al.*, 2021). In the absence of fasting, elevated [β-HBA] would likely be associated with chronic pancreatitis or other conditions impacting the function of the pancreas (Colegrove, 2018; Field *et al.*, 2018). A comparison of serum chemistry panels of clinically-normal and -abnormal California sea lion (CSL; *Zalophus californianus*) of all age classes (undergoing rehabilitation at the Marine Mammal Center, Sausalito, California), found serum [BUN] to be significantly lower for CSL diagnosed with parasite infestation or verminous pneumonia and higher in CSL diagnosed with both pneumonia and gastrointestinal infections or septicemia related to peritonitis due to perforated gastric/duodenal ulcers, as compared to the clinically-normal animals (Roletto, 1993); results were not subset with respect to age class. In a study of serum chemistries of both wild and stranded CSL with known morbidities, a marked increase (6.5-fold)

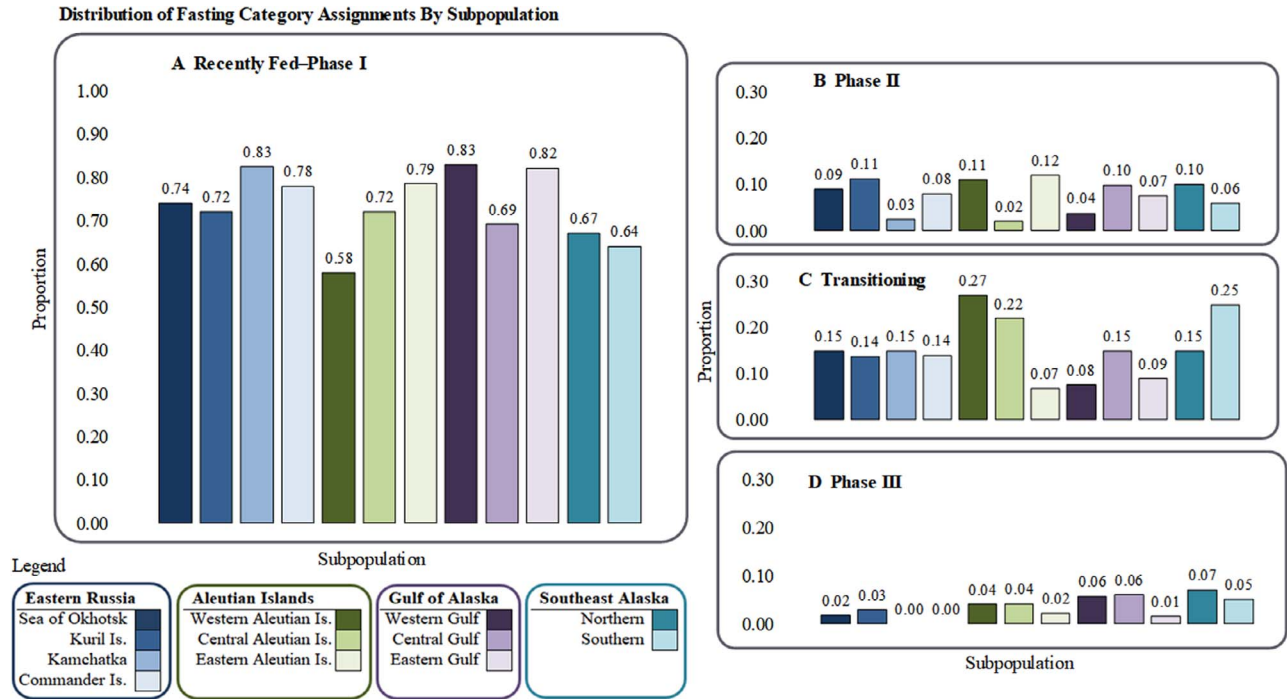


Figure 6: Proportion of pups categorized as (A) Recently Fed/Phase I fasting, (B) Phase II, (C) Transitioning Phase II–III, and (D) Phase III for each subpopulation, organized from the northwestern to northeastern Pacific Ocean. Subpopulations significantly different from others within each fasting category are denoted by lowercase letters. Note, the y-axis in (A, range 0.0–1.0) is different than that shown for (B), (C), and (D) with range 0.0–0.3. Sum of proportions for each subpopulation in (A), (B), (C), and (D) sums to 1.

Table 5: Variables evaluated using backward selection for the binomial model comparing *Short* and *Long* fasts

Binomial Model Selection		
Explanatory Variables		
Model	Additive	Interactive
Full	Subpopulation	Subpopulation*Sex
	Sex	Subpopulation*Body Condition Index
	Timing	Subpopulation*Timing
	Body Condition Index	Sex*Body Condition Index
Top	Metapopulation	
	Sex	

Table 6: Parameter estimates and 95% confidence interval for the mean proportion of pups categorized as *Long* fasting duration by subpopulation from the binomial regression model

Region	Metapopulation	n	Mean	95% Confidence Interval	
				Lower	Upper
Russia	Sea of Okhotsk	121	0.17	0.10	0.23
	Kuril Islands	314	0.17	0.13	0.21
	Kamchatka	40	0.15	0.03	0.27
	Commander Islands	37	0.14	0.02	0.25
Aleutian Islands	Western Aleutian Islands	53	0.36	0.23	0.49
	Central Aleutian Islands	84	0.27	0.18	0.37
Southeast Alaska	Northern Southeast Alaska	128	0.23	0.15	0.30
	Southern Southeast Alaska	307	0.32	0.26	0.37

Table 7: Proportion of ♂ pups by subpopulation and overall, within each fasting of four fasting categories, and classified into *Long* fasting duration

Region	Subpopulation	Sub-population	Proportion of ♂ Pups By Fasting Category				Long Fast Duration
			<i>Fed-I</i>	<i>II</i>	<i>II-III</i>	<i>III</i>	
Russia	Sea of Okhotsk	0.50	0.53	0.55	0.33	0.50	0.35
	Kuril Islands	0.53	0.56	0.43	0.45	0.44	0.45
	Kamchatka Peninsula	0.55	0.55	1.00	0.50	N/A	0.50
	Commander Islands	0.58	0.62	0.67	0.33	N/A	0.33
Aleutian Islands	Western Aleutian Islands	0.45	0.53	0.33	0.40	0.00	0.35
	Central Aleutian Islands	0.56	0.56	0.50	0.50	1.00	0.57
	Eastern Aleutian Islands	0.58	0.54	0.71	0.85	0.50	0.76
Gulf of Alaska	Western Gulf of Alaska	0.55	0.55	0.50	0.75	0.33	0.57
	Central Gulf of Alaska	0.52	0.54	0.69	0.25	0.63	0.36
	Eastern Gulf of Alaska	0.54	0.53	0.60	0.50	1.00	0.57
Southeast Alaska	Northern Southeast Alaska	0.55	0.60	0.54	0.32	0.56	0.39
	Southern Southeast Alaska	0.54	0.57	0.50	0.48	0.50	0.48
All		0.54	0.56	0.58	0.47	0.55	0.46

italicized proportions indicate < 5 pups in fasting category to calculate proportion

italicized values indicate that the proportion was calculated from < 5 individual SSL pups assigned into that category for that subpopulation.

in [BUN] in animals with *Leptospira* infection was identified, but no difference in [BUN] was noted in those diagnosed with malnutrition, pneumonia, or trauma (Williams, 2013). Studies, such as those referenced above, involved wild pinnipeds evaluated in rehabilitation centers, available for additional sample collections and other diagnostic tests to evaluate their condition and further validate the diagnosis.

Significant exposure to *Leptospira* by SSL was not supported by serologic investigations (Burek *et al.*, 2003; 2005). Further, prior studies of SSL pup blood chemistry, hematology and metabolites all support that free-ranging pups maintain adequate hydration (Castellini *et al.*, 1993; Rea *et al.*, 1998; Lander *et al.*, 2013). Fasting-adapted pinnipeds maintain water balance during fasting through water contributions from lipid catabolism (Crocker and Champagne, 2018). While there is some overlap between pups evaluated in the aforementioned SSL studies and the current study, these additional data were not available to be evaluated for all pups with metabolite measurements. The field physical examinations of SSL pups included in this study indicate that nearly all were externally physically normal, with exception of those noted as skinny, thin, emaciated, starving, or orphaned ($n=13$); these individuals were used in our evaluation of fasting phase categories (Table 3). Further, our analysis of a subset of SSL pups included in this study found no differences between pups that were physically and chemically restrained prior to blood sampling (see Supplemental data).

Some plasma samples in this study were archived at -80°C for several years (maximal interval of 8 years) prior to analyses; no available studies assess plasma metabolite stability at this storage temperature and duration. Any degradation of plasma samples that may have occurred during storage

would likely result in reduced concentrations of the two metabolites we evaluated; this would conservatively result in a bias towards classification into *Short* rather than *Long* fasts. The serum [BUN] was found to be stable following storage at -80°C for 28 weeks after collection from CSL (Williams, 2013). Our internal evaluation identified no anticoagulant or freeze-thaw effects on SSL plasma [β -HBA] (Crawford *et al.*, In Press). Other studies evaluating stability of rat or bovine plasma [BUN] or [β -HBA] with respect to anticoagulant (EDTA, heparin), storage time (up to one month), temperature (minimal -40°C) and freeze-thaw events also found no significant changes in metabolite concentration (Stokel and Nydam, 2005; Singh *et al.*, 2014). Additionally, a nuclear magnetic resonance study of bovine plasma samples stored for up to 15 years at -20°C found no significant difference in [BUN] or [β -HBA] (Trabi *et al.*, 2013).

