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Large and in charge: cortisol levels vary with sex, diet, and body mass in an Antarctic predator, the leopard seal

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Evaluating physiological responses in the context of a species' life history, demographics, and ecology is essential to understanding the health of individuals and populations. Here, we measured the main mammalian glucocorticoid, cortisol, in an elusive Antarctic apex predator, the leopard seal (*Hydrurga leptonyx*). We also examined intraspecific variation in cortisol based on life history (sex), morphometrics (body mass, body condition), and ecological traits ($\delta^{15}\text{N}$, $\delta^{13}\text{C}$). To do this, blood samples, life history traits, and morphometric data were collected from 19 individual leopard seals off the Western Antarctic Peninsula. We found that adult leopard seals have remarkably high cortisol concentrations (100.35 ± 16.72 mg/dL), showing the highest circulating cortisol concentration ever reported for a pinniped: 147 mg/dL in an adult male. Leopard seal cortisol concentrations varied with sex, body mass, and diet. Large adult females had significantly lower cortisol (94.49 ± 10.12 mg/dL) than adult males (120.85 ± 6.20 mg/dL). Similarly, leopard seals with higher isotope values (i.e., adult females, $\delta^{15}\text{N}$: $11.35 \pm 0.69\text{‰}$) had lower cortisol concentrations than seals with lower isotope values (i.e., adult males, $\delta^{15}\text{N}$: $10.14 \pm 1.65\text{‰}$). Furthermore, we compared cortisol concentrations across 26 closely related Arctoid taxa (i.e., mustelids, bears, and pinnipeds) with comparable data. Leopard seals had the highest mean cortisol concentrations that were 1.25 to 50 times higher than other Arctoids. More broadly, Antarctic ice seals (Lobodontini: leopard seal, Ross seal, Weddell seal, crabeater seal) had higher cortisol concentrations compared to other pinnipeds and Arctoid species. Therefore, high cortisol is a characteristic of all lobodontines and may be a specialized adaptation within this Antarctic dwelling clade. Together, our results highlight exceptionally high cortisol concentrations in leopard seals (and across lobodontines) and reveal high

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1 Introduction

Cortisol, the main mammalian glucocorticoid, is an energy mobilization hormone that regulates a range of biological processes, including immune function, metabolism, and the stress response (Sapolsky, 1999; Palme et al., 2005; Reeder and Kramer, 2005; Wingfield and Romero, 2011; Haase et al., 2016). Cortisol levels can increase in response to a variety of intrinsic and extrinsic stressors (Kitaysky et al., 1999; Levine, 2002; Anestis et al., 2006; Wingfield, 2013a; Wingfield, 2013b; Aurich et al., 2015; Lafferty et al., 2015). Hence, it is commonly referred to as the “stress hormone” and has traditionally been measured in mammals as a means to evaluate stress levels (Creel, 2001; Abbott et al., 2003; Sheriff et al., 2011; Bryan et al., 2013; Kershaw and Hall, 2016; Beaulieu-McCoy et al., 2017). However, recent work has highlighted the diverse and complex functions that cortisol plays in mammals, many of which are not associated with stress (MacDougallShackleton et al., 2019; Romero and Beattie, 2022). As a result of its role in energy-mobilization, cortisol is an essential mammalian hormone that regulates key physiological processes; consequently, measuring and comparing cortisol concentrations within and between species provides important context for understanding the physiological responses of mammals to different abiotic and biotic stimuli.

Many factors affect baseline cortisol values, including life history stages (Kitaysky et al., 1999; Levine, 2002; Anestis et al., 2006; Aurich et al., 2015; Lafferty et al., 2015;

Azevedo et al., 2019), interspecific interactions (Bartsh et al., 1992; Ashley et al., 2011;

variability in cortisol concentrations among individuals from a single location. This information provides the context for understanding how leopard seal physiology changes with life history, ecology, and morphology and sets the foundation for assessing their physiology in the context of a rapidly changing Antarctic environment.

KEYWORDS

leopard seal, marine mammals, intraspecific variation, pinniped, cortisol, physiology, body size, stable isotope

Creel et al., 2013; Levy et al., 2020), and environmental variables (Wingfield, 2013a; Wingfield, 2013b). For example, sex-specific life history events (i.e., reproductive status, lactation, competition for breeding) can lead to sex-based differences in cortisol, with animals showing sex-based changes in cortisol seasonally (Levine, 2002; Lafferty et al., 2015). Further, intraspecific differences in body size can lead to variation in cortisol due to its influence on behavior interactions or dominance status (Creel, 2001; Abbott et al., 2003; Creel et al., 2013). Generally, larger and more dominant animals display lower cortisol concentrations compared to their smaller and subordinate counterparts (Creel, 2001; Abbott et al., 2003; Levy et al., 2020). Similarly, animals in poor body condition often have higher cortisol than animals in healthy body condition (Macbeth et al., 2012; Shero et al., 2015; Lemos et al., 2022), and this is often linked to dietary differences. In many animals, diet is measured with stable isotope analysis, where isotope ratios of nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) measured in animal tissues are used to indicate the trophic level and food sources (Gannes et al., 1998; Tykot, 2004). Animals with higher $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values, indicating a more nutrient-enriched diet, can have lower cortisol concentrations than those with lower isotope values (Lafferty et al., 2015; Karpovich et al., 2019). Understanding how cortisol changes in response to life history, ecological, and physiological factors is important for interpreting the significance of high (or low) cortisol concentrations within and between taxa.

Cortisol is commonly measured in pinnipeds (seals, sea lions, and walrus) to evaluate the relationship between their physiology and other biological metrics (Constable et al., 2006; du Dot et al., 2009; Trumble et al., 2013; Keogh and Atkinson, 2015; Shero et al., 2015; Kershaw and Hall, 2016; Peck et al., 2016; Beaulieu-McCoy et al., 2017; Karpovich et al., 2019; Thompson and Romano, 2019; Keogh et al., 2020; Sperou, 2020; Ogloff et al., 2022). Among pinnipeds, Southern Ocean pinnipeds (six species: Ross seals, *Ommatophoca rossii*; Weddell seals, *Leptonychotes weddellii*; crabeater seals, *Lobodon carcinophaga*; leopard seals, *Hydrurga leptonyx*; Antarctic fur seals, *Arctocephalus gazella*; and southern elephant seals, *Mirounga leonina*) have some of the highest cortisol concentrations ever recorded (Liggins et al., 1979; Barrell and Montgomery, 1989; Bartsh et al., 1992; Liggins et al., 1993; Harcourt, 2001; Tryland et al., 2012). There is some evidence that cortisol concentrations in Southern Ocean pinnipeds vary as a function of reproductive status (Liggins et al., 1979; Bartsh et al., 1992; Shero et al., 2015), territoriality (Bartsh et al., 1992), age class (Liggins et al., 1979; Barrell and Montgomery, 1989; Bartsh et al., 1992; Tryland et al., 2012), size (Bartsh et al., 1992), and between species (Liggins et al., 1993; Tryland et al., 2012). However, many of these previous studies were conducted over 25 years ago, had small sizes, and provided little (or no) information about life history or body condition parameters; this limits our ability to compare and assess causes of intraspecific variation in cortisol in these pinnipeds.

Here, our study focuses on an Antarctic apex predator, the leopard seal. Only one study to date measured cortisol concentrations in leopard seals (Liggins et al., 1993). Like other Antarctic pinnipeds, Liggins et al. (1993) found that leopard seals had high cortisol concentrations only slightly lower than the closely related Weddell seal. However, interpretation of their results is

limited by the small sample size ($n=4$) and the lack of data on life history covariates. Numerous factors likely influence intraspecific differences in leopard seal cortisol concentrations. For example, leopard seals show strong female-biased sexual dimorphism, where adult females are up to 50% larger than adult males (Laws, 1977; Bonner, 1994; Kienle et al., 2022). Leopard seals also feed across trophic levels with a diet ranging from large endothermic mesopredators (e.g., penguins, Antarctic fur seals) to small ectothermic prey (e.g., Antarctic fish, krill; (Øritsland, 1977; Boveng et al., 1998; Hall-Aspland and Rogers, 2004; Botta et al., 2018; Krause et al., 2020). Furthermore, leopard seal diet appears to be related to body size. Larger females often acquire larger, more energy-dense endothermic prey and have higher $\delta^{15}\text{N}$ values than individuals of both sexes (Krause et al., 2020). However, it is currently unknown how differences in life history, body size, and diet affect leopard seal cortisol concentrations.

Therefore, the primary goals of this study were to measure cortisol in leopard seals from a single population and examine intraspecific variation in cortisol concentrations as a function of sex, body size (mass), body condition (scaling body mass index), and isotopic values associated with diet ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$). We hypothesized that large bodied leopard seals, specifically females, would exhibit lower cortisol values than smaller leopard seals (e.g., adult males and smaller females). Additionally, we hypothesized that individuals feeding at higher trophic levels (higher $\delta^{15}\text{N}$) would exhibit lower cortisol concentrations and be in better body condition than those feeding at lower trophic levels. Finally, we compared cortisol concentrations measured in leopard seals with comparable data for related terrestrial and marine carnivorous mammals (i.e., Arctoids) to contextualize our findings.

2 Materials and methods

2.1 Animal handling and sample collection

Field work was conducted at the U.S. Antarctic Marine Living Resources (AMLR) Program research station on Cape Shirreff, Livingston Island, Antarctic Peninsula (62.47°S, 60.77°W). Nineteen individual leopard seals were chemically immobilized during two field seasons (January and April-May 2018; April-May 2019; [Table 1](#)). One female seal (#397) was captured and sampled in both 2018 and 2019, for a total of 20 samples. Immobilization and sample

collection followed established protocols and are detailed in [Charapata et al. \(2023\)](#) and [Kienle et al. \(2022\)](#). In brief, leopard seals were sedated with a midazolam-butorphanol protocol administered through a jab stick as established in [Pussini and Goebel, 2015](#); there was no manual restraint prior to capture.

TABLE 1 Life history traits and morphometric data for the 20 leopard seals in this study.

Seal ID	Sex	Sample Date	Age Class	Mass (kg)	Standard Length (cm)	Scaled Body Mass Index
12	F	4/28/18	Adult	476	300	455
37	F	5/9/19	Adult	500	295	507
57	F	4/24/18	Adult	540	319	417
138	F	4/18/18	Adult	333	298	326
140	M	4/19/18	Adult	285	265	419
141	M	4/21/18	Adult	282	268	399
142	F	4/23/18	Adult	394	293	409
143	F	4/25/18	Adult	368	284	426
144	M	4/30/18	Adult	324	286	366
145	F	5/6/18	Juvenile	147	213	–
153	F	4/29/19	Adult	496	315	400
156	F	5/1/19	Adult	463	292	486
157	M	4/28/19	Adult	317	283	371
158	F	4/21/19	Adult	487	312	406
159	F	5/2/19	Adult	456	309	393
160	F	5/13/19	Adult	355	320	271
161	F	5/13/19	Adult	436	293	453
162	F	5/21/19	Adult	437	297	433
397	F	4/22/19	Adult	486	298	476
397*	F	4/20/18	Adult	497	300	475

*Repeat individual sample used for analysis.

While sedated, morphometric data (e.g., lengths, mass), life history data (e.g., sex, age class), and blood samples were collected. Body mass (kg) was measured using a sling, digital scale (DynaLink Measurement Systems International, Rice Lake, WI), hand winch, and tripod. Standard length was recorded (cm; tip of nose to tip of tail along the dorsal side of the seal). Sex was visually assessed based on genitalia. Age was estimated based on the relationship between standard length and age (Laws, 1957); seals <260 cm in length were classified as juveniles (n=1) and seals >260 cm were classified as adults (n=19; including repeat sample). After blood samples were collected, they were immediately stored on ice until they were centrifuged in the lab within 3 hours of collection. Vacutainers were centrifuged at ~1,200 rpms for ~15 mins to separate serum, plasma, and red blood cells and stored at -80°C until analysis. Research was approved by the Institutional Animal Care and Use Committee (IACUC) at the University of California, Santa Cruz and conducted under federal authorizations for marine mammal research under National Marine Fisheries Service permit #19439 and Antarctic Conservation Act permit #2018-016.

