Research paper

Corticosterone in central North Pacific male humpback whales (*Megaptera novaeangliae*): Pairing sighting histories with endocrine markers to assess stress

Kelly A. Cates[a,*], Shannon Atkinson[a], Adam A. Pack[b,c], Janice M. Straley[d], Christine M. Gabriele[e], Suzanne Yin[f]

[a] *University of Alaska Fairbanks, College of Fisheries and Ocean Sciences, Fisheries Department, Juneau Center, 17101 Pt. Lena Loop Road, Juneau, Alaska 99801, USA*
[b] *Departments of Psychology and Biology, University of Hawai'i at Hilo, 200 West Kawili Street, Hilo, Hawai'i 96720, USA*
[c] *The Dolphin Institute, P.O. Box 6279, Hilo, Hawai'i 96720, USA*
[d] *University of Alaska Southeast Sitka Campus, 1332 Seward Ave., Sitka, Alaska 99835, USA*
[e] *Glacier Bay National Park, Gustavus, Alaska 99826, USA*
[f] *Hawai'i Marine Mammal Consortium, P.O. Box 6107, Kamuela, Hawai'i 96743, USA*

ARTICLE INFO

ABSTRACT

Developing a better understanding of the stress response is critical to ensuring the health and sustainability of marine mammal populations. However, accurately measuring and interpreting a stress response in free-ranging, large cetaceans is a nascent field. Here, an enzyme immunoassay for corticosterone was validated for use in biopsy samples from male humpback whales (*Megaptera novaeangliae*). Analyses were conducted on 247 male North Pacific humpback whale blubber samples, including 238 non-calves and 9 calves that were collected on the Hawaiian breeding and Southeast Alaskan feeding grounds from 2004 to 2006. Significant relationships were found when corticosterone concentrations were examined by year, age class and distribution between locations. When examined by year, corticosterone concentrations for male humpback whales were higher in Hawaii in 2004 than in 2005 and 2006 ($p < 0.05$). Corticosterone concentration also varied by age class with initially high concentrations at birth which subsequently tapered off and remained relatively low until sexual maturity was reached around age 8–10 years. Corticosterone concentrations appeared to peak in male humpback whales around 15–25 years of age. Blubber biopsies from Alaska and Hawaii had similar mean corticosterone concentrations, yet the variability in these samples was much greater for whales located in Hawaii. It is clear that much work remains to be done in order to accurately define or monitor a stress response in male humpback whales and that specific attention is required when looking at age, sex, and yearly trends. Our results suggest that a stress response may be most impacted by age and yearly oceanographic conditions and needs to be initially examined at the individual level.

1. Introduction

The increasing use of the marine environment for various anthropogenic activities has prompted recent research efforts to better understand the stress response in marine mammals to these events as well as to establish baseline response levels of the natural stressors in their lives (Atkinson et al., 2015; Champagne et al., 2018; Houser et al., 2016; Hunt et al., 2019; Rolland et al., 2012). In all mammals, glucocorticoids (GCs), such as cortisol and corticosterone, are produced in response to a stressor and are frequently used as an index of an individual's overall well-being (Atkinson et al., 2015). GCs have been shown to vary by species, age, sex, time of day, location, body condition and social status. Generally, GCs increase with age, peak during breeding seasons, and are elevated in females, especially those that are pregnant and lactating, although there is species-specific variation in all of these biological classifications (Atkinson et al., 2015; Champagne et al., 2018; Wingfield, 2013). However, broad variability in the mammalian stress response highlights the need for targeted studies that examine natural variability within individual marine mammal species due to changes in season, life history or individual sensitivities (Atkinson et al., 2015; Boonstra, 2004). Not only is such information essential for an appreciation of how individual marine mammal species

---

* Corresponding author.
  E-mail address: kacates@alaska.edu (K.A. Cates).

respond to stressors during natural activities such as fasting, pregnancy, migration, and competing for mates, but it is also essential for understanding the impacts of anthropogenic activities (Burek et al., 2008; Rolland et al., 2012; Wright et al., 2007), as well as changes in the marine ecosystem from shifts in climate (Henson et al., 2017; Huntington et al., 2017; Wernberg et al., 2016). Moreover, to fully appreciate the stress response of a marine mammal species to anthropogenic activities and to significant shifts in climate that affect marine ecosystems, it is essential to establish a baseline profile of 'normal' concentrations of GCs during natural activities with which to compare. In recognition of this, detailed studies to examine how marine mammals respond to certain environmental stressors and the costs of these stressors at the individual level have been the subjects of several recent large scale research initiatives (ONR, 2009; Wartzok et al., 2005). The present study examines the variability in GC concentrations during natural activities in the central breeding and feeding areas of North Pacific humpback whales.

Most GC studies on marine mammals have focused on pinnipeds and captive or by-caught cetaceans. These studies have established that GCs can be examined in a variety of sample matrices and that there are indeed age, time of day, season, location, social and reproductive state differences (Di Poi et al., 2015; Gulland et al., 2018; Houser et al., 2016, 2011; Hunt et al., 2014; Mashburn and Atkinson, 2004; Oki and Atkinson, 2004; Petrauskas et al., 2008; Rolland et al., 2012). In contrast, few studies have examined stress in free ranging cetaceans, especially in mysticetes, due to their difficulty in detection and/or capture. Where capture is not possible, the most successful studies have examined a suite of steroid hormones in feces, respiratory vapor, and blubber (Atkinson et al., 2019; Burgess et al., 2016; Cates et al., 2019; Rolland et al., 2012; Valenzuela-Molina et al., 2018; Wasser et al., 2000). Out of these sample matrices, blubber is the most studied and dependable sample matrix for understanding hormonal trends in free-ranging, large cetaceans. From studies on small, captive cetaceans, blubber is thought to be a good proxy of current circulating hormones in blood serum (Champagne et al., 2017). In fact, due to the lipophilic nature of steroid hormones, they tend to accumulate in blubber and do not turn over as quickly as hormones present in blood (Norman and Litwack, 1997). In captive bottlenose dolphins (*Tursiops truncatus*), circulating hormones in the blood were documented as showing up in the blubber layer within 2 h of a ACTH stress challenge (Champagne et al., 2018). Given the slower metabolism (Ball et al., 2017; Williams et al., 2001) and thicker blubber layer of mysticetes compared to odontocetes (Gaspar et al., 2000; Lockyer, 1986) it is reasonable to assume that the deposition of hormones in blubber from circulating blood would occur on the order of days rather than hours in mysticetes. As such, studies of hormones in the blubber layer of mysticetes should provide a broad scale index of health and is not likely affected by any stress-related effects of biopsy sample acquisition.

Humpback whales (*Megaptera novaeangliae*) are arguably the most extensively studied of the mysticetes (see summaries in Clapham, 2000, 1996; Gabriele et al., 2017; Herman, 2017; Herman et al., 2011). Yet, relatively little is known about variations in humpback whale stress physiology as compared to other parameters and life history traits, such as abundance, migratory trends, reproduction, behavior and communication (Allen et al., 2018; Baker et al., 1985; Barlow et al., 2011; Cartwright and Sullivan, 2009; Cates et al., 2019; Chittleborough, 1965; Cholewiak et al., 2018; Christiansen et al., 2016; Clapham et al., 1992; Clapham and Mayo, 1990; Craig et al., 2003, 2002; Darling et al., 2006; Gabriele et al., 2007; Helweg and Herman, 1994; Pack et al., 2017; Tyack and Whitehead, 1983). Indeed, studies of humpback whale responses to anthropogenic activities have historically been conducted using behavioral responses as a measure of impact without considering physiological measures of the stress response (Blair et al., 2016; Cerchio et al., 2014; Dunlop et al., 2015).

Recently, several studies have examined GC's in humpback whales from feces, blubber and blow spray showing that GC's are present in

these sample matrices and that they are able to be measured using standard endocrinology techniques (Dalle Luche et al., 2019; Hunt et al., 2019; Mingramm et al., 2020; Teerlink et al., 2018). Dalle Luche et al. (2019) validated the use of liquid chromatography tandem mass spectrometry for the assessment of multiple hormones from humpback whale blubber simultaneously, while Hunt et al. (2019) validated the use of feces from humpback whales in the assessment of reproduction and stress by comparison to known life history traits and found that progesterone and glucocorticoids were elevated in pregnant females. Teerlink et al. (2018) examined the relationship between cortisol and whale watching vessels finding regional differences in cortisol concentrations but no overall correlation to the amount of tourism traffic present; while Mingramm et al. (2020) examined blubber cortisol levels from live and dead humpback whales, finding that blubber from deceased whales exhibited much higher cortisol concentrations than samples from live animals. While these studies contribute to the growing field of mysticete endocrine assessment, it bears mention that particular care should be given to GC metabolite expression by sex, sample matrix and the source of the sample matrix (e.g. blubber from stranded vs live animals) as early investigations have shown that metabolite expression is likely to vary by these criteria (Atkinson et al., 2020). To date, no study has conducted a temporal and spatial assessment of stress by sex in live, free ranging North Pacific humpback whales.

As GCs are closely linked with reproductive cycles (Burgess et al., 2013) and female humpback whales exhibit different reproductive costs than males (Christiansen et al., 2016), the present study focused exclusively on the range of variability in GCs in male humpback whales in varying temporal, spatial, social and ecological contexts. Humpback whales typically undergo relatively long migrations of several thousand kilometers between high latitude feeding grounds and low latitude breeding grounds (Barlow et al., 2011; Chittleborough, 1965; Dawbin, 1966; Katona and Beard, 1990). On the breeding grounds all but suckling calves fast, and activities are largely devoted to calving, calf rearing, and mating, as well as to behaviors related to these activities. While on the breeding grounds, males produce long elaborate repeated vocal sequences termed "song" (reviewed in Herman, 2017) and also escort single females or single mother-calf pairs (Craig et al., 2002). When two or more males escort a female, the males compete for physical proximity (and presumably mating access) to that female (Clapham, 1996; Tyack and Whitehead, 1983). Contest competition between males can range in aggressiveness from physical displays, to blocking behavior, to chases and body strikes (Baker and Herman, 1984; Herman et al., 2007). While both singing and competing require energy, the latter is likely more energetically costly and consequently a greater stressor than the former. Nonetheless, fasting over an extended period should in theory provide the most persistent source of stress on a male humpback during the breeding season. Residency duration for male humpback whales on the breeding grounds may extend 13 weeks or longer depending on an individual's body resources and age class (Craig et al., 2001). Over this period, individuals must rely on metabolized fat stores for energy. Consequently, over the course of a breeding season, males incur a loss in the thickness of their blubber (Christiansen et al., 2016; Nishiwaki, 1960). Thus, at any moment on the breeding grounds, a variety of natural factors may contribute to the diversity of stress levels found within the matrix of male humpback whales including the length of time a male has already been present on the breeding grounds, and whether a male actively participates in competitive groups or avoids these types of associations. Arguably, male humpback whales should demonstrate less diversity in stress levels on the feeding grounds than on the breeding grounds inasmuch as the whales are no longer fasting and are no longer competing physically for mates, although the extent to which food resources are readily available to all individuals and the degree to which they have gone without food may complicate the picture. Nonetheless, it would appear that male humpbacks would experience on average greater stress

during the breeding season compared to pre- and post-breeding periods, as has been shown in some terrestrial vertebrates (Romero, 2002).

