

A longitudinal study of endocrinology and foraging ecology of subadult gray whales prior to death based on baleen analysis

Alejandro Fernández Ajó^{a,*}, Clarissa Teixeira^b, Daniela M.D. de Mello^c, Danielle Dillon^{d,1}, James M. Rice^e, C. Loren Buck^d, Kathleen E. Hunt^f, Matthew C. Rogers^g, Leigh G. Torres^a

^a Geospatial Ecology of Marine Megafauna Lab, Marine Mammal Institute, Department of Fisheries, Wildlife and Conservation Sciences, Oregon State University, Newport 97365, OR, USA

^b Marine Mammal Institute, Department of Fisheries, Wildlife and Conservation Sciences, Oregon State University, Newport 97365, OR, USA

^c Department of Physiology, Institute of Bioscience, University of São Paulo, São Paulo 05508090, SP, Brazil

^d Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ 86011, USA

^e Oregon Marine Mammal Stranding Network, Marine Mammal Institute, Oregon State University, Newport 97365, OR, USA

^f George Mason University & Smithsonian-Mason School of Conservation, 1500 Remount Rd, Front Royal, VA 22630, USA

^g NOAA, National Marine Fisheries Service, Alaska Fisheries Science Center Auke Bay Laboratories, Juneau, AK 99801, USA

ARTICLE INFO

Keywords:

Mysticetes

Stable isotopes

Enzyme immunoassays

Baleen

Longitudinal profiles

ABSTRACT

Individual-level assessments of wild animal health, vital rates, and foraging ecology are critical for understanding population-wide impacts of exposure to stressors. Large whales face multiple stressors, including, but not limited to, ocean noise, pollution, and ship strikes. Because baleen is a continuously growing keratinized structure, serial extraction, and quantification of hormones and stable isotopes along the length of baleen provide a historical record of whale physiology and foraging ecology. Furthermore, baleen analysis enables the investigation of dead specimens, even decades later, allowing comparisons between historic and modern populations. Here, we examined baleen of five sub-adult gray whales and observed distinct patterns of oscillations in $\delta^{15}\text{N}$ values along the length of their baleen plates which enabled estimation of baleen growth rates and differentiation of isotopic niche widths of the whales during wintering and summer foraging. In contrast, no regular patterns were apparent in $\delta^{13}\text{C}$ values. Prolonged elevation of cortisol in four individuals before death indicates that chronic stress may have impacted their health and survival. Triiodothyronine (T3) increased over months in the whales with unknown causes of death, simultaneous with elevations in cortisol, but both hormones remained stable in the one case of acute death attributed to killer whale predation. This parallel elevation of cortisol and T3 challenges the classic understanding of their interaction and might relate to increased energetic demands during exposure to stressors. Reproductive hormone profiles in subadults did not show cyclical trends, suggesting they had not yet reached sexual maturity. This study highlights the potential of baleen analysis to retrospectively assess gray whales' physiological status, exposure to stressors, reproductive status, and foraging ecology in the months or years leading up to their death, which can be a useful tool for conservation diagnostics to mitigate unusual mortality events.

1. Introduction

Individual-level assessments of changes in health, vital rates, movements, and foraging ecology of wild animals in response to disturbance events are key for identifying potential impacts on the broader population (Pirodda et al., 2022), as well as for gaining insights needed for effective, targeted conservation and management strategies.

Large whales, for example, are exposed to an increasing number of stressors, including ocean noise (e.g., vessel traffic, military sonar, seismic oil and gas exploration, and construction; Lemos et al., 2022; Rolland et al., 2012), contaminants such as plastics, heavy metals and chemicals (Lowe et al., 2022; Reckendorf, 2023; Torres et al., 2023), harmful algal blooms (D'Agostino et al., 2022), ship strikes and entanglement in fishing gear (Clapham, 2016; Derville et al., 2023), marine

* Corresponding author.

E-mail address: fernaale@oregonstate.edu (A. Fernández Ajó).

¹ Present address: New England Aquarium, 1 Central Wharf, Boston, MA 02110.

heatwaves (Suryan et al., 2021), and prey shifts (Derville et al., 2023; Pallin et al., 2023). Yet, conservation efforts to mitigate threats to whale populations are hindered by the challenges of monitoring and obtaining repeated samples from individuals due to the large size, mobility, and remote marine habitats of baleen whales (Hunt et al., 2013).