Evaluating the thresholds used in fasting categorization

With respect to our *a priori* expectations of fasting category (Table 4) for the subset of pups ($n=87$) with additional information to inform our hypotheses, we have considered many explanations for the cases that met or failed to meet our expectations. The words used by field biologists and veterinarians to describe more dire pup conditions (i.e. emaciated, starveling, orphaned) were associated with pups that met our *a priori* expectations, reaffirming our interpretation of the field notes. Less specific descriptors (i.e. skinny, thin) were likely applied more subjectively in the field, and our interpretation and expectation of fasting category did not meet the outcomes. We were surprised to find that only 59% of pups identified as neonates were categorized as *Fed-I*; these pups were expected to be with the dam and still within the perinatal

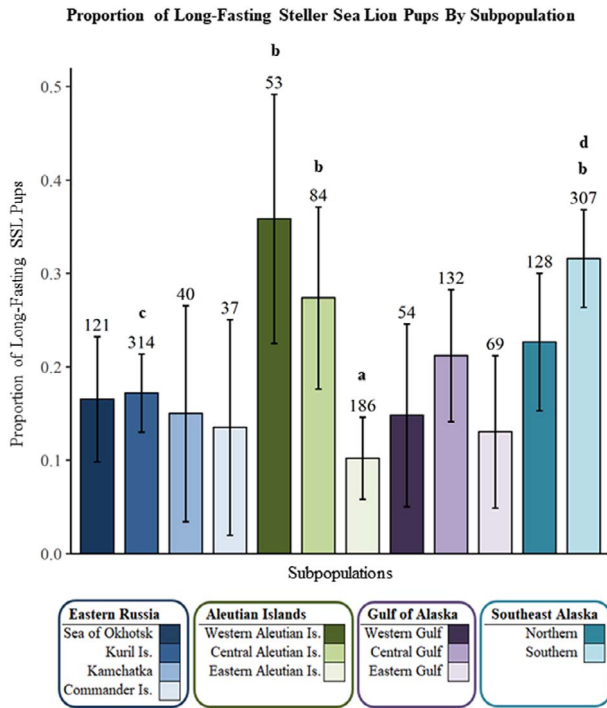


Figure 7: Long Fast duration binomial model parameter estimates by subpopulation, corrected for sex and ordered from northwestern to northeastern Pacific Ocean. Sample sizes are displayed above error bars. Subpopulations labeled “a” were significantly different than those labeled “b”, and those labeled “c” were significantly different from those labeled “d” ($P < 0.03$).

period. Further, we anticipated that the field identification of neonates would be highly accurate, based upon presence and condition of a retained umbilicus. The well-documented variability in the duration of SSL perinatal periods (Table 1), as well as the frequency/volume of feedings during the perinatal period (largely unknown or undocumented) could explain why our expectations were not fully met.

The evaluation of pups with milk in their stomachs at the time of handling illustrates the need for a more detailed understanding of the turnover time for the metabolites we evaluated, especially through the post-prandial into post-absorptive states. Similarly, the video surveillance record indicated that dams and pups were in each other’s proximity, but not specifically that nursing occurred at that time. The dam’s exact time of departure from the rookery was not available for consideration. Additionally dam-pup reunions could have occurred outside of the video frame.

While our expectations were not fully met in comparison to our *a priori* expectations, we found reasonable explanations for the discrepancies and no consistent differences in classification that would indicate that the thresholds utilized to assign fasting category need revision.

Potential drivers for extended foraging trip durations

Our study approach was not designed to identify *causes* of variability in fasting duration, but rather to examine the existence of variability across much of the SSL breeding range. Together with management authorities, we can now investigate the variability detected in pup fasting duration in some subpopulations more thoroughly. As Otariid dams function as central place foragers during lactation, we suggest the simplest proximate explanation for longer pup fasting intervals is an increased duration of dam foraging trips, which further suggests an overall increased foraging effort (foraging effort includes all aspects of foraging–traveling, searching, pursuing, catching, consuming). The ultimate factors, however, that influence foraging duration likely include one or more variables related to local conditions (i.e. prey availability, competition, predation) and/or the health of an individual SSL. Studies of many of these factors exist in the literature as researchers extensively searched for the cause(s) of the wDPS SSL population decline that began in the 1970s (NMFS, 2008). We submit that each of these aspects affecting SSL life history are worthy of consideration; non-lethal and/or sub-clinical interactions may have significant effects on dam foraging behavior, which may manifest as measurable effects to pup health, body condition and viability.

The available prey base composition may vary in species abundance, diversity, distribution and/or quality. Previous pinniped maternal attendance studies have found that dams extended their foraging trips when prey resources were limited or had an atypical geographic distribution (i.e. during El Niño southern oscillations); this phenomenon is well summarized for Otariids by Milette and Trites (2003), Trites and Donnelly (2003), Maniscalco *et al.* (2006) and Burkanov *et al.* (2011). Understanding local trophic structure is complicated by the cascading effects of dynamic oceanographic regimes and climate change which can influence timing of phytoplankton blooms, fish distribution, winter ice extent in adjacent waters and storm frequency and intensity (Benson and Trites, 2002; Peña *et al.*, 2019; Hipfner *et al.*, 2020; Gulland *et al.*, 2022). The nutritional stress hypothesis was suggested as an explanation for the continued population decline in some portions of the SSL range (Calkins and Goodwin, 1988). While studies have produced mixed results, empirical evidence does not support that nutritional stress was a leading cause (see Fritz and Hinckley, 2005; Atkinson *et al.*, 2008; and Trites, 2021 for discussion of nutritional stress hypothesis studies).

Several sympatric piscivores, including other pinnipeds, cetaceans, sharks and seabirds, have dietary overlap with SSL. Conspecific competition may be significant in some portions of the SSL’s range. The impact of commercial fisheries has also been debated as a source of competition to SSL (see NRC, 2003 and Atkinson *et al.*, 2008 for a thorough review of fisheries and related studies on SSL). While some suggest that commercial fisheries were not a significant factor in the initial

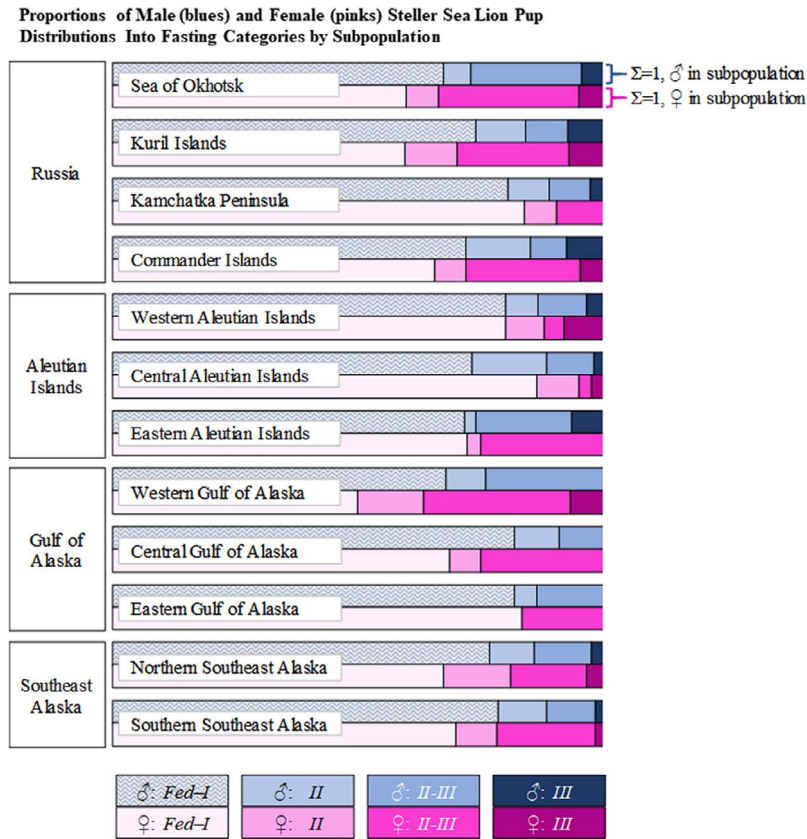


Figure 8: Comparison of the proportions of male (blues) and female (pinks) SSL pups distributed into the four fasting categories by subpopulation (white labels over each pair of rows). The sum of the proportions for each row equals one, standardizing comparisons of the frequency of pups within each fasting category between sexes.

population decline of SSL (Hennen, 2006), others suggest that changes in fish biomass and/or fish size class structure due to commercial fishing have effectively altered local carrying capacity for some meso/apex predators (McHuron *et al.*, 2020).