In the current study, we compared corticosterone concentrations in the Hawaii Distinct Population Segment (DPS) of humpback whales, specifically those that feed in Southeast Alaska and breed in Hawaii (Barlow et al., 2011; Calambokidis et al., 2008). Since male humpback whales fast on the breeding grounds, have variable residency durations depending on age class and body resources, and engage in variable energetically demanding behaviors, we predicted that corticosterone concentrations would be more variable on the breeding grounds versus the feeding grounds with the highest observed corticosterone concentrations occurring during the breeding season and that corticosterone concentrations would be positively correlated with age. We also tested the assumption that corticosterone concentrations would differ between blubber and skin tissue collected from the same individual and lastly, that individual corticosterone concentrations should show an increased stress response from initial to final sampling on the breeding grounds and should show a decreased stress response from the initial to the final sampling in Alaska.

2. Materials and methods

2.1. Study areas

Male humpback whales of the Hawaii DPS that exhibit feeding site fidelity to Southeast Alaska (SEAK) were examined in the present study

(Fig. 1). Blubber and skin biopsy samples were collected from two locales: 1) Southeast Alaska, including Sitka Sound (57.0°N 135.5 W°), Chatham Strait (56.95°N 134.62°W), Frederick Sound (57.13°N 134.10°W), Lynn Canal (58.4°N 134.8°W) and waters west of Prince of Wales (55.95°N 132.48°W), and 2) the main Hawaiian islands, specifically the Au'au, Kalohi and Pailolo channels between Maui (20.89°N 156.68°W), Moloka'i (21.14°N 157.03°W), Lana'i (20.83°N 156.91°W) and Kaho'olawe (20.55°N 156.60°W) and waters off the North Kohala Coast of Hawai'i Island (19.98°N 155.87°W).
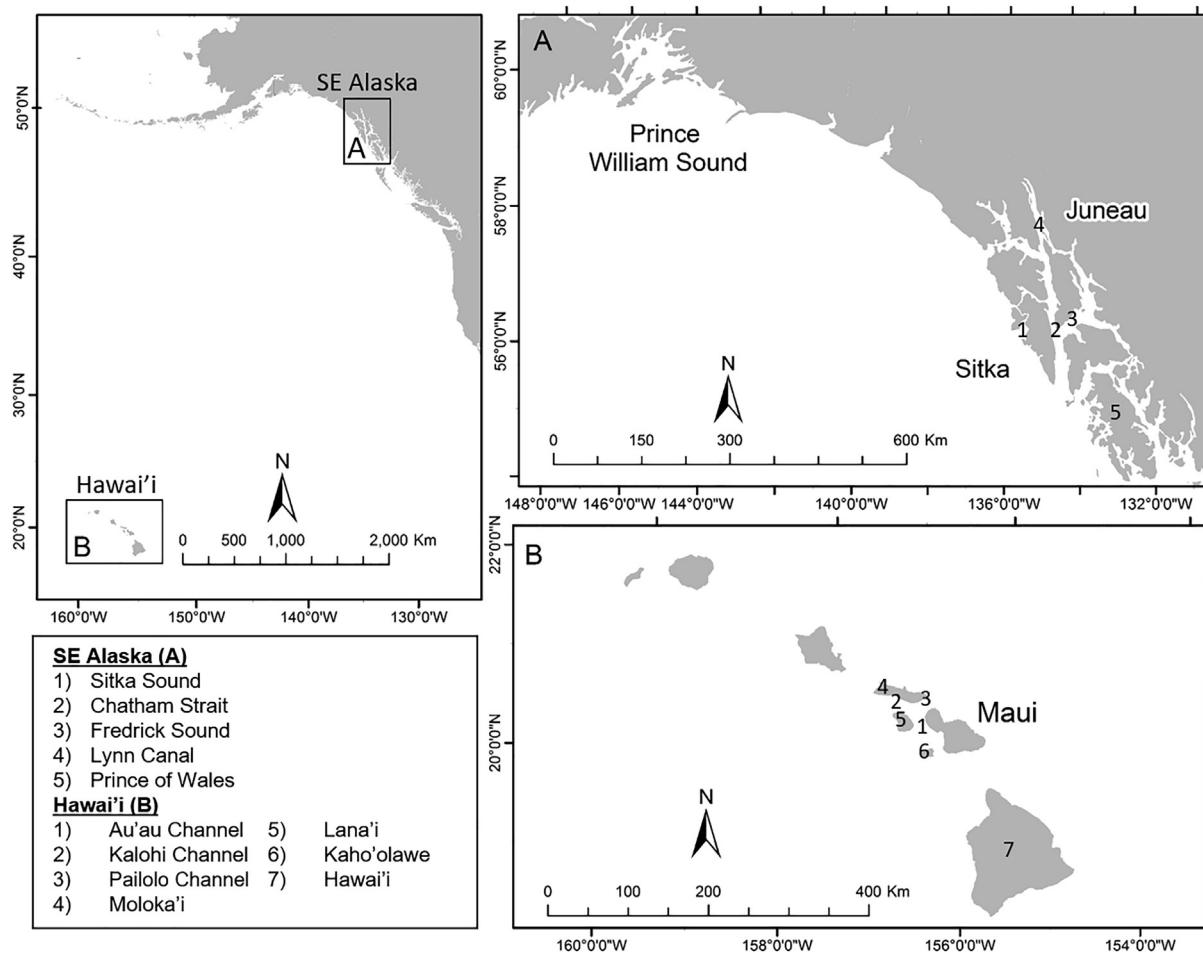
2.2. Sample collection

2.2.1. Biopsy sampling

Samples were collected from four research groups. Two were based in Hawaii during 2004–2006 and two were based in Southeast Alaska during 2004–2005. All samples were collected during an international, collaborative study of humpback whales across the North Pacific called Structure of Populations, Levels of Abundance, and Status of Humpbacks (SPLASH) (Calambokidis et al., 2008). Samples were collected following SPLASH protocols and are described in Cates et al. (2019).

2.2.2. Sample selection

Samples used in this study were randomly selected by month from available humpback whale blubber samples archived at National Marine Fisheries Service's (NMFS) Southwest Fisheries Science Center

Fig. 1. Blubber and skin biopsy samples were collected from two locales; 1) Southeast Alaska, including Sitka Sound (57.0°N 135.5 W°), Chatham Strait (56.95°N 134.62°W), Frederick Sound (57.13°N 134.10°W), Lynn Canal (58.4°N 134.8°W) and waters west of Prince of Wales (55.95°N 132.48°W), and 2) the main Hawaiian islands, specifically the Au'au, Kalohi and Pailolo channels between Maui (20.89°N 156.68°W), Moloka'i (21.14°N 157.03°W), Lana'i (20.83°N 156.91°W) and Kaho'olawe (20.55°N 156.60°W) and waters off the North Kohala Coast of Hawai'i Island (19.98°N 155.87°W). Map used with permission from Cates et al. (2019).

(SWFSC) in order to capture the cyclical variation in physiology. Samples were classified according to the sample type (skin or blubber), location where the biopsy was obtained (Alaska or Hawaii) and date of collection. Following Cates et al. (2019), seasons were defined as follows: fall (September 16-January 15), winter (January 16-March 15), spring (March 16-June 15), and summer (June 16-September 15).

2.3. Data collected about each whale

2.3.1. Photographic identification using natural markings

Humpback whales can be identified by the unique black and white pigmentation patterns on the ventral surface of their flukes, along with the distinctive trailing edge (Katona and Whitehead, 1981). Identification photographs (photo-id) of the tail flukes of tissue-sampled humpback whales were collected either prior to or after the biopsy was obtained and were matched to tissue samples following the protocol outlined in Cates et al. (2019).

2.3.2. Determining age-class and reproductive status for an individual whale

The age-class of whales was determined from field notes that accompanied the samples. Calves were designated based on their small size (ca. < 5 m) (Pack et al., 2017, 2009) and close spatial association with an adult-sized whale (i.e. its mother) that displayed nurturant behavior (e.g. shielding the small-sized whale with its pectoral fin) (Gabriele et al., 2017; Glockner-Ferrari and Ferrari, 1985). All other whales were considered non-calves. Sighting histories from the Southeast Alaska collaborative databases (maintained by co-authors Straley and Gabriele) were used to determine whales of known age or a minimum age for whales whose exact age was unknown. Whales of known age were first sighted as calves. The minimum age of a whale which was photographed prior to the SPLASH project as an adult was calculated as the number of years from the earliest sighting to the most recent sighting plus two years (to account for the individual's year as a calf and year as a yearling when it would have appeared notably smaller than an adult-sized whale). For example, the known age of a whale photographed during the study in 2006 that was originally photographed in 1994 as a calf would be 12 years, whereas the calculated minimum age of a whale photographed in 2006 that was originally photographed as a non-calf in 1994 would be 14 years. Minimum age thus represents a conservative estimate of age.

2.4. Sex and genetic identification

Oregon State University's Cetacean Conservation and Genomics Laboratory conducted genetic analyses and sex determination on the samples as part of the post-collection aims of the SPLASH effort (Baker et al., 2013). Each whale was given a unique genetic ID which was used in conjunction with photo-IDs to match whales under one SPLASH ID where necessary.