The use of combined analytical methods in different biological tissue types collected from free-ranging whales and/or stranded carcasses has allowed researchers to overcome some of these limitations and increased our ability to disentangle different aspects of the foraging ecology and physiology of large whales (Fleming et al., 2018; Hunt et al., 2020, 2013; Teixeira et al., 2022). Baleen, for example, is a unique structure that forms the filter-feeding apparatus in mysticetes whales and is perhaps the best biological tissue for acquiring longitudinal ecological and physiological data to assess the responses of whales to stressors and their changing environment (Caraveo-Patiño et al., 2007; Fernández Ajó et al., 2020, 2018; Hunt et al., 2014; Lysiak et al., 2018). Like other keratinized epidermal tissues (e.g. claws, hair, spines, and whiskers), baleen is a continuously growing structure that extends from a well-vascularized dermal zone. The slow growth rate of baleen allows for the simultaneous incorporation of intrinsic biomarkers, including stable isotopes and hormones with sufficient temporal resolution to examine seasonal patterns spanning the time of baleen growth (Best and Schell, 1996; Hunt et al., 2018, 2017a, 2017b; Lysiak et al., 2023, 2018; Schell et al., 1989). For mysticetes with shorter baleen (e.g., humpback whales, *Megaptera novaeangliae*, and gray whales, *Eschrichtius robustus*), this period is 1–5 years (Caraveo-Patiño et al., 2007; Gabriele et al., 2021; Lowe et al., 2021b, 2021a) versus a decade or more in species with longer baleen (e.g., bowheads, *Balaena mysticetus*; Hunt et al., 2022, 2014; Lysiak et al., 2023; Matthews and Ferguson, 2015; and right whales, *Eubalaena* sp.; Hunt et al., 2017a, 2016; Lysiak et al., 2018).

Measurements of bulk carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotopes in baleen has been successfully applied to assess time-integrated diet and movements of different cetacean species (e.g., Best and Schell, 1996; Pomerleau et al., 2018; Silva et al., 2019). $\delta^{15}\text{N}$ values are often used as an indicator of trophic position (Fry, 1988), whereas $\delta^{13}\text{C}$ values reflect the sources of primary production that fuel the food webs and are generally used to distinguish movements across isotopically distinct food webs. Typically, consumers have higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values by 0.5–2 ‰ and 0.5–5 ‰, respectively, compared with their diet (Post, 2002), but these tissue-to-diet discrimination factors can vary with growth rates, diet quality, and nutritional or reproductive status (Ben-David et al., 2012; Borrell et al., 2012; Rita et al., 2019). $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ assimilate longitudinally along baleen plates and generally form oscillation patterns that may reflect changes in either feeding status (e.g., active foraging vs. fasting) along their annual migration (Best and Schell, 1996; Eisenmann et al., 2016; Sang et al., 2005); and/or latitudinal variation in prey isotope values between summering and wintering grounds (Busquets-Vass et al., 2017; Matthews and Ferguson, 2015; Mitani et al., 2006).

On the other hand, endocrine assessments along the baleen longitudinal axis can aid in reconstructing the endocrine history of whales leading up to their death (Fernández Ajó et al., 2020; Lowe et al., 2021a; Lysiak et al., 2018). For instance, an increased secretion of glucocorticoids from the hypothalamic–pituitary–adrenal (HPA) axis signifies the activation of the vertebrate stress response (Romero and Wingfield, 2016). Assessments on glucocorticoid hormones (cortisol and corticosterone) in baleen have been utilized to infer the physiological response of whales to stressors and known causes of death (Fernández Ajó et al., 2020, 2018; Lowe et al., 2021a; Lysiak et al., 2018). The hypothalamic–pituitary–thyroid axis (HPT) regulates the synthesis and secretion of thyroxine (T₄), which undergoes enzymatic conversion to the more active form, T₃. Both T₃ and T₄ modulate basal metabolic rate, growth and development, thermogenesis, along with other permissive actions (Romero and Wingfield, 2016). As T₃ is generally recognized as the most biologically active thyroid hormone, it is considered a relevant biomarker to inform nutritional status (Eales, 1988; Flamant et al.,

2017). In baleen, T₃ has been used to infer nutritional status (Fernández Ajó et al., 2020). Additionally, the analysis of reproductive hormones within baleen has proven valuable for assessing pregnancy and inter-calving cycles in females, as well as testosterone cycles in males (Hunt et al., 2022, 2018, 2016; Lowe et al., 2021b; Lysiak et al., 2023).