2.2 Cortisol analysis and validation

We measured cortisol concentrations in individual leopard seals from the blood serum samples (n=20, including repeat individual and juvenile). Cortisol concentrations were assessed in duplicate using a commercially available radioimmunoassay (RIA, MP Biomedical). Samples were run neat, and no lipid extraction was performed. Due to the high concentration of cortisol in the leopard seal samples, all serum samples were diluted 0.6x with zero calibrator solution to fit on the standard curve, and the dilution factor was applied to the raw concentrations.

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A pooled sample was serially diluted five times (1:2, 1:4, 1:8, 1:16, 1:32) and compared to the standard curve. Serially diluted samples yielded a curve that was parallel to the standard curves. To assess accuracy, samples were spiked with kit standards, and the percent recovery of added hormone was calculated ($99.2 \pm 11\%$). Mean intra-assay coefficient of variation was calculated for all samples ($2.1 \pm 1.5\%$). All samples were analyzed in one run.

2.3 Stable isotope analysis

Plasma samples were used for bulk carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope analysis. The half-life of isotopes is typically short-lived in plasma, reflecting ~1 weeks of information prior to data collection as shown in bears and birds (Hobson and Clark, 1993; Hilderbrand et al., 1996). The relative abundance of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ can provide information on individuals' diet, where $\delta^{15}\text{N}$ reflects trophic position (Hobson and Welch, 1992; Rau et al., 1992; Lesage et al., 2001) and $\delta^{13}\text{C}$ is used for carbon resources exploited by consumers (Ramsay and Hobson, 1991).

Aliquots of plasma samples (500 ml) were separated into individual cryovials, freeze dried for ~24 hours, and homogenized with a metal spatula. Dried homogenized samples were packaged (~0.7 mg) into 5 x 9 mm tin capsules for stable isotope analysis.

Lipids can affect $\delta^{13}\text{C}$ values in animal tissues (DeNiro and Epstein, 1977). The typical atomic C/N ratios of lipid-extracted marine mammal prey ranges between 3 and 4 (Newsome et al., 2010). A previous study found carbon and nitrogen ratios from nonextracted leopard seal plasma were below 3.5 and thus were considered reliable indicator of the animal's diet (Krause et al., 2020). Therefore, to directly compare our $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between these studies, we also did not perform a lipid extraction or correction (Folch et al., 1957; Krause et al., 2020).

Carbon and nitrogen stable isotope analysis was performed at Baylor University using Elemental Analyzer (EA) Costech 4010 Elemental Combustion System (ECS) paired with a Conflow IV interphase (Thermo Scientific) and Thermo Delta V Advantage continuous flow Isotope Ratio Mass Spectrometer (EA-IRMS). Plasma nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) isotope values are expressed in delta notation (δ) in units of per mil (‰). Additionally, isotope values are reported as the ratio of the heavy to light isotope relative to international standards; atmospheric nitrogen and Vienna Pee Dee Belemnite (VPDB), respectively, using the following equation:

$$\delta X = \frac{R_{\text{sample}}}{R_{\text{standard}}} - R_{\text{standard}} * 1000$$

where X is the ^{13}C or ^{15}N and R is the corresponding ratio of $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$. The R_{standard} value is set by Pee Dee Belemnite for $\delta^{13}\text{C}$ and atmospheric N_2 for $\delta^{15}\text{N}$.

2.4 Body size and body condition

We assessed both body size and body condition to ensure our analysis accounted for the possibility that individuals may be in healthy body condition even though they are small in size and vice versa (Traylor and Alisauskas, 2006; Macbeth et al., 2012). Body mass (kg) was used as a proxy for body size. Body condition was visually assessed in the field and from high-resolution photographs showing leopard seals with a full-body profile taken from a perpendicular angle. We followed a qualitative protocol evaluating bony protrusions for body condition assessment as described in Hupman et al., 2020. Individuals were classified as in severe, poor, good, or excellent body condition based

on this evaluation method (Hupman et al., 2020). A body condition index was also calculated for each individual using the following scaled body mass (SBM) index equation (Peig and Green, 2009):

$$Mi = Mi ! \frac{Lo^{bsma}}{Li^C}$$

where Mi and Li are the mass and standard length of the individual, Lo is the mean standard length of the different age classes, $bsma$ is the scaling exponent from plotting natural log transformed mass by standard length for all seals, and the Mic was the predicted mass of the individual when standardized to Lo . We calculated a SBM index for all individuals except for the one juvenile seal since there was only a single representative in this age class. This SBM index allows for quantitative comparison of body condition between individuals

directly compare absolute hormone concentrations across different studies, we implemented guidelines to obtain average cortisol values from studies that measured cortisol in the same tissue (blood) and similar assay type. Previous studies have demonstrated that cortisol concentrations measured from both serum and plasma samples are directly comparable but other tissue samples (e.g., whiskers, hair, blubber) are not (Foster and Dunn, 1974; Sheriff et al., 2011). Therefore, our literature search only included studies for species where cortisol concentrations were measured in serum and/or plasma. Cortisol concentrations can also vary based on the analytical method (e.g., liquid chromatography–mass spectrometry (LC/MS) vs. RIA; (Faupel-Badger et al., 2010); as a result, we narrowed our search to only include studies where cortisol concentrations in plasma and serum were measured with an immunoassay (e.g., radioimmunoassay, enzyme-linked immunosorbent assay, chemiluminescence immunoassay; Webster et al., 1990; Shimada et al., 1995; Graham et al., 2015; Franco-Martinez et al., 2019).

We performed a comprehensive literature review using Google Scholar and Web of Science, specifically using the words ‘serum’, ‘plasma’, and ‘cortisol’ in our search terms. Individuals of both sexes and any age class were included in the comparison. From each relevant paper, we extracted the cortisol mean and median values and ranges, if provided. If multiple cortisol means and median values were reported within a study, such as between sexes, locations, age classes, and seasons, we included all the values in our comparison, and then averaged all values from the literature to get one value for each species. Since some papers only included a mean value, we provide a range based on the lowest and highest mean values reported across all papers for each species (henceforth: “mean range”). All cortisol concentrations were converted to mg/dL for comparison. Studies that were conducted on individuals living in human care facilities (e.g., zoos, aquariums) were not included in this review due to significant hormonal differences in captive and wild individuals (Gardiner and Hall, 1997; Creel, 2001). Some studies report serial cortisol concentrations as part of an experimental design (e.g., ACTH administrations); in these cases, we only used values that were collected as baseline measurements prior to the start of the experiment. Studies where animals were chemically immobilized were included in this review; previous work on other carnivorans has shown that cortisol values are not influenced by immobilization (Champagne et al., 2012). For Ross seals, the only study with serum cortisol concentrations stated values were “above the threshold of cortisol analysis” ($>1380\text{nmol/L}$; Tryland et al., 2012), thus the threshold value ($1380\text{nmol/L} = 50\text{ mg/dL}$) was used in this analysis. Mean values from each publication were combined to calculate one average value for each species.

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while accounting for differences in age class, length, and mass (Peig and Green, 2009; DeRango et al., 2019b; Charapata et al., 2023).

2.5 Metadata analysis

We conducted a metadata analysis of the peer-reviewed literature to compare cortisol concentrations among extant species of the mammalian clade Arctoidea for which comparable data were available. The Arctoidea clade includes three extant families of carnivorans: (1) Mustelidae (e.g., sea otters, ferrets, badgers), (2) Ursidae (e.g., bears), and (3) Pinnipedia. To ensure that we could

2.6 Statistical analyses

We conducted all data analyses in R version 1.4.1103 (R Core Team, 2022). Since there was only a single juvenile, age class was not assessed among this data and the juvenile was excluded from all analyses. Additionally, we only included one value chosen randomly from the one repeat individual (#397; season 2018). To reduce model complexity and account for our small sample size, we first ran univariate models to assess the associations between each predictor variable (sex, mass, $d^{15}\text{N}$, and $d^{13}\text{C}$, season, and SBM index) and the response variable (cortisol). Pearson’s correlation was used to measure the strength of the relationships and then we plotted and visualized the relationships (ggplot2, tidyverse; Wickham, 2016; Wickham et al., 2019). Variables that did not show significant associations were excluded from the multivariate model, specifically season and SBM index. We used variance inflation factors (VIF) to assess for multicollinearity among the predictor variables in our regression model. VIFs were calculated for each predictor variable, with values > 5 indicating multicollinearity (Hair et al., 2011). Given the imbalance between sexes and the multicollinearity observed between sex and mass ($\text{VIF} > 5$), sex was not included in the multivariate model. After removing sex as a predictor variable, no multicollinearity existed between the remaining

variables ($VIF < 5$). We used analysis of variance (ANOVAs) to assess the relationship between sex and cortisol and evaluated sex-specific differences in SBM index, mass, $\delta^{15}\text{N}$, and $\delta^{13}\text{C}$.

We then ran linear models to determine relationships between cortisol and all other variables (i.e., $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, mass). A previous leopard seal study identified a relationship between mass and $\delta^{15}\text{N}$ (Krause et al., 2020), thus we tested for a relationship and a significant interaction between mass and $\delta^{15}\text{N}$ with a linear regression and visualized the interaction plot (Figure S1). The full model included mass, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and the biologically relevant interaction between mass and $\delta^{15}\text{N}$. We ran all relevant combinations of the full model to compare with the full model (Table S1). Each candidate model was compared to the null model using likelihood ratio tests of null and residual deviances. Model residuals were visually assessed for approximate normality and homoscedasticity (Winter, 2013). Models were ranked based on Akaike information criterion corrected for small sample sizes (AICc) using the aictab function in the AICcmodavg package (Burnham, 2011; Mazerolle, 2020). The model with the lowest AICc represented the model with the highest support. We then examined the contribution of each fixed effect of our top model by looking at the estimated coefficients and p-value. All reported values are derived from the linear models. For our metadata analysis, we assessed if cortisol varied between groups of Arctoid (i.e., mustelids, ursids, pinnipeds) and pinnipeds (lobodontines and other pinnipeds) using an ANOVA followed by a Tukey's test to adjust the p-value for multiple comparisons.

3 Results

We measured cortisol concentrations, body mass, body condition (qualitative assessment and SBM index), and $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in 19 individual leopard seals (Tables 1, 2). The mean cortisol concentration for adult leopard seals was 100.35 ± 16.72 mg/dL, with a range from 74.93 to 147.40 mg/dL.

3.1 Sex-specific differences in leopard seals

We found sex-specific differences in cortisol, body mass, $\delta^{15}\text{N}$, and $\delta^{13}\text{C}$. Specifically, adult female leopard seals were significantly larger (445 ± 61 kg) than adult males (302 ± 21 kg; $F_{1,16} = 20.03$, $p < 0.001$). Adult males, the smaller sex, had significantly higher cortisol concentrations (120.86 ± 20.52 mg/dL) than adult females (94.49 ± 10.12 mg/dL; $F_{1,16} = 13.32$, $p = 0.002$; Figure 1A). Mean $\delta^{15}\text{N}$ was $11.08 \pm 1.05\text{‰}$ and ranged from 8.56 to 12.62‰ and differed between sexes ($F_{1,16} = 5.04$, $p = 0.03$; Figure 1B). Females had higher $\delta^{15}\text{N}$ values ($11.35 \pm 0.69\text{‰}$) than males ($10.14 \pm 1.65\text{‰}$; $F_{1,16} = 5.04$, $p = 0.03$). Additionally, there was a significant relationship between mass and $\delta^{15}\text{N}$; larger seals had higher $\delta^{15}\text{N}$ than smaller seals ($F_{1,16} = 6.90$, $p = 0.018$; Figure 2A). Mean plasma $\delta^{13}\text{C}$ was $-24.13 \pm 0.55\text{‰}$ and ranged from -23.12‰ to -25.18‰ and varied by sex ($F_{1,16} = 4.68$, $p = 0.04$), where females had slightly higher $\delta^{13}\text{C}$ ($-24.00 \pm 0.43\text{‰}$) than males ($-24.94 \pm 0.73\text{‰}$). We did not find sex-specific differences in body condition. Rather, all leopard seals were in 'excellent' body condition based on the qualitative assessment of bony protrusions (Hupman et al., 2020), and we found no differences based on the SBM index ($p < 0.05$).