The presence of predators has a well-known effect of increasing vigilance behaviors which consequently subtract time allocated towards other behaviors, such as foraging (Ripple *et al.*, 2001, 2016; Fortin *et al.*, 2005; Wirsing *et al.*, 2008; Ripple and Beschta, 2012; Suraci *et al.*, 2016; Gaynor *et al.*, 2019; Palmer *et al.*, 2022). Killer whales (*Orcinus orca*) are known predators of SSL (Barrett-Lennard *et al.*, 1995; Saulitis *et al.*, 2000; Heise *et al.*, 2003; Maniscalco *et al.*, 2007; Permyakov and Burkanov, 2009; Dahlheim and White, 2010). Prior energetics studies even modeled the potential for killer whales to be solely responsible for the decline of the wDPS SSL, as part of the hypothesis regarding a large megafauna collapse in the north Pacific Ocean in the last century (Springer *et al.*, 2003, 2008; DeMaster *et al.*, 2006; Estes *et al.*, 2009, 2011, 2016). More recent studies have identified a functional range expansion of killer whales associated with absence of sea ice (Higdon and Ferguson, 2009;

Lefort *et al.*, 2020); increasing availability of suitable habitat and prey resources could result in increased killer whale populations and/or changes in their seasonal distribution adjacent to sea lion rookeries. Fate studies of juvenile SSL and other studies have provided convincing evidence of predation on SSL by Pacific sleeper sharks (*Somniosus pacificus*; Sigler *et al.*, 2006; Horning and Mellish, 2014; Bishop *et al.*, 2019). It is plausible that predatory pressure may have been a significant contributory factor in the decline or lack of recovery of SSL in some subpopulations, both directly and indirectly through increased vigilance and avoidance behaviors, culminating in measurable changes in dam foraging behavior (Wirsing *et al.*, 2008; Palmer *et al.*, 2022).

The impacts of morbidities caused by environmental contaminants are very difficult to demonstrate, quantify and interpret. Organochlorines, heavy metals and plastics have been identified in marine wildlife within the SSL’s range; collectively these contaminants are categorized as neurotoxins, carcinogens and/or endocrine disruptors. Several studies have identified an elevated presence of organochlorines in SSL tissues (Myers *et al.*, 2008; Wang *et al.*, 2011; Beckmen *et al.*, 2016; Keogh *et al.*, 2020). Additionally, higher

proportions of elevated total mercury concentrations have been found in SSL pups (Rea *et al.*, 2020) and some fish (Cyr *et al.*, 2019) within the same discrete portion of the Aleutian Islands (western and central Aleutian Islands) where we identified greater proportions of pups in *Long* duration fasts. A sublethal impact to the acute phase inflammatory response of the immune system was found to be depressed in free-ranging SSL pups with higher total mercury concentrations (Kennedy *et al.*, 2019). Plastics have been observed in sediments, invertebrates and vertebrate species in the subarctic North Pacific (Doyle *et al.*, 2011; Desforges *et al.*, 2015; Mu *et al.*, 2019a, 2019b; Padula *et al.*, 2020; Zhang *et al.*, 2020). Though detailed, controlled studies on morbidities related to varying exposures of these contaminants to SSL or other pinnipeds have not been conducted, sublethal effects of contaminants on behaviors such as foraging should be considered as potential factors influencing dam foraging trip duration. For example, reduced foraging efficiency associated with increased dietary or maternal-derived mercury exposures have been documented across vertebrate species, including fish (Fjeld *et al.*, 1998; Grippo and Heath, 2003; Armstrong *et al.*, 2020), reptiles (Chin *et al.*, 2013) and birds (Adams and Frederick, 2009; Bouton *et al.*, 2009; Swaddle *et al.*, 2017; Grunst *et al.*, 2023).

Breeding female age structure and pup adiposity

Prior studies have shown that maternal attendance factors were influenced by the dam's parity history and age (Maniscalco *et al.*, 2002, 2006; Milete and Trites, 2003; Burkanov *et al.*, 2011; Hastings and Jemison, 2016). Burkanov *et al.* (2011) found that younger dams had perinatal periods of approximately 30% shorter duration than dams > 6 years of age on rookeries in Russia. These studies suggest that dam age, prior experience and condition would be positively correlated with pup condition and would therefore influence pup fasting physiology. Population assessments have considered adults, juveniles and pups separately, but little information is available about the age-structure of breeding females at the subpopulation level.

Pups with greatly reduced adiposity could move in an accelerated fashion through Phases *I* and *II* into the later fasting categories (*II-III*, *III*), resulting in a metabolite profile of a *Long* fasting pup over a shorter elapsed time than expected. Such a scenario could develop following subsequent long fasts, but without prior knowledge of a pup's fasting history and condition, we are unable to evaluate this further. We do expect SSL pup adiposity prior to the first fast to vary as a broad range of perinatal period durations (Table 1) have been observed, ranging from 3.2 days (Año Nuevo, CA in 1973; Hood and Ono, 1997) to 17 days (Medny Island, Russia in 2007; Burkanov *et al.*, 2011). Pup suckling efficiency and stomach volume increase as a pup ages, placing upper limits upon the quantity of milk ingested (Higgins *et al.*, 1988). Female Antarctic fur seal (*Arcto-*

cephalus gazella) pups lost mass at a significantly greater rate than male pups (−2.55% and −2.12%, respectively) during fasting periods; male pups also had significantly higher growth rates, suggesting that at this age, sexual-dimorphism may account for mass-specific rates of decline in body mass during fasting periods (Guinet *et al.*, 1999). Collectively, the variability in these pup characteristics may result in differences in the onset of fasting Phases *II* and *III*.

Biological significance of pup sex differences

The difference noted in fasting duration between sexes was not unexpected for a sexually-dimorphic, polygynous species. Parental investment theory suggests that the increased investment in male offspring is a means to maximizing lifetime fitness (Trillmich, 1996). Consistent with that theory, we found that the presence of female pups was significantly greater in *Long* fasts than male pups, irrespective of subpopulation, suggesting that the energy reserves of a greater proportion of male pups were adequate to sustain the protein conservation phase for the duration of the dam's absence. Male pups of sexually-dimorphic pinnipeds tended to be born larger than females of the same species (within same geographical region), the sex ratio of pups was more-commonly skewed towards males, male pups had a faster growth rates and male pups consumed greater absolute milk volumes (before mass-correction) than female pups (Kerley, 1985; Anderson and Fedak, 1987; Oftedal *et al.*, 1987; Trillmich, 1990; Lunn *et al.*, 1993; Ono and Boness, 1996; Boltnev and York, 2001; Winship *et al.*, 2001; Bradsaw *et al.*, 2003). New Zealand fur seal (*Arctocephalus forsteri*) dams had significantly longer foraging trips when rearing male pups than females (Goldsworthy, 2006). Anderson and Fedak (1987) further calculated that the energetic investment in rearing male grey seal (*Halichoerus grypus*) pups was 10% greater than that of females for the combined gestation and lactation periods.

The sex-ratio trend typical of sexually-dimorphic pinnipeds held true for 10 of 12 SSL subpopulations in our study. The largest deviation from this trend was observed in pups in the western Aleutian Islands, an area where population abundance has not stabilized and continues to decline and where elevated mercury concentrations have been repeatedly documented in young pups, as previously discussed. A female-skewed sex ratio could signal that the extra costs associated with rearing male pups were significant for some SSL dams. Our evaluation of this beyond speculation is limited as 1) the sex ratio of pups we provided was based off of a subsample of the pups born into a subpopulation, and 2) handling events aimed to occur after the peak of the pupping period, but before the pupping period commences such that additional pups were likely born post-handling. The plasticity of these sex-specific differences in maternal investment with respect to environmental conditions is not well understood for SSL. We

suspect that with increased and more balanced sample sizes among subpopulations, a more distinct pattern may emerge.

Conclusions

Plasma analyses used to characterize fasting phase provide valuable insight into the variability in maternal attendance at the subpopulation level. Where archived plasma samples exist, the distribution of fasting phases for a snapshot in time can be created retrospectively where traditional maternal attendance studies were not conducted. We suggest this application of plasma metabolite comparisons, used in combination with other health assessment metrics, is a valuable long-term monitoring tool for examining variability in fasting duration and condition in pups at a given rookery.

While results for the majority of pups from most subpopulations in this study are not a cause for concern, this study highlights three subpopulations where further investigations into maternal attendance and resultant pup condition are warranted. These three subpopulations have had limited to no prior maternal attendance studies conducted: none conducted in the western Aleutian Islands, Seguam Island in the central Aleutian Islands was studied in 1997 and rookeries in southern Southeast Alaska were monitored from 1992 to 1997 (Table 1). Due to vast differences in population growth within the western and central Aleutian Islands and the southern portion of Southeast Alaska, we suspect the elevated proportions of *Long* fasts in these areas are influenced by different drivers. Conspecific competition should be considered as a leading contributing factor in Southeast Alaska, while a density independent factor(s), such as mercury exposure, is more likely within the western and central Aleutian Islands. Broader scale maternal attendance studies at these locations would provide additional information regarding timing of the parturition period at the rookery, perinatal period duration, fasting duration and shore visit frequency and durations, and may aid interpretation of our findings. Although our interpretation of causes of longer fasts is limited from available data on the potential explanatory factors discussed herein, potential conservation implications could result from fasting for extended periods, compromising lean body mass and impeding allocation to somatic growth, development and lipid storage by pups.