Therefore, the paired quantification of hormones and stable isotope values along the longitudinal axis of the baleen plate has the potential to provide integrated information about individuals' foraging ecology, movements, and endocrinology preceding their death. Notably, baleen is routinely recovered at necropsies, and its inherent strength, durability, and minimal storage requirements (i.e., these samples can be preserved dry at room temperature) ensure the preservation of the analytes of interest within the keratin matrix. Consequently, detection of hormones and isotopes remains feasible in dried samples for decades (Fernández Ajó et al., 2022; Hunt et al., 2017b). These remarkable properties of baleen not only capture multi-year timeframes, enabling the determination of individuals' seasonal endocrine and foraging patterns, but also facilitate comparisons between historic and modern populations of whales (Hunt et al., 2014).

Eastern North Pacific (ENP) gray whales migrate between their wintering grounds along the Baja California, Mexico, coastline, and their summer foraging grounds in the Bering, Chukchi, and Beaufort Seas. Within the ENP gray whale population, a distinctive group known as the Pacific Coast Feeding Group (PCFG; Calambokidis et al., 2002; Rice and Wolman, 1971), consists of around 200–250 individuals (Calambokidis et al., 2019) and is known for shortening its migration to forage along the Pacific coast, between northern California, USA, to British Columbia, CA, during the summer months remaining in close proximity to the shore (Mate and Urban-Ramirez, 2003). Gray whales are considered generalists (Nerini, 2012), while the ENP whales forage primarily on benthic amphipods (family Ampeliscidae) along the Bering and Chukchi Seas (Brower et al., 2017; Moore et al., 2003; Nerini, 2012), the PCFG whales forage on a variety of prey types, including mysids (family Mysidae), crab larvae (Cancer magister, Porcellanidae sp.), ghost shrimp (*Callinassa californiensis*), and benthic amphipods (Darling et al., 1998; Dunham and Duffus, 2002, 2001; Hildebrand et al., 2021).

The ENP population has experienced at least two recorded Unusual Mortality Events (UMEs), in 1999–2000 and from 2019 to the present, during which an unusually high number of gray whales were found dead along the Pacific coast from northern Mexico to the Alaskan Arctic, USA (Martínez-Aguilar et al., 2019; Urbán, 2020). Several factors have been considered as possible causes for the high number of gray whale strandings, including variation in Arctic prey availability and the duration of their feeding season caused by the timing of sea ice formation and breakup (Stewart et al., 2023), starvation, anthropogenically derived toxicants, biotoxins, infectious diseases, parasites, fisheries interactions, and ship strikes (Eguchi et al., 2023; Gulland et al., 2005). In the current UME, dead whales are frequently emaciated, indicating nutritional limitation as a causal factor of death (Christiansen et al., 2021). While the poor condition of many of the stranded whales supports the idea that starvation could be a significant contributing factor in these mortalities, the underlying causes of starvation during these events are unknown, and it is also unclear whether the whales' decline in body condition was rapid or gradual.

In this study, we analyzed patterns across time of stable isotopes and five hormones within baleen plates recovered postmortem from five subadult gray whales (4 males, 1 female) that stranded during the 2019-present UME. Our goal is twofold: first, to retrospectively examine the hormone and isotopic profiles in gray whales prior to mortality; and second, to assess potential factors contributing to mortality and the onset timing of chronic illness leading to death. Our isotopic analysis includes the longitudinal profiles of bulk $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in baleen, as they are well-established markers of seasonal changes in diet and foraging grounds in large whales enabling to estimate baleen growth rates (Best and Schell, 1996; Busquets-Vass et al., 2017; Matthews and Ferguson, 2015). Our hormonal analysis quantifies two adrenal

Table 1

Biological information for individual gray whales, *Eschrichtius robustus*, collected along the Oregon Coast and sampled for both hormone and stable isotope analysis.

Whale Code	Whale ID	Strand Date	Cause of Death	Sex	TL (cm)	Total Samples	BGR (mm/week)	GS (days)	Age Class
Er_1	HMSC_190424_Er	2019-04-24	Unk	F	1080	12*	3.2	~242	Subadult
Er_2	HMSC_200331_Er	2020-03-31	Unk	M	1086	26	3.2	~550	Subadult
Er_3	HMSC_200515_Er	2020-05-15	Unk	M	996	20	3.2	~418	Subadult
Er_4	HMSC_210529_Er	2021-05-29	Unk	M	1060	25	3.2	~528	Subadult
Er_5	HMSC_210816_Er	2021-08-16	Orca	M	1000	27	4.7	~390	Subadult

Cause of death: Unk = undetermined, Orca = evidence of Killer whale, *Orcinus orca*, predation as acute cause of death; **Sex** = Female (F), Male (M); **TL** = Total Length from snout to fluke notch in cm; **Total samples** = number of subsamples obtained from each baleen plate; **BGR** = baleen growth rate estimated in days per cm (from stable isotope analysis); **GS** = Growth span, estimated timespan represented by the entire baleen plate, in days, derived from baleen growth rate and total length of plate; * only the erupted portion of the baleen was collected during the necropsy.