2.5. Enzyme immunoassay (EIA)

Hormone extraction methods were conducted as described in Cates et al. (2019). Corticosterone was chosen to analyze male humpback whale stress response as it is the main blubber glucocorticoid metabolite expressed (Atkinson et al. 2020, unpublished data). Extracted hormone was analyzed using Arbor Assay kit K014-H1 for corticosterone and procedures were performed according to the manufacturer's protocol. Manufacturer cross-reactivity with other steroids was as follows: desoxycorticosterone (12.3%), tetrahydrocorticosterone (0.76%), aldosterone (0.62%), cortisol (0.38%), progesterone (0.24%), dexamethasone (0.12%), corticosterone-21-hemisucciante (< 0.1%) and < 0.008% for all other steroids analyzed. Assay parallelism and accuracy tests were performed in order to validate use of humpback whale blubber for measuring corticosterone in EIA using a pooled

blubber sample for male humpback whales. Serial dilutions (neat to 1:8) of the pool exhibited displacement parallel to that of the standard curve and proved accurate ($y = 7.62 + 0.95x$, $r^2 = 0.99$) in the amount of corticosterone measured. Inter-assay coefficient of variation for one assay control was 11.68% and intra-assay coefficient of variation was below 10%. The lower limit of detection (LD) was 4.88 pg/ml with 40 out of 247 samples (16%) falling below this threshold. Substitution in the form of $LD/\sqrt{2}$ was performed for these 40 samples, a process that is accepted if < 25% of samples are substituted and there is only one LD (Croghan and Egeghy, 2003; LaFleur et al., 2011; EPA, 2000).

2.6. Statistical analyses

All statistical analyses were con1ducted in the program R (R Core Team, 2018, version 3.5.1. Available at https://www.R-project.org). Hormone concentration data were first examined for normality and variance. Finding that the data were left skewed a Box Cox analysis was performed to determine a suitable transformation. A fourth root transformation was deemed the most appropriate transformation and was applied to the corticosterone data. General additive models (GAM) (Hastie and Tibshirani, 1986) were then constructed with the most parsimonious model determined as that with the lowest Akaike information criterion (AIC) value. The full GAM model was of the form:

$$gam(corticosterone \; jul: grounds + s(testosterone, k = 3) + s(age, k = 3) + year, data = cort\_, method = "ML")$$

where testosterone and age were smoothed effects and year and Julian day were treated as linear effects. The transformed data were then used to examine the temporal and spatial differences in blubber corticosterone concentrations using a Welch's t-test, a two-sample Kolmogorov-Smirnov test, or a one-way ANOVA. If a significant result ($p < 0.05$) was found in the ANOVA test, a Dunn's test was performed to determine which groups differed significantly from each other. The spatial and temporal range of variation in corticosterone concentration were depicted by boxplots which show the mean and nominal range of the data inferred from the upper and lower quartiles, as well as outliers in the data. T-tests (Welch's t-test and paired t-test), ANOVA, Dunn's test and boxplot analyses were also performed to examine any difference between calves and non-calves and between blubber and skin sample types. Additionally, a Pearson Correlation Test was conducted to determine any potential relationships between blubber and skin corticosterone concentrations, as well as testosterone and corticosterone concentrations.

3. Results

A total of 247 tissue samples (238 male non-calves, 9 male calves) were analyzed for corticosterone (Table 1). Four individually identified

Table 1
Distribution of humpback whale blubber samples by year, location and season. Bracketed numbers indicate calves. No samples, as a part of the SPLASH effort, were collected in 2006 in Alaska. Seasons were defined as follows: fall (September 16-January 15), winter (January 16-March 15), spring (March 16-June 15), and summer (June 16-September 15).

Alaska	Spring	Summer	Fall	Winter	Total
2004	2	33[2]	21	0	56[2]
2005	2[3]	14[2]	1	0	17[5]
				Alaska Total	**73[7]**
Hawaii					
2004	19	0	1	43[1]	63[1]
2005	13	0	2	39	54
2006	9	0	4[1]	35	48[1]
				Hawaii Total	**165[2]**

whales were sampled in consecutive years in Alaska and Hawaii and ten were sampled twice during the same season (six in AK during the feeding season and four in Hawaii during the breeding season).
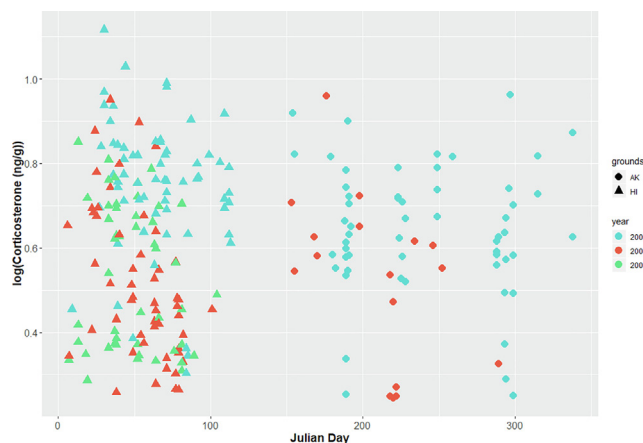
3.1. Corticosterone models

Generalized additive models were constructed and model selection used to determine what variables would best predict corticosterone concentration in male humpback whales. Variables including Julian day, season, month, location, year, grounds, age and testosterone concentration where hypothesized to explain observed variation in corticosterone concentration. Due to the inherent collinearity of Julian day, season and month, season and month were removed as candidate predictor variables from model selection. Values for candidate predictor variables were not available for all response variable (i.e. corticosterone concentration) samples. Therefore, models were fit to two subsets of the data: (1) the full dataset with a limited number of variables, and (2) a reduced dataset that incorporated all the available variables, as age information was not available for all samples. Models fit to the full dataset included Julian day, year, grounds, and testosterone as predictor variables. AIC-based model selection and visual inspection of the output from the GAMs suggests that year ($p < 0.05$ for all years) and an interaction between Julian day and grounds ($p < 0.05$ for AK and HI) were the two most important variables in predicting corticosterone concentration. When model specifying Julian day as a linear or non-linear smoothed effect (approximated as a thin plate spline), a linear relationship was preferred (edf = 1). For the reduced dataset, where the available variables were Julian day, year, grounds, testosterone, and age, model selection among competing GAMs suggests that year ($p < 0.05$ for all years) and testosterone ($p = 0.05$) were the two most important variables in predicting corticosterone concentration. However, the next two models showed a marginal difference in AIC and included Julian day (model 2, delta-AIC = 1) and age (model 3, delta-AIC = 3) as important variables in predicting corticosterone concentration (Table 2). When model specifying testosterone as a linear or non-linear smoothed effect in the reduced dataset (approximated as a thin plate spline), a non-linear relationship was preferred (edf = 1.61).

Table 2
General additive models for two datasets, 1) a full dataset with a subset of variables and 2) a reduced dataset with all available variables. Available variables for the full dataset (n = 248) were Julian day, grounds, year, and testosterone. Available variables for the reduced dataset (n = 64) were Julian day, grounds, year, testosterone and age. The best and most parsimonious model for predicting corticosterone concentrations using the full dataset (AIC = -141.47) was year and an interaction between Julian day and grounds. The best and most parsimonious model for predicting corticosterone concentrations using the reduced dataset (AIC = -35.10) was testosterone and year. The top 5 models (based on AIC) constructed for each dataset are presented below.

Full Dataset Models	df	R2	logLik	AIC	Δ AIC
jul:grounds + year	6.00	0.20	76.70	−141.47	0.00
jul:grounds + testosterone + year	7.82	0.20	78.10	−140.57	0.90
testosterone + year	5.00	0.16	70.40	−130.88	10.59
year	3.00	0.15	68.80	−129.52	11.95
testosterone	2.00	0.01	47.60	−89.19	52.28

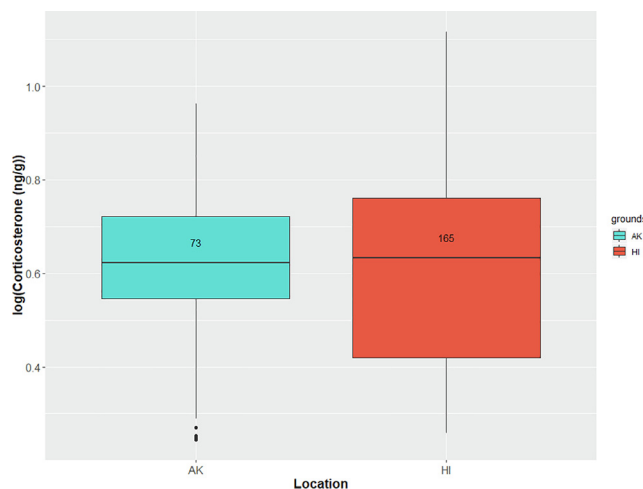| Reduced Dataset Models	df	R2	logLik	AIC	Δ AIC
testosterone + year	4.61	0.24	23.40	−35.10	0.00
jul:grounds + testosterone + year	6.75	0.25	25.00	−34.10	1.00
jul:grounds + testosterone + year + age	7.98	0.24	25.40	–32.10	3.00
year	6.37	0.16	21.80	−28.40	6.70
jul:grounds + year	2.00	0.17	15.00	−24.10	11.00

Fig. 2. Corticosterone concentrations (ng/g) across Julian day. Data is color coded by year and shape represents location. Julian day influences the spatial location of humpback whales and corticosterone concentrations were significantly higher in Hawaii (HI) in 2004 than in 2005 or 2006 ($p < 0.05$).
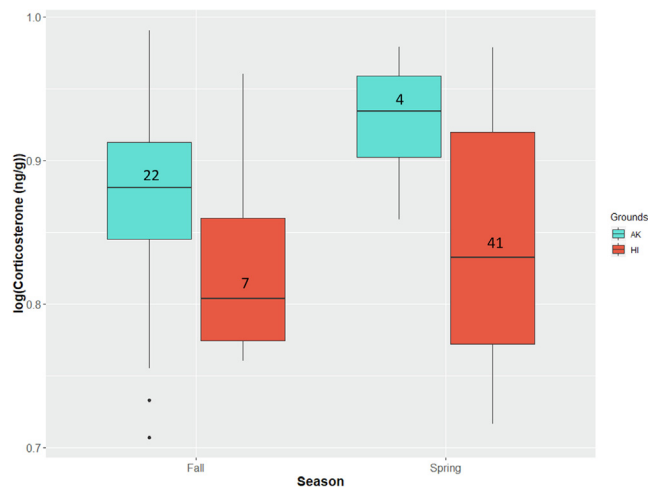
3.2. Spatial and temporal corticosterone concentration trends

Corticosterone concentrations in blubber samples from non-calf humpback whales were examined by location (Alaska and Hawaii), year (2004–2006), season (Spring, Summer, Fall and Winter) and Julian day. When corticosterone concentrations were compared by year, a significant result (Kruskal-Wallis, $p < 0.05$) was found, with 2004 being statistically different from 2005 and 2006. When binned by location, a significant difference between years was not found for Alaska samples (Welch's Two Sample T-Test, $p = 0.29$ (no data for 2006)), but was found for Hawaii samples (Kruskal-Wallis, $p < 0.05$) with 2004 having significantly greater corticosterone concentrations than 2005 and 2006 (Fig. 2).

