DOI: 10.7589/2017-10-257

Journal of Wildlife Diseases, 55(1), 2019, pp. 91–104

REGIONAL AND AGE-RELATED VARIATIONS IN HAPTOGLOBIN CONCENTRATIONS IN STELLER SEA LIONS (*EUMETOPIAS JUBATUS*) FROM ALASKA, USA

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ABSTRACT: Varying concentrations of the highly conserved acute phase response protein, haptoglobin, can indicate changes to the health and disease status of mammals, including the Steller sea lion (SSL; *Eumetopias jubatus*). To better understand factors relating to acute phase response in SSLs, circulating haptoglobin concentrations (Hp) were quantified in plasma collected from 1,272 individuals sampled near rookeries and haulouts off the coast of Alaska, US. We compared Hp in SSLs between sexes and among different age classes (young pups, young-of-the-year, yearlings, subadults, and adults) sampled within distinct regions in Alaska (Aleutian Islands, Gulf of Alaska, Southeast Alaska). Regional and age-related differences were observed, particularly in younger SSLs. No sex-related differences were detected. We identified weakly significant relationships between Hp and hematology measurements including white blood cell counts and hematocrit in young pups from the Aleutian Islands and Southeast Alaska. No relationship between Hp and body condition was found. Lastly, a nonlinear relationship of plasma Hp and whole blood total mercury concentrations (THg) was observed in SSLs from the endangered western distinct population segment in Alaska. These results demonstrated that regional variation in Hp, especially in younger SSLs, may reflect regional differences in health and circulating THg.

Key words: Biomarker, haptoglobin, hematology, inflammation, Steller sea lion, total mercury.

INTRODUCTION

Haptoglobin, a protein biomarker of inflammation in mammals (Murata et al. 2004), is upregulated during the primary inflammatory response and concentrations vary with physical and environmental stressors (Petersen et al. 2004). More specifically, haptoglobin binds free reactive hemoglobin released from damaged red blood cells and prevents damaging redox reactions, minimizing oxidative stress (Alayash et al. 2011; Bertaggia et al. 2014). This function is likely conserved considering the genetic and structural homology of haptoglobin among mammals (Polticelli et al. 2008; Andersen et al. 2012). The health of some species of free-ranging wildlife, including the Steller sea lion (SSL; *Eumetopias jubatus*), may be assessed using haptoglobin (Zenteno-Savin et al. 1997; Bertelsen et al. 2009).

Unlike the recently delisted eastern distinct population segment (eDPS) of SSLs, the endangered western distinct population segment (wDPS) has been slow to recover from population decline (Loughlin and York 2000; Fritz et al. 2013, 2014). Mean haptoglobin concentrations (Hp) in SSLs from the wDPS

were reported to be significantly greater than from the eDPS (Zenteno-Savin et al. 1997). Thomton and Mellish (2007) reported that Hp in SSLs increased with inflammation, infection, and trauma.

Contaminants may adversely impact the health of the wDPS SSLs (Rea et al. 2013; Beckmen et al. 2016). Concentrations of mercury (Hg) are significantly greater and more variable in young SSLs from the declining wDPS than from the eDPS (Castellini et al. 2012; Rea et al. 2013). Subclinical effects of Hg in pinnipeds may occur (Das et al. 2008; Van Hoomissen et al. 2015). Greater than 20% of young SSL pups from the wDPS had total mercury concentrations (THg) in lanugo above benchmarks for neurologic or reproductive effects for fish-eating mammals (Health Canada 2007; Basu et al. 2009; Rea et al. 2013). The majority (8/15) of the SSL pups sampled at Agattu Island, Alaska, US in 2011 (Rea et al. 2013) had blood THg greater than concentrations that stimulate the proinflammatory response and alter the blood proteome inflammatory pathway in humans (Gardner et al. 2009; Birdsall et al. 2010). We aimed to determine whether high THg is associated with increased Hp in SSLs.

We measured Hp in plasma collected over 21 yr from SSLs in the eDPS and wDPS of Alaska. We tested for differences in Hp among SSLs from the Aleutian Islands (AI), Gulf of Alaska (GOA), and Southeast Alaska (SEA) and metapopulations within these regions (defined as western AI, central AI, eastern AI, western GOA, central GOA, and eastern GOA, northern SEA, and southern SEA; York et al. 1996) based on sex and age class. A reference range of Hp was determined to be used as a baseline for comparing Hp in SSLs. We also identified relationships between Hp and physiologic measurements including white blood cell counts (WBC), hematocrit (Hct), and body condition index (BCI). Finally, we explored the relationship of Hp with whole blood THg in young pups from regions that had sufficient data for statistical analysis.

MATERIALS AND METHODS

Sample collection and hematology

From 1992 to 2013, SSL young pups (<1.5 mo), young-of-the-year (>1.5–12 mo), yearlings (>12–24 mo), subadults (>24–44 mo), and adult females of similar reproductive status were captured and sampled on or near rookery or haulout sites (Fig. 1) in the AI (n=452), GOA (n=377), and the SEA (n=443). Age classification followed other SSL studies (King et al. 2007; Rea et al. 2016). Routine capture, restraint, and sampling methodologies were employed to collect whole blood samples (Heath et al. 1996; Raum-Suryan et al. 2004). Blood and plasma aliquots were frozen (–10 C while shipboard), shipped on dry ice, and then frozen (–80 C) until analysis. Data such as sex, age class, metapopulations (as defined earlier) and regions of capture (AI, GOA, SEA), and morphometrics (mass, axillary girth and dorsal standard length), were recorded. The BCI was calculated as axillary girth/standard length × 100 using morphometric data. White blood cells were manually counted aboard the research vessel, and Hct was measured using manual or automated methods. Geographic, morphometric, BCI, and hematologic data collected in Alaska from 1992–96 and 1998–2011 were previously reported (Rea et al. 1998; Lander et al. 2013).