glucocorticoid steroids, cortisol and corticosterone, as biomarkers of stress. The thyroid hormone triiodothyronine (T3), as a biomarker of nutritional state, i.e., a proxy for foraging success, given its role in regulating metabolic rate in mammals, as reviewed in Behringer et al., 2018. And two gonadal steroids, progesterone and testosterone. The two gonadal steroids are assessed here as markers of reproductive status, and to assess sexual maturity. All our specimens are from individuals classified as subadults at necropsy, although subadult whales may initiate gonadal secretion of reproductive hormones well in advance of full reproductive competence, and stress is known to delay sexual maturity in many mammals (Dettmer and Chusyd, 2023; Hunt et al., 2022). Further, the individual baselines for each hormone and each individual whale are assessed to monitor individual variability in response to potential stressors. Through the integration of isotopic and hormone methodologies, we demonstrate that baleen analysis provides a comprehensive narrative detailing the endocrine and trophic ecology of individual whales across time, aiding to fill knowledge gaps between individual physiology and population impacts. Ultimately, these biomarkers can make significant contributions to management and conservation efforts by informing the complex physiological dynamics that underlie whale's mortality.

2. Materials & methods

2.1. Sample collection

From April 2019 to August 2021, a baleen plate from each of five stranded gray whales (n = 4 males and n = 1 female) was collected by the Oregon Marine Mammal Stranding Network (OMMSN, NMFS MMPA/ESA permit No. 18786-06) along the Oregon, USA coast, between Whaleshead Beach in Brookings (42.15°N, -124.35°W) and Cape Mears (45.54°N, -123.96°W). All males had complete baleen plates (i.e., including the most recent growth within the gum), while the female's baleen plate was missing the most recently grown baleen at the root of the baleen plate (i.e., the baleen was cut at the gumline when recovered at necropsy). All specimens were removed from the right side of the rostrum and the center of the rack, where the longest baleen plates are located. The whale's total length (TL, measured as snout-to-fluke-notch), presence of scars, general body condition, and presumed cause of death were also recorded (Table 1). All individuals were classed as "subadults" based on the size categories (i.e., female TL 9–11.7 m, male TL 9–11.1 m; Rice and Wolman, 1971), i.e., at least 24 months old but not yet sexually mature.

2.2. Preparation of baleen plates for hormone extraction and quantification

To remove any soft tissues adhered to the base of the baleen plates (proximal end near the gum line with the newest baleen), we rehydrated and softened the tissues by submerging the baleen plates in freshwater, and subsequently scrapped the soft tissues off with a metal scraper or scalpel. We then freeze-dried the baleen plates under vacuum (LabConco FreeZone 6L system with Stoppering Tray Dryer, Kansas City, MO, USA),

until the pressure reading of the lyophilizer stabilized for at least 12 h, indicating that the samples were dry. Dried, cleaned plates were then stored at room temperature in individual sealed plastic bags, each with a 50 g silica gel desiccant pack (Arbor Assays, Ann Arbor, MI, USA).

We collected 20–50 mg of powder from sampling points spaced every 1 cm along the labial edge of the plate, using a hand-held electric rotary grinder (Dremel® model 395 type 5) fitted with a tungsten carbide ball-tip, with each sample collected from a < 1.5 cm transverse groove across the posterior face of the plate. The proximal-most point on the base of the baleen plate was designated as the 0 cm point.

Sampling started 1 cm from the base and continued every 1 cm to the tip (distal end) until the baleen became too thin to collect the minimum required sample mass for hormone extraction (20 mg); thus, we typically excluded the distal-most two centimeters of each plate (i.e., the oldest growth). To avoid cross contamination, during sampling we shielded other regions of the plate with adhesive tape, and between samples the entire baleen plate, sampling equipment, and fume hood were cleaned with compressed air, and the work surface and all equipment were also cleaned with 70 % ethanol. Powder samples were weighed to the nearest 0.1 mg on an Ohaus Explorer Pro EP214C analytical balance (Ohaus, Pine Brook, NJ, USA), with a nearby workstation ionizer (SPI No. 94000, SPIwestek.com) placed next to the scale to minimize any effects of static electric charge. Weighed samples were placed in 16 × 100 mm borosilicate glass tubes and securely capped until hormone extraction, which took place within 72 h of drilling. In total, 110 powder samples were produced, with each whale's plate producing between 12 and 27 samples.