3.2 Variability in cortisol concentrations

Intraspecific variability in cortisol concentrations was explained by body mass, $\delta^{15}\text{N}$, and the interaction of body mass and $\delta^{15}\text{N}$ ($F_{3,14} = 19.91$, $p < 0.001$; Table S2) and explained the most variance in cortisol levels ($R^2 = 0.81$, Adjusted $R^2 = 0.77$). Cortisol varied by mass ($t = -5.28$, $p < 0.001$); smaller individuals exhibiting higher cortisol concentrations (Figure 2B). Similarly, cortisol varied by $\delta^{15}\text{N}$ ($t = -5.46$, $p < 0.001$); seals with higher $\delta^{15}\text{N}$ values had lower cortisol concentrations than

seals with lower $\delta^{15}\text{N}$ values. Finally, cortisol varied based on the interaction between mass and $\delta^{15}\text{N}$ ($t = 5.04$, $p < 0.001$), indicating that body size and diet ($\delta^{15}\text{N}$) collectively influence cortisol levels (Figure 2C). Neither body condition (SBM index), season, nor plasma $\delta^{13}\text{C}$ contributed to variability in cortisol concentrations.

TABLE 2 Leopard seal cortisol concentrations measured in blood serum and $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values measured in blood plasma samples for the 20 leopard seals samples in this study.

Seal ID	Sex	Cortisol ($\mu\text{g/dL}$)	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)
12	F	101.64	11.78	-23.79
37	F	93.52	11.19	-23.88
57	F	106.21	12.30	-23.84
138	F	100.26	12.62	-23.12
140	M	147.40	8.56	-25.19
141	M	126.51	8.87	-24.91
142	F	99.45	10.90	-23.57
143	F	99.55	9.81	-24.68
144	M	102.72	11.65	-23.53
145	F	128.63	12.79	-23.64
153	F	81.22	11.46	-24.08
156	F	92.10	11.32	-23.81
157	M	106.79	11.51	-24.83
158	F	111.76	11.87	-23.93
159	F	92.56	11.42	-24.23
160	F	99.12	10.88	-24.85
161	F	86.53	11.43	-24.14
162	F	84.12	10.72	-24.18
397	F	88.03	12.08	-24.12
397*	F	74.93	11.32	-24.14

*Repeat individual sample used for analysis.

3.3 Comparison of cortisol concentrations across Arctoidea

We compared cortisol concentrations among 26 Arctoid species (pinnipeds, $n=20$; ursids, $n=3$; mustelids, $n=3$) from 41 peer-reviewed studies (Table 3; Figure 3). For pinnipeds, the mean cortisol concentration among the 20 species was 25.79 ± 30.49 mg/dL (mean range: 0.01 – 120.85 mg/dL). For ursids, the mean cortisol concentration among the three species was $8.94 \pm$

6.23 mg/dL (mean range: 1.55 – 19 mg/dL). For mustelids, the mean cortisol concentration among the three species was 4.72 ± 2.08 mg/dL (mean range: 2.17 – 9.34 mg/dL). Mean cortisol concentrations significantly differed among the three Arctoid groups ($F_{2,113} = 5.0$, $p = 0.008$, Figure 4A). Specifically, pinnipeds had significantly higher cortisol concentrations compared to mustelids ($p = 0.02$). However, pinniped cortisol concentrations did not differ from ursids ($p = 0.09$), nor between ursids and mustelids ($p = 0.91$). Across taxa, leopard seals had the highest cortisol concentration (mean: 93.01 ± 17.03 mg/dL; mean range: 61.95 – 120.86 mg/dL; this study, Liggins et al, 1993). More broadly, Antarctic seals, specifically the Lobodontini [Antarctic seals: crabeater, leopard, Ross, and Weddell seals] had the highest mean cortisol concentrations (76.94 ± 21.59 mg/dL, mean range: 38.82 – 120.86 mg/dL) compared to the other 16 pinniped taxa (mean: 11.17 ± 9.79 mg/dL; $F_{1,88} = 17.95$, $p < 0.001$; Figure 4B).

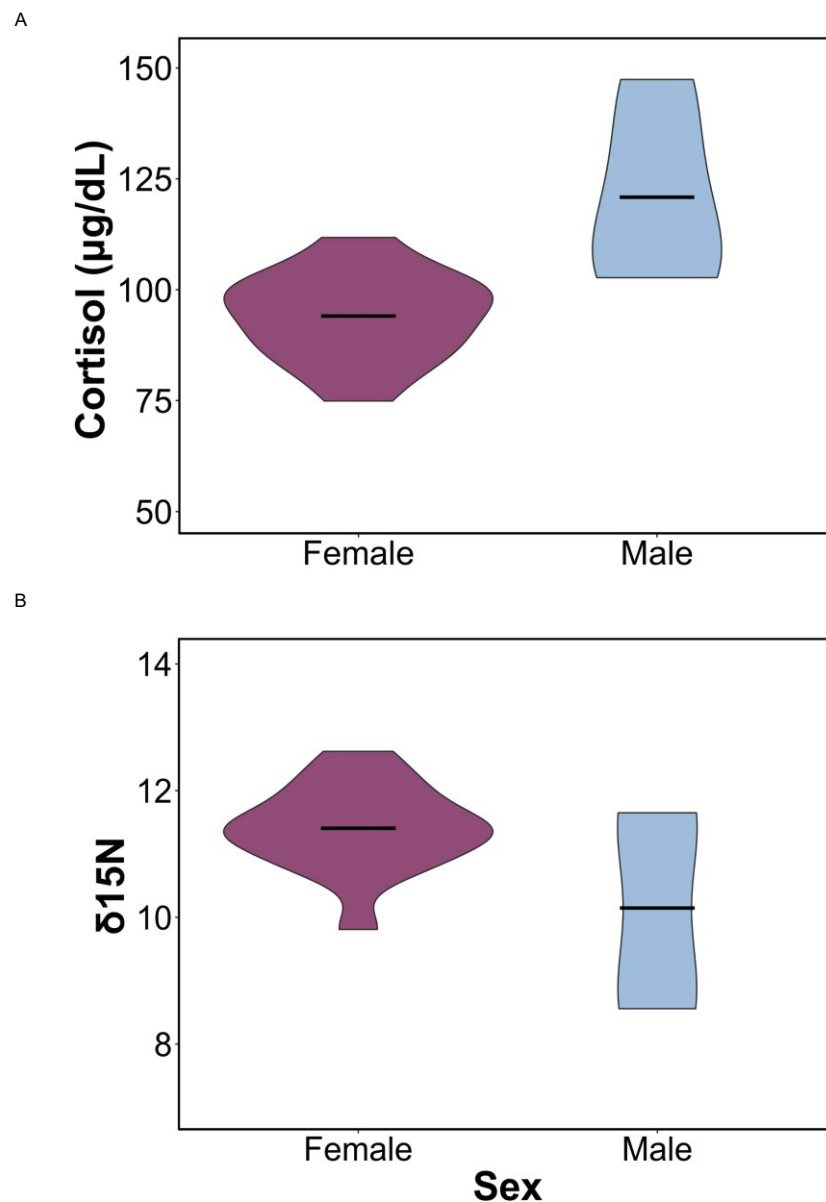


FIGURE 1

Sex-specific differences in cortisol and $\delta^{15}\text{N}$ values. (A) Violin plot comparing differences in mean cortisol concentrations between males and females ($F_{1,16} = 13.31$, $p = 0.002$). (B) Violin plot comparing in $\delta^{15}\text{N}$ between males and females ($F_{1,16} = 5.04$, $p = 0.03$). In both plots, horizontal bars represent the mean concentrations for each sex and the ends represent the range. Females are shown in purple, and males in light blue.

4 Discussion

Leopard seals have high cortisol concentrations compared to other related pinniped and Arctoid taxa. This finding aligns with the one previous study that measured cortisol concentrations in four leopard seals (Liggins et al., 1993). Our study expands our understanding of leopard

4.1 Variability in cortisol concentrations (sex, mass, and diet)

Our first hypothesis was that larger leopard seals, specifically females, would have lower cortisol concentrations than smaller seals (e.g., adult males and smaller females), and our results support this hypothesis. We find that larger bodied leopard seals have lower cortisol values compared to smaller seals. In fact, one of the smallest adult seals (male, 285 kg) had cortisol concentrations 58% higher than one of the largest seals (female, 500 kg). These size-based differences are inherently linked to sex. Leopard seals exhibit female-biased sexual dimorphism, where adult female leopard seals are up to 1.5 times larger than males (Kienle et al., 2022; this study). As a result, adult female leopard seals, the larger

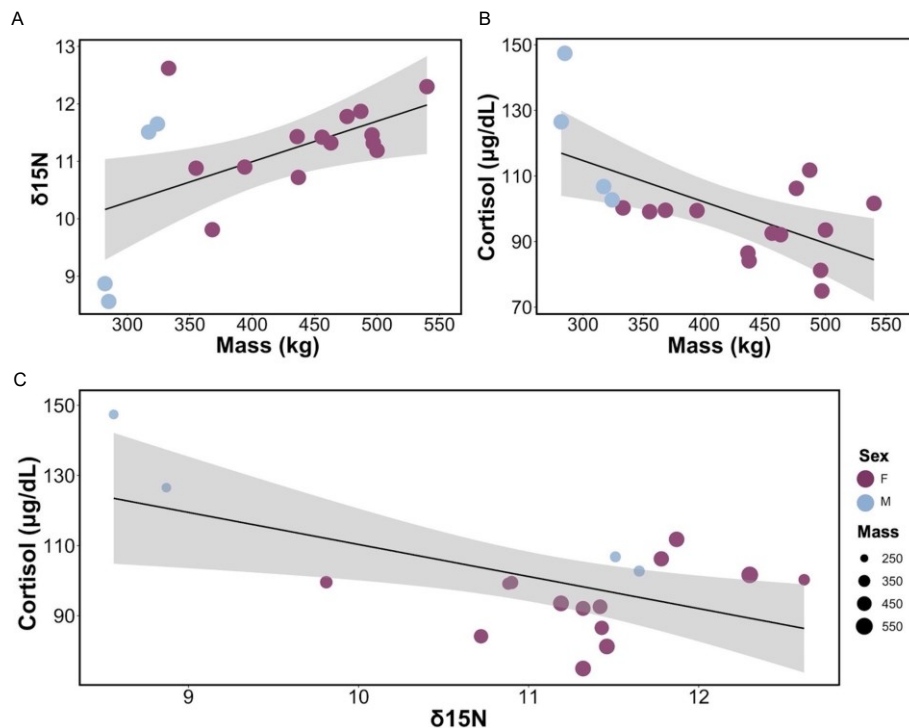


FIGURE 2

Relationships between cortisol, body mass, and $\delta^{15}\text{N}$ in leopard seals. (A) Linear regression between $\delta^{15}\text{N}$ and body mass for adult leopard seals ($y = 8.17 + 0.007x$, $R^2 = 0.30$, $p = 0.01$). (B) Linear regression of the relationship between cortisol and body mass for adult leopard seals ($y = 152.49 - 0.12x$, $R^2 = 0.39$, $p = 0.006$). (C) Bubble plot showing the linear regression between cortisol and $\delta^{15}\text{N}$, with bubble size representing the mass of each seal and their sex ($y = 201.7 - 9.14x$, $R^2 = 0.33$, $p = 0.01$). For all graphs, females are shown in purple, and males are in light blue.

seal physiology by measuring and comparing intraspecific variation in cortisol concentrations in one of the largest samples of leopard seals to date. As we predicted, leopard seal cortisol concentrations vary based on sex, morphometrics, and diet. Together, this study provides the foundation for evaluating the physiology of leopard seals in the context of a rapidly changing Antarctic environment.

sex, are the ones that have lower cortisol compared to adult males, the smaller sex.

Additionally, we hypothesized that leopard seals feeding at higher trophic levels (higher $\delta^{15}\text{N}$ values) would have lower cortisol concentrations and be in better body condition than seals feeding at lower trophic levels. Our findings were consistent with our hypothesis to some extent; leopard seals that have higher $\delta^{15}\text{N}$ isotopic values have lower cortisol values and are primarily large-bodied adult females. However, while there was a size-based difference in $\delta^{15}\text{N}$ values, there was no difference in body condition between any of the seals. While we had hypothesized that seals in better body condition would have higher $\delta^{15}\text{N}$ values, all of our seals were in excellent body condition and showed little variation. This finding may be location-specific, as leopard seals in other locations appear to show more variation in body condition (Hupman et al., 2020); in these areas, there may be a relationship between $\delta^{15}\text{N}$ and body condition that was not seen in this study. Furthermore, although we did not see a complete shift in trophic position, we did observe difference in isotopic values. Together these results showcase the importance of the interplay of sex, body size, and diet ($\delta^{15}\text{N}$ isotopic values) on cortisol regulation in leopard seals. These findings also align with our current knowledge on leopard seals' foraging ecology and behavior.