Haptoglobin analysis

We measured Hp in plasma using the Tridelta Phase Haptoglobin Assay (Tridelta Development, Maynooth, Ireland). Samples and standard control Hp dilutions were prepared for each 96-well plate (Immulon Microtiter Plate, Thermo Fisher Scientific, Waltham, Massachusetts, USA). Absorbance values at a wavelength of 630 nm at 37 C were detected using an ultraviolet spectrophotometer (Spectramax, 340PC, Molecular Devices, San Jose, California, USA) and transcribed (SoftMax Pro 4.8, San Jose, California, USA). Measurements were taken following a 5-min and a 10-min incubation, and readings corresponding with the best fit calibration curve were used. Mean was calculated from triplicate measurements. Samples were reanalyzed if the coefficient of variance was greater than 10%. If Hp was higher than the upper range of the standard curve (linear range: 0.005–1.75 mg/mL), the out-of-range samples were diluted and reanalyzed.

Total mercury concentration determination

Whole blood THg was measured in duplicate using a DMA-80 direct mercury analyzer (Milestone Inc., Shelton, Connecticut, USA; US Environmental Protection Agency Method 7473)

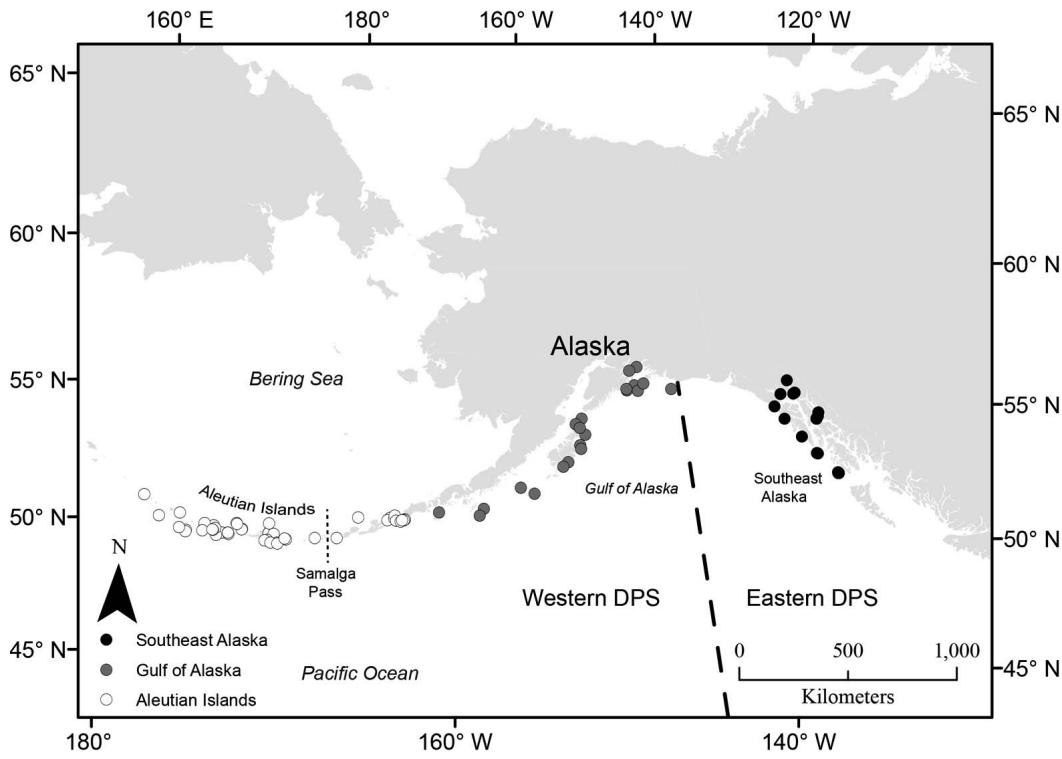

FIGURE 1. Steller sea lion (*Eumetopias jubatus*) sampling locations for haptoglobin within rookeries across the coastal Alaska, USA. The eastern distinct population segment (DPS) and western DPS for Alaskan Steller sea lions are designated by longitude 144°W.

at the Wildlife Toxicology Laboratory at the University of Alaska Fairbanks (Rea et al. 2013; Peterson et al. 2016). Certified reference materials (DORM-3, National Research Council, Ottawa, Ontario, Canada; Seronorm, Westbury, New York, USA), calibration verifications, and system and method blanks were included in each run for quality assurance. Recoveries for certified reference materials and liquid standards were previously reported for samples analyzed from 15 pups from Agattu Island in 2011 (90–106%; Rea et al. 2013), and recoveries for samples analyzed from rookery pups in SEA, AI, and GOA in 2000, 2012, and 2013 reported in this study were 103.24±0.02% (DORM-3), 102.00±0.01% (Seronorm), and 96.00±0.01% (1 mg/kg mercury chloride).