Mean ($\pm$ stdv) corticosterone concentrations were not significantly different between Alaska (n = 73, 0.62 ± 0.17 ng/g) and Hawaii (n = 165, 0.61 ± 0.20 ng/g), yet Hawaii had a significantly greater distribution than Alaska (Two-sample Kolmogorov-Smirnov test, $p = 0.02$, Fig. 3). There were no significant differences (Kruskal Wallis, $p = 0.07$) between the four seasons; Spring (n = 45, 0.56 ± 0.20 ng/g), Summer (n = 47, 0.61 ± 0.17 ng/g), Fall (n = 29, 0.57 ± 0.18 ng/g) and Winter (n = 117, 0.64 ± 0.20 ng/g). Spring and Fall were the only two seasons during which biopsies were collected from whales in both Alaska and Hawaii. The median dates of

Fig. 3. Corticosterone concentrations were examined between Hawaii and Alaska with no significant difference in mean corticosterone concentration detected between locations.
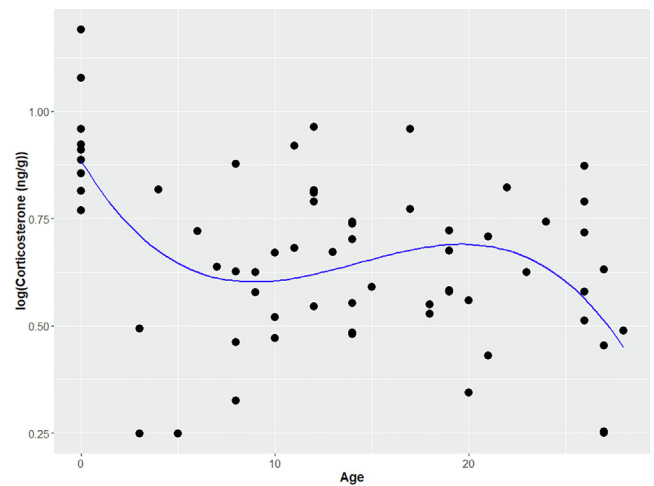
Fig. 4. Spring and fall were the only two seasons during which biopsies were collected from whales in both Alaska and Hawaii. Whales in spring (n = 45) located in Alaska (n = 4, 0.74 ± 0.16 ng/g) and Hawaii (n = 41, 0.54 ± 0.19 ng/g), did not have significantly different corticosterone concentrations (Welch's *t*-test, p = 0.07). Likewise, whales in fall (n = 29) did not have significantly different corticosterone concentrations between locations (Alaska, n = 22, 0.59 ± 0.18 ng/g; Hawaii, n = 7, 0.49 ± 0.19 ng/g; Welch's *t*-test, p = 0.23).

collection for whales biopsied in the spring and fall in Alaska were June 2nd and October 21st, respectively, whereas the median dates of collection in Hawaii were March 31st and January 7th. Whales in spring (n = 45) located in Alaska (n = 4, 0.74 ± 0.16 ng/g) and Hawaii (n = 41, 0.54 ± 0.19 ng/g), did not have significantly different corticosterone concentrations (Welch's *t*-test, p = 0.07). Likewise, whales in fall (n = 29) did not have significantly different corticosterone concentrations between locations (Alaska, n = 22, 0.59 ± 0.18 ng/g; Hawaii, n = 7, 0.49 ± 0.19 ng/g; Welch's *t*-test, p = 0.23) (Fig. 4). However, due to the small sample size in some of the locations (i.e., Alaska in spring and Hawaii in fall), the relative power of these tests is low and caution should be exercised when using these data in physiological assessments.

Lastly, corticosterone concentrations were examined by Julian day. When Julian day was compared to corticosterone concentration for each location a weak, non-significant correlation was observed (r = -0.03 & −0.10 for HI and AK, respectively). However, when 2004 data points were removed, the strength of the correlation between Julian day and corticosterone concentration increased (r = -0.32 & −0.62 for HI and AK, respectively) providing a significant result (p = 0.001 & 0.002 for HI and AK, respectively).

3.3. Corticosterone by age

Age information for 64 individual whales (exact age or minimum age) was available and pooled for analysis (Exact age: mean age = 5yrs, min = 0yrs, max = 24yrs; Minimum age: mean age = 16yrs, min = 3yrs, max = 28yrs). A significant, weak relationship between age and corticosterone concertation was found (Pearson Correlation, r = -0.36, p < 0.05), however, when calves (n = 9) were removed from the dataset no correlation was found (Pearson Correlation, r = -0.03, p = 0.83). From model fitting, a 3rd order polynomial was found to best represent this relationship (r² = 0.26, Fig. 5) capturing more of the data than 1st and 2nd order polynomials and as much data as a 4th order polynomial (ANOVA, p = 0.007, 0.004, 0.06 for 1st (order model) vs 3rd, 2nd vs 3rd, and 3rd vs 4th, respectively). The general trend suggests that corticosterone concentrations are initially high at birth then taper off and remain relatively low until sexual maturity is reached around age 8–10 year. Corticosterone concentration appears to

Fig. 5. Corticosterone concentration plotted against age for 64 whales. Paired age and corticosterone concentration data suggest that corticosterone concentrations are initially high at birth then taper off and remain relatively low until sexual maturity is reached around age 8–10. Corticosterone concentration appears to peak in humpback whales around 15–25 years. A 3rd order parabolic curve best fit the data (r² = 0.26).

peak in adult humpback whales around 15–25 years of age. Calves exhibit the highest concentrations of corticosterone (n = 9, 0.93 ± 0.13 ng/g) and were significantly greater than non-calves (n = 238, 0.61 ± 0.19 ng/g, Welch's *t*-test, p < 0.05).

3.4. Corticosterone concentration from individual whales biopsied in both Hawaii and Alaska

Four whales were biopsied within the same year in Alaska and Hawaii and while no consistent trend was observed, three out of the four whales showed higher corticosterone concentrations in Alaska than when in Hawaii (Fig. 6). In addition, ten whales were biopsied during the same season in the same location. Mean duration between sampling events was 42 ± 20 days in Alaska and 20 ± 7 days in Hawaii. Comparison of these paired biopsies did not yield a clear trend and instead showed a variable trend with some whales exhibiting higher corticosterone concentrations in the first biopsy (n = 3) and some in the second (n = 7, Fig. 7).

3.5. Corticosterone concentration in blubber and skin

There was no significant difference in corticosterone concentration between blubber and skin matrices from the same individuals (n = 36 blubber and 36 skin samples, Paired T-Test, p = 0.38). When samples were further binned by geographic collection location, no significant result was found (Paired T-Test, p = 0.33 and p = 0.80 for Alaska and Hawaii samples, respectively). A Pearson Correlation test showed that these two tissue matrices are correlated (n = 36 pairs, r = 0.56, p < 0.05) and when binned by location, Alaska samples were not significantly correlated (n = 16 pairs, r = 0.48, p = 0.06), whereas Hawaii samples were significantly correlated (p = 20 pairs, r = 0.61, p < 0.05).

3.6. Corticosterone and testosterone concentrations

Testosterone and corticosterone concentrations from the same blubber biopsies were compared with testosterone concentrations obtained from Cates et al. (2019). There was no significant correlation between corticosterone and testosterone concentrations (Pearson correlation test, r = 0.07, p = 0.31). When split by location, the result was still non-significant (r = 0.07 & 0.17, p = 0.35 &0.13 for Hawaii and
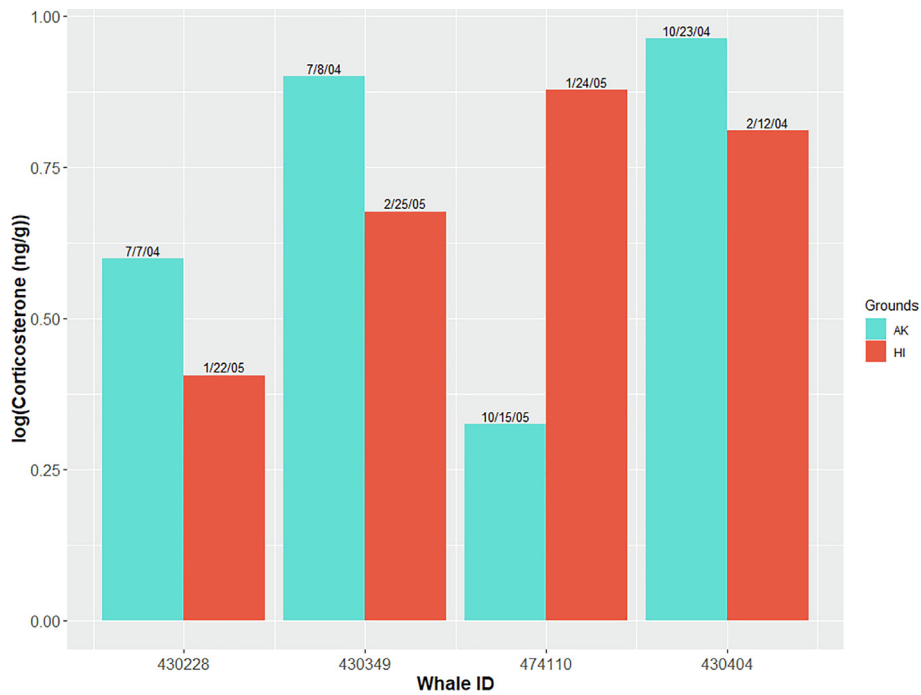
Fig. 6. Four whales were biopsied within the same year in Alaska and Hawaii. No consistent trend was observed in corticosterone concentrations, yet three out of the four whales showed higher corticosterone concentrations in Alaska than when in Hawaii.

Alaska, respectively)

4. Discussion

Our findings present a complex picture of stress in the central North Pacific population of humpback whales. We tested the hypotheses that corticosterone concentrations would be more variable on the breeding grounds versus the feeding grounds, positively correlated with age, would differ between blubber and skin tissue and lastly, that concentrations should increase from initial to final sampling on the breeding grounds and should decrease from the initial to the final sampling in Alaska. While we did find that corticosterone concentrations were more variable on the breeding grounds than on the feeding grounds, our other hypotheses were not supported, suggesting that the relationship between corticosterone level and other biologically

important factors in humpback whale ecology may be more nuanced than previously thought.