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Author Contributions

This study was initiated and conceptualized by L.R. many years ago, and later S.C. expanded and refined the objectives for the project. L.R. and R.C. supervised this project. L.R. and S.C. managed and coordinated the metabolite research. T.G., B.F. and V.B. procured funding to support the field component and provided samples analysed in this project. L.R. and S.C. procured funds for laboratory analyses, and L.R. additionally provided laboratory space, basic laboratory supplies and spectrophotometer for metabolite analyses. The initial methodology, assays and validation of data for the metabolite investigations were developed, directed and conducted by L.R., P.R. and S.C. Demographic and morphometric data utilized by this study were curated by B.F., T.G., V.B. Metabolite data were curated by P.R. and S.C. S.C. further compiled all data types into a relational database and produced the data set used in quantitative analyses. Statistical analyses were conducted through computer code (Program R) produced by S.C., with consultation and supervision by G.B. Metabolite data interpretation leaned on the expertise of R.C., L.R. and S.C. Ecological interpretations were collaboratively reached by S.C., L.R., T.O'H. and G.B. Extensive literature reviews were conducted by S.C. and L.R. S.C. produced the original draft of the manuscript and produced the visualization of the work. Critical reviews and commentary were provided by L.R., R.C., T.O'H., G.B. and B.F. prior to submission as well as in the revision stages.

Conflicts of Interest

The authors declare there are no conflicts of interest. Furthermore, the findings and conclusions in the paper are those of the author(s) and do not necessarily represent the views of the National Marine Fisheries Service. Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

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Data Availability

Data from this project are publicly available through the dryad data repository website: <https://doi.org/10.5061/dryad.66t1g1k76>.

Supplementary Material

Supplementary material is available at *Conservation Physiology* online.

References

- Adams EM, Frederick PC (2009) Effects of methylmercury and spatial complexity on foraging behavior and foraging efficiency in juvenile white ibises (*Eudocimus albus*). *Environ Toxicol Chem* 27: 1708–1712.
- Allen BM, Angliss RP (2013) Alaska marine mammal stock assessments. U.S. Dept Commerce, NOAA Tech Mem:NOAA-TM-AFSC-245: 1–22.
- Altukhov AV, Andrews RD, Calkins DG, Gelatt TS, Gurarie ED, Loughlin TR, Mamaev EG, Nikulin VS, Permyakov PA, Ryazanov SD *et al.* (2015) Age specific survival rates of Steller Sea lions at rookeries with divergent population trends in the Russian Far East. *PLoS One* 10(5): 1–15, e0127292. doi.org/10.1371/journal.pone.0127292.
- Anderson SS, Fedak MA (1987) Grey seal, *Halichoerus grypus*, energetics: females invest more in male offspring. *J Zool Lond* 211: 667–679. <https://doi.org/10.1111/j.1469-7998.1987.tb04478.x>.
- Armstrong BM, Mora-Zamoano FX, Carvan MJ III, McNaught S, Basu N, Head J, Klingler RH, Ivan L, Murphy CA (2020) Exploring the impacts of methylmercury-induced behavioral alterations in larval yellow perch in Lake Michigan using an individual-based model. *T Am Fish Soc* 149: 664–680. <https://doi.org/10.1002/tafs.10263>.
- Arnould JPY, Green JA, Rawlins DR (2001) Fasting metabolism in Antarctic fur seal (*Arctocephalus gazella*) pups. *Comp Biochem Physiol A* 129: 829–841. [https://doi.org/10.1016/S1095-6433\(01\)00339-7](https://doi.org/10.1016/S1095-6433(01)00339-7).
- Atkinson S, DeMaster DP, Calkins DG (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>.
- Barrett-Lennard LG, Heise K, Saulitis E, Ellis G, Matkin C (1995) The impact of killer whale predation on Steller Sea lion populations in British Columbia and Alaska. University of British Columbia, Fisheries Centre, Vancouver, BC, Canada, 2204: 71p.
- Beckmen KB, Keogh MJ, Burek-Huntington KA, Ylitalo GM, Fadely BS, Pitcher KW (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>.
- Benson AJ, Trites AW (2002) Ecological effects of regime shifts in the Bering Sea and eastern North Pacific Ocean. *Fish Fish* 3: 95–113. <https://doi.org/10.1046/j.1467-2979.2002.00078.x>.
- Bishop AM, Dubel AK, Sattler R, Brown CL, Horning M (2019) Wanted dead or alive: characterizing likelihood of juvenile Steller Sea lion predation from diving and space use patterns. *Endanger Species Res* 40: 357–367. <https://doi.org/10.3354/esr00999>.
- Boateng AA, Sriram K, Meguid MM, Crook M (2010) Refeeding syndrome: treatment considerations based on collective analysis of literature case reports. *Nutrition* 26: 156–167. <https://doi.org/10.1016/j.nut.2009.11.017>.
- Boltnev AI, York AE (2001) Maternal investment in northern fur seals (*Callorhinus ursinus*): interrelationships among mothers' age, size, parturition date, offspring size and sex ratios. *J Zool* 254: 219–228. <https://doi.org/10.1017/S0952836901000735>.
- Bouton SN, Frederick PC, Spalding MG, McGill H (2009) Effects of chronic, low concentrations of dietary methylmercury on the behavior of juvenile great egrets. *Environ Toxicol Chem* 18: 1934–1939.
- Bradsaw CJA, Harcourt RG, Davis LS (2003) Male-biased sex ratios in New Zealand fur seal pups relative to environmental variation. *Behav Ecol Sociobiol* 53: 297–307. <https://doi.org/10.1007/s00265-003-0580-9>.
- Brandon EEA (2000) *Maternal Investment in Steller sea lions in Alaska* (Publication No. 10169573) [Ph.D. Dissertation, Texas A&M University, College Station, Texas]. ProQuest Dissertations and Theses Global, Ann Arbor, MI, USA. 137 p.
- Brandon EEA, Calkins DG, Loughlin TR, Davis RW (2005) Neonatal growth of Steller Sea lion (*Eumetopias jubatus*) pups in Alaska. *Fish Bull* 103: 246–257.
- Brown R, Calkins DG, Merrick RL, Loughlin TR (1995) A comparison of Steller Sea lion, *Eumetopias jubatus*, pup masses between rookeries with increasing and decreasing populations. *Fish Bull Natl Oc At* 93: 753–758.
- Burek KA, Gulland FM, Sheffield G, Beckmen KB, Keyes E, Spraker TR, Smith AW, Skilling DE, Evermann JF, Stott JL *et al.* (2005) Infectious disease and the decline of Steller Sea lions (*Eumetopias jubatus*) in Alaska, USA: insights from serologic data. *J Wildlife Dis* 41: 512–524. <https://doi.org/10.7589/0090-3558-41.3.512>.
- Burek KA, Gulland FM, Sheffield G, Keyes E, Spraker TR, Smith AW, Skilling D, Evermann JF, Stott JL, Trites AW (2003) Disease agents in Steller Sea lions in Alaska: a review and analysis of serology data from 1975–2000. *Fisheries Centre Research Reports* 11(4): 26p, University of British Columbia, Vancouver, BC, Canada.
- Burkanov VN, Gurarie ED, Altukhov AV, Mamaev EG, Permyakov PA, Trukhin A, Waite J, Gelatt TA (2011) Environmental and biological factors influencing maternal attendance patterns of Steller Sea lions (*Eumetopias jubatus*) in Russia. *J Mammal* 92: 352–366. <https://doi.org/10.1644/10-MAMM-A-194.1>.
- Calkins DG, Goodwin E (1988) Investigation of the declining sea lion population in the Gulf of Alaska. *Alaska Department of Fish and Game, Marine Mammals Scientific Publication*, Anchorage, Alaska, USA, 88p.