Statistical analyses

Central tendency, summary statistics, statistical analyses, and graphics were computed using the statistical program R version 3.1.2 (lme4, MuMIn, MASS, referenceIntervals, rpart, and ggplot2 packages; R Development Core Team 2014) to compare the variation of Hp (mg/mL) in SSLs with sex, age class, metapopulation, region, and the hematologic measurements (WBC, Hct), BCI, and whole blood THg. To meet the assumptions of normality and homogeneity of variances required for parametric statistical testing, Hp was logarithmically transformed prior to further analyses. Mean Hp is reported with SE, and statistical differences were considered significant with an alpha value of <0.05.

A generalized linear mixed model (GLMM) was used to identify descriptive factors affecting the variability in Hp for SSLs. To account for temporal variability, capture year was incorporated into the GLMM as a random factor. The appropriate geographic scale used for analysis was determined by comparing mean Hp among metapopulations for each age class using analysis of variance (ANOVA). Age classes where sample sizes were not adequately represented across metapopulations ($n<10$) were excluded from analyses. When no differences were detected among metapopulations, data were pooled. Criteria for subsequent analyses for SSLs were determined from the most parsimonious model based on the change in Akaike Information Criterion. For important factors, two sample *t*-

tests, ANOVAs, and multiple comparisons (Tukey's test) were used to identify groups contributing to the differences observed. We also reported the proportion of pups with Hp at least double or greater the regional mean concentration, criteria indicating acute phase response in other mammalian species (Petersen et al. 2004). Outliers were detected and removed using methods described by Horn et al. (2001) and reference ranges were computed using a nonparametric 95% reference limit criterion and 90% confidence intervals.

Simple linear regression models were used to determine if Hct, WBC, or BCI explained the variability in Hp for a subset of young pups. The relationship of Hp with THg was assessed for young pups in AI (n=186, years 2011–13) and GOA (n=25, years 2000 and 2010) with matched plasma Hp and whole blood THg data. Classification and regression tree analysis (Loh et al. 2011) was employed to estimate mean Hp of groups based on the variability in THg for data with sufficient sample sizes.

RESULTS

Comparison of regional and age class–specific Hp

The range of Hp in the plasma of SSLs off the coast of Alaska (n=1,272) was 0.01–11.03 mg/mL (Table 1 and Fig. 1). Adult female and subadult SSLs were excluded from the GLMM because sample sizes did not meet our criteria. Considering there were no significant differences in mean Hp among metapopulations ($P \geq 0.050$) for young pups, young-of-the-year, and yearlings, region was considered the appropriate geographic scale to include for the GLMM. Therefore, the full GLMM for SSLs included region, age class, sex, and their interactions with capture year included as a random effect on the variability of Hp for young pups, young-of-the-year, and yearlings. The most parsimonious model included region, age class, and their interaction. The inclusion of sex and its interactive effects was negligible when comparing the change in Akaike Information Criterions and r-squared values among models. Further, the model including sex as a main factor did not statistically differ (ANOVA, $F_{11,1201}$=2.77, P=0.249) from the model without sex (see Supplementary Material Table 1). Therefore, Hp for males and females was pooled for each age class within a region. Given these findings, subsequent analyses were conducted to separately compare the differences in mean Hp among all age classes within each region and regional differences for each age class.

Significant differences in mean Hp were detected among age classes when comparing within each region: AI (t-test, t_{80}=–8.22, P<0.001), GOA (ANOVA, $F_{3,367}$=3.00, P=0.031), and SEA (ANOVA, $F_{3,428}$=21.92, P<0.001). Mean Hp in SSLs from AI was similar among most age classes, with the exception that young-of-the-year had a significantly lower mean Hp (0.49±0.06, n=69) compared with mean Hp in young pups (1.33±0.07, n=373; Table 1). Multiple comparisons tests showed that for SSLs in the GOA, mean Hp in young pups (1.57±0.12, n=155), young-of-the-year (1.35±0.24, n=124), and yearlings (1.13±0.08, n=72) were not different from one another, and all were significantly less (2.04±0.24, n=20) than mean Hp observed for adult females. Adult females from SEA had similar mean Hp (2.22±0.16, n=25) to young pups (2.93±0.15, n=210) and young-of-the-year (1.52±0.09, n=99) in this region, and all were significantly greater (1.30±0.09, n=98) than mean Hp in yearlings in SEA (Table 1). Subadults were excluded from analyses due to insufficient sample sizes.

When comparing mean Hp among regions for each age class of SSLs, significant regional differences were observed for young pups (ANOVA, $F_{2,735}$=57.38, P<0.001) and young-of-the-year (ANOVA, $F_{2,289}$=49.60, P<0.001) whereas no significant regional differences were detected for yearlings (t_{165}=0.40, P=0.691) or adult females (t-test, t_{43}=–0.25, P=0.800; Fig. 2). We did not have sufficient sample sizes of subadult SSLs for statistical comparisons. Multiple comparisons tests revealed that mean Hp measured for young pups in the AI (1.33±0.07, n=373) was similar (1.57±0.12, n=155) to GOA; however, both were significantly lower (2.93±0.15, n=210; P<0.001) than in SEA (Fig.2A). For young-of-the-year, the difference was largely driven by significantly lower mean Hp (0.49±0.06, n=69; P<0.001) measured for AI compared

TABLE 1. Mean±SE and sample size of haptoglobin concentrations (mg/mL) for Steller sea lions (*Eumetopias jubatus*) of different age classes that were sampled from each of three regions in Alaska, USA (1992–2013). Bold indicates significantly (*P=0.031, **P<0.001) lower haptoglobin concentrations when comparing among age classes within a region. Statistical comparisons were made when n>10.