Modeling suggested that the most influential variables on corticosterone concentration were year, Julian day and grounds (i.e. feeding or breeding location), and to a lesser extent testosterone and age (Table 2). However, when these variables were examined independent of other variables only year and age were found to have significant results. For example, when corticosterone concentrations were compared to year, 2004 had significantly higher corticosterone levels than 2005 and 2006 (Fig. 2). These differences may be a result of increases in anthropogenic activities, abiotic influences, fluctuations in suitable prey, disease, body condition or any number of other factors (Atkinson et al., 2015; Burek et al., 2008; Christiansen et al., 2016; Henson et al., 2017; Rolland et al., 2012; Wernberg et al., 2016). Sea surface temperature (SST) did differ between years in this study, with a warmer average sea surface
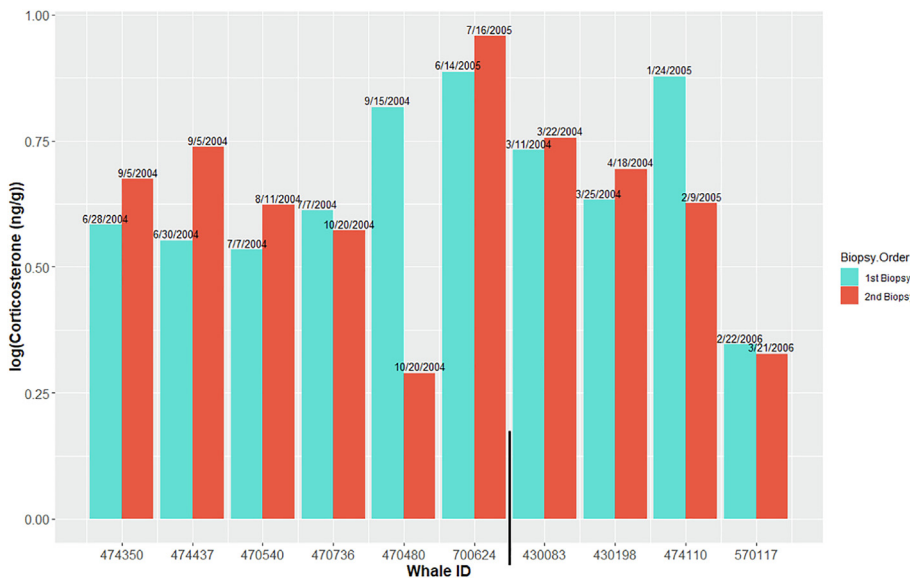

Fig. 7. Ten whales were biopsied during the same year in the same location. Comparison of these paired biopsies did not yield a clear trend and instead showed a variable trend with some whales exhibiting higher corticosterone concentrations in the first biopsy (n = 3) and some in the second (n = 7). Samples collected in Alaska are to the left of the black bar and Hawaii samples are to the right.

temperature experienced in Hawaii in 2004 than in 2005 and 2006 (PacIOOS, 2019). However, 2003 SST temperatures in Alaska (the feeding season immediately preceding the 2004 breeding season) were not significantly warmer than 2004 or 2005 (CoastWatch, 2020) and 2003 herring spawning biomass in southeast Alaska was not significantly lower than spawning biomass in 2004 and 2005 (Hebert, 2017). In addition, the Glacier Bay National Park recorded crude birth rate (6.1%), while lower than 2004 (11.6%) and 2005 (6.9%), was well within the documented range of values (3.4–17.9% between 1984 and 2006) for this extremely variable parameter (Neilson and Gabriele, 2003, 2006). Moreover, despite the annual difference in corticosterone concentration and SST in Hawaii, the whales evidenced an estimated annual increase of 6% (Calambokidis et al. 2008). Thus, the factors underlying significantly higher corticosterone concentrations in Hawaii in 2004 remain unclear. Corticosterone concentration as a function of year may be impacted by prevalent oceanographic conditions (Cartwright et al., 2019), yet to better understand the role that SST may play as a stressor to humpback whales, additional biopsies are needed across spatial, temporal and temperature gradients.

We also found a significant relationship when corticosterone was examined by age class which was driven largely by calves exhibiting higher corticosterone concentrations than non-calves (Fig. 2). This parallels findings which found that when human infants are first born their reproductive and stress hormone levels begin relatively high and gradually decrease, remaining low until they near puberty (Forest et al., 1973; Quigley, 2002). In Hawaii, calves may range from neonates to those seven or more weeks old (Craig et al., 2001; Pack et al., 2017). Mothers often segregate themselves and their calves into shallow water to avoid energetically costly associations with males prospecting for mating opportunities and engaging in competition with other males (Craig et al., 2014). However, as calves grow and age, mother-calf pairs may be found in deeper waters where these energetically stressful encounters may occur more often (Pack et al., 2017). Calves in Alaska are older and spend more time separated from their mothers (Szabo and Duffus, 2008) than in Hawaii, thus having to be more vigilant for potential predators or other potential threats. In both locations, calves experience a suite of additional challenges such as the cost of locomotion, impacts of close approaches by tourism vessels and meeting the nutrient demands of rapid growth. It is perhaps not surprising that they have relatively higher stress levels than non-calves.

Age class may also play a role in the greater variation of corticosterone concentrations seen in whales located in Hawaii versus Alaska. While these two locations had similar mean corticosterone concentrations, the spread of these values was much greater for whales located in Hawaii (Fig. 3). To the degree that there is an equivalent opportunity among males in Alaska to locate and secure food resources (i.e. low levels of competition for these resources), it is likely that most males on the feeding grounds experience similar levels of nutritional stress as they are all searching for prey and seeking to replenish fat stores. In contrast, in Hawaii differences in individual male stress may reflect differences in fasting period as well as differences in engagement in social activities associated with stress. For example, as noted earlier, depending on their body resources, males may vary their residency on the breeding grounds with longer residencies in theory being more stressful than those that are shorter. In addition, corticosterone concentrations appear to peak in male humpback whales between 15 and 25 years of age (Fig. 5). This mimics a similar trend seen in testosterone concentrations (Cates et al., 2019) where testosterone appears to peak between 8 and 25 years of age. This makes biological sense as sexually mature males are likely to experience higher levels of physical competition as a consequence of activities related to mating whereas sexually immature males, although present in some competitive groups (Spitz et al., 2002) may be less likely to physically engage in contest competition that may cause injury. Indeed, Pack et al. (2012) documented size assortative pairing in humpback whales in Hawaiian waters showing that immature males tend to associate with immature

females without calves rather than mature females without calves. Factors such as these could contribute to the greater variability in corticosterone concentrations observed in Hawaii compared with Alaska. However more information on corticosterone as a function of male residency and role is needed to test this hypothesis.

Examination of the relationship between corticosterone in blubber and skin from the same individuals showed that these two matrices are similar in concentration, whereas for testosterone, blubber and skin concentrations were significantly different (Cates et al., 2019). In light of the similarity between blubber and skin corticosterone concentrations, it could be argued that skin could be used as proxy for blubber corticosterone concentrations. However, this substitution is likely ill-advised as the similarity between blubber and skin is not consistent and varies by location, with a greater similarity occurring in Hawaii than in Alaska. This could be the result of any number of influences including warmer water temperatures or differences in metabolic rates between Hawaii and Alaska which would affect the diffusion rate of steroid hormones moving into and through the lipophilic blubber layer (Campbell et al., 2008; Purves et al., 2003). As such, our study does not support using skin as a substitute for blubber in the analysis of steroid hormones.

There was no significant difference in mean corticosterone concentrations between Alaska and Hawaii, and likewise, there was no overall trend by season (Figs. 3 & 4). These findings were further supported at the individual level; individually identified whales who were biopsied in both Alaska and Hawaii did not yield a clear trend in higher corticosterone concentrations favoring one area over another as individuals who were biopsied in different locations did not yield a clear trend (Fig. 6) and whales who were biopsied twice in the same season did not consistently increase or decrease in corticosterone levels (Fig. 7).

Overall, these results are somewhat surprising as one might expect biologically taxing periods like breeding where males are fasting, declining in body condition, and physically competing for mates to result in greater levels of stress than would occur while feeding. For example, male Northern elephant seals (Mirounga angustirostris) also fast during the breeding season and engage is seasonal migration. They provide evidence for oxidative stress as a physiological cost of reproduction, where both sexes strongly up-regulate antioxidant defenses during breeding (Sharick et al., 2015). During the winter fast, polar bears (Ursus maritimus) initially experience low levels of GCs, but once fat stores are depleted, protein stores become the main energy source and GCs increase markedly (Boonstra, 2004; Stirling et al., 1999). However, at the population level, it appears that male humpback whales experience a consistent mean concentration of corticosterone throughout the year whether they are feeding or breeding. One factor that may contribute to this finding is that the collection of males at any one time on the feeding or breeding grounds is likely to include individuals that have been present for some time and others that are newly arrived (Craig et al., 2003). On the feeding grounds, this equates to males in various stages of replenishing their fat stores and on the breeding grounds to males in various stages of fasting (Straley, 1990). To the extent that body condition is related to stress, measures of stress across males in an area or between areas may thus show large variability but no overall differences.