Large-bodied female leopard seals have some of the highest $\delta^{15}\text{N}$ values and lowest cortisol concentrations. Similarly, a recent study also reported higher $\delta^{15}\text{N}$ values in adult female leopard seals at

TABLE 3 Cortisol concentrations in 26 Arctoid species (e.g., pinnipeds, ursids, mustelids) from 41 peer-reviewed studies.

Species	N	Cortisol (mg/dL)	Reference
PINNIPEDS			
Antarctic fur seal (<i>Arctocephalus gazella</i>)	90	8.30 ± 2.2	1,3
Australian fur seal (<i>Arctocephalus pusillus doriferus</i>)	114	5.94	26
Bearded seal (<i>Erignathus barbatus</i>)	74	25.24 ± 13.3	16
California sea lion (<i>Zalophus californianus</i>)	266	9.00 ± 3.25	31-32
Crabeater seal (<i>Lobodon carcinophaga</i>)	13	45.54 ± 4.75	1,3
Galapagos sea lion (<i>Zalophus wolfebacki</i>)	34	1.98	33
Grey seal (<i>Halichoerus grypus</i>)	67	8.26 ± 6.51	13,20-22
Guadalupe fur seal (<i>Arctocephalus townsendi</i>)	36	8.32 ± 3.31	27
Harbor seal (<i>Phoca vitulina</i>)	219	21.08 ± 12.59	23-25
Harp seal (<i>Pagophilus groenlandicus</i>)	39	7.29 ± 2.82	13-14
Leopard seal (<i>Hydrurga leptonyx</i>)	24	93.01 ± 29.58	1-2
Northern elephant seal (<i>Mirounga angustirostris</i>)	35	6.44 ± 1.85	17-19
Ringed seal (<i>Pusa hispida</i>)	74	1.35 ± 0.07	11-12
Ross seal (<i>Ommatophoca rossii</i>)	20	50.02	3
South American fur seal (<i>Arctocephalus australis</i>)	36	0.01 ± 0.00	28
Southern elephant seal (<i>Mirounga leonine</i>)	106	16.19 ± 16.39	1,7-8
Stellar sea lion (<i>Eumetopias jubatus</i>)	1002	13.05 ± 2.71	29-30
Subantarctic fur seal (<i>Arctocephalus tropicalis</i>)	91	6.69 ± 3.31	9-10
Walrus (<i>Odobenus rosmarus</i>)	12	2.17	15
Weddell seal (<i>Leptonychotes weddellii</i>)	159	79.90 ± 16.39	1,3-6
URSIDS			
Black bear (<i>Ursus americanus</i>)	62	6.06 ± 3.95	38
Brown bear (<i>Ursus arctos</i>)	251	7.89 ± 7.92	35-37
Polar bear (<i>Ursus maritimus</i>)	125	12.66 ± 3.40	34-35
MUSTELIDS			
American marten (<i>Martes americana</i>)	40	8.60 ± 1.07	41
Eurasian badger (<i>Meles meles</i>)	42	2.17	40
Sea otter (<i>Enhydra lutris</i>)	161	4.19 ± 1.15	39

REFERENCES: (Liggins et al., 1993)¹; this study²; (Tryland et al., 2012)³; (Shero et al., 2015)⁴; (Hogg and Rogers, 2009)⁵; (Bartsh et al., 1992)⁶; (Engelhard et al., 2002)⁷; (Ferreira et al., 2005)⁸; (Verrier et al., 2012)⁹; (Guinet et al., 2004)¹⁰; (Tryland et al., 2006)¹¹; (Rainer Engelhardt, 1982)¹²; (Engelhardt and Ferguson, 1980)¹³; (Nordøy et al., 1993)¹⁴; (Tryland et al., 2009)¹⁵; (Tryland et al., 2021)¹⁶; (Champagne et al., 2005)¹⁷; (Ensminger et al., 2014)¹⁸; (Crocker et al., 2012)¹⁹; (Lidgard et al., 2008)²⁰; (Bennett et al., 2012)²¹; (Nordøy et al., 1990)²²; (Gardiner and Hall, 1997)²³; (Gulland et al., 1999)²⁴; (Di Poi et al., 2015)²⁵; (Atkinson et al., 2011)²⁶; (DeRango et al., 2019a)²⁷; (Seguel et al., 2019)²⁸; (Myers et al., 2010)²⁹; (Keogh et al., 2013)³⁰; (Pedemera-Romano and Auriolles-Gamboa, 2010)³¹; (DeRango et al., 2019b)³²; (DeRango et al., 2021)³³; (Haave et al., 2003)³⁴; (Chow, 2013)³⁵; (Macbeth et al., 2010)³⁶; (Brannon, 1985)³⁷; (Harlow et al., 1990)³⁸; (Larson et al., 2009)³⁹; (George et al., 2014)⁴⁰; (Boonstra et al., 2018)⁴¹.

Species mean cortisol concentrations represent the combined cortisol average from each referenced study. N refers to the cumulative sample size across all studies, representing the total number of samples. Values reported as mean ± standard deviation. Species are arranged in alphabetical order for each section.

Cape Shirreff (individuals not included in this study) than adult males (Krause et al., 2020). The authors suggested that females' higher nitrogen values reflected an increased consumption of prey with higher $\delta^{15}\text{N}$ (or nitrogen-enriched prey), such as Antarctic fur seals and penguins, compared to males (Krause et al., 2020). At Cape Shirreff, female

leopard seals routinely feed on penguins and Antarctic fur seal pups from December to March (Krause et al., 2015; Krause et al., 2020; Krause et al., 2022). Long-term studies at Cape Shirreff have documented that large adult female leopard seals haul-out more frequently and in higher concentrations near Antarctic fur seal and penguin breeding colonies than anywhere else in the region (Krause et al., 2015; Krause et al., 2016; Kienle et al., 2022). Further, large female leopard seals exclude males and less dominate females from these nutrient rich resources (Krause et al., 2015; Krause and Rogers, 2019). These large female leopard seals are so successful at predating on pups that they are driving the decline of

Antarctic fur seals at Cape Shirreff (Hiruki et al., 1999; Krause et al., 2015; Krause et al., 2020). Similarly, behavioral observations during feeding events have shown that female leopard seals occasionally engage in kleptoparasitism and steal prey from conspecifics (Krause et al., 2015). Video footage confirms that large adult females are the ones that are successful when defending or stealing prey (Krause et al., 2015). Success during these behavioral interactions seems to be

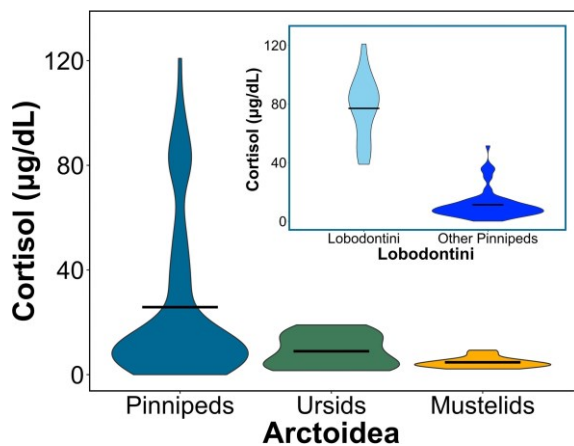


FIGURE 4

Circulating cortisol concentrations vary between pinnipeds (seals, sea lions, and walruses), ursids (bears), and mustelids (i.e., skunks, minks, ferrets). (Large plot) Violin plot comparing mean cortisol concentrations between the groups of Arctoidea ($F_{2,113} = 5$, $p = 0.008$). Horizontal bars represent the mean cortisol concentration for each group, and the ends of each violin plot represent the range. Pinniped (blue) cortisol concentrations differed from mustelids (yellow; $p = 0.02$) but not ursids (green; $p > 0.05$), and ursids and mustelids did not significantly differ from each other. (Sub plot) Violin plot comparing mean cortisol concentrations between lobodontine, or Antarctic seals (light blue), to all other pinnipeds (dark blue; $F_{1,88} = 17.95$, $p < 0.001$). Horizontal bars represent the mean cortisol concentration for each group, and the ends of the violin plot represent the range.

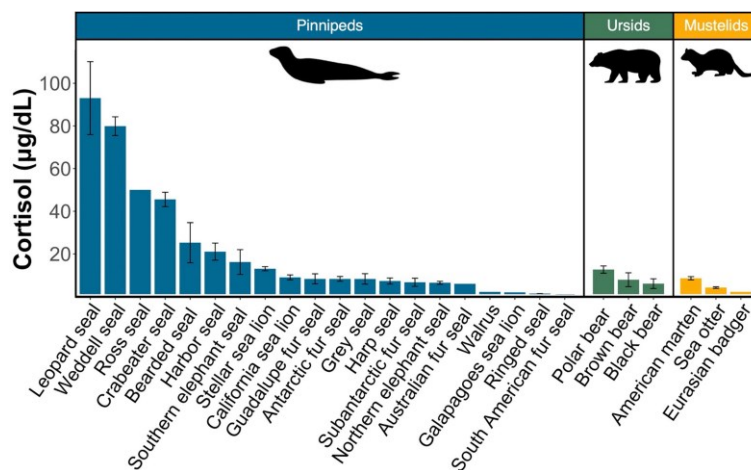


FIGURE 3

Comparison of mean circulating cortisol concentrations in pinnipeds (blue), ursids (green), and mustelids (yellow) calculated from peer-reviewed studies and this study. Bars represent the combined mean cortisol concentration for each species. Error bars represent the standard error of the mean. References for each species can be found in Table 3.

driven by the combination of both sex and body size, suggesting that females' large body size confers a competitive advantage in these interspecific foraging interactions, especially when feeding on endothermic prey.

Behavioral interactions—such as aggressive and competitive interactions among conspecifics—can have severe physical and physiological consequences (Bronson and Eleftheriou, 1964; Manogue et al., 1975; Creel et al., 1997; Creel, 2001; Creel, 2005). During competitive interactions over resources, the 'losers' often have higher cortisol concentrations (Bronson and Eleftheriou, 1964; Manogue et al., 1975; Creel, 2001; Abbott et al., 2003), while the 'winners' typically have lower cortisol concentrations and are often the larger individuals (Clutton-Brock et al., 1988; Rutberg and Greenberg, 1990; Archie et al., 2006; Stockley and Bro-Jørgensen, 2011). Large adult females seem to outcompete smaller individuals of both sexes (e.g., adult males, smaller females, and juveniles), resulting in access to high quality, nitrogen-rich prey resources which is reflected in their high $d^{15}N$ values [(Krause et al., 2020); this study]. Physiologically, this may lead to larger leopard seals, specifically females, having lower cortisol values than smaller conspecifics.

Similarly, animals that consume less food, lower-quality food, and/or have a less nutritious diet can exhibit nutritional stress, an imbalance of the intake of essential nutrients, that can lead to higher cortisol concentrations (McEwen and Lasley, 2002; Bryan et al., 2013); this pattern is well documented in terrestrial mammals (Barboza et al., 2004; Bryan et al., 2013; George et al., 2014; Lafferty et al., 2015) and birds (Kitaysky et al., 1999; Cockrem, 2007). In harbor (*Phoca vitulina*), ringed (*Pusa hispida*), and spotted (*Phoca largha*) seals, for example, studies have shown an inverse relationship between cortisol and diet ($d^{15}N$); as $d^{15}N$ values increase, cortisol decreases ((Karpovich et al., 2019; Ogloff et al., 2022)). Furthermore, in spotted seals, measurements of cortisol and $d^{15}N$ along whiskers (which represents a longitudinal time-series) showed that cortisol increased concurrently with a decrease in $d^{15}N$;

this shows that higher cortisol is associated with feeding at a lower trophic level (Karpovich et al., 2019). In this study, we see the same pattern. Adult leopard seals with higher $d^{15}N$ had lower cortisol concentrations than leopard seals with lower $d^{15}N$; this suggests diet regulates cortisol differences in this species.