	Age classes[a]									
Region	n	Young pups	n	Young-of-the-year	n	Yearlings	n	Subadults	n	Adult females
Aleutian Islands	373	1.33±0.07	69	**0.49±0.06****	4	1.20±0.39	1	5.18	5	2.66±0.45
Gulf of Alaska	155	**1.57±0.12***	124	**1.35±0.10***	72	**1.13±0.08***	5	1.68±0.28	20	2.04±0.24
Southeast Alaska	210	2.93±0.15	99	1.52±0.09	98	**1.30±0.09****	10	1.34±0.24	25	2.22±0.16

[a] Young pups (<1.5 mo), young-of-the-year (>1.5–12 mo), yearlings (>12–24 mo), subadults (>24–44 mo), adult females (>44 mo).

with (1.35±0.10, n=124) for GOA and (1.52±0.09, n=99) for SEA (Fig. 2B).

Following the detection and removal of 15 outliers, the lower and upper reference thresholds of Hp for SSLs were calculated as 0.13 mg/mL and 5.06 mg/mL, respectively (n=1,272). The percentage of individuals falling outside this range in each region was low (2–5%). However, the greatest percentage of SSLs with Hp less than the lower reference threshold were from AI and GOA. The percentage of SSLs with Hp greater than the upper threshold of Hp in SEA was more than double that of GOA and AI (Table 2). Lastly, a greater proportion of young pups from GOA (13.5%) had Hp that was at least two times the regional mean compared with young pups from AI (9.6%) and SEA (9.7%).

Relationships of Hp with WBC, Hct, and BCI

Considering the regional differences observed, subsequent analyses to investigate relationships of Hp with WBC, Hct, and BCI were performed on young pups for each region separately. The mean and SE for WBC, Hct, and BCI were determined for young pups sampled from the AI, GOA, and SEA over various years between 1992 and 2013 (Table 3). A statistically significant positive relationship of Hp with WBC was observed in young pups from the AI (r^2=0.05, P<0.009; Fig. 3A) and SEA (r^2=0.36, P<0.014; Fig. 3B). There was a significant negative relationship (r^2=0.09, P<0.001) between Hp and Hct in young pups from the GOA (Fig. 3C). Haptoglobin concentrations in young pups from other regions were not related to corresponding WBC or Hct measurements (P>0.050). Lastly, BCI was not related to Hp for young pups, and this finding was consistent across all regions (P>0.050). Two individuals with the lowest Hct (11.75% and 17.5%) from SEA were both underweight for their age (mass of 26 kg and 32 kg for ~2 mo old), although both were within normal ranges for total protein (6.2 g/dL and 5.5 g/dL; Lander et al. 2013). Young pups from SEA had lower mean Hct than did young pups from other regions; however, no relationship of Hp with Hct in SEA animals was observed, regardless of the removal of the two individuals.

Variations in Hp with whole blood THg

Whole blood THg ranged from 0.01–0.35 mg/kg in a subset of young pups with matched Hp in SEA, the AI, and GOA, and the mean THg for these regions was 0.01±0.01 mg/kg, 0.06±0.01 mg/kg, and 0.04±0.01 mg/kg, respectively (Table 3). No statistical analysis could be performed for young SEA pups. No linear relationship between Hp and THg was identified for GOA young pups (years 2000 and 2010), and regression tree analysis was not performed on this subset of young pups from GOA given the relatively small sample