- Castellini JM, Rea LD, Lieske CL, Beckmen KB, Fadely BS, Maniscalco JM, O'Hara TM (2012) Mercury concentrations in hair from neonatal and juvenile Steller Sea lions (*Eumetopias jubatus*): implications based on age and region in this northern Pacific marine sentinel piscivore. *Ecohealth* 9: 267–277. <https://doi.org/10.1007/s10393-012-0784-4>.
- Castellini MA, Costa DP (1990) Relationships between plasma ketones and fasting duration in neonatal elephant seals. *Am J Physiol* 259: R1086–R1089.
- Castellini MA, Davis RW, Loughlin TR, Williams TM (1993) Blood chemistries and body condition of Steller Sea lion pups at Marmot Island, Alaska. *Mar Mamm Sci* 9: 202–208. <https://doi.org/10.1111/j.1748-7692.1993.tb00446.x>.
- Castellini MA, Rea LD (1992) The biochemistry of natural fasting at its limits. *Experientia* 48: 575–582. <https://doi.org/10.1007/BF01920242>.
- Champagne CD, Crocker DE, Fowler MA, Houser DS (2012) Fasting Physiology of the Pinnipeds: The Challenges of Fasting While Maintaining High Energy Expenditure and Nutrient Delivery for Lactation. In M McCue, ed, *Comparative Physiology of Fasting, Starvation, and Food Limitation*. Springer, Berlin, Heidelberg, pp. 309–336.
- Chan DL (2015) Refeeding Syndrome in Small Animals. In DL Chan, ed, *Nutritional Management of Hospitalized Small Animals*. John Wiley & Sons, Chichester, pp. 159–164.
- Cherel Y, Robin J-P, Maho YL (1988) Physiology and biochemistry of long-term fasting in birds. *Can J Zool* 66: 159–166. <https://doi.org/10.1139/z88-022>.
- Chin SY, Willson JD, Cristol DA, Drewett DVV, Hopkins WA (2013) Altered behavior of neonatal watersnakes (*Nerodia sipedon*) exposed to maternally transferred mercury. *Environ Pollut* 176: 144–150. <https://doi.org/10.1016/j.envpol.2013.01.030>.
- Chinnadurai SK, VanWettere A, Linder KE, Harms CA, DeVoe RS (2008) Secondary amyloidosis and renal failure in a captive California Sea lion (*Zalophus californianus*). *J Zoo Wildlife Med* 39: 274–278. <https://doi.org/10.1638/2007-0096R.1>.
- Colegrove KM (2018) Noninfectious Diseases. In FMD Gulland, LA Dierauf, KL Whitman, eds, *CRC Handbook of Marine Mammal Medicine*, Ed3rd. CRC Press, Boca Raton, FL, USA, pp. 267–296
- Colegrove KM, Gulland FMD, Harr K, Naydan DK, Lowenstine LJ (2009) Pathological features of amyloidosis in stranded California Sea lions (*Zalophus californianus*). *J Comp Pathol* 140: 105–112. <https://doi.org/10.1016/j.jcpa.2008.08.003>.
- Costa DP (1991) Reproduction and Foraging Energetics of Pinnipeds: Implications for Life History Patterns. In D Renouf, ed, *The Behavior of Pinnipeds*. Springer, Dordrecht, pp. 300–344.
- Crawford SG, Coker RH, Rea LD (In Review) Assessment of a point-of-care ketometer using Steller Sea lion pup whole blood and plasma. *Conserv Physiol*.
- Crocker DE, Champagne CD (2018) Pinniped Physiology. In B Wursig, JGM Thewissen, KM Kovacs, eds, *Encyclopedia of Marine Mammals*, Ed3rd. Academic Press, London, UK, pp. 725–733.
- Crocker DE, McDonald BI (2016) Post-Partum. In MA Castellini, J-A Mellish, eds, *Marine Mammal Physiology: Requisite for Ocean Living*, Ed1st. CRC Press, Boca Raton, FL, pp. 219–241.
- Crook MA (2014) Refeeding syndrome: problems with definition and management. *Nutrition* 30: 1448–1455. <https://doi.org/10.1016/j.nut.2014.03.026>.
- Crook MA, Hally V, Panteli J (2001) The importance of the refeeding syndrome. *Nutrition* 17: 632–637. [https://doi.org/10.1016/S0899-9007\(01\)00542-1](https://doi.org/10.1016/S0899-9007(01)00542-1).
- Cyr A, Lopez JA, Rea LD, Wooller MJ, Loomis T, McDermott S, O'Hara TM (2019) Mercury concentrations in marine species from the Aleutian Islands: spatial and biological determinants. *Sci Total Environ* 664: 761–770. <https://doi.org/10.1016/j.scitotenv.2019.01.387>.
- Dahlheim ME, White PA (2010) Ecological aspects of transient killer whales *Orcinus orca* as predators in southeastern Alaska. *Wildlife Biol* 16: 308–322. <https://doi.org/10.2981/09-075>.
- Davis RW, Brandon EA, Calkins DG, Loughlin TR (2006) Female Attendance and Neonatal Pup Growth in Steller Sea Lions (*Eumetopias jubatus*). In AW Trites, SK Atkinson, DM DP, LW Fritz, TS Gelatt, LD Rea, KM Wynne, eds, *Sea Lions of the World*. Alaska Sea Grant Publications, Fairbanks, Alaska AK-SG-06-01, pp. 13–29.
- DeMaster DP, Trites AW, Clapham P, Mizroch S, Wade P, Small RJ, Ver Hoef J (2006) The sequential megafaunal collapse hypothesis: testing with existing data. *Prog Oceanogr* 68: 329–342. <https://doi.org/10.1016/j.pocean.2006.02.007>.
- Desforges J-PW, Galbraith M, Ross PS (2015) Ingestion of microplastics by zooplankton in the Northeast Pacific Ocean. *Arch Environ Con Tox* 69: 320–330. <https://doi.org/10.1007/s00244-015-0172-5>.
- Doyle MJ, Watson W, Bowlin NM, Sheavly SB (2011) Plastic particles in coastal pelagic ecosystems of the Northeast Pacific Ocean. *Mar Environ Res* 71: 41–52. <https://doi.org/10.1016/j.marenvres.2010.10.001>.
- Estes JA, Doak D, Springer A, Williams TM (2009) Causes and consequences of marine mammal population declines in Southwest Alaska: a food-web perspective. *Philos T R Soc B* 364: 1647–1658. <https://doi.org/10.1098/rstb.2008.0231>.
- Estes JA, Heithaus M, McCauley DJ, Rasher DB, Worm B (2016) Megafaunal impacts on structure and function of ocean ecosystems. *Ann Rev of Env Res* 41: 83–116. <https://doi.org/10.1146/annurev-environ-110615-085622>.
- Estes JA, Terborgh J, Brashares JS, Power ME, Berger J, Bond WJ, Carpenter SR, Essington TE, Holt RD, Jackson JB et al. (2011) Trophic downgrading of planet earth. *Science* 333: 301–306. <https://doi.org/10.1126/science.1205106>.
- Field CL, Gulland FMD, Johnson SP, Simeone CA, Whoriskey ST (2018) Seal and Sea Lion Medicine. In FMD Gulland, LA Dierauf, KL Whitman,