Together, leopard seals show intraspecific variation in cortisol concentrations based on the interplay of sex, body mass, and $d^{15}N$. Specifically, female leopard seals are larger, have lower cortisol concentrations, higher $d^{15}N$, and show behaviors consistent with foraging at the top of the food web. Conversely, males are smaller, have higher cortisol concentrations, lower $d^{15}N$, and likely feed lower on the food web. While our sample size is small, our results reveal high variability in life history, ecological, morphological, and physiological traits in leopard seals from a single location. We predict that future studies with increased sample sizes and geographic coverage will further confirm high variability in leopard seal ecophysiological traits, as well as drivers of cortisol differences between individuals and populations.

4.2 Comparison of cortisol concentrations among arctoidea

Adult leopard seals in our study had mean cortisol concentration ~100 mg/dL, which is 1.6 times higher than previously reported for the species (Liggins et al., 1993). Here, the highest measured cortisol value was 147 mg/dL in a male leopard seal; this is the highest circulating cortisol concentration ever reported for a pinniped. Previously, the highest measured pinniped cortisol value was 104.8 mg/dL in a Weddell seal (Bartsh et al., 1992). Nearly half of the leopard seals in this study (47%, $n=9$) had cortisol concentrations that exceeded 100 mg/dL. Leopard seals also had the highest cortisol concentrations among 26 related mammals (e.g., pinnipeds, ursids, and mustelids) with values that ranged from 1.25 (Weddell seals) to 50 times higher (Galapagos sea lion). These

findings showcase just how remarkably high leopard seal cortisol concentrations are.

More broadly, we found that lobodontines (Antarctic seals; crabeater, leopard, Ross, and Weddell seals) had much higher cortisol concentrations than other pinnipeds, ursids, and mustelids. Lobodontines have cortisol concentrations up to 7 times higher than other pinnipeds. Previous studies have also noted high cortisol levels in Antarctic seals (Liggins et al., 1979; Bartsh et al., 1992; Hogg and Rogers, 2009; Tryland et al., 2012). One hypothesis for high cortisol levels in lobodontines is that most of these species are deep divers and that high cortisol helps protect against high pressure syndrome (Liggins et al., 1993; Hogg and Rogers, 2009). Weddell seals were used to support this hypothesis; the species is characterized by both high cortisol (mean: ~80 mg/dL) and relatively deep, long dives (mean dive depth: 100–350 m, mean dive duration: 15–20 min; Kooyman et al., 1981; Castellini et al., 1992; Liggins et al., 1993; Davis and Kanatous, 1999). However, southern elephant seals (*Mirounga leonina*), another Southern Ocean pinniped, are deeper, longer divers (mean dive depth: 300–600 m, mean dive duration: 25 mins; Hindell et al., 1991; Jonker and Bester, 1998; Engelhard et al., 2002) but do not exhibit high cortisol (mean: ~16 mg/dL). Leopard seals also do not support this hypothesis; leopard seals are short, shallow divers (mean dive depth: 16–29 m, mean dive duration: 2–3 min; Krause et al., 2016; Kienle et al., 2022), yet have the highest cortisol concentrations.

We suggest that high cortisol is a specialized adaption within the lobodontines clade. There are six species of Southern Ocean pinnipeds, which are split into phylogenetically distinct clades: lobodontines (4 species), southern elephant seals (*Mirounga*), and Antarctic fur seals (*Otariinae*; Arnason et al., 2006). This clade of lobodontines diverged from elephant seals approximately 6.9 Ma (Berta et al., 2022). Southern elephant seals and Antarctic fur seals are often distributed farther north in subpolar habitats and rely on land, rather than ice, during the breeding season (Leboeuf et al., 1986; Siniff, 1991; Laws, 1994; Lunn et al., 1994). In contrast, lobodontines have a more southern distribution, inhabit subAntarctic and Antarctic waters, and breed on pack-ice (Siniff, 1991; Berta et al., 2022). These different life histories, distribution patterns, and evolutionary pressures may explain why lobodontines exhibit significantly higher cortisol compared to other pinnipeds.

One method that can be used to better assess the role of high cortisol in lobodontines is to measure the amount of free cortisol. Free cortisol is the active cortisol that can be utilized by tissues during presumably stressful events (Desantis et al., 2013). Cortisol is bound by a protein in the blood, the corticosteroid binding globulin (CBG), and when cortisol is bound by CBG, it becomes inactive and has no biological effect. Free cortisol is unbound, biologically active, and available for uptake and use by tissues. Under chronic stress, individuals will exhibit high levels of glucocorticoids, a reduction in CBG, and an increase in free cortisol (Mendel, 1989; Boonstra et al., 1998; Boonstra, 2005). Most studies, including this one, have analyzed cortisol concentrations in blood serum or plasma samples, thereby measuring total (free and bound). Only a handful of studies have compared free and bound

cortisol separately in lobodontines. For example, Liggins et al. (1979) reported that more than 90% of cortisol was bound in Weddell seals and reported a similar result in a single leopard seal sample (Liggins et al., 1993). A recent study measured free glucocorticoids by assessing the maximum corticosteroid binding capacity (MCBC); the authors found that two lobodontines (crabeater and Weddell seals) had MCBC concentrations 4 to 15 times higher than other non-lobodontine pinnipeds (e.g., California sea lions, Antarctic and Australian fur seals; Delehanty et al., 2020). These results suggest that lobodontines have a ‘reservoir’ of available, but not biologically active, cortisol. Based on these findings, we would expect that cortisol in leopard seals would be mostly bound and not active, suggesting leopard seals (as a species) are not physiologically stressed. Therefore, we suggest that high cortisol levels may be an evolutionary adaption that is specific to leopard seals and, more broadly, all lobodontine seals, rather than a reflection of high stress levels. However, additional research comparing free versus bound cortisol among pinnipeds and within lobodontines species is needed to elucidate the role of high cortisol in these Antarctic-dwelling predators.

4.3 Conclusions and considerations

The primary goal of our study was to analyze cortisol levels of leopard seals and evaluate how they compared to other Arctoid species. Additionally, we aimed to assess how cortisol varies within a

population of leopard seals, based on the factors sex, body mass and condition, and isotopic values. We demonstrate that leopard seals have high cortisol concentrations that vary with body mass, sex, and diet ($\delta^{15}\text{N}$). This is the most comprehensive study of leopard seal cortisol concentrations and the first study to investigate the relationship between cortisol and ecophysiological traits. Leopard seals show high variability in ecological, morphological, and physiological traits, even from a single location, like Cape Shirreff.

Because leopard seals are difficult to study, our study’s sample size is relatively small, especially for males and juveniles. While this limits our ability to determine the mechanistic drivers of intraspecific variability in cortisol, future studies will likely build on this work to confirm and expand our understanding of leopard seal physiology. Future research should target leopard seals across their core and extended range throughout the Southern Ocean and increase sampling across different life history stages. Furthermore, our metaanalysis showcased a comparison of cortisol concentrations across various carnivoran taxa. For our metadata analysis, we implemented stringent criteria for a study to be included in our analysis; however, we acknowledged that our metaanalysis should not serve as a definitive benchmark for cortisol values across different species, as each study had its own limitations regarding sample sizes, collection and analysis methods, and results should be interpreted accordingly. Nevertheless, to our knowledge this is the first metaanalysis to conduct inter-species comparisons in cortisol concentrations, and these types of comparisons are valuable for

comparative studies investigating drivers and evolutionary patterns of physiological traits.

In conclusion, evaluating the physiology of leopard seals in regard to their life history and ecology provides crucial baseline knowledge on the health of individuals and the population. Leopard seals are one of the least studied apex predators on Earth but play a disproportionately large role in Antarctic ecosystem structure and function. Therefore, our study provides a strong foundation for assessing leopard seals' physiology, which is fundamental for understanding their vulnerability to climate change.

Data availability statement

The original contributions presented in the study are included in the article/[Supplementary Material](#). Further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was reviewed and approved by Institutional Animal Care and Use Committee at the University of California, Santa Cruz, federal authorizations for marine mammal research under National Marine Fisheries Service permit #19439, and an Antarctic Conservation Act permit #2018-016.

Author contributions

ES: conceptualization, investigation, methodology, data visualization, formal analysis, writing (original, review, and editing). DEC: conceptualization, investigation, methodology, sampling acquisition, funding, writing (review and editing). RB-C: sample acquisition, formal analysis, writing (review and editing). DK: formal analysis, writing (review and editing). DPC, MG, SBK, and ST: conceptualization, sample acquisition, funding, project administration, writing (review and editing). SSK: conceptualization, sample acquisition, project administration, funding, supervision, writing (review and editing). All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at:

<https://www.frontiersin.org/articles/10.3389/fmars.2023.1179236/full#supplementary-material>

References

- Abbott, D. H., Keverne, E. B., Bercovitch, F. B., Shively, C. A., Mendoza, S. P., Saltzman, W., et al. (2003). Are subordinates always stressed? a comparative analysis of rank differences in cortisol levels among primates. *Horm. Behav.* 43, 67–82. doi: 10.1016/s0018-506x(02)00037-5
- Anestis, S. F., Bribiescas, R. G., and Hasselschwert, D. L. (2006). Age, rank, and personality effects on the cortisol sedation stress response in young chimpanzees. *Physiol. Behav.* 89, 287–294. doi: 10.1016/j.physbeh.2006.06.010
- Archie, E. A., Morrison, T. A., Foley, C. A. H., Moss, C. J., and Alberts, S. C. (2006). Dominance rank relationships among wild female African elephants, *Loxodonta africana*. *Anim. Behav.* 71, 117–127. doi: 10.1016/j.anbehav.2005.03.023
- Armason, U., Gullberg, A., Janke, A., Kullberg, M., Lehman, N., Petrov, E. A., et al. (2006). Pinniped phylogeny and a new hypothesis for their origin and dispersal. *Mol. Phylogenet. Evol.* 41, 345–354. doi: 10.1016/j.ympev.2006.05.022
- Ashley, N. T., Wingfield, J. C., Demas, G. E., and Nelson, R. J. (2011). Sickness behavior in vertebrates. *Ecoimmunology* (New York, NY:Oxford University Press) 296, 45–91.
- Atkinson, S., Arnould, J. P. Y., and Mashburn, K. L. (2011). Plasma cortisol and thyroid hormone concentrations in pre-weaning Australian fur seal pups. *Gen. Comp. Endocrinol.* 172, 277–281. doi: 10.1016/j.ygcen.2011.03.014
- Aurich, J., Wulf, M., Ille, N., Erber, R., von Lewinski, M., Palme, R., et al. (2015). Effects of season, age, sex, and housing on salivary cortisol concentrations in horses. *Domest. Anim. Endocrinol.* 52, 11–16. doi: 10.1016/j.domaniend.2015.01.003
- Azevedo, A., Bailey, L., Bandeira, V., Dehnhard, M., Fonseca, C., de Sousa, L., et al. (2019). Correction: age, sex and storage time influence hair cortisol levels in a wild mammal population. *PloS One* 14, e0222963. doi: 10.1371/journal.pone.0222963
- Barboza, P. S., Hartbauer, D. W., Hauer, W. E., and Blake, J. E. (2004). Polygynous mating impairs body condition and homeostasis in male reindeer (*Rangifer tarandus tarandus*). *J. Comp. Physiol. B* 174, 309–317. doi: 10.1007/s00360-004-0416-6
- Barrell, G. K., and Montgomery, G. W. (1989). Absence of circadian patterns of secretion of melatonin or cortisol in weddell seals under continuous natural daylight. *J. Endocrinol.* 122, 445–449. doi: 10.1677/joe.0.1220445
- Bartsh, S. S., Johnston, S. D., and Siniff, D. B. (1992). Territorial behavior and breeding frequency of male weddell seals (*Leptonychotes weddellii*) in relation to age, size, and concentrations of serum testosterone and cortisol. *Can. J. Zool.* 70, 680–692. doi: 10.1139/z92-102