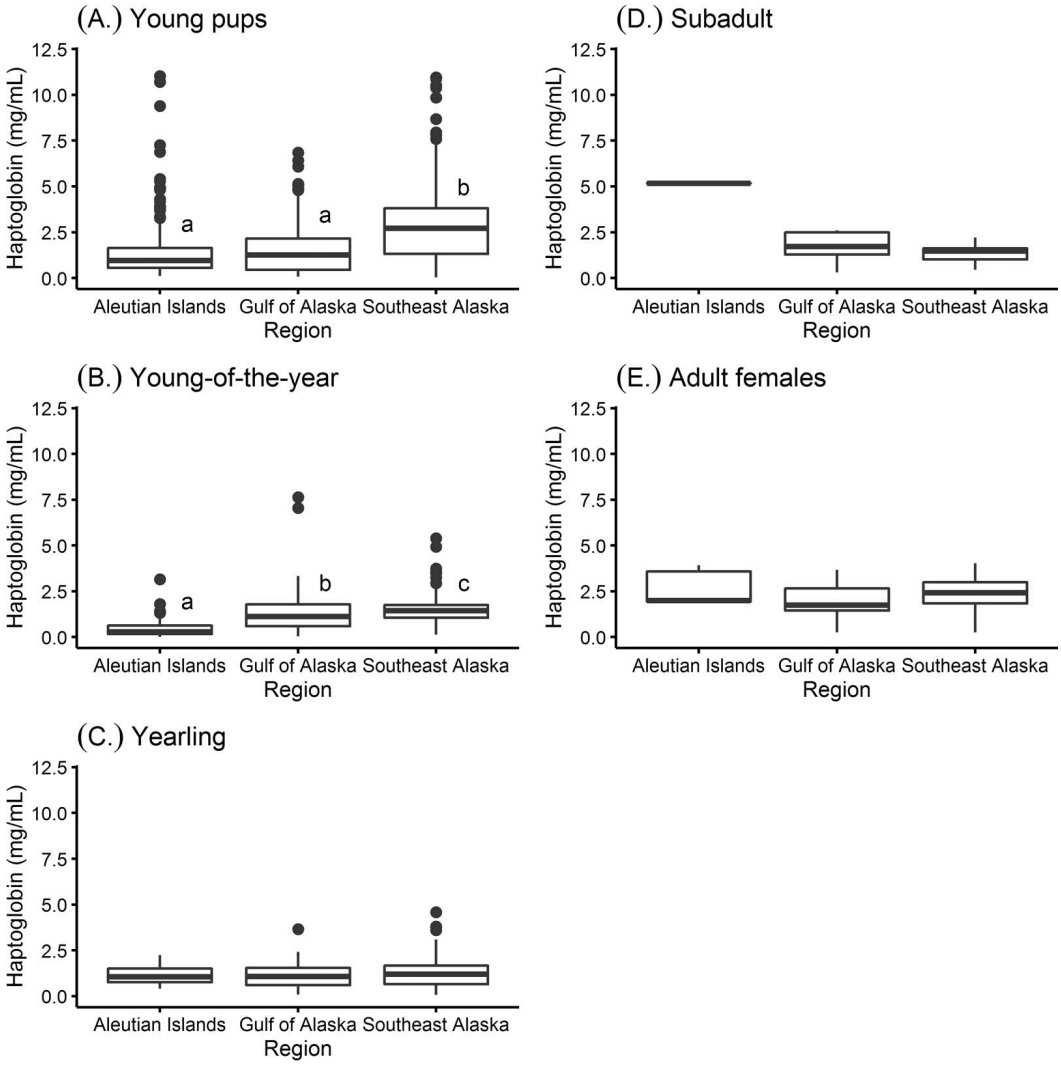

FIGURE 2. A comparison of haptoglobin concentrations (mg/mL) among regions in Alaska, USA for each age class; (A) young pups, (B) young-of-the-year, (C) yearlings, (D) subadults, and (E) adult females of Steller sea lions (*Eumetopias jubatus*). Lowercase letters (a–c) designate significant differences ($P<0.05$) and groupings from multiple comparisons tests. Sample sizes of subadults in the Aleutian Islands, Gulf of Alaska, and Southeast Alaska did not meet the criterion ($n<10$) for comparison.

size. Haptoglobin concentrations in young pups from AI (years 2011–13) varied significantly with THg; however, the hyperbolic relationship did not fit a linear model (Fig. 4A). Regression tree analysis computationally assigned a node at a THg of 0.11 mg/kg (Fig. 4B). The resulting regression tree model indicated that young pups from the AI with whole blood THg below 0.11 mg/kg had an average Hp of 1.54 mg/mL. Conversely, young pups from AI with whole blood THg equal to or exceeding 0.11 mg/kg had a lower predicted average Hp of 0.95 mg/mL (Fig. 4B). On average, Hp was predicted to be 62% greater in pups below 0.11 mg/kg THg.

DISCUSSION

Regardless of possible individual and regional variability, haptoglobin may provide

TABLE 2. Sample size and the number of Steller sea lions (*Eumetopias jubatus*) with haptoglobin concentrations (Hp) outside the upper and lower thresholds of the reference interval for haptoglobin in plasma by region in Alaska, USA. The calculated reference range for Hp for Steller sea lions (n=1, 272) was 0.13–5.06 mg/mL following the removal of 15 outliers (Horn et al. 2001).

		Hp levels outside of reference interval			
		≤0.13 mg/mL		≥5.06 mg/mL	
Region	Total n	n	%	n	%
Aleutian Islands	452	13	2.9	9	2.0
Gulf of Alaska	377	12	3.2	7	1.9
Southeast Alaska	443	9	2.0	24	5.4

insight to general physiologic changes when taken into consideration with key descriptive factors and other important physiologic indicators (Kakuschke et al. 2010). From an ecological perspective, using baseline Hp as an index of health has the strength of repeatability and of being predictive of inducible changes to acute phase response (Matson et al. 2012).

A greater range of Hp was observed in SSLs than in most marine mammal species (Beckmen et al. 2003; Krafft et al. 2006; Frouin et al. 2013), and mean Hp was greatest in SSLs from SEA. In domestic animals, an acute phase response occurs when peak Hp is 1.5–10 times greater than baseline, indicating changes to general health status (Petersen et al. 2002, 2004; Cray and Belgrave 2014). In hospitalized dogs, the range and median of Hp were greater compared with healthy individuals, except that dogs with liver disease had significantly lower median Hp compared to dogs with other illnesses (Crawford et al. 2013). Humans and other species with hemolytic disease or liver cirrhosis also had lower Hp (<0.3 mg mL^{-1}), indicating a compromised acute phase response (Marchand et al. 1980; Dobryszycka 1997; Kormoczi et al. 2006). For SSLs with confirmed infection or trauma-induced acute phase response, Hp was greater than 5 mg/mL (Thomton et al. 2007), similar to our upper reference threshold value (5.06 mg/mL). In our study, a small proportion of young SSL pups from each region (9–13%) had Hp that was at least two times the regional mean or greater. A greater proportion of young-of-the-year SSLs in the AI were below the lower limit of the normal range for other mammalian species, yet the number of SSLs with Hp greater than the upper reference threshold was more than doubled in SEA. Abnormally low Hp or abnormally high Hp may indicate a compromised or active acute phase response.