2.3. Stable isotope analyses (SIA)

We weighed approximately 1 mg of baleen powder from each sampling location (i.e., every 1 cm along the longitudinal axis on each baleen plate) directly into tin capsules. Bulk $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were measured using a Thermo FlashSmart elemental analyzer coupled to a Thermo Finnigan Delta Plus XP continuous-flow isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany). Results are expressed in parts per thousand (‰) and delta notation (δ) using the equation: $\delta_{\text{sample}} = [\text{R}_{\text{sample}}/\text{R}_{\text{standard}} - 1] \times 1000$, where R_{sample} and $\text{R}_{\text{standard}}$ are the $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$ ratios of the sample and standard, respectively (Peterson and Fry, 1987). The isotopic reference materials used were supplied by the International Atomic Energy Agency (IAEA-N-1, $\delta^{15}\text{N} = 0.4 \pm 0.2$ ‰; IAEA-CH-7, $\delta^{13}\text{C} = -32.1 \pm 0.05$ ‰; IAEA-CH-3, $\delta^{13}\text{C} = -24.7 \pm 0.04$ ‰) and the United States Geological Survey (USGS25, $\delta^{13}\text{C} = -34.58 \pm 0.06$ ‰, $\delta^{15}\text{N} = -0.94 \pm 0.16$ ‰; USGS40, $\delta^{13}\text{C} = -26.3 \pm 0.04$ ‰, $\delta^{15}\text{N} = -4.5 \pm 0.1$ ‰; USGS41, $\delta^{13}\text{C} = +37.6 \pm 0.04$ ‰, $\delta^{15}\text{N} = 47.6 \pm 0.2$ ‰). Internal standards were included with all samples as quality controls; all error data are SD (purified methionine, Alfa Aesar, $\delta^{13}\text{C} = -34.5 \pm 0.06$ ‰, $\delta^{15}\text{N} = -0.9 \pm 0.1$ ‰; homogenized Chinook salmon muscle, NOAA Auke Bay Laboratories, $\delta^{13}\text{C} = -19.2 \pm 0.05$ ‰, $\delta^{15}\text{N} = 15.5 \pm 0.1$ ‰). The analytical precision based on the standard deviation of the standard laboratory replicas was < 0.1 ‰ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. To ensure that our samples did not contain any ^{13}C -depleted lipids, we also measured