- Beaulieu-McCoy, N. E., Sherman, K. K., Trego, M. L., Crocker, D. E., and Kellar, N. M. (2017). Initial validation of blubber cortisol and progesterone as indicators of stress response and maturity in an otariid; the California sea lion (*Zalophus californianus*). *Gen. Comp. Endocrinol.* 252, 1–11. doi: 10.1016/j.ygcen.2017.07.003
- Bennett, K. A., Moss, S. E. W., Pomeroy, P., Speakman, J. R., and Fedak, M. A. (2012). Effects of handling regime and sex on changes in cortisol, thyroid hormones and body mass in fasting grey seal pups. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 161, 69–76. doi: 10.1016/j.cbpa.2011.09.003
- Berta, A., Churchill, M., and Boessenecker, R. W. (2022). "The origin of phocid seals and evolution of key behavioral character traits," in *Ethology and behavioral ecology of phocids*. Eds. D. P. Costa and E. A. McHuron (Cham: Springer International Publishing), 3–30. doi: 10.1007/978-3-030-88923-4_1
- Bonner, W. N. (1994). Seals and sea lions of the world (Facts on File).
- Boonstra, R. (2005). Equipped for life: the adaptive role of the stress axis in Male mammals. *J. Mammal.* 86, 236–247. doi: 10.1644/BHE-001.1
- Boonstra, R., Dusek, A., and Flynn, R. W. (2018). DHEA and territoriality during the nonbreeding season in male American martens (*Martes americana*). *J. Mammal.* 99, 826–835.
- Boonstra, R., Hik, D., Singleton, G. R., and Tinnikov, A. (1998). The impact of predator-induced stress on the snowshoe hare cycle. *Ecol. Monogr.* 68, 371–394. doi: 10.1890/0012-9615(1998)068[0371:tiopis]2.0.co;2
- Botta, S., Secchi, E. R., Rogers, T. L., Prado, J. H. F., de Lima, R. C., Carlini, P., et al. (2018). Isotopic niche overlap and partition among three Antarctic seals from the Western Antarctic peninsula. *Deep Sea Res. Part 2 Top. Stud. Oceanogr.* 149, 240–249. doi: 10.1016/j.dsr2.2017.11.005
- Boveng, P. L., Hiruki, L. M., Schwartz, M. K., and Bengtson, J. L. (1998). Population growth of antarctic fur seals: limitation by a top predator, the leopard seal? *Ecology* 79, 2863–2877. doi: 10.1890/0012-9658(1998)079[2863:pgoafs]2.0.co;2
- Brannon, R. D. (1985). Serum chemistry of central and northern Alaska grizzly bears. *J. Wildl. Manage.* 49, 893–900. doi: 10.2307/3801365
- Bronson, F. H., and Eleftheriou, B. E. (1964). Chronic physiological effects of fighting in mice. *Gen. Comp. Endocrinol.* 4, 9–14. doi: 10.1016/0016-6480(64)90033-4
- Bryan, H. M., Darimont, C. T., Paquet, P. C., Wynne-Edwards, K. E., and Smits, J. E. G. (2013). Stress and reproductive hormones in grizzly bears reflect nutritional benefits and social consequences of a salmon foraging niche. *PloS One* 8, e80537. doi: 10.1371/journal.pone.0080537
- Burnham, K. P., Anderson, D. R., and Huyvaert, K. P. (2011). AIC model selection and multimodel inference in behavioral ecology: some background, observations, and comparisons. *Behav. Ecol. Sociobiol.* 65, 23–35. doi: 10.1007/s00265-010-1029-6
- Castellini, M. A., Kooyman, G. L., and Ponganis, P. J. (1992). Metabolic rates of freely diving weddell seals: correlations with oxygen stores, swim velocity and diving duration. *J. Exp. Biol.* 165, 181–194. doi: 10.1242/jeb.165.1.181
- Champagne, C. D., Houser, D. S., Costa, D. P., and Crocker, D. E. (2012). The effects of handling and anesthetic agents on the stress response and carbohydrate metabolism in northern elephant seals. *PloS One* 7, e38442. doi: 10.1371/journal.pone.0038442
- Champagne, C. D., Houser, D. S., and Crocker, D. E. (2005). Glucose production and substrate cycle activity in a fasting adapted animal, the northern elephant seal. *J. Exp. Biol.* 208, 859–868. doi: 10.1242/jeb.01476
- Charapata, P., Clark, C. T., Miller, N., Kienle, S. S., Costa, D. P., Goebel, M. E., et al. (2023). Whiskers provide time-series of toxic and essential trace elements, Se:Hg molar ratios, and stable isotope values of an apex Antarctic predator, the leopard seal. *Sci. Total Environ.* 854, 158651. doi: 10.1016/j.scitotenv.2022.158651
- Chow, B. A. (2013). Stress physiology of bears: cortisol dynamics and identification of novel serum proteins. (University of Waterloo)
- Clutton-Brock, T. H., Green, D., Hiraiwa-Hasegawa, M., and Albon, S. D. (1988). Passing the buck: resource defence, lek breeding and mate choice in fallow deer. *Behav. Ecol. Sociobiol.* 23, 281–296. doi: 10.1007/bf00300575
- Cockrem, J. F. (2007). Stress, corticosterone responses and avian personalities. *J. fur Ornithol.* 148, 169–178. doi: 10.1007/s10336-007-0175-8
- Constable, S., Parslow, A., Dutton, G., Rogers, T., and Hogg, C. (2006). Urinary cortisol sampling: a non-invasive technique for examining cortisol concentrations in the weddell seal, *Leptonychotes weddellii*. *Zoo Biol.* 25, 137–144. doi: 10.1002/zoo.20088
- Creel, S. (2001). Social dominance and stress hormones. *Trends Ecol. Evol.* 16, 491–497. doi: 10.1016/S0169-5347(01)02227-3
- Creel, S. (2005). Dominance, aggression, and glucocorticoid levels in social carnivores. *J. Mammal.* 86, 255–264. doi: 10.1644/BHE-002.1
- Creel, S., Creel, N. M., Mills, M. G. L., and Monfort, S. L. (1997). Rank and reproduction in cooperatively breeding African wild dogs: behavioral and endocrine correlates. *Behav. Ecol.* 8, 298–306. doi: 10.1093/beheco/8.3.298
- Creel, S., Dantzer, B., Goymann, W., and Rubenstein, D. R. (2013). The ecology of stress: effects of the social environment. *Funct. Ecol.* 27, 66–80. doi: 10.1111/j.13652435.2012.02029.x
- Crocker, D. E., Ortiz, R. M., Houser, D. S., Webb, P. M., and Costa, D. P. (2012). Hormone and metabolite changes associated with extended breeding fasts in male northern elephant seals (*Mirounga angustirostris*). *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 161, 388–394. doi: 10.1016/j.cbpa.2011.12.013
- Davis, R. W., and Kanatous, S. B. (1999). Convective oxygen transport and tissue oxygen consumption in weddell seals during aerobic dives. *J. Exp. Biol.* 202, 1091–1113. doi: 10.1242/jeb.202.9.1091
- Delehanty, B., Bossart, G. D., Champagne, C., Crocker, D. E., Elliott, K. H., Fair, P. A., et al. (2020). Measurement of free glucocorticoids: quantifying corticosteroid binding capacity and its variation within and among mammal and bird species. *Conserv. Physiol.* 8, coaa057. doi: 10.1093/conphys/coaa057
- DeNiro, M. J., and Epstein, S. (1977). Mechanism of carbon isotope fractionation associated with lipid synthesis. *Science* 197, 261–263. doi: 10.1126/science.327543
- DeRango, E. J., Greig, D. J., Gálvez, C., Norris, T. A., Barbosa, L., Elorriaga-Verplancken, F. R., et al. (2019a). Response to capture stress involves multiple corticosteroids and is associated with serum thyroid hormone concentrations in Guadalupe fur seals (*Arctocephalus philippii townsendi*). *Mar. Mamm. Sci.* 35, 72–92. doi: 10.1111/mms.12517
- DeRango, E. J., Prager, K. C., Greig, D. J., Hooper, A. W., and Crocker, D. E. (2019b). Climate variability and life history impact stress, thyroid, and immune markers in California sea lions (*Zalophus californianus*) during El Niño conditions. *Conserv. Physiol.* 7, cozo10. doi: 10.1093/conphys/cozo10
- DeRango, E. J., Schwarz, J. F. L., Piedrahita, P., Pérez-Rosas, D., Crocker, D. E., and Krüger, O. (2021). Hormone-mediated foraging strategies in an uncertain environment: insights into the at-sea behavior of a marine predator. *Ecol. Evol.* 11, 7579–7590. doi: 10.1002/ecs3.7590
- Desantis, L. M., Delehanty, B., Weir, J. T., and Boonstra, R. (2013). Mediating free glucocorticoid levels in the blood of vertebrates: are corticosteroid-binding proteins always necessary? *Funct. Ecol.* 27, 107–119. doi: 10.1111/1365-2435.12038
- Di Poi, C., Atkinson, S., Hoover-Miller, A., and Blundell, G. (2015). Maternal buffering of stress response in free-ranging pacific harbor seal pups in Alaska. *Mar. Mamm. Sci.* 31, 1098–1117. doi: 10.1111/mms.12217
- Dot, T. J., Rosen, D. A. S., Richmond, J. P., Kitaysky, A. S., Zinn, S. A., and Trites, A. W. (2009). Changes in glucocorticoids, IGF-I and thyroid hormones as indicators of nutritional stress and subsequent refeeding in steller sea lions (*Eumetopias jubatus*). *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 152, 524–534. doi: 10.1016/j.cbpa.2008.12.010
- Engelhard, G. H., Brasseur, S. M. J. M., Hall, A. J., Burton, H. R., and Reijnders, P. J. H. (2002). Adrenocortical responsiveness in southern elephant seal mothers and pups during lactation and the effect of scientific handling. *J. Comp. Physiol. B* 172, 315–328. doi: 10.1007/s00360-002-0257-0
- Engelhardt, F. R., and Ferguson, J. M. (1980). Adaptive hormone changes in harp seals, phoca groenlandica, and gray seals, halichoerus grypus, during the postnatal period. *Gen. Comp. Endocrinol.* 40, 434–445. doi: 10.1016/0016-6480(80)90006-4
- Ensminger, D. C., Somo, D. A., Houser, D. S., and Crocker, D. E. (2014). Metabolic responses to adrenocorticotrophic hormone (ACTH) vary with life-history stage in adult male northern elephant seals. *Gen. Comp. Endocrinol.* 204, 150–157. doi: 10.1016/j.ygcen.2014.04.024
- Faupel-Badger, J. M., Fuhrman, B. J., Xu, X., Falk, R. T., Keefer, L. K., Veenstra, T. D., et al. (2010). Comparison of liquid chromatography-tandem mass spectrometry, RIA, and ELISA methods for measurement of urinary estrogens. *Cancer Epidemiol. Biomarkers Prev.* 19, 292–300. doi: 10.1158/1055-9965.EPI-09-0643
- Ferreira, A. P. S., Martinez, P. E., Colares, E. P., Robaldo, R. B., Berne, M. E. A., Miranda Filho, K. C., et al. (2005). Serum immunoglobulin G concentration in southern elephant seal, mirounga leonina (Linnaeus), from elephant island (Antarctica): sexual and adrenal steroid hormones effects. *Veterinary Immunol. Immunopathology* 106, 239–245. doi: 10.1016/j.vetimm.2005.02.024
- Folch, J., Lees, M., and Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 226, 497–509. doi: 10.1016/S0021-9258(18)64849-5
- Foster, L. B., and Dunn, R. T. (1974). Single-antibody technique for radioimmunoassay of cortisol in unextracted serum or plasma. *Clin. Chem.* 20, 365–368. doi: 10.1093/clinchem/20.3.365
- Franco-Martinez, L., Tvarijonavičute, A., Martínez-Subiela, S., Teles, M., and Tort, L. (2019). Chemiluminescent assay as an alternative to radioimmunoassay for the measurement of cortisol in plasma and skin mucus of oncorhynchus mykiss. *Ecol. Indic.* 98, 634–640. doi: 10.1016/j.ecolind.2018.11.046
- Gannes, L. Z., Martinez del Rio, C., and Koch, P. (1998). Natural abundance variations in stable isotopes and their potential uses in animal physiological ecology. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 119, 725–737. doi: 10.1016/S1095-6433(98)01016-2
- Gardiner, K. J., and Hall, A. J. (1997). Diel and annual variation in plasma cortisol concentrations among wild and captive harbor seals (*Phoca vitulina*). *Can. J. Zool.* 75, 1773–1780. doi: 10.1139/z97-806