Elevated Hp in harbor seals (*Phoca vitulina*) was attributed to the phocine distemper virus epidemic, but no age nor regional differences in Hp were found (Kakuschke et al. 2010). Unlike

TABLE 3. Mean±SE and sample size for the parameters of white blood cell count (WBC), hematocrit (Hct), body condition (BCI), and whole blood total mercury (THg) in Steller sea lion (*Eumetopias jubatus*) young pups from three regions in Alaska, USA. Significant relationships between haptoglobin concentrations are indicated in bold, and the level of significance is denoted as *P=0.009, **P<0.001, and ***P=0.014. Whole blood THg represents a subset of data previously reported by Rea et al. (2013) and Peterson et al. (2016).

			Parameters						
Region	Total	n	WBC	n	Hct	n	BCI	n	THg
Aleutian Islands	373	150	**13,890±255***	167	40.16±0.22	362	70.15±0.21	186	0.06±0.01
Gulf of Alaska	155	119	12,510±513	143	**39.64±0.43****	146	68.43±0.27	25	0.04±0.01
Southeast Alaska	210	15	**11,083±249*****	108	33.17±0.32	205	70.37±0.22	3	0.01±0.01

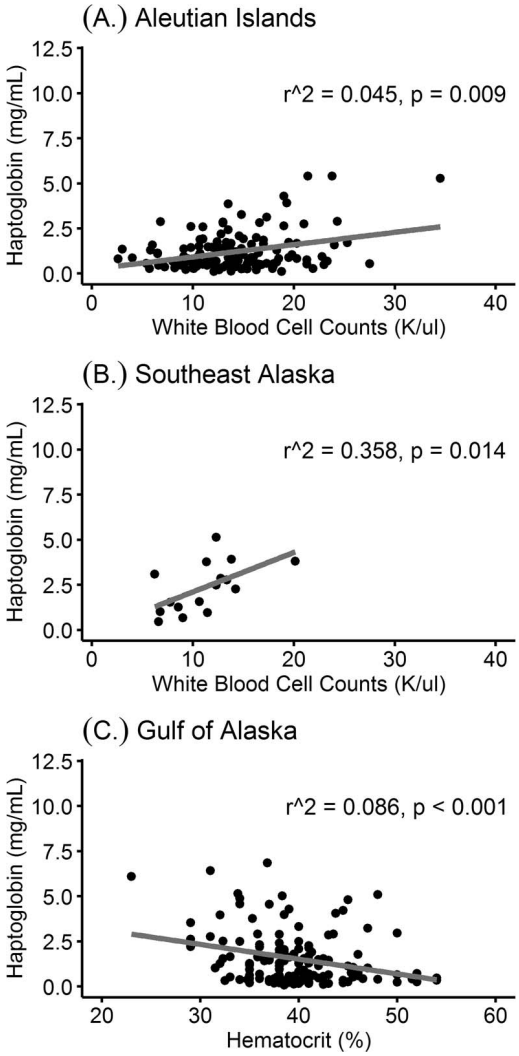

FIGURE 3. The relationships of plasma haptoglobin concentrations with white blood cell counts (total cells$\times 10^3$) in (A) young pups of Steller sea lions (*Eumetopias jubatus*) from the Aleutian Islands and (B) Southeast Alaska, and relationships of haptoglobin concentrations with hematocrit (%) in young pups from (C) Gulf of Alaska.

harbor seals, the variation in Hp in young SSL pups and young-of-the-year depended on region and age. In general, Hp in younger SSLs from the wDPS was lower compared with the eDPS, which was the opposite of findings previously reported for a smaller number of SSLs (Zenteno-Savin et al. 1997). Furthermore, we found no regional differences in mean Hp for yearlings and adults.

Poor agreement among commercialized Hp assays is not uncommon (Czopowicz et al. 2017), and newer commercial assays for measuring Hp in marine mammals (Thomton et al. 2007) may be responsible for differing results among studies on SSLs over the past decades. Plasma samples previously assayed using gel electrophoresis (Zenteno-Savin et al. 1997) were reanalyzed using the colorimetric assay, and higher Hp was measured for some SEA animals than had been previously reported, suggesting potential inconsistency of the gel assay (see Supplementary Fig. 1). These presumed gel issues likely resulted in the discrepancy between the previously reported low Hp in SEA and our current regional pattern of Hp. Several findings in this study are in agreement to those previously reported for SSLs using colorimetric assays. For example, no differences in mean Hp were found between sexes of all pre-reproductive age classes, and Hp in juveniles (1.33±0.17 mg/mL) was similar (1.13±0.08 mg/mL) in similarly aged individuals we sampled (Thomton et al. 2007). Prolonged freezing also may contribute to significant changes in measures of protein biomarkers when using archived samples; however, this is unlikely for the SSL archive Hp plasma samples. Martins et al. (2017) found that Hp remained stable over time regardless of freezing duration or freeze-thaw cycles.

Given that Hp increases following pregnancy, parturition, and lactation in other mammals (Berkova et al. 2001; Hiss et al. 2009), the greater mean Hp for adult females sampled a few months after the breeding season may be a result of reproductive or metabolic status. Furthermore, Hp compared among AI, GOA, and SEA was not statistically different for yearlings and adult females. Therefore, the range of Hp observed for older SSLs in this study is likely representative of normal variability for those age classes.