Based on the 14 individual humpback whales who were sampled twice in the same season or between locations in the same year, our results suggest that stress is better studied at the individual level rather than at the population level as individuals encounter external stressors at different rates, times, frequencies and intensities. Unlike reproductive cycles, where hormones increase and decrease cyclically throughout the year (Cates et al., 2019), stress is more likely to vary at an individual level and may be either acute or chronic. Individual variation could be the result of nutritional condition, breeding pressures, climatic shifts, disease, reproductive state and/or anthropogenic stressors such as fishing gear entanglement (Atkinson et al., 2015;

Burek et al., 2008; Henson et al., 2017; Huntington et al., 2017; Rolland et al., 2012; Wernberg et al., 2016). For example, on the feeding grounds, humpback whales search for prey alone, in pairs, or in co-ordinated groups, each with their associated benefits and costs (Clapham, 2009). Coordinated foraging may increase the likelihood of prey detection or capture thus increasing fat stores and theoretically decreasing stress levels. Conversely, coordinated feeding groups are also a primary target for whale watching tourism and may attract tourism vessels and thus an additional stressor. On the breeding grounds, non-calf humpback whale males may be observed in a variety of different behavioral roles that in theory would likely contribute to variations in energy expenditure, metabolic well-being or general stress levels (Cartwright and Sullivan, 2009; Herman et al., 2011; Spitz et al., 2002). These include singly escorting a mother-calf pair or a single female without calf (Mobley and Herman, 1985; Pack et al., 2012). Males can also be observed singing, often alone but sometimes while escorting a mother-calf pair, lone female, or being joined by another male (Darling et al., 2006; Darling and Bérubé, 2001; Herman et al., 2013), and physically competing with other males for access to female within competitive groups (Tyack and Whitehead, 1983). Within competitive groups (which may number from 2 to 15 + males), the so-called "principal escort" actively defends the position closest to the female from challengers through displays, chases and body strikes while other secondary escorts adopt a non-challenging tactic (Baker and Herman, 1984; Clapham et al., 1992; Pack et al., 2009; Spitz et al., 2002). Thus, whether a male humpback whale is on the feeding or breeding grounds, undergoing migration or experiencing some other external pressure, it seems that whales encounter different types of potential stressors on a nearly daily basis and that when these experiences are grouped together may equate to similar mean corticosterone concentrations across spatial and temporal boundaries.

Measuring stress response in any organism is complicated. It may be that accurate assessment of the stressor may need to involve several metrics and that blubber may not be the best matrix with which to analyze stress. Steroid hormones are lipophilic and accumulate in adipose tissue (i.e. blubber), however the degree to which they accumulate and the length that they reside in the tissue is dependent on several factors including metabolic rate (Kershaw and Flier, 2004). For humpback whales, which as mysticetes, have relatively slow metabolic rates (Ball et al., 2017), this means that studies of hormones found in blubber represent health over a longer time frame than the snapshot of condition that blood would provide. As such, blubber is a good medium for analyzing chronic stress but examination of acute stress may require additional biological metrics. Examination of the stress response in humpback whales would benefit from a coordinated study on behavior, body condition and steroid hormones with a focus on resightings of individuals within and across season in order to determine the impact of potential stressors. It may also require the examination of additional hormones. While GCs are generally thought of as good indicators of a stress response, other hormones such as aldosterone, DHEA-S and thyroid hormones may also play a role in the regulation of stress (Atkinson et al., 2015). For the most part, endocrine systems in marine mammals follow the basic organization and chemical characteristics of other mammals, yet due to the pressures imposed by an aquatic lifestyle they have adapted in numerous ways. Aldosterone is the major mineralocorticoid and is primarily responsible for increasing sodium reabsorption from the renal tubules. It may also play several other important roles in marine mammals, including regulation of water retention during extended natural fasts (Ortiz et al., 2006). Aldosterone secretion is typically elevated coincident with cortisol increases in a variety of situations, including cold water exposure (Houser et al., 2011), restraint and handling (Champagne et al., 2012), and with an ACTH challenge (Champagne et al., 2018; Keogh and Atkinson, 2015). Thus, aldosterone appears to serve a role in the stress response in marine mammals (Gulland et al., 2018) and may be a very useful indicator of the stress response, particularly in regard to salt balance. It

also bears mentioning that studies focusing on evaluating stress in female humpbacks should concentrate their efforts of examination of cortisol, as preliminary studies have found a sex-based difference in the primary glucocorticoid metabolite expressed (Atkinson et al. 2020). It is likely that non-pregnant females will mimic the trends in stress expression observed in male humpbacks, yet stress levels are likely to increase with pregnant females (Hunt et al., 2019).

Regardless of how the stress response is measured, it is critical to ensuring the health and sustainability of marine mammal populations. Should some event disrupt the normal physiology of humpback whales, it would be advantageous to have a baseline to compare to and identify the root cause of these changes. Such an event did occur recently in the Hawaii DPS. In 2016, the Hawaii DPS was delisted from an endangered status under the United States Endangered Species Act as a result of this population reaching estimated pre-commercial whaling levels of abundance in conjunction with an annual healthy population growth rate of approximately 6% (Calambokidis et al. 2008). Observed rates of whale sightings including calves, however, began to decrease significantly beginning in 2014 (Cartwright et al., 2019; NOAA, 2019). Due to a "perfect cocktail" of environmental conditions (e.g. negative Pacific Decadal Oscillation (PDO), a North Pacific Heatwave and a strong El Nino), normal climatic conditions for humpbacks, and the rest of the species in the eastern North Pacific, altered. Shifts in the distribution and range of species were widespread and widescale mortality events of several species, such as Common Murres (*Uria aalge*), Crested Auklets *(Aethia cristatella)* and California sea lions (*Zalophus californianus*), occurred (Cornwall, 2019; NMFS, 2019; NPS, 2019). Humpback whales also exhibited unusual behavior, with a severe decline in reproductive rates documented, an increase in the amount of emaciated or skinny whales seen on the feeding grounds, an increase in skin abnormalities and an unusual number of whales whose residence time on the feeding grounds increased (Cartwright et al., 2019; Neilson et al., 2017; Straley et al., 2018). In order to understand the root cause of these physiological and behavioral changes, baseline indices of health are needed from which to compare.

Endocrine markers in combination with behavioral and longitudinal data on individually identified humpback whales provide a powerful tool in the assessment of physiology and life history states for responsible management and conservation of humpback whales. As the chemical messengers in the body, hormones are an essential element in the smooth functioning of any organism and an excellent place to begin to examine the health of an individual. With an emerging catalog of baseline concentrations for steroid hormones in humpback whales, we are increasing our ability and efficacy in documenting, calculating, and addressing changes in normal humpback whale physiology. Not only is such information essential for an appreciation of how individual marine mammal species respond to natural stressors, but it is also essential for understanding the impacts of anthropogenic activities and climatic shifts on humpback whales.

CRediT authorship contribution statement

Kelly A. Cates: Conceptualization, Lab Work, Data Analysis, Writing - original draft. **Shannon Atkinson:** Conceptualization, Writing - review & editing, Supervision. **Adam A. Pack:** Conceptualization, Writing - review & editing, Data curation. **Janice M. Straley:** Conceptualization, Writing - review & editing, Data curation. **Christine M. Gabriele:** Writing - review & editing, Data curation. **Suzanne Yin:** Writing - review & editing.

Acknowledgements

References

Allen, J.A., Garland, E.C., Dunlop, R.A., Noad, M.J., 2018. Cultural revolutions reduce complexity in the songs of humpback whales. Proc. R. Soc. B. 285, 20182088.

Atkinson, S., Crocker, D., Houser, D., Mashburn, K., 2015. Stress physiology in marine mammals: how well do they fit the terrestrial model? J. Comp. Physiol. B. 185, 463–486.

Atkinson, S., Gendron, D., Branch, T.A., Mashburn, K.L., Melica, V., Enriquez-Paredes, L.E., Brownell Jr, R.L., 2019. Pregnancy rate and biomarker validations from the blubber of eastern North Pacific blue whales. Mar. Mammal Sci. 36, 6–28.

Atkinson, S., Mashburn, K., Cates, K., Melica, V., 2020. Stress in large whales: dominate glucocorticoid varies by sex, species and sample material. In Prep.

Baker, C.S., Herman, L.M., 1984. Aggressive behavior between humpback whales (Megaptera novaeangliae) wintering in Hawaiian waters. Can. J. Zool. 62, 1922–1937.

Baker, C.S., Herman, L.M., Perry, A., Lawton, W.S., Straley, J.M., Straley, J.H., 1985. Population characteristics and migration of summer and late-season humpback whales (Megaptera novaeangliae) in southeastern Alaska. Mar. Mammal Sci. 1, 304–323.

Baker, C.S., Steel, D., Calambokidis, J., Falcone, E., González-Peral, U., Barlow, J., Burdin, A.M., Clapham, P.J., Ford, J.K.B., Gabriele, C.M., 2013. Strong maternal fidelity and natal philopatry shape genetic structure in North Pacific humpback whales. Mar. Ecol. Prog. Ser. 494, 291–306.

Ball, H.C., Londraville, R.L., Prokop, J.W., George, J.C., Suydam, R.S., Vinyard, C., Thewissen, J.G.M., Duff, R.J., 2017. Beyond thermoregulation: metabolic function of cetacean blubber in migrating bowhead and beluga whales. J. Comp. Physiol. B. 187, 235–252.

Barlow, J., Calambokidis, J., Falcone, E.A., Baker, C.S., Burdin, A.M., Clapham, P.J., Ford, J.K.B., Gabriele, C.M., LeDuc, R., Mattila, D.K., 2011. Humpback whale abundance in the North Pacific estimated by photographic capture-recapture with bias correction from simulation studies. Mar. Mammal Sci. 27, 793–818.

Blair, H.B., Merchant, N.D., Friedlaender, A.S., Wiley, D.N., Parks, S.E., 2016. Evidence for ship noise impacts on humpback whale foraging behaviour. Biol. Lett. 12, 20160005.

Boonstra, R., 2004. Coping with changing northern environments: the role of the stress axis in birds and mammals. Integr. Comp. Biol. 44, 95–108.

Burek, K.A., Gulland, F., O'Hara, T.M., 2008. Effects of climate change on Arctic marine mammal health. Ecol. Appl. 18, 126–134.

Burgess, E.A., Brown, J.L., Lanyon, J.M., 2013. Sex, scarring, and stress: understanding seasonal costs in a cryptic marine mammal. Conserv. Physiol. 1, cot014.

Burgess, E.A., Hunt, K.E., Kraus, S.D., Rolland, R.M., 2016. Get the most out of blow hormones: validation of sampling materials, field storage and extraction techniques for whale respiratory vapour samples. Conserv. Physiol. 4, cow024.

Calambokidis, J., Falcone, E.A., Quinn, T.J., Burdin, A.M., Clapham, P.J., Ford, J.K.B., Gabriele, C.M., LeDuc, R., Mattila, D., Rojas-Bracho, L., 2008. SPLASH: Structure of populations, levels of abundance and status of humpback whales in the North Pacific. Unpubl. Rep. Submitt. by Cascadia Res. Collect. to USDOC, Seattle, WA under Contract AB133F-03-RP-0078.

Campbell, N.A., Reece, J.B., Urry, L.A., Cain, M.L., Wasserman, S.A., Minorsky, P. V., Jackson, R.B., 2008. Energy requirements are related to animal size, activity, and environment, in: Campbell Biology. San Francisco: Benjamin Cumming, p. 870.