- George, S. C., Smith, T. E., Mac Cana, P. S. S., Coleman, R., and Montgomery, W. I. (2014). Physiological stress in the Eurasian badger (*Meles meles*): effects of host, disease and environment. *Gen. Comp. Endocrinol.* 200, 54–60. doi: 10.1016/j.ygcen.2014.02.017
- Graham, M. L., Gresch, S. C., Hardy, S. K., Mutch, L. A., Janeczek, J. L., and Hegstad-Davies, R. L. (2015). Evaluation of commercial ELISA and RIA for measuring porcine cpeptide: implications for research. *Xenotransplantation* 22, 62–69. doi: 10.1016/j.xen.2014.02.017
- Guinet, C., Servera, N., Mangin, S., Georges, J.-Y., and Lacroix, A. (2004). Change in plasma cortisol and metabolites during the attendance period ashore in fasting lactating subantarctic fur seals. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 137, 523–531. doi: 10.1016/j.cbpa.2003.11.006
- Gulland, F. M. D., Haulena, M., Lowenstine, L. J., Munro, C., Graham, P. A., Bauman, J., et al. (1999). Adrenal function in wild and rehabilitated pacific harbor seals (*Phoca vitulina richardii*) and in seals with phocine herpesvirus-associated adrenal necrosis. *Mar. Mamm. Sci.* 15, 810–827. doi: 10.1111/j.1748-7692.1999.tb00844.x
- Haase, C. G., Long, A. K., and Gilooly, J. F. (2016). Energetics of stress: linking plasma cortisol levels to metabolic rate in mammals. *Biol. Lett.* 12, 20150867. doi: 10.1098/rsbl.2015.0867
- Haave, M., Ropstad, E., Derocher, A. E., Lie, E., Dahl, E., Wiig, Ø., et al. (2003). Polychlorinated biphenyls and reproductive hormones in female polar bears at Svalbard. *Environ. Health Perspect.* 111, 431–436. doi: 10.1289/ehp.5553
- Hair, J. F., Ringle, C. M., and Sarstedt, M. (2011). PLS-SEM: indeed a silver bullet. *J. Marketing Theory Pract.* 19, 139–152. doi: 10.2753/MTP1069-6679190202
- Hall-Aspland, S. A., and Rogers, T. L. (2004). Summer diet of leopard seals (*Hydrurga leptonyx*) in Prydz Bay, Eastern Antarctica. *Polar Biol.* 27, 729–734. doi: 10.1007/s00300-004-0662-9
- Harcourt, R. G. (2001). Advances in new Zealand mammalogy 1990–2000: pinnipeds. *J. R. Soc. N. Z.* 31, 135–160. doi: 10.1080/03014223.2001.9517644
- Harlow, H. J., Beck, T. D. L., Walters, L. M., and Greenhouse, S. S. (1990). Seasonal serum glucose, progesterone, and cortisol levels of black bears (*Ursus americanus*). *Can. J. Zool.* 68, 183–187. doi: 10.1139/z90-025
- Hilderbrand, G. V., Farley, S. D., Robbins, C. T., Hanley, T. A., Titus, K., and Servheen, C. (1996). Use of stable isotopes to determine diets of living and extinct bears. *Can. J. Zool.* 74, 2080–2088. doi: 10.1139/z96-236
- Hindell, M. A., Slip, D. J., and Burton, H. R. (1991). The diving behavior of adult male and female southern elephant seals, *mirounga-leonina* (Pinnipedia, phocidae). *Aust. J. Zool.* 39, 595–619. doi: 10.1071/z9910595
- Hiruki, L. M., Schwartz, M. K., and Boveng, P. L. (1999). Hunting and social behaviour of leopard seals (*Hydrurga leptonyx*) at seal island, south Shetland islands, Antarctica. *J. Zool.* 249, 97–109. doi: 10.1111/j.1469-7998.1999.tb01063.x
- Hobson, K. A., and Clark, R. G. (1993). Turnover of ^{13}C in cellular and plasma fractions of blood: implications for nondestructive sampling in avian dietary studies. *Auk* 110, 638–641. doi: 10.2307/4088430
- Hobson, K. A., and Welch, H. E. (1992). Determination of trophic relationships within a high Arctic marine food web using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis. *Mar. Ecol. Prog. Ser.* 9–18.
- Hogg, C. J., and Rogers, T. L. (2009). “Measuring stress in Antarctic seals,” in *Health of Antarctic wildlife: a challenge for science and policy*. Eds. K. R. Kerry and M. Riddle (Berlin, Heidelberg: Springer Berlin Heidelberg), 263–270. doi: 10.1007/978-3-54093923-8_15
- Hupman, K., Visser, I. N., Fyfe, J., Cawthorn, M., Forbes, G., Grabham, A. A., et al. (2020). From vagrant to resident: occurrence, residency and births of leopard seals (*Hydrurga leptonyx*) in new Zealand waters. *N. Z. J. Mar. Freshw. Res.* 54, 1–23. doi: 10.1080/00288330.2019.1619598
- Jonker, F. C., and Bester, M. N. (1998). Seasonal movements and foraging areas of adult southern female elephant seals, *mirounga-leonina*, from Marion island. *Antarct. Sci.* 10, 21–30. doi: 10.1017/S0954102098000042
- Karpovich, S. A., Skinner, J. P., Kapronczai, L. A., Smith, J. A., and Janz, D. M. (2019). Examination of relationships between stable isotopes and cortisol concentrations along the length of phocid whiskers. *Mar. Mamm. Sci.* 35, 395–415. doi: 10.1111/mms.12546
- Keogh, M. J., and Atkinson, S. (2015). Endocrine and immunological responses to adrenocorticotrophic hormone (ACTH) administration in juvenile harbor seals (*Phoca vitulina*) during winter and summer. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 188, 22–31. doi: 10.1016/j.cbpa.2015.06.011
- Keogh, M. J., Atkinson, S., and Maniscalco, J. M. (2013). Body condition and endocrine profiles of steller sea lion (*Eumetopias jubatus*) pups during the early postnatal period. *Gen. Comp. Endocrinol.* 184, 42–50. doi: 10.1016/j.ygcen.2012.12.016
- Keogh, M. J., Gastaldi, A., Charapata, P., Melin, S., and Fadely, B. S. (2020). Stress-related and reproductive hormones in hair from three north pacific otariid species: steller sea lions, California sea lions and northern fur seals. *Conserv. Physiol.* 8, coaa069. doi: 10.1093/conphys/coaa069
- Kershaw, J. L., and Hall, A. J. (2016). Seasonal variation in harbour seal (*Phoca vitulina*) blubber cortisol - a novel indicator of physiological state? *Sci. Rep.* 6, 21889. doi: 10.1038/srep21889
- Kienle, S. S., Goebel, M. E., LaBrecque, E., Borrás-Chavez, R., Trumble, S. J., Kanatous, S. B., et al. (2022). Plasticity in the morphometrics and movements of an Antarctic apex predator, the leopard seal. *Front. Mar. Sci.* 9. doi: 10.3389/fmars.2022.976019
- Kitaysky, A. S., Wingfield, J. C., and Piatt, J. F. (1999). Dynamics of food availability, body condition and physiological stress response in breeding black-legged kittiwakes. *Funct. Ecol.* 13, 577–584. doi: 10.1046/j.1365-2435.1999.00352.x
- Kooyman, G. L., Castellini, M. A., and Davis, R. W. (1981). Physiology of diving in marine mammals. *Annu. Rev. Physiol.* 43, 343–356. doi: 10.1146/annurev.ph.43.030181.002015
- Krause, D. J., Goebel, M. E., Marshall, G. J., and Abernathy, K. (2016). Summer diving and haul-out behavior of leopard seals (*Hydrurga leptonyx*) near mesopredator breeding colonies at Livingston Island, Antarctic Peninsula. *Mar. Mamm. Sci.* 32, 839–867. doi: 10.1111/mms.12309
- Krause, D. J., Bonin, C. A., and Goebel, M. E. (2022). The rapid population collapse of a key marine predator in the northern Antarctic peninsula endangers genetic diversity and resilience to climate change. *Front. Marine.* doi: 10.3389/fmars.2021.796488
- Krause, D. J., Goebel, M. E., and Kurl, C. M. (2020). Leopard seal diets in a rapidly warming polar region vary by year, season, sex, and body size. *BMC Ecol.* 20, 32. doi: 10.1186/s12898-020-00300-y
- Krause, D. J., Goebel, M. E., Marshall, G. J., and Abernathy, K. (2015). Novel foraging strategies observed in a growing leopard seal (*Hydrurga leptonyx*) population at Livingston island, Antarctic peninsula. *Anim. Biotelemetry* 3, 24. doi: 10.1186/s40317-015-0059-2
- Krause, D. J., and Rogers, T. L. (2019). Food caching by a marine apex predator, the leopard seal (*Hydrurga leptonyx*). *Can. J. Zool.* 97, 573–578. doi: 10.1139/cjz-20180203
- Lafferty, D. J. R., Laudenslager, M. L., Mowat, G., Heard, D., and Belant, J. L. (2015). Sex, diet, and the social environment: factors influencing hair cortisol concentration in free-ranging black bears (*Ursus americanus*). *PLoS One* 10, e0141489. doi: 10.1371/journal.pone.0141489
- Larson, S., Monson, D., Ballachey, B., Jameson, R., and Wasser, S. K. (2009). Stress-related hormones and genetic diversity in sea otters (*Enhydra lutris*). *Mar. Mamm. Sci.* 25, 351–372. doi: 10.1111/j.1748-7692.2008.00260.x
- Laws, R. M. (1957). On the growth rates of the leopard seal, *hydrurga leptonyx* Vol. 1820 (De Blainville: Saugtierkundliche Mitt).
- Laws, R. M. (1977). The significance of vertebrates in the Antarctic marine ecosystem (Accessed November 7, 2022).
- Laws, R. M. (1994). “History and present status of southern elephant seal populations,” in *Elephant seals: population ecology, behavior, and physiology* (Berkeley: University of California Press), 49–65.
- Leboeuf, B. J., Costa, D. P., Huntley, A. C., Kooyman, G. L., and Davis, R. W. (1986). Pattern and depth of dives in northern elephant seals, *mirounga angustirostris*. *J. Zool.* 208, 1–7. doi: 10.1111/j.1469-7998.1986.tb04705.x
- Lemos, L. S., Olsen, A., Smith, A., Burnett, J. D., Chandler, T. E., Larson, S., et al. (2022). Stressed and slim or relaxed and chubby? a simultaneous assessment of gray whale body condition and hormone variability. *Mar. Mamm. Sci.* 38, 801–811. doi: 10.1111/mms.12877
- Lesage, V., Hammill, M. O., and Kovacs, K. M. (2001). Marine mammals and the community structure of the estuary and gulf of St Lawrence, Canada: evidence from stable isotope analysis. *Mar. Ecol. Prog. Ser.* 210, 203–221. doi: 10.3354/meps210203
- Levine, J. E. (2002). Editorial: stressing the importance of sex. *Endocrinology* 143, 4502–4504. doi: 10.1210/en.2002-221041
- Levy, E. J., Gesquiere, L. R., McLean, E., Franz, M., Warutere, J. K., Sayialel, S. N., et al. (2020). Higher dominance rank is associated with lower glucocorticoids in wild female baboons: a rank metric comparison. *Horm. Behav.* 125, 104826. doi: 10.1016/j.yhbeh.2020.104826
- Lidgard, D. C., Boness, D. J., Bowen, W. D., and McMillan, J. I. (2008). The implications of stress on male mating behavior and success in a sexually dimorphic polygynous mammal, the grey seal. *Horm. Behav.* 53, 241–248. doi: 10.1016/j.yhbeh.2007.10.003
- Liggins, G. C., France, J. T., Knox, B. S., and Zapol, W. M. (1979). High corticosteroid levels in plasma of adult and foetal weddell seals (*Leptonychotes weddellii*). *Acta Endocrinol.* 90, 718–726. doi: 10.1530/acta.0.0900718
- Liggins, G. C., France, J. T., Schneider, R. C., Knox, B. S., and Zapol, W. M. (1993). Concentrations, metabolic clearance rates, production rates and plasma binding of cortisol in Antarctic phocid seals. *Acta Endocrinol.* 129, 356–359. doi: 10.1530/acta.0.1290356
- Lunn, N. J., Boyd, I. L., and Croxall, J. P. (1994). Reproductive performance offshore Antarctic fur seals: the influence of age, breeding experience, environmental variation and individual quality. *J. Anim. Ecol.* 63, 827–840. doi: 10.2307/5260