- eds, *CRC Handbook of Marine Mammal Medicine*, Ed3rd. CRC Press, Boca Raton, FL, USA, pp. 909–934.
- Fjeld E, Haugen TO, Vollestad LA (1998) Permanent impairment in the feeding behavior of grayling (*Thymallus thymallus*) exposed to methylmercury during embryogenesis. *Sci Total Environ* 213: 247–254. [https://doi.org/10.1016/S0048-9697\(98\)00097-7](https://doi.org/10.1016/S0048-9697(98)00097-7).
- Fortin D, Beyer HL, Boyce MS, Smith DW, Duchesne T, Mao JS (2005) Wolves influence elk movements: behavior shapes a trophic cascade in Yellowstone National Park. *Ecology* 86: 1320–1330. <https://doi.org/10.1890/04-0953>.
- Fravel VA, Van Bonn W, Gulland F, Rios C, Fahlman A, Graham JL, Havel PJ (2016) Intraperitoneal dextrose administration as an alternative emergency treatment for hypoglycemic yearling California Sea lions (*Zalophus californianus*). *J Zoo Wildlife Med* 47: 76–82. <https://doi.org/10.1638/2014-0187.1>.
- Fritz LW, Hinckley S (2005) A critical review of the regime shift–“junk food”–nutritional stress hypothesis for the decline of the Western stock of Steller Sea lion. *Mar Mamm Sci* 21: 476–518. <https://doi.org/10.1111/j.1748-7692.2005.tb01245.x>.
- Fritz LW, Towell R, Gelatt TS, Johnson DS, Loughlin TR (2014) Recent increases in survival of western Steller Sea lions in Alaska and implications for recovery. *Endanger Species Res* 26: 13–24. <https://doi.org/10.3354/esr00634>.
- Gaynor KM, Brown JS, Middleton AD, Power ME, Brashares JS (2019) Landscapes of fear: spatial patterns of risk perception and response. *Trends Ecol Evol* 34: 355–368. <https://doi.org/10.1016/j.tree.2019.01.004>.
- Gerlach TJ, Sadler VM, Ball RL (2013) Conservative management of pneumothorax and pneumoperitoneum in two Florida manatees (*Trichechus manatus latirostris*). *J Zoo Wildlife Med* 44: 996–1001. <https://doi.org/10.1638/2012-0136R3.1>.
- Goldsworthy SD (2006) Maternal strategies of the New Zealand fur seal: evidence for interannual variability in provisioning and pup growth strategies. *Aust J Zoo* 54: 31–44. <https://doi.org/10.1071/ZO05041>.
- Grippo MA, Heath AG (2003) The effect of mercury on the feeding behavior of fathead minnows (*Pimephales promelas*). *Ecotox Environ Safe* 55: 187–198. [https://doi.org/10.1016/S0147-6513\(02\)00071-4](https://doi.org/10.1016/S0147-6513(02)00071-4).
- Grunst AS, Grunst ML, Gremillet D, Kato A, Bustamante P, Albert C, Brisson-Curadeau E, Claribaux M, Cruz-Flores M, Gentes S *et al.* (2023) Mercury contamination challenges the behavioral response of keystone species to Arctic climate change. *Environ Sci Technol* 57: 2054–2063. <https://doi.org/10.1021/acs.est.2c08893>.
- Guinet C, Goldsworthy SD, Robinson S (1999) Sex differences in mass loss rate and growth efficiency in Antarctic fur seal (*Arctocephalus gazella*) pups at Macquarie Island. *Behav Ecol Sociobiol* 46: 157–163. <https://doi.org/10.1007/s002650050605>.
- Gulland FMD, Baker JD, Howe M, LaBrecque E, Leach L, Moore SE, Reeves RR, Thomas PO (2022) A review of climate change effects on marine mammals in United States waters: past predictions, observed impacts, current research and conservation imperatives. *Climate Change Ecol* 3: 100054. <https://doi.org/10.1016/j.ecochg.2022.100054>.
- Hastings KK, Jemison LA (2016) Age-specific variation in timing of parturition in Steller Sea lions at Forrester Island complex, Alaska. *Mar Mamm Sci* 32: 777–785. <https://doi.org/10.1111/mms.12288>.
- Hastings KK, Johnson DS, Pendleton GW, Fadely BS, Gelatt TS (2020a) Investigating life-history traits of Steller Sea lions with multistate hidden Markov mark-recapture models: age at weaning and body size effects. *Ecol and Evol* 11: 714–734. <https://doi.org/10.1002/ece3.6878>.
- Hastings KK, Rehberg MJ, O’Corry-Crowe GM, Pendleton GW, Jemison LA, Gelatt TS (2020b) Demographic consequences and characteristics of recent population mixing and colonization in Steller Sea lions. *Eumetopias jubatus J Mamm* 101: 107–120. <https://doi.org/10.1093/jmammal/gyz192>.
- Haulena M (2014) Otariid Seals. In G West, DJ Heard, N Caulkett, eds, *Zoo Animal and Wildlife Immobilization and Anesthesia*, Ed2nd. John Wiley & Sons, Inc., Hoboken, NJ, USA, pp. 661–672
- Heath RB, DeLong R, Jameson V, Bradley D, Spraker T (1997) Isoflurane anesthesia in free ranging sea lion pups. *J Wildlife Dis* 33: 206–210. <https://doi.org/10.7589/0090-3558-33.2.206>.
- Heise K, Barrett-Lennard LG, Saulitis E, Matkin C, Bain D (2003) Examining the evidence for killer whale predation on Steller Sea lions in British Columbia and Alaska. *Aquat Mamm* 29: 325–334. <https://doi.org/10.1578/01675420360736497>.
- Hennen D (2006) Associations between the Alaska Steller Sea lion decline and commercial fisheries. *Ecol Appl* 16: 704–717. [https://doi.org/10.1890/1051-0761\(2006\)016\[0704:ABTASS\]2.0.CO;2](https://doi.org/10.1890/1051-0761(2006)016[0704:ABTASS]2.0.CO;2).
- Higdon JW, Ferguson SH (2009) Loss of Arctic Sea ice causing punctuated change in sightings of killer whales (*Orcinus orca*) over the past century. *Ecol Appl* 19: 1365–1375. <https://doi.org/10.1890/07-1941.1>.
- Higgins LV, Costa D, Huntley A, Le Boeuf BJ (1988) Behavioral and physiological measurements of maternal investment in the Steller Sea lion, *Eumetopias jubatus*. *Mar Mamm Sci* 4: 44–58. <https://doi.org/10.1111/j.1748-7692.1988.tb00181.x>.
- Hipfner JM, Galbraith M, Bertram DF, Green DJ (2020) Basin-scale oceanographic processes, zooplankton community structure, and diet and reproduction of a sentinel North Pacific seabird over a 22-year period. *Prog Oceanogr* 182: 1–11, 102290. <https://doi.org/10.1016/j.pocean.2020.102290>.
- Holmes EE, Fritz LW, York AE, Sweeney K (2007) Age-structured modeling reveals long-term declines in the natality of western Steller Sea lions. *Ecol Appl* 17: 2214–2232. <https://doi.org/10.1890/07-0508.1>.
- Hood WR, Ono KA (1997) Variation in maternal attendance patterns and pup behaviour in a declining population of Steller Sea lions (*Eumetopias jubatus*). *Can J Zool* 75: 1241–1246. <https://doi.org/10.1139/z97-146>.
- Horning M, Mellish J-AE (2014) In cold blood: evidence of Pacific sleeper shark (*Somniosus pacificus*) predation on Steller Sea lions (*Eumetopias*

- jubatus*) in the Gulf of Alaska. *Fish Bull Natl Oc At* 112: 297–310. <https://doi.org/10.7755/FB.112.4.6>.
- Houser DS, Costa DP (2003) Entrance into stage III fasting by starveling northern elephant seal pups. *Mar Mamm Sci* 19: 186–197. <https://doi.org/10.1111/j.1748-7692.2003.tb01101.x>.
- Jemison LA, Pendleton GW, Fritz LW, Hastings KK, Maniscalco JM, Trites AW, Gelatt TS (2013) Inter-population movements of Steller Sea lions in Alaska with implications for population separation. *PLoS One* 8: 1–18, e70167. <https://doi.org/10.1371/journal.pone.0070167>.
- Jemison LA, Pendleton GW, Hastings KK, Maniscalco JM, Fritz LW (2018) Spatial distribution, movements, and geographic range of Steller Sea lions (*Eumetopias jubatus*) in Alaska. *PLoS One* 13: 1–36, e0208093. <https://doi.org/10.1371/journal.pone.0208093>.
- Kennedy SN, Castellini JM, Hayden AB, Fadely BS, Burkanov VN, Dajles A, O'Hara TM, Rea LD (2019) Regional and age-related variations in haptoglobin concentrations in Steller Sea lions (*Eumetopias jubatus*) from Alaska, USA. *J Wildlife Dis* 55: 91–104. <https://doi.org/10.7589/2017-10-257>.
- Keogh MJ, Atkinson S, Maniscalco JM (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 MJ, Taras B, Beckmen KB, Burek-Huntington KA, Ylitalo GM, Fadely BS, Rea LD, Pitcher KW (2020) Organochlorine contaminant concentrations in blubber of young Steller Sea lion (*Eumetopias jubatus*) are influenced by region, age, sex, and lipid stores. *Sci Total Environ* 698: 1–14, 134183. <https://doi.org/10.1016/j.scitotenv.2019.134183>.
- Kerley GIH (1985) Pup growth in the fur seals *Arctocephalus tropicalis* and *A. Gazella* on Marion Island. *J Zool Lond A* 205: 315–324. <https://doi.org/10.1111/j.1469-7998.1985.tb05619.x>.
- Kuhn CE, Chumbley K, Johnson D, Fritz L (2017) A re-examination of the timing of pupping for Steller Sea lions *Eumetopias jubatus* breeding on two islands in Alaska. *Endanger Species Res* 32: 213–222. <https://doi.org/10.3354/esr00796>.
- Lander ME, Fadely BS, Gelatt TS, Rea LD, Loughlin TR (2013) Serum chemistry reference ranges for Steller Sea lion (*Eumetopias jubatus*) pups from Alaska: stock differentiation and comparisons within a North Pacific sentinel species. *Eco Health* 10: 376–393. <https://doi.org/10.1007/s10393-013-0891-x>.
- Lawler DF, Tangredi B, Matassa K, Hunter M, Evans RH (2021) Proximate aspects of starvation-related morbidity and mortality among young California Sea lions (*Zalophus californianus*). *J Vet Anat* 14: 39–56. <https://doi.org/10.21608/jva.2021.197200>.
- Le Maho Y, Vu Van Kha H, Koubi H, Dewasmes G, Girard J, Ferri P, Cagnard M (1981) Body composition, energy expenditure and plasma metabolites in long-term fasting geese. *Am J Physiol* 241: E342–E354. <https://doi.org/10.1152/ajpendo.1981.241.5.E342>.
- Lefort KJ, Matthews CJ, Higdon JW, Petersen SD, Westdal KH, Garroway CJ, Ferguson SH (2020) A review of Canadian Arctic killer whale (*Orcinus orca*) ecology. *Can J Zool* 98: 245–253. <https://doi.org/10.1139/cjz-2019-0207>.
- Lignot J-H, LeMaho Y (2012) A History of Modern Research into Fasting, Starvation, and Inanition. In MD McCue, ed, *Comparative Physiology of Fasting, Starvation, and Food Limitation*. Springer, Berlin Heidelberg, Berlin, pp. 7–23.
- Luethy D, Stefanovski D, Sweeney RW (2020) Refeeding syndrome in small ruminants receiving parenteral nutrition. *J Vet Intern Med* 34: 1674–1679. <https://doi.org/10.1111/jvim.15840>.
- Lunn NJ, Boyd IL, Barton T, Croxall JP (1993) Factors affecting the growth rate and mass at weaning of Antarctic fur seals at Bird Island, South Georgia. *J Mamm* 74: 908–919. <https://doi.org/10.2307/1382429>.
- Maniscalco JM, Atkinson S, Armato P (2002) Early maternal care and pup survival in Steller Sea lions: a remote video monitoring project in the northern Gulf of Alaska. *Arct Res US* 16: 36.
- Maniscalco JM, Matkin CO, Maldini D, Calkins DG, Atkinson S (2007) Assessing killer whale predation on Steller Sea lions from field observations in Kenai fjords, Alaska. *Mar Mamm Sci* 23: 306–321. <https://doi.org/10.1111/j.1748-7692.2007.00103.x>.
- Maniscalco JM, Parker P (2018) Maternal and offspring effects on the timing of parturition in western Steller Sea lions (*Eumetopias jubatus*). *Can J Zool* 96: 333–339. <https://doi.org/10.1139/cjz-2017-0058>.
- Maniscalco JM, 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>.
- Mathews EA, Womble JN, Pendleton GW, Jemison LA, Maniscalco JM, Streveler G (2011) Population growth and colonization of Steller Sea lions in the Glacier Bay region of southeastern Alaska: 1970s–2009. *Mar Mamm Sci* 27: 852–880. <https://doi.org/10.1111/j.1748-7692.2010.00455.x>.
- McHuron EA, Luxa K, Pelland NA, Holsman K, Ream R, Zeppelin T, Sterling JT (2020) Practical application of a bioenergetic model to inform management of a declining fur seal population and their commercially important prey. *Front Mar Sci* 7: 1–17, 597973. <https://doi.org/10.3389/fmars.2020.597973>.
- McKnight CL, Newberry C, Sarav M, Martindale R, Hurt R, Daley B (2019) Refeeding syndrome in the critically ill: a literature review and clinician's guide. *Curr Gastroenterol Rep* 21: 58. <https://doi.org/10.1007/s11894-019-0724-3>.
- McRuer D, Ingraham H (2019) Natural History and Medical Management of Ursids. In SM Hernandez, HW Barron, EA Miller, RF Aguilar, MJ Yabsley, eds, *Medical Management of Wildlife Species: A Guide for Practitioners*, Ed1st. John Wiley & Sons, Inc., Hoboken, NJ, USA, pp. 327–341
- Mehanna HM, Moledina J, Travis J (2008) Refeeding syndrome: what it is, and how to prevent and treat it. *BMJ* 336: 1495–1498. <https://doi.org/10.1136/bmj.a301>.