Cartwright, R., Sullivan, M., 2009. Behavioral ontogeny in humpback whale (Megaptera novaeangliae) calves during their residence in Hawaiian waters. Mar. Mammal Sci. 25, 659–680.

Cartwright, R., Venema, A., Hernandez, V., Wyels, C., Cesere, J., Cesere, D., 2019. Fluctuating reproductive rates in Hawaii's humpback whales, Megaptera novaeangliae, reflect recent climate anomalies in the North Pacific. R. Soc. Open Sci. 6, 181463.

Cates, K.A., Atkinson, S., Gabriele, C.M., Pack, A.A., Straley, J.M., Yin, S., 2019. Testosterone Trends Within and Across Seasons in Male Humpback Whales

(Megaptera novaeangliae) from Hawaii and Alaska. Gen. Comp. Endocrinol. 279, 164–173.

Cerchio, S., Strindberg, S., Collins, T., Bennett, C., Rosenbaum, H., 2014. Seismic surveys negatively affect humpback whale singing activity off northern. Angola. PLoS One 9.

Champagne, C.D., Houser, D.S., Costa, D.P., 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.

Champagne, C.D., Kellar, N.M., Crocker, D.E., Wasser, S.K., Booth, R.K., Trego, M.L., Houser, D.S., 2017. Blubber cortisol qualitatively reflects circulating cortisol concentrations in bottlenose dolphins. Mar. Mammal Sci. 33, 134–153.

Champagne, C.D., Kellar, N.M., Trego, M.L., Delehanty, B., Boonstra, R., Wasser, S.K., Booth, R.K., Crocker, D.E., Houser, D.S., 2018. Comprehensive endocrine response to acute stress in the bottlenose dolphin from serum, blubber, and feces. Gen. Comp. Endocrinol. 266, 178–193.

Chittleborough, R.G., 1965. Dynamics of two populations of the humpback whale, Megaptera novaeangliae (Borowski). Mar. Freshw. Res. 16, 33–128.

Cholewiak, D.M., Cerchio, S., Jacobsen, J.K., Urbán-R, J., Clark, C.W., 2018. Songbird dynamics under the sea: acoustic interactions between humpback whales suggest song mediates male interactions. R. Soc. Open Sci. 5, 171298.

Christiansen, F., Dujon, A.M., Sprogis, K.R., Arnould, J.P.Y., Bejder, L., 2016. Noninvasive unmanned aerial vehicle provides estimates of the energetic cost of reproduction in humpback whales. Ecosphere. 7, e01468.

Clapham, P.J., 2009. Humpback whale: Megaptera novaeangliae, in: Encyclopedia of Marine Mammals (Second Edition). Elsevier, pp. 582–585.

Clapham, P.J., 2000. The humpback whale in: Mann, J., Connor, R.C., Tyack, P.L., Whitehead, H., Cetacean Societies: Field Studies of Dolphins and Whales. The University of Chicago Press, Chicago, pp. 173–196.

Clapham, P.J., 1996. The social and reproductive biology of humpback whales: an ecological perspective. Mamm. Rev. 26, 27–49.

Clapham, P.J., Mayo, C.A., 1990. Reproduction of humpback whales (Megaptera novaeangliae) observed in the Gulf of Maine. Rep. Int. Whal. Commn. Spec. 171–175.

Clapham, P.J., Palsbøll, P.J., Mattila, D.K., Vasquez, O., 1992. Composition and dynamics of humpback whale competitive groups in the West Indies. Behaviour. 122, 182–194.

CoastWatch, 2020. Providing satellite data for the coastal ocean [WWW Document]. NOAA. URL https://coastwatch.pfeg.noaa.gov/.

Cornwall, W., 2019. A new 'Blob' menaces Pacific ecosystems. Sci. Mag. 365, 1233–1233.

Craig, A., Herman, L.M., Pack, P.A.A., 2001. Estimating residence times of humpback whales in Hawaii. Rep. Hawaiian Islands Natl. Mar. Sanctuary. Natl. Ocean. Atmos. Adm, Washington, DC.

Craig, A.S., Herman, L.M., Gabriele, C.M., Pack, A.A., 2003. Migratory timing of humpback whales (Megaptera novaeangliae) in the central North Pacific varies with age, sex and reproductive status. Behaviour. 140, 981–1001.

Craig, A.S., Herman, L.M., Pack, A.A., 2002. Male mate choice and male-male competition coexist in the humpback whale (Megaptera novaeangliae). Can. J. Zool. 80, 745–755.

Craig, A.S., Herman, L.M., Pack, A.A., Waterman, J.O., 2014. Habitat segregation by female humpback whales in Hawaiian waters: avoidance of males? Behaviour. 151, 613–631.

Croghan, C., Egeghy, P.P., 2003. Methods of dealing with values below the limit of detection using SAS. Present. Southeast. SAS User Group, Sept. 22-24. St. Petersburg, FL.

Dalle Luche, G., Bengtson Nash, S., Kucklick, J.R., Mingramm, F.M.J., Boggs, A.S.P., 2019. Liquid chromatography tandem mass spectrometry for the quantification of steroid hormone profiles in blubber from stranded humpback whales (Megaptera novaeangliae). Conserv. Physiol. 7, coz030.

Darling, J.D., Bérubé, M., 2001. Interactions of singing humpback whales with other males. Mar. Mammal Sci. 17, 570–584.

Darling, J.D., Jones, M.E., Nicklin, C.P., 2006. Humpback whale songs: Do they organize males during the breeding season? Behaviour 143, 1051–1102.

Dawbin, W.H., 1966. The seasonal migratory cycle of humpback whales, in: Norris, K.S., Whales, Dolphins and Porpoises. University of California Press, Berkeley, pp. 145–170.

Di Poi, C., Atkinson, S., Hoover-Miller, A., Blundell, G., 2015. Maternal buffering of stress response in free-ranging Pacific harbor seal pups in Alaska. Mar. Mammal Sci. 31, 1098–1117.

Dunlop, R.A., Noad, M.J., McCauley, R.D., Kniest, E., Paton, D., Cato, D.H., 2015. The behavioural response of humpback whales (Megaptera novaeangliae) to a 20 cubic inch air gun. Aquat. Mamm. 41, 412.

EPA, U.S., 2000. Guidance for data quality assessment: Practical methods for data analysis. Off. Environ. Prot. Section 4, 42–53.

Forest, M.G., Cathiard, A.M., Bertrand, J.A., 1973. Evidence of testicular activity in early infancy. J. Clin. Endocrinol. Metab. 37, 148–151.

Gabriele, C.M., Neilson, J.L., Straley, J.M., Baker, C.S., Cedarleaf, J.A., Saracco, J.F., 2017. Natural history, population dynamics, and habitat use of humpback whales over 30 years on an Alaska feeding ground. Ecosphere. 8, e01641.

Gabriele, C.M., Straley, J.M., Neilson, J.L., 2007. Age at first calving of female humpback whales in southeastern Alaska. Mar. Mammal Sci. 23, 226–239.

Gaspar, C., West, K., Atkinson, S., Sweeney, J., 2000. Variations in blubber thickness of individual dolphins and between species. Annu. Conf. Am. Assoc. Zoo Vet. 505.

Glockner-Ferrari, D.A., Ferrari, M.J., 1985. Individual Identification, behavior, reproduction, and distribution of humpback whales, Megaptera novaeangliae, in Hawaii. NTIS Report No. MMC-83 106, Springfield, VA.

Gulland, F.M.D., Dierauf, L.A., Whitman, K.L., 2018. CRC handbook of marine mammal medicine. CRC Press, Boca Raton, FL.

Hastie, T.J., Tibshirani, R.J., 1986. Generalized additive models. Statist. Sci. 1, 297–318.

Hebert, K.P., 2017. Southeast Alaska 2017 Herring Stock Assessment Surveys. Alaska

Department of Fish and Game, Division of Sport Fish, Research and Technical Services. Annual Report, 1-119.

Helweg, D.A., Herman, L.M., 1994. Diurnal patterns of behaviour and group membership of humpback whales (Megaptera novaeangliae) wintering in Hawaiian waters. Ethology. 98, 298–311.

Henson, S.A., Beaulieu, C., Ilyina, T., John, J.G., Long, M., Séférian, R., Tjiputra, J., Sarmiento, J.L., 2017. Rapid emergence of climate change in environmental drivers of marine ecosystems. Nat. Commun. 8, 14682.

Herman, E.Y.K., Herman, L.M., Pack, A.A., Marshall, G., Shepard, M.C., Bakhtiari, M., 2007. When whales collide: Crittercam offers insight into the competitive behavior of humpback whales on their Hawaiian wintering grounds. Mar. Technol. Soc. J. 41, 35–43.

Herman, L.M., 2017. The multiple functions of male song within the humpback whale (Megapatera novaeangliae) mating system: Review, evaluation, and synthesis. Biol. Rev. 92, 1795–1818.

Herman, L.M., Pack, A.A., Rose, K., Craig, A., Herman, E.Y.K., Hakala, S., Milette, A., 2011. Resightings of humpback whales in Hawaiian waters over spans of 10–32 years: site fidelity, sex ratios, calving rates, female demographics, and the dynamics of social and behavioral roles of individuals. Mar. Mammal Sci. 27, 736–768.

Herman, L.M., Pack, A.A., Spitz, S.S., Herman, E.Y.K., Rose, K., Hakala, S., Deakos, M.H., 2013. Humpback whale song: who sings? Behav. Ecol. Sociobiol. 67, 1653–1663.

Houser, D.S., Champagne, C.D., Crocker, D.E., Kellar, N.M., Cockrem, J., Romano, T., Booth, R.K., Wasser, S.K., 2016. Natural variation in stress hormones, comparisons across matrices, and impacts resulting from induced stress in the bottlenose dolphin, in: The Effects of Noise on Aquatic Life II. Springer, pp. 467–471.

Houser, D.S., Wasser, S., Cockrem, J.F., Kellar, N., Romano, T., 2011. Variability of Hormonal Stress Markers Collected from a Managed Dolphin Population. DTIC Document.

Hunt, K.E., Robbins, J., Buck, C.L., Bérubé, M., Rolland, R.M., 2019. Evaluation of fecal hormones for noninvasive research on reproduction and stress in humpback whales (Megaptera novaeangliae). Gen. Comp. Endocrinol. 280, 24–34.

Hunt, K.E., Stimmelmayr, R., George, C., Hanns, C., Suydam, R., Brower, H., Rolland, R.M., 2014. Baleen hormones: a novel tool for retrospective assessment of stress and reproduction in bowhead whales (Balaena mysticetus). Conserv. Physiol. 2, cou030.