The relationship of Hp with other physiologic parameters involved with the acute phase response gives insight into the potential physiologic status of each animal (Hanthorn et al. 2014). White blood cell counts are an important indicator of changes to immune

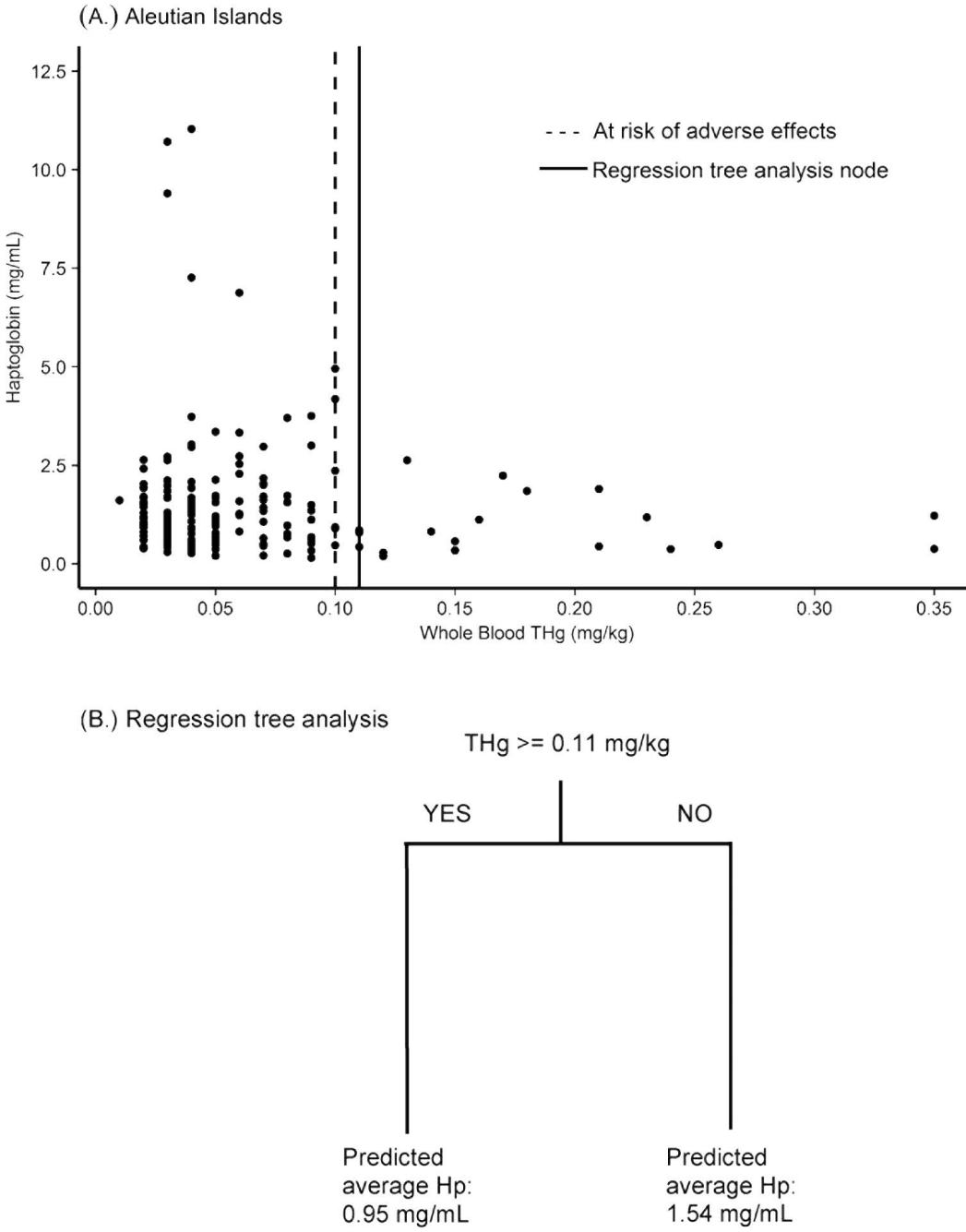

FIGURE 4. The distribution of plasma haptoglobin concentrations (Hp) with matched whole blood total mercury concentrations (THg; mg/kg). (A) Young pups of Steller sea lions (*Eumetopias jubatus*) from the Aleutian Islands. (B) A regression tree demonstrating a statistically derived bifurcation of the data. The "at risk" adverse effects threshold for mammals (Health Canada 2007) and the regression tree analysis node are also denoted as dashed lines in (A).

response and survival (Shuert et al. 2015). The majority of hematologic measurements, including WBC, in young pups from the AI and GOA were within reference ranges (Lander et al. 2013), and mean Hp was not significantly different among these regions.

The relationship of Hp with hemoglobin was assessed using Hct as a proxy for hemoglobin (Quintó et al. 2006; O'Brien et al. 2014). The Hct values for the majority of young pups were within normal ranges and similar to the subset of these animals previously reported (Rea et al. 1998; Lander et al. 2013). A weak, negative relationship of Hp with increasing Hct was observed for young pups in GOA. A similar relationship was previously reported for juveniles from the wDPS (Thomton et al. 2007). The mean Hct for SEA young pups was lower on average than in GOA and AI, although no relationship of Hp with Hct in SEA animals was observed. Finally, body condition had no effect on the variability of Hp, and this finding was consistent among regions. Therefore, young pups with poor body condition have the same variability in Hp as do young pups with good body condition, supporting that other extrinsic factors may be involved with changes to Hp. We caution that Hp should not be used as an indicator of nutritional stress and that BCI estimates may not accurately represent total body fat content (Rea et al. 2016), especially in young pups (Rea et al. 1998).