- Merrick RL, Gearin P, Osmek S, Withrow D (1988) Field studies of northern sea lions at Ugamak Island, Alaska during the 1985 and 1986 breeding seasons. *U.S. Dept Commerce NOAA Technical Memo. NMFS FINWC-143*, 65 p.
- Merrick RL, Loughlin TR (1997) Foraging behavior of adult female and young-of-the-year Steller Sea lions in Alaskan waters. *Can J Zool* 75: 776–786. <https://doi.org/10.1139/z97-099>.
- Milette LL, Trites AW (2003) Maternal attendance patterns of Steller Sea lions (*Eumetopias jubatus*) from stable and declining populations in Alaska. *Can J Zool* 81: 340–348. <https://doi.org/10.1139/z03-008>.
- Mu J, Qu L, Jin F, Zhang S, Fang C, Ma X, Zhang W, Huo C, Cong Y, Wang J (2019a) Abundance and distribution of microplastics in the surface sediments from the northern Bering and Chukchi seas. *Environ Pollut* 245: 122–130. <https://doi.org/10.1016/j.envpol.2018.10.097>.
- Mu J, Zhang S, Qu L, Jin F, Fang C, Ma X, Zhang W, Wang J (2019b) Microplastics abundance and characteristics in surface waters from the Northwest Pacific, the Bering Sea, and the Chukchi Sea. *Mar Pollut Bull* 143: 58–65. <https://doi.org/10.1016/j.marpolbul.2019.04.023>.
- Myers MJ, Ylitalo GM, Krahn MM, Boyd D, Calkins DG, Burkanov VN, Atkinson S (2008) Organochlorine contaminants in endangered Steller Sea lion pups (*Eumetopias jubatus*) from western Alaska and the Russian Far East. *Sci Total Environ* 396: 60–69. <https://doi.org/10.1016/j.scitotenv.2008.02.008>.
- NMFS (2008) Recovery plan for the Steller Sea lion: Eastern and Western Distinct Population Segments (*Eumetopias jubatus*), Revision. *National Marine Fisheries Service Office of Protected Resources, National Oceanic and Atmospheric Administration, Silver Spring, MD, USA*, 325 p.
- NMFS (2013) Endangered and threatened species; delisting of the eastern distinct population segment of Steller Sea lion under the endangered species act; amendment to special protection measures for endangered marine mammals. *Fed Reg* 78(213): 66140–66199. US Dept Comm, Washington, D.C. <https://www.govinfo.gov/content/pkg/FR-2013-11-04/pdf/2013-25261.pdf>
- NRC (2003) *Decline of the Steller Sea Lion in Alaskan Waters: Untangling Food Webs and Fishing Nets*. National Academy of Sciences Press, Washington, D.C., USA, 204 pages.
- Oftedal OT, Iverson SJ, Boness DJ (1987) 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>.
- Olivier P, Andrews R, Burkanov V, Davis RV (2022) Diving behavior, foraging strategies, and energetics of female Steller Sea lions during early lactation. *J Exp Mar Bio* 550: 151707. <https://doi.org/10.1016/j.jembe.2022.151707>.
- Ono KA, Boness DJ (1996) Sexual dimorphism in sea lion pups: differential maternal investment, or sex-specific differences in energy allocation? *Behav Ecol Sociobiol* 38: 31–41. <https://doi.org/10.1007/s002650050214>.
- Padula V, Beaudreau AH, Hagedorn B, Causey D (2020) Plastic-derived contaminants in Aleutian archipelago seabirds with varied foraging strategies. *Mar Pollut Bull* 158: 1–10, 111435. <https://doi.org/10.1016/j.marpolbul.2020.111435>.
- Palmer MS, Gaynor KM, Becker JA, Abraham JO, Mumma MA, Pringle RM (2022) Dynamic landscapes of fear: understanding spatiotemporal risk. *Trends Ecol Evol* 37: 911–925. <https://doi.org/10.1016/j.tree.2022.06.007>.
- Peña MA, Nemcek N, Robert M (2019) Phytoplankton responses to the 2014–2016 warming anomaly in the northeast subarctic Pacific Ocean. *Limnol Oceanogr* 64: 515–525. <https://doi.org/10.1002/lno.11056>.
- Permyakov P, Burkanov VN (2009) Interactions between killer whales (*Orcinus orca*) and Steller Sea lions (*Eumetopias jubatus*) in the vicinity of brat Chirpoev Island, Kuril Islands. *Russ J Mar Biol* 35: 255–258. <https://doi.org/10.1134/S1063074009030109>.
- Pitcher KW, Burkanov VN, Calkins DG, Le Boeuf BJ, Mamaev EG, Merrick RL, Pendleton GW (2001) Spatial and temporal variation in the timing of births of Steller Sea lions. *J Mammal* 82: 1047–1053. [https://doi.org/10.1644/1545-1542\(2001\)082<1047:SATVIT>2.0.CO;2](https://doi.org/10.1644/1545-1542(2001)082<1047:SATVIT>2.0.CO;2).
- Pitcher KW, Calkins DG (1981) Reproductive biology of Steller Sea lions in the Gulf of Alaska. *J Mammal* 62: 599–605. <https://doi.org/10.2307/1380406>.
- R Core Team (2019) *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Rea LD (1995) *Prolonged Fasting in Pinnipeds*. University of Alaska Fairbanks, Fairbanks, Alaska, PhD Dissertation, 153 p.
- Rea LD, Castellini JM, Avery JP, Fadely BS, Burkanov VN, Rehberg MJ, O'Hara TM (2020) Regional variations and drivers of mercury and selenium concentrations in Steller Sea lions. *Sci Total Environ* 744: 1–10, 140787. <https://doi.org/10.1016/j.scitotenv.2020.140787>.
- Rea LD, Castellini MA, Fadely BS, Loughlin TR (1998) Health status of young Alaska Steller Sea lion pups (*Eumetopias jubatus*) as indicated by blood chemistry and hematology. *Comp Biochem Phys A* 120: 617–623. [https://doi.org/10.1016/S1095-6433\(98\)10074-0](https://doi.org/10.1016/S1095-6433(98)10074-0).
- Rea LD, Fadely BS, Farley SD, Avery JP, Dunlap-Harding WS, Stegall VK, Eischens CA, Gelatt TS, Pitcher KW (2016) Comparing total body lipid content of young-of-the-year Steller Sea lions among regions of contrasting population trends. *Mar Mamm Sci* 32: 1200–1218. <https://doi.org/10.1111/mms.12327>.
- Rea LD, Rosen DAS, Trites AW (2000) Metabolic response to fasting in 6-week-old Steller Sea lion pups (*Eumetopias jubatus*). *Can J Zool* 78: 890–894. <https://doi.org/10.1139/z00-007>.
- Rehberg MJ, Andrews RD, Swain UG, Calkins DG (2009) Foraging behavior of adult female Steller Sea lions during the breeding season in Southeast Alaska. *Mar Mamm Sci* 25: 588–604. <https://doi.org/10.1111/j.1748-7692.2008.00278.x>.