Huntington, H.P., Quakenbush, L.T., Nelson, M., 2017. Evaluating the effects of climate change on indigenous marine mammal hunting in Northern and Western Alaska using traditional knowledge. Front. Mar. Sci. 4, 319.

Katona, S.K., Beard, J.A., 1990. Population size, migrations and feeding aggregations of the humpback whale (Megaptera novaeangliae) in the western North Atlantic Ocean. Rep. Int. Whal. Comm. Special Is 295–306.

Katona, S.K., Whitehead, H.P., 1981. Identifying humpback whales using their natural markings. Polar Rec. 20, 439–444.

Keogh, M.J., 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. Part A Mol. Integr. Physiol. 188, 22–31.

Kershaw, E.E., Flier, J.S., 2004. Adipose tissue as an endocrine organ. J. Clin. Endocrinol. Metab. 89, 2548–2556.

LaFleur, B., Lee, W., Billhiemer, D., Lockhart, C., Liu, J., Merchant, N., 2011. Statistical methods for assays with limits of detection: Serum bile acid as a differentiator between patients with normal colons, adenomas, and colorectal cancer. J. Carcinog. 10, 1–18.

Lockyer, C., 1986. Body fat condition in Northeast Atlantic fin whales, Balaenoptera physalus, and its relationship with reproduction and food resource. Can. J. Fish. Aquat. Sci. 43, 142–147.

Mashburn, K.L., Atkinson, S., 2004. Evaluation of adrenal function in serum and feces of Steller sea lions (Eumetopias jubatus): influences of molt, gender, sample storage, and age on glucocorticoid metabolism. Gen. Comp. Endocrinol. 136, 371–381.

Mingramm, F.M.J., Keeley, T., Whitworth, D.J., Dunlop, R.A., 2020. Blubber cortisol levels in humpback whales (Megaptera novaeangliae): A measure of physiological stress without effects from sampling. Gen. Comp. Endocrinol. 291, 113436.

Mobley, J.R., Herman, L.M., 1985. Transience of social affiliations among humpback whales (Megaptera novaeangliae) on the Hawaiian wintering grounds. Can. J. Zool. 63, 762–772.

Neilson, J.L., Gabriele, C.M., 2003. Results of humpback whale population monitoring in Glacier Bay and adjacent waters: 2006. Rep. to Natl. Park Serv. Glacier Bay Natl. Park Preserv. Gustavus, Alaska.

Neilson, J.L., Gabriele, C.M., 2006. Results of humpback whale population monitoring in Glacier Bay and adjacent waters: 2006. Rep. to Natl. Park Serv. Glacier Bay Natl. Park Preserv, Gustavus, Alaska.

Neilson, J., Gabriele, C.M., Vanselow, P.B.S., 2017. Humpback whale monitoring in Glacier Bay and adjacent waters 2017: annual progress report. Natural Resource Report: NPS/GLBA/NRR-2018/1660. US Department of the Interior, National Park Service. Fort Collins, Colorado.

Nishiwaki, M., 1960. Ryukyuan Humpback Whaling in 1960. Inst. Cetacean Res. 1–15.

NMFS, 2019. Looking Back at The Blob: Record Warming Drives Unprecedented Ocean Change [WWW Document]. URL https://www.fisheries.noaa.gov/feature-story/looking-back-blob-record-warming-drives-unprecedented-ocean-change?utm_medium=email&utm_source=govdelivery (accessed 1.11.19).

NOAA, 2019. Trends in humpback whale (Megaptera novaeangliae) abundance, distribution, and health in Hawaii and Alaska: Report from a meeting held on November 27-28, 2019. NOAA National Ocean Service, Office of National Marine Sanctuaries, Hawaiian Islands Humpback W.

Norman, A.W., Litwack, G., 1997. Hormones. Academic Press.

NPS, 2019. Seabird Die-Offs [WWW Document]. URL https://www.nps.gov/subjects/aknatureandscience/commonmurrewreck.htm (accessed 1.11.19).

Oki, C., Atkinson, S., 2004. Diurnal patterns of cortisol and thyroid hormones in the Harbor seal (Phoca vitulina) during summer and winter seasons. Gen. Comp. Endocrinol. 136, 289–297.

ONR, 2009. Office of Naval Research Marine Mammal and Biology Program Annual Meeting. Arlington, Virginia.

Ortiz, R.M., Crocker, D.E., Houser, D.S., Webb, P.M., 2006. Angiotensin II and aldosterone increase with fasting in breeding adult male northern elephant seals (Mirounga angustirostris). Physiol. Biochem. Zool. 79, 1106–1112.

PacIOOS, 2019. Water Temperature Buoy Observations [WWW Document]. URL http://www.pacioos.hawaii.edu/water-category/buoy/.

Pack, A.A., Herman, L.M., Craig, A.S., Spitz, S.S., Waterman, J.O., Herman, E.Y.K., Deakos, M.H., Hakala, S., Lowe, C., 2017. Habitat preferences by individual humpback whale mothers in the Hawaiian breeding grounds vary with the age and size of their calves. Anim. Behav. 133, 131–144.

Pack, A.A., Herman, L.M., Spitz, S.S., Craig, A.S., Hakala, S., Deakos, M.H., Herman, E.Y.K., Milette, A.J., Carroll, E., Levitt, S., 2012. Size-assortative pairing and discrimination of potential mates by humpback whales in the Hawaiian breeding grounds. Anim. Behav. 84, 983–993.

Pack, A.A., Herman, L.M., Spitz, S.S., Hakala, S., Deakos, M.H., Herman, E.Y.K., 2009. Male humpback whales in the Hawaiian breeding grounds preferentially associate with larger females. Anim. Behav. 77, 653–662.

Petrauskas, L., Atkinson, S., Gulland, F., Mellish, J.A., Horning, M., 2008. Monitoring glucocorticoid response to rehabilitation and research procedures in california and steller sea lions. J. Exp. Zool. Part A Ecol. Genet. Physiol. 309, 73–82.

Purves, W.K., Sadava, D., Orians, G.H., Heller, C., 2003. Thermoregulation in Endotherms, in: Life: The Science of Biology. Sinauer Associates, Sunderland, p. 792.

Quigley, C.A., 2002. The postnatal gonadotropin and sex steroid surge—insights from the androgen insensitivity syndrome. J. Clin. Endocrinol. Metab. 87, 24–28.

R Core Team, 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria https://www.R-projectorg/.

Rolland, R.M., Parks, S.E., Hunt, K.E., Castellote, M., Corkeron, P.J., Nowacek, D.P., Wasser, S.K., Kraus, S.D., 2012. Evidence that ship noise increases stress in right whales. Proc. R. Soc. London B Biol. Sci. 279, 2363–2368.

Romero, L.M., 2002. Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. Gen. Comp. Endocrinol. 128, 1–24.

Sharick, J.T., Vazquez-Medina, J.P., Ortiz, R.M., Crocker, D.E., 2015. Oxidative stress is a potential cost of breeding in male and female northern elephant seals. Funct. Ecol. 29, 367–376.

Spitz, S.S., Herman, L.M., Pack, A.A., Deakos, M.H., 2002. The relation of body size of male humpback whales to their social roles on the Hawaiian winter grounds. Can. J. Zool. 80, 1938–1947.

Stirling, I., Lunn, N.J., Iacozza, J., 1999. Long-term trends in the population ecology of polar bears in western Hudson Bay in relation to climatic change. Arctic. 52, 294–306.

Straley, J.M., 1990. Fall and winter occurrence of humpback whales (Megaptera novaeangliae) in southeastern Alaska. Rep. Int. Whal. Comm. Spec. 319–323.

Straley, J.M., Moran, J.R., Boswell, K.M., Vollenweider, J.J., Heintz, R.A., Quinn II, T.J., Witteveen, B.H., Rice, S.D., 2018. Seasonal presence and potential influence of humpback whales on wintering Pacific herring populations in the Gulf of Alaska. Deep Sea Res. Part II Top. Stud. Oceanogr. 147, 173–186.

Szabo, A., Duffus, D., 2008. Mother–offspring association in the humpback whale, Megaptera novaeangliae: following behaviour in an aquatic mammal. Anim. Behav. 75, 1085–1092.

Teerlink, S., Horstmann, L., Witteveen, B., 2018. Humpback whale (Megaptera novaeangliae) blubber steroid hormone concentration to evaluate chronic stress response from whale-watching vessels. Aquat. Mamm. 44.

Tyack, P., Whitehead, H., 1983. Male competition in large groups of wintering humpback whales. Behaviour. 83, 132–154.

Valenzuela-Molina, M., Atkinson, S., Mashburn, K., Gendron, D., Brownell, R.L., 2018. Fecal steroid hormones reveal reproductive state in female blue whales sampled in the Gulf of California. Mexico. Gen. Comp. Endocrinol. 261, 127–135.

Wartzok, D., Altmann, J., Au, W., Ralls, K., Starfield, A., Tyack, P.L., 2005. Marine mammal populations and ocean noise: determining when noise causes biologically significant effects. The National Academies Press, Washington, DC.

Wasser, S.K., Hunt, K.E., Brown, J.L., Cooper, K., Crockett, C.M., Bechert, U., Millspaugh, J.J., Larson, S., Monfort, S.L., 2000. A generalized fecal glucocorticoid assay for use in a diverse array of nondomestic mammalian and avian species. Gen. Comp. Endocrinol. 120, 260–275.

Wernberg, T., Bennett, S., Babcock, R.C., De Bettignies, T., Cure, K., Depczynski, M., Dufois, F., Fromont, J., Fulton, C.J., Hovey, R.K., 2016. Climate-driven regime shift of a temperate marine ecosystem. Science. 353, 169–172.

Williams, T.M., Haun, J., Davis, R.W., Fuiman, L.A., Kohin, S., 2001. A killer appetite: metabolic consequences of carnivory in marine mammals. Comp Biochem. Physiol. Part A Mol. Integr. Physiol. 129, 785–796.

Wingfield, J.C., 2013. Ecological processes and the ecology of stress: the impacts of abiotic environmental factors. Funct. Ecol. 27, 37–44.

Wright, A.J., Soto, N.A., Baldwin, A.L., Bateson, M., Beale, C.M., Clark, C., Deak, T., Edwards, E.F., Fernández, A., Godinho, A., 2007. Do marine mammals experience stress related to anthropogenic noise? Int. J. Comp. Psychol. 20, 274–316.