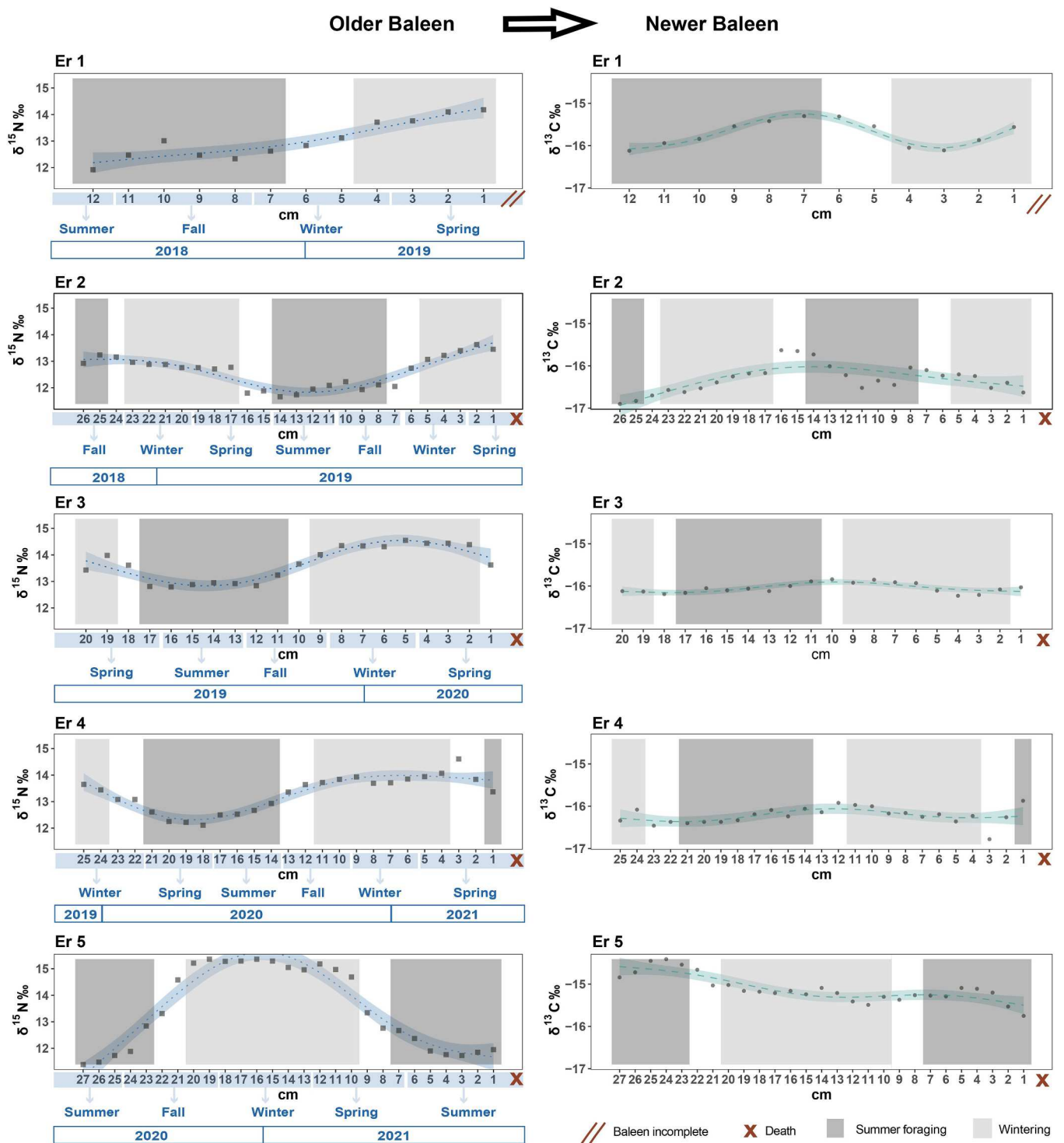


Fig. 1. Longitudinal profiles of $\delta^{15}\text{N}$ (left) and $\delta^{13}\text{C}$ (right) in baleen plates of stranded subadult gray whales collected along the Oregon Coast. X-axes show sample location along the baleen, in cm from base of the baleen plate (i.e., newest baleen = 1 cm), with 1 cm (newest baleen) at far right, i.e., time runs from left to right. Y-axes show $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values (‰). Estimated season and year of growth is shown below the x-axes, and the time of death is noted with a red X on the x-axis. Migration phenology is denoted by dark gray (putative summer foraging phase) and light gray (putative wintering phase) shading, estimated based on $\delta^{15}\text{N}$ fluctuations. Squares and closed circles depict actual values of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, respectively; the dotted and dashed lines depict the fit of the GAM models, with the blue ($\delta^{15}\text{N}$) and green ($\delta^{13}\text{C}$) fringe illustrating the 95% confidence intervals. Only the erupted portion of the baleen plate from Er 1 (top) was available, i.e., the proximal-most portion of the base of the plate was missing, denoted with two parallel red lines on the x-axis. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2

Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values $\pm$ SD, and niche size for each of the five baleen plates of stranded subadult gray whales sampled by phenology phase (summer foraging vs. wintering).

	Summer Foraging		Wintering			
	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	Niche Region (‰ ²)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	Niche Region (‰ ²)
Er_1	-15.6 $\pm$ 0.3	12.4 $\pm$ 0.3	2.05	-15.8 $\pm$ 0.2	13.9 $\pm$ 0.2	0.47
Er_2	-16.3 $\pm$ 0.4	12.2 $\pm$ 0.5	1.98	-16.3 $\pm$ 0.2	13.0 $\pm$ 0.3	0.98
Er_3	-16.0 $\pm$ 0.1	12.9 $\pm$ 0.2	0.17	-16.0 $\pm$ 0.1	14.2 $\pm$ 0.3	0.84
Er_4	-16.2 $\pm$ 0.1	12.4 $\pm$ 0.3	0.51	-16.1 $\pm$ 0.2	13.7 $\pm$ 0.2	0.52
Er_5	-15.0 $\pm$ 0.4	11.9 $\pm$ 0.4	3.57	-15.2 $\pm$ 0.1	15.1 $\pm$ 0.2	0.49

the C:N ratio of each sub-sample; all of which were within the range expected for pure protein (2.7–3.5) (Ambrose, 1990); see [Supplementary Material, Table S2](#)).