- Ripple WJ, Beschta RL (2012) Trophic cascades in Yellowstone: the first 15 years after wolf reintroduction. *Biol Conserv* 145: 205–213. <https://doi.org/10.1016/j.biocon.2011.11.005>.
- Ripple WJ, Estes JA, Schmitz OJ, Constant V, Kaylor MJ, Lenz A, Motley JL, Self KE, Taylor DS, Wolf C (2016) What is a trophic cascade? *Trends Ecol Evol* 31: 842–849. <https://doi.org/10.1016/j.tree.2016.08.010>.
- Ripple WJ, Larsen EJ, Renkin RA, Smith DW (2001) Trophic cascades among wolves, elk and aspen on Yellowstone National Park's northern range. *Biol Conserv* 102: 227–234. [https://doi.org/10.1016/S0006-3207\(01\)00107-0](https://doi.org/10.1016/S0006-3207(01)00107-0).
- Robin J-P, Frain M, Sardet C, Groscolas R, Le Maho Y (1988) Protein and lipid utilization during long-term fasting in emperor penguins. *Am J Physiol-Regl* 23: R61–R68.
- Roletto J (1993) Hematology and serum chemistry values for clinically healthy and sick pinnipeds. *J Zoo Wildlife Med* 24: 145–157.
- Rosen DAS (2009) Steller Sea lions *Eumetopias jubatus* and nutritional stress: evidence from captive studies. *Mammal Rev* 39: 284–306. <https://doi.org/10.1111/j.1365-2907.2009.00150.x>.
- Rosen DAS, Worthy GA (2018) Nutrition and Energetics. In FMD Guland, LA Dierauf, KL Whitman, eds, *CRC Handbook of Marine Mammal Medicine*, Ed3rd. CRC Press, Boca Raton, FL, USA, pp. 695–737.
- Sandegren FE (1970) *Breeding and Maternal Behavior of the Steller Sea Lion (Eumetopias jubatus) in Alaska*. University of Alaska Fairbanks, Fairbanks, AK, MSc Thesis, 138 p.
- Saulitis E, Matkin C, Barrett-Lennard L, Heise K, Ellis G (2000) Foraging strategies of sympatric killer whale (*Orcinus orca*) populations in Prince William sound, Alaska. *Mar Mamm Sci* 16: 94–109. <https://doi.org/10.1111/j.1748-7692.2000.tb00906.x>.
- Schulz TM, Bowen WD (2004) Pinniped lactation strategies: evaluation of data on maternal and offspring life history traits. *Mar Mamm Sci* 20: 86–114. <https://doi.org/10.1111/j.1748-7692.2004.tb01142.x>.
- Secor SM, Carey HV (2016) Integrative physiology of fasting. *Compre Physiol* 6: 773–825.
- Sigler MF, Hulbert LB, Lunsford CR, Thompson N, Burek K, O'Corry-Crowe G, Hirons AC (2006) Diet of Pacific sleeper shark, a potential Steller Sea lion predator, in the Northeast Pacific Ocean. *J Fish Biol* 69: 392–405. <https://doi.org/10.1111/j.1095-8649.2006.01096.x>.
- Singh M, Pandya R, Chandra S, Sharma S (2014) Stability of clinical chemistry and hematological analytes in preserved plasma and blood obtained from Wistar rats. *Scand J Lab Anim Sci* 40: 1–6.
- Springer AM, Estes JA, van Vliet GB, Williams TM, Doak DF, Danner EM, Forney KA, Pfister B (2003) Sequential megafaunal collapse in the North Pacific Ocean: an ongoing legacy of industrial whaling? *P Natl Acad Sci USA* 100: 12223–12228. <https://doi.org/10.1073/pnas.1635156100>.
- Springer AM, Estes JA, van Vliet GB, Williams TM, Doak DF, Danner EM, Pfister B (2008) Mammal-eating killer whales, industrial whaling, and the sequential megafaunal collapse in the North Pacific Ocean: a reply to critics of Springer *et al.* 2003. *Mar Mamm Sci* 24: 414–442. <https://doi.org/10.1111/j.1748-7692.2008.00185.x>.
- Stokel T, Nydam DV (2005) Effect of anticoagulant and storage conditions on bovine nonesterified fatty acid and β -hydroxybutyrate concentrations in blood. *J Dairy Sci* 88: 3139–3144. [https://doi.org/10.3168/jds.S0022-0302\(05\)72996-9](https://doi.org/10.3168/jds.S0022-0302(05)72996-9).
- Suraci JP, Clinchy M, Dill LM, Roberts D, Zanette LY (2016) Fear of large carnivores causes a trophic cascade. *Nat Commun* 7: 1–7. <https://doi.org/10.1038/ncomms10698>.
- Swaddle JP, Diehl TR, Taylor CE, Fanaee AS, Benson JL, Huckstep NR, Cristol DA (2017) Exposure to dietary mercury alters cognition and behavior of zebra finches. *Curr Zool* 63: 213–219. <https://doi.org/10.1093/cz/zox007>.
- Trabi M, Keller MD, Jonsson NN (2013) NMR-based metabonomics of bovine blood: an investigation into the effects of long term storage on plasma samples. *Metabolomics* 9: 1041–1047. <https://doi.org/10.1007/s11306-013-0520-2>.
- Trillmich F (1990) The behavioral ecology of maternal effort in fur seals and sea lions. *Behaviour* 114: 3–20. <https://doi.org/10.1163/156853990X00022>.
- Trillmich F (1996) Parental investment in pinnipeds. In JA Rosenblatt, CT Snowdon, eds, *Advances in the Study of Behavior*, Vol 25. Academic Press, San Diego, CA, USA, pp. 533–577.
- Trites AW (2021) Behavioral Insights into the Decline and Natural History of Steller Sea Lions. In C Campagna, R Harcourt, eds, *Ethology and Behavioral Ecology of Otariids and the Odobenid*. Springer, Cham, Switzerland, pp. 489–519.
- Trites AW, Donnelly CP (2003) The decline of Steller Sea lions *Eumetopias jubatus* in Alaska: a review of the nutritional stress hypothesis. *Mammal Rev* 33: 3–28. <https://doi.org/10.1046/j.1365-2907.2003.00009.x>.
- Trites AW, Jonker RAH (2000) Morphometric measurements and body condition of healthy and starveling Steller Sea lion pups (*Eumetopias jubatus*). *Aquat Mamm* 26: 151–157.
- Verrier D, Atkinson S, Guinet C, Groscolas R, Arnould JP (2012) Hormonal responses to extreme fasting in subantarctic fur seal (*Arctocephalus tropicalis*) pups. *Am J Physiol-Regl* 302: R929–R940. <https://doi.org/10.1152/ajpregu.00370.2011>.
- Verrier D, Groscolas R, Guinet C, Arnould JP (2009) Physiological response to extreme fasting in subantarctic fur seal (*Arctocephalus tropicalis*) pups: metabolic rates, energy reserve utilization, and water fluxes. *Am J Physiol-Regl* 297: R1582–R1592. <https://doi.org/10.1152/ajpregu.90857.2008>.
- Verrier D, Groscolas R, Guinet C, Arnould JPY (2011) Development of fasting abilities in subantarctic fur seal pups: balancing the demands of growth under extreme nutritional restrictions. *Funct Ecol* 25: 704–717. <https://doi.org/10.1111/j.1365-2435.2010.01823.x>.

- Wang J, Hulck K, Hong SM, Atkinson S, Li QX (2011) Accumulation and maternal transfer of polychlorinated biphenyls in Steller Sea lions (*Eumetopias jubatus*) from Prince William sound and the Bering Sea, Alaska. *Environ Pollut* 159: 71–77. <https://doi.org/10.1016/j.envpol.2010.09.022>.
- Warton DI, Hui FK (2011) The arcsine is asinine: the analysis of proportions in ecology. *Ecology* 92: 3–10. <https://doi.org/10.1890/10-0340.1>.
- Williams KM (2013) *Clinical values of blood variables in wild and stranded California sea lions (Zalophus californianus) and blood sample storage stability*. M.S. Thesis California State University & Moss Landing Marine Laboratory, Monterey Bay, California, 85 p.
- Winn D (2006) When food can be fatal: recovery from emaciation. *Wildl Rehab Bull* 24: 26–29. <https://doi.org/10.53607/wrb.v24.187>.
- Winship AJ, Trites AW, Calkins DG (2001) Growth in body size of the Steller Sea lion (*Eumetopias jubatus*). *J Mamm* 82: 500–519. [https://doi.org/10.1644/1545-1542\(2001\)082<0500:GIBSOT>2.0.CO;2](https://doi.org/10.1644/1545-1542(2001)082<0500:GIBSOT>2.0.CO;2).
- Wirsing AJ, Heithaus MR, Frid A, Dill LM (2008) Seascales of fear: evaluating sublethal predator effects experienced and generated by marine mammals. *Mar Mamm Sci* 24: 1–15. <https://doi.org/10.1111/j.1748-7692.2007.00167.x>.
- Zhang D, Liu X, Huang W, Li J, Wang C, Zhang D, Zhang C (2020) Microplastic pollution in deep-sea sediments and organisms of the Western Pacific Ocean. *Environ Pollut* 259: 1–9, 113948. <https://doi.org/10.1016/j.envpol.2020.113948>.