- Macbeth, B. J., Cattet, M. R. L., Obbard, M. E., Middel, K., and Janz, D. M. (2012). Evaluation of hair cortisol concentration as a biomarker of long-term stress in free-ranging polar bears. *Wildl. Soc. Bull.* 36, 747–758. doi: 10.1002/wsb.219
- Macbeth, B. J., Cattet, M. R. L., Stenhouse, G. B., Gibeau, M. L., and Janz, D. M. (2010). Hair cortisol concentration as a noninvasive measure of long-term stress in free-ranging grizzly bears (*Ursus arctos*): considerations with implications for other wildlife. *Can. J. Zool.* 88, 935–949. doi: 10.1139/z10-057
- MacDougall-Shackleton, S. A., Bonier, F., Romero, L. M., and Moore, I. T. (2019). Glucocorticoids and “Stress” are not synonymous. *Integr. Org. Biol.* 1, obz017. doi: 10.1093/iob/obz017
- Manogue, K. R., Leshner, A. I., and Candland, D. K. (1975). Dominance status and adrenocortical reactivity to stress in squirrel monkeys (*Saimiri sciureus*). *Primates* 16, 457–463. doi: 10.1007/bf02382742
- Mazerolle, M. J. (2020). AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c). R package version 2.3-1. Available at: <https://cran.r-project.org/package=AICcmodavg>.
- McEwen, B. S., and Lasley, E. (2002). The end of stress as we know it (Dana Press). Available at: <https://psycnet.apa.org/fulltext/2002-06297-000.pdf>.
- Mendel, C. M. (1989). The free hormone hypothesis: a physiologically based mathematical model. *Endocr. Rev.* 10, 232–274. doi: 10.1210/edrv-10-3-232
- Myers, M. J., Litz, B., and Atkinson, S. (2010). The effects of age, sex, season and geographic region on circulating serum cortisol concentrations in threatened and endangered steller sea lions (*Eumetopias jubatus*). *Gen. Comp. Endocrinol.* 165, 72–77. doi: 10.1016/j.ygcen.2009.06.006
- Newsome, S. D., Ralls, K., Van Horn Job, C., Fogel, M. L., and Cypher, B. L. (2010). Stable isotopes evaluate exploitation of anthropogenic foods by the endangered San Joaquin kit fox (*Vulpes macrotis mutica*). *J. Mammal.* 91, 1313–1321. doi: 10.1111/j.1748-7692.2009.00354.x
- Nordoy, E. S., Aakvaag, A., and Larsen, T. S. (1993). Metabolic adaptations to fasting in harp seal pups. *Physiol. Zool.* 66, 926–945. doi: 10.1086/physzool.66.6.30163747
- Nordoy, E. S., Ingebrechtsen, O. C., and Blix, A. S. (1990). Depressed metabolism and low protein catabolism in fasting grey seal pups. *Acta Physiol. Scand.* 139, 361–369. doi: 10.1111/j.1748-1716.1990.tb08935.x
- Ogloff, W. R., Anderson, R. A., Yurkowski, D. J., Debets, C. D., Anderson, W. G., and Ferguson, S. H. (2022). Spatiotemporal variation of ringed seal blubber cortisol levels in the Canadian Arctic. *J. Mammal.* 103, 1208–1220. doi: 10.1093/jmammal/gyac047
- Øritsland, T. (1977). Food consumption of seals in the Antarctic pack ice. In: Llano GA (ed) Adaptations within Antarctic ecosystems. Proc 3rd Symp Antarct Biol. (Washington DC: Smithsonian Institution)
- Palme, R., Rettenbacher, S., Touma, C., El-Bahr, S. M., and Möstl, E. (2005). Stress hormones in mammals and birds: comparative aspects regarding metabolism, excretion, and noninvasive measurement in fecal samples. *Ann. N. Y. Acad. Sci.* 1040, 162–171. doi: 10.1196/annals.1327.021
- Peck, H. E., Costa, D. P., and Crocker, D. E. (2016). Body reserves influence allocation to immune responses in capital breeding female northern elephant seals. *Funct. Ecol.* 30, 389–397. doi: 10.1111/1365-2435.12504
- Pedernera-Romano, C., and Aurióles-Gamboa, D. (2010). Serum cortisol in California sea lion pups (*Zalophus californianus californianus*). *Animal* 19.3(2010), 275–280. doi: 10.1017/S0962728600001652
- Peig, J., and Green, A. J. (2009). New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. *Oikos* 118, 1883–1891. doi: 10.1111/j.1600-0706.2009.17643.x
- Pussini, N., and Goebel, M. E. (2015). A safer protocol for field immobilization of leopard seals (*Hydrurga leptonyx*). *Mar. Mamm. Sci.* 31, 1549–1558. doi: 10.1111/mms.12232
- Rainer Engelhardt, F. (1982). Hydrocarbon metabolism and cortisol balance in oil-exposed ringed seals, *Phoca hispida*. *Camp. Biochem. Physiol.* 72C, 133–136. doi: 10.1016/0306-4492(82)90219-2
- Ramsay, M. A., and Hobson, K. A. (1991). Polar bears make little use of terrestrial food webs: evidence from stable-carbon isotope analysis. *Oecologia* 86, 598–600. doi: 10.1007/BF00318328
- Rau, G. H., Takahashi, T., Des Marais, D. J., Repeta, D. J., and Martin, J. H. (1992). The relationship between $\delta^{13}\text{C}$ of organic matter and $[\text{CO}_2(\text{aq})]$ in ocean surface water: data from a JGOFS site in the northeast Atlantic ocean and a model. *Geochim. Cosmochim. Acta* 56, 1413–1419. doi: 10.1016/0016-7037(92)90073-R
- R Core Team (2022) R: a language and environment for statistical computing. R foundation for statistical computing. Available at: <https://www.R-project.org/>.
- Reeder, D. M., and Kramer, K. M. (2005). Stress in free-ranging mammals: integrating physiology, ecology, and natural history. *J. Mammal.* 86, 225–235. doi: 10.1644/BHE-003.1
- Romero, L. M., and Beattie, U. K. (2022). Common myths of glucocorticoid function in ecology and conservation. *J. Exp. Zool. A Ecol. Integr. Physiol.* 337, 7–14. doi: 10.1002/jez.2459
- Rutberg, A. T., and Greenberg, S. A. (1990). Dominance, aggression frequencies and modes of aggressive competition in feral pony mares. *Anim. Behav.* 40, 322–331. doi: 10.1016/S0003-3472(05)80927-3
- Sapolsky, R. M. (1999). Glucocorticoids, stress, and their adverse neurological effects: relevance to aging. *Exp. Gerontol.* 34, 721–732. doi: 10.1016/s0531-5565(99)00047-9
- Seguel, M., Perez-Venegas, D., Gutierrez, J., Crocker, D. E., and DeRango, E. J. (2019). Parasitism elicits a stress response that allocates resources for immune function in south American fur seals (*Arctocephalus australis*). *Physiol. Biochem. Zool.* 92, 326–338. doi: 10.1086/702960
- Sheriff, M. J., Dantzer, B., Delehanty, B., Palme, R., and Boonstra, R. (2011). Measuring stress in wildlife: techniques for quantifying glucocorticoids. *Oecologia* 166, 869–887. doi: 10.1007/s00442-011-1943-y
- Shero, M. R., Krotz, R. T., Costa, D. P., Avery, J. P., and Burns, J. M. (2015). How do overwinter changes in body condition and hormone profiles influence Weddell seal reproductive success? *Funct. Ecol.* 29, 1278–1291. doi: 10.1111/1365-2435.12434
- Shimada, M., Takahashi, K., Ohkawa, T., Segawa, M., and Higurashi, M. (1995). Determination of salivary cortisol by ELISA and its application to the assessment of the circadian rhythm in children. *Horm. Res.* 44, 213–217. doi: 10.1159/000184628
- Siniff, D. B. (1991). An overview of the ecology of Antarctic seals. *Am. Zool.* 31, 143–149. doi: 10.1093/icb/31.1.143
- Sperou, E. S. (2020). A comprehensive analysis of sex hormones and their role in reproductive suppression in female northern elephant seals (*Mirounga angustirostris*). scholarworks.calstate.edu
- Stockley, P., and Bro-Jørgensen, J. (2011). Female competition and its evolutionary consequences in mammals. *Biol. Rev. Camb. Philos. Soc.* 86, 341–366. doi: 10.1111/j.1469-185X.2010.00149.x
- Thompson, L. A., and Romano, T. A. (2019). Effects of health status on pressure-induced changes in phocid immune function and implications for dive ability. *J. Comp. Physiol. B* 189, 637–657. doi: 10.1007/s00360-019-01228-6
- Traylor, J. J., and Alisauskas, R. T. (2006). Effects of intrinsic and extrinsic factors on survival of white-winged scoter (*Melanitta fusca deglandi*) ducklings. *Auk* 123, 67–81. doi: 10.1093/auk/123.1.67
- Trumble, S. J., O’Neil, D., Cornick, L. A., Gulland, F. M. D., Castellini, M. A., and Atkinson, S. (2013). Endocrine changes in harbor seal (*Phoca vitulina*) pups undergoing rehabilitation. *Zoo Biol.* 32, 134–141. doi: 10.1002/zoo.21036
- Tryland, M., Krafft, B. A., Lydersen, C., Kovacs, K. M., and Thoresen, S. I. (2006). Serum chemistry values for free-ranging ringed seals (*Pusa hispida*) in Svalbard. *Vet. Clin. Pathol.* 35, 405–412. doi: 10.1111/j.1939-165x.2006.tb00156.x
- Tryland, M., Lydersen, C., Kovacs, K. M., Rafter, E., and Thoresen, S. I. (2021). Serum biochemistry and haematology in wild and captive bearded seals (*Erignathus barbatus*) from Svalbard, Norway. *Acta Vet. Scand.* 63, 33. doi: 10.1186/s13028-02100598-8
- Tryland, M., Lydersen, C., Kovacs, K. M., and Thoresen, S. I. (2009). Serum chemistry reference values in free-ranging north Atlantic male walrus (*Odobenus rosmarus rosmarus*) from the Svalbard archipelago. *Vet. Clin. Pathol.* 38, 501–506. doi: 10.1111/j.1939-165X.2009.00154.x
- Tryland, M., Nymo, I. H., Nielsen, O., Nordoy, E. S., Kovacs, K. M., Krafft, B. A., et al. (2012). Serum chemistry and antibodies against pathogens in antarctic fur seals, weddell seals, crabeater seals, and Ross seals. *J. Wildl. Dis.* 48, 632–645. doi: 10.7589/0090-3558-48.3.632
- Tykot, R. H. (2004). “Stable isotopes and diet: you are what you eat,” in *Physics methods in archaeometry* (IOS press), 433–444. doi: 10.3254/978-1-61499-010-9-433
- Verrier, D., Atkinson, S., Guinet, C., Groscolas, R., and Arnould, J. P. Y. (2012). Hormonal responses to extreme fasting in subantarctic fur seal (*Arctocephalus tropicalis*) pups. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 302, R929–R940. doi: 10.1152/ajpregu.00370.2011
- Webster, H. V., Bone, A. J., Webster, K. A., and Wilkin, T. J. (1990). Comparison of an enzyme-linked immunosorbent assay (ELISA) with a radioimmunoassay (RIA) for the measurement of rat insulin. *J. Immunol. Methods* 134, 95–100. doi: 10.1016/0022-1759(90)90116-d
- Wickham, H. (2016). ggplot2: elegant graphics for data analysis (Springer International Publishing).
- Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., François, R., et al. (2019). Welcome to the tidyverse. *J. Open Source Software* 4, 1686. doi: 10.21105/joss.01686
- Wingfield, J. C. (2013a). Ecological processes and the ecology of stress: the impacts of abiotic environmental factors. *Funct. Ecol.* 27, 37–44. doi: 10.1111/1365-2435.12039
- Wingfield, J. C. (2013b). The comparative biology of environmental stress: behavioural endocrinology and variation in ability to cope with novel, changing environments. *Anim. Behav.* 85, 1127–1133. doi: 10.1016/j.anbehav.2013.02.018
- Wingfield, J. C., and Romero, L. M. (2011). Adrenocortical responses to stress and their modulation in free-living vertebrates. *Compr. Physiol.* doi: 10.1002/cphy.cp070411

Winter, B. (2013) Linear models and linear mixed effects models in r with linguistic applications. Available at: <http://arxiv.org/abs/1308.5499>.