2.4. Baleen growth rates and timelines

To assign an estimated season of growth to each part of the baleen plate, we inspected the $\delta^{15}\text{N}$ data for evidence of seasonal changes. Specifically, based on the patterns observed in other baleen whales (Best and Schell, 1996; Lysiak et al., 2018; Matthews and Ferguson, 2015), we assumed that the areas of baleen with lower $\delta^{15}\text{N}$ were grown during summer when whales are most actively foraging, while the regions of baleen with higher $\delta^{15}\text{N}$ were assumed to have grown during winter. Similarly, points with intermediate $\delta^{15}\text{N}$ values (i.e., between summer $\delta^{15}\text{N}$ troughs and winter $\delta^{15}\text{N}$ peaks) were assumed to represent spring and fall migrations. However, because gray whale baleen is relatively short and hence expected to only capture a single full annual cycle, these potential timelines may be imprecise. Thus, we also compared each whale's $\delta^{15}\text{N}$ data to published estimates of baleen growth rate (BGR) for gray whales, which vary from 3.2 mm/week (Sumich, 2001) to 4.7 mm/week (Caraveo-Patiño et al., 2007). Therefore, for each plate we calculated two potential timelines, counting cm from the base of the baleen plate, using the two published BGR estimates, i.e., assuming the

proximal-most point on the plate was grown near the day the whale was found dead, with all other points on the plate then assigned an estimated date of growth based on that BGR (either 3.2 or 4.7 mm/week). These two timelines bracket a range of potential plausible BGRs. The two BGR-derived timelines were then compared to the $\delta^{15}\text{N}$ timeline for that whale, i.e., to verify that our $\delta^{15}\text{N}$ interpretations involve a plausible BGR for this species.

2.5. Hormone extraction and quantification

We extracted hormones from pre-weighed baleen powder samples using 1.6 mL of absolute methanol per 20 mg powder, i.e., keeping a constant ratio of 80:1 mL of solvent to g of sample. This solvent:sample ratio yields good detectability with low variation (inter-sample coefficient of variation < 10 %; (Fernández Ajó et al., 2022)). The solvent: sample mixture was vortexed 2 h at room temperature (Large Capacity Mixer, Glas-Col, Terre Haute, IN, USA; speed set on 40) and centrifuged for 1 min at 4025 g. The supernatant from each tube was transferred to individual 13 $\times$ 100 mm borosilicate tubes and dried at 45 °C for a minimum of 4 h in a sample evaporator (SpeedVac 121P, Thermo Fisher Scientific, Waltham, MA, USA) under vacuum. We reconstituted the dried samples in 0.50 mL of assay buffer (X065 buffer; Arbor Assays, Ann Arbor, MI, USA), sonicated for 5 min, vortexed for 5 min, and transferred the sample to 1.5 mL vapor proof O-ring-capped cryovials. We stored the tubes overnight at -80 °C and decanted the extract into a new cryovial the following day. This was considered the "1:1" (full-strength, neat) extract and was stored at -80C until assay.

We used commercial enzyme immunoassay (EIA) kits to quantify immunoreactive corticosterone, cortisol, progesterone, testosterone, and T3 in baleen extracts (Arbor Assays kits: corticosterone #K014, cortisol #K003, progesterone #K025, testosterone #K032, and T3 #K056, Ann Arbor, MI, USA). These five kits have previously been validated for gray whale baleen extracts (Hunt et al., 2017b). We assayed all samples at a 1:2 dilution, which in this species produces acceptable detectability and percent-bounds while also allowing assay of multiple hormones from a single 500ul extract. Final data are expressed as ng of hormone per g of dried baleen powder. All assays adhered to standard QA/QC criteria, which included a full standard curve, NSB (non-specific binding), zero dose ("blank"), and an independent control in every EIA microplate. All samples, standards, controls, NSBs, and zeros were assayed in duplicate. Any sample that exhibited a coefficient of variation exceeding 10 % between duplicates

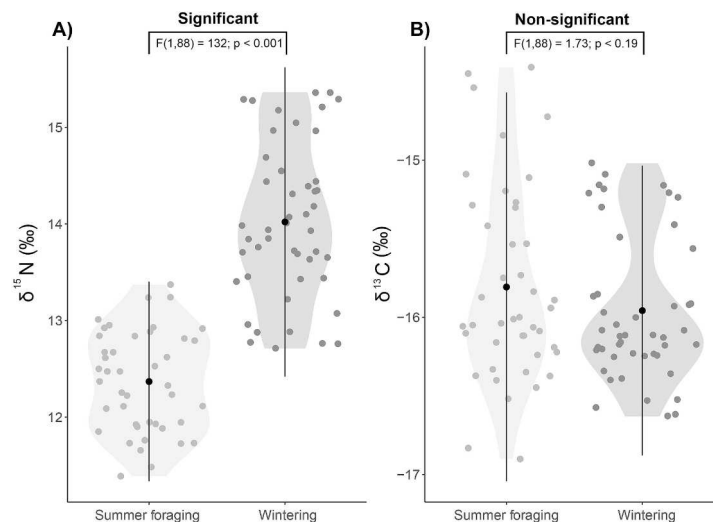


Fig. 2. Violin plots for A) $\delta^{15}\text{N}$ and B) $\delta^{13}\text{C}$ values in baleen plates of stranded subadult gray whales sampled by phenology phase (i.e., summer foraging in dark grey and wintering in light grey). Circles depict actual $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values. The black dot represents the mean, and whiskers indicate the standard deviation; statistically significant differences between groups are shown at the top with F and p values from ANOVA.