A greater proportion of SSLs in the wDPS have THg in blood and hair (Rea et al. 2013) that exceed established threshold levels for adverse effects in humans and some wildlife (Clarkson and Magos 2006; Arctic Monitoring and Assessment Programme 2011; Dietz et al. 2013), although it is uncertain if exposure to Hg adversely affects SSLs. In harbor seals, subclinical effects of Hg may occur above these threshold levels (Das et al. 2008; Van Hoomissen et al. 2015). It is possible that Hg above thresholds for adverse effects in young SSLs may contribute to the lack of recovery of SSLs in the AI and GOA (Holmes et al. 2008; Castellini et al. 2012; Rea et al. 2013). These findings warrant investigation of physiologic factors that may be influenced by Hg exposure, including protective proteins like haptoglobin. Considering contaminants can influence acute phase proteins (Yiangou et al. 1991), we investigated the relationship of whole blood THg with Hp in a subset of young wDPS pups and found that individuals with greater concentrations of THg have lower Hp compared with young pups with lower THg. The cut-off node (THg of 0.11 mg/kg) statistically assigned from the regression tree analysis of Hp and THg from AI young pups is similar to the critical level determined for risk of adverse effects of Hg exposure in humans (0.1 mg/kg; Health Canada 2007). The mean Hp for the group of individuals with THg greater than 0.11 mg/kg THg is almost half that of Hp in pups with lower concentrations of THg. These individuals with greater THg were also well above the lower limit (58 μg/L) benchmark in maternal cord blood for adverse effects in humans (National Research Council 2006). Exposure to Hg exceeding critical, at-risk thresholds may have indirect effects on the Hp pathway leading to decreased Hp. However, the biological significance of decreased Hp of this magnitude in SSLs is unknown. We are cautious to make any conclusions about the adverse effects of Hg on Hp in SSLs, given the distribution of the data (i.e., fewer young pups with THg>0.11 mg/kg than with <0.11 mg/kg) and lack of clinically validated reference ranges of Hp for SSLs.

Acute phase response can be modified by contaminant exposure via fish consumption (Gump et al. 2012), and Hp may be altered if oxidative stress from contaminant exposure damages hepatocytes where the majority of Hp is produced. Controlled feeding studies with sled dogs (Sonne et al. 2007) and river otters (Ben-David et al. 2001) showed that exposure to naturally accumulated contaminants such as polychlorinated biphenyls and polycyclic aromatic hydrocarbons resulted in markedly decreased levels of expression or Hp, compared with the control group, and was suggested to be the result of damaged hepatocytes incurred from toxicant-related oxidative stress. The greatest THg in young SSLs is observed in liver tissue compared to

other tissues (Correa et al. 2014), and individuals that experience high levels of exposure in utero may be most vulnerable to toxic levels during development (Rea et al. 2013; Oliveira et al. 2015). Therefore, it is possible that contaminants like Hg, that accumulate in hepatocytes where it is manufactured and regulated (Andersen et al. 1966), could also influence Hp in SSLs.

Regional differences in Hp observed in young pups and young of the year may indicate differences in acute phase response among the regions in the wDPS and eDPS. Given that the Hp pathway depends on the interaction of the Hp and hemoglobin complex with macrophages (Thomsen et al. 2013), and that WBCs and Hp share a positive relationship during acute phase response (Thomton et al. 2007), it is not surprising that a positive relationship of WBC and Hp was observed in some cases. However, the lack of this relationship in young pups from GOA may indicate a difference in acute phase response, or lack thereof, in this group. Although the health status relevance of the noted statistically significant relationship of THg and Hp is unclear, these results lend support to the possibility that there may be an underlying biological mechanism worthy of further exploration. Determining Hp in endangered SSLs can be useful for inferring changes to acute phase response that may correlate with changes to general health in SSL groups among different geographic regions, especially when considered in conjunction with other physiologic measurements.

ACKNOWLEDGMENTS

We thank the field research teams of the Alaska Department of Fish and Game (ADFG), Marine Mammal Laboratory of the National Oceanographic and Atmospheric Administration, Kamchatka Branch of the Pacific Geographical Institution, and the North Pacific Wildlife Consulting, LLC as well as crews of the *R/V Medeia*, *P/V Stimson*, *P/V Wolstad*, *R/V Tiglax*, *M/V Pacific Star*, and the *R/V Norseman I* and *II* for sample collection, animal measurements, and hematology analysis in the field. We thank P. Rivera for laboratory assistance with Hp analysis and L. Correa, S. Piersalowski, and G. Johnson for measurement of THg. We also thank M. Johns for statistical support, J. Harley for computational coding assistance, M. Courtney for ArcGIS map support, T. Becker and S. Becker for word reduction edits, and T. Kuhn and A. Ferrante for constructive reviews of this manuscript. Funding supporting this research was provided through National Oceanographic and Atmospheric Administration Cooperative Agreements NA17FX1079, NA04NMF4390170, NA08NMF4390544, and NA13NMF4720041 and the Biomedical Learning and Student Training program under National Institute of General Medical Sciences of the National Institutes of Health Awards UL1GM118991, TL4GM118992, or RL5GM118990. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. Alaska Department of Fish and Game research was conducted under Marine Mammal Protection Act (MMPA) permits 358-1564, 358-1769, and 358-1888 and ADFG ACUC 03-002 and 06-07.

SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at http://dx.doi.org/10.7589/2017-10-257.

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Submitted for publication 18 October 2017.
Accepted 24 March 2018.