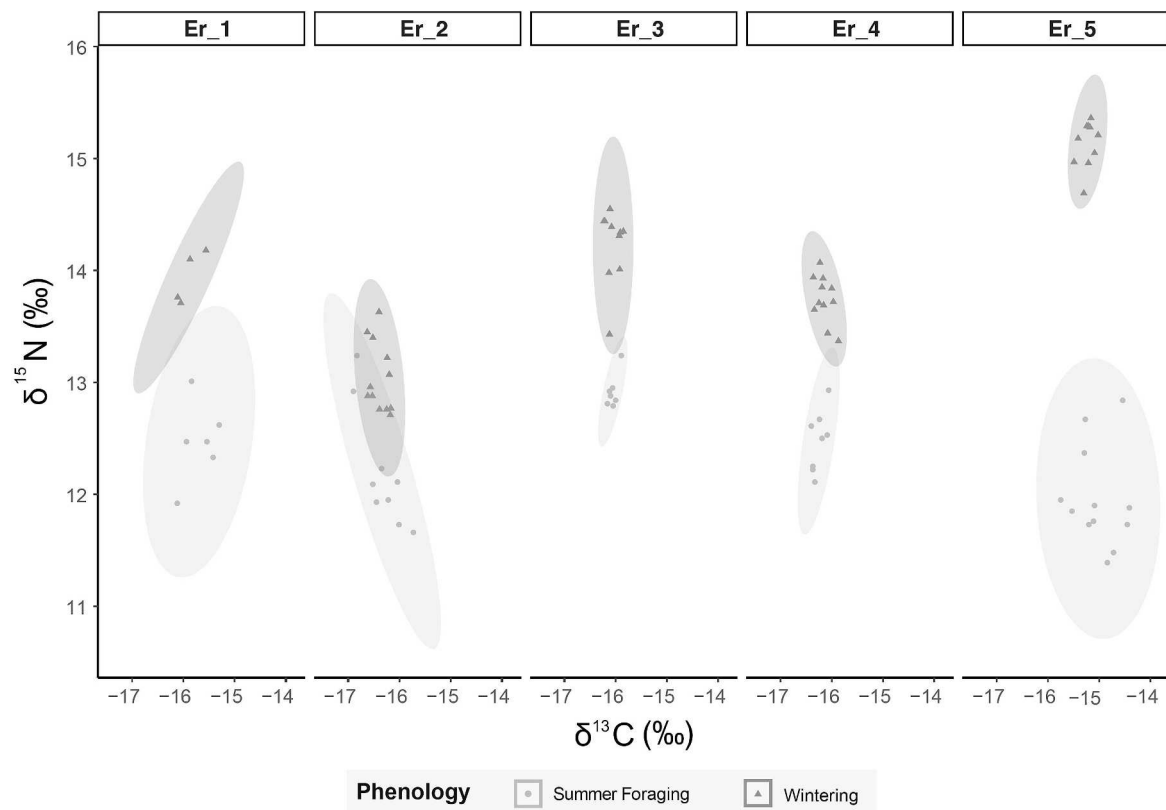


Fig. 3. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ biplot illustrating the isotopic niche width of five subadult gray whales stranded along the Oregon Coast, divided by phenology phase (summer foraging vs. wintering). Points within each ellipse represent sub-samples within each sampled baleen plate, and ellipses represent the niche region for each phenology within individuals (light grey = summer foraging; dark grey = wintering).

was re-analyzed. For antibody cross-reactivities, assay sensitivities, and other methodological details, see Hunt et al., (2017a) and the manufacturer's protocols (<https://www.arborassays.com>).

We evaluated the complete longitudinal profiles for both glucocorticoids, cortisol and corticosterone, in only two individuals (Er_1 and Er_4) to determine the dominant (most abundant) glucocorticoid and to compare the longitudinal profiles of the two hormones. As cortisol was at higher concentration than corticosterone for these two whales, corticosterone was not assayed for the other baleen specimens (see Results). We assayed all other hormones (progesterone, testosterone, T3) for all samples from all whales.

2.6. Statistical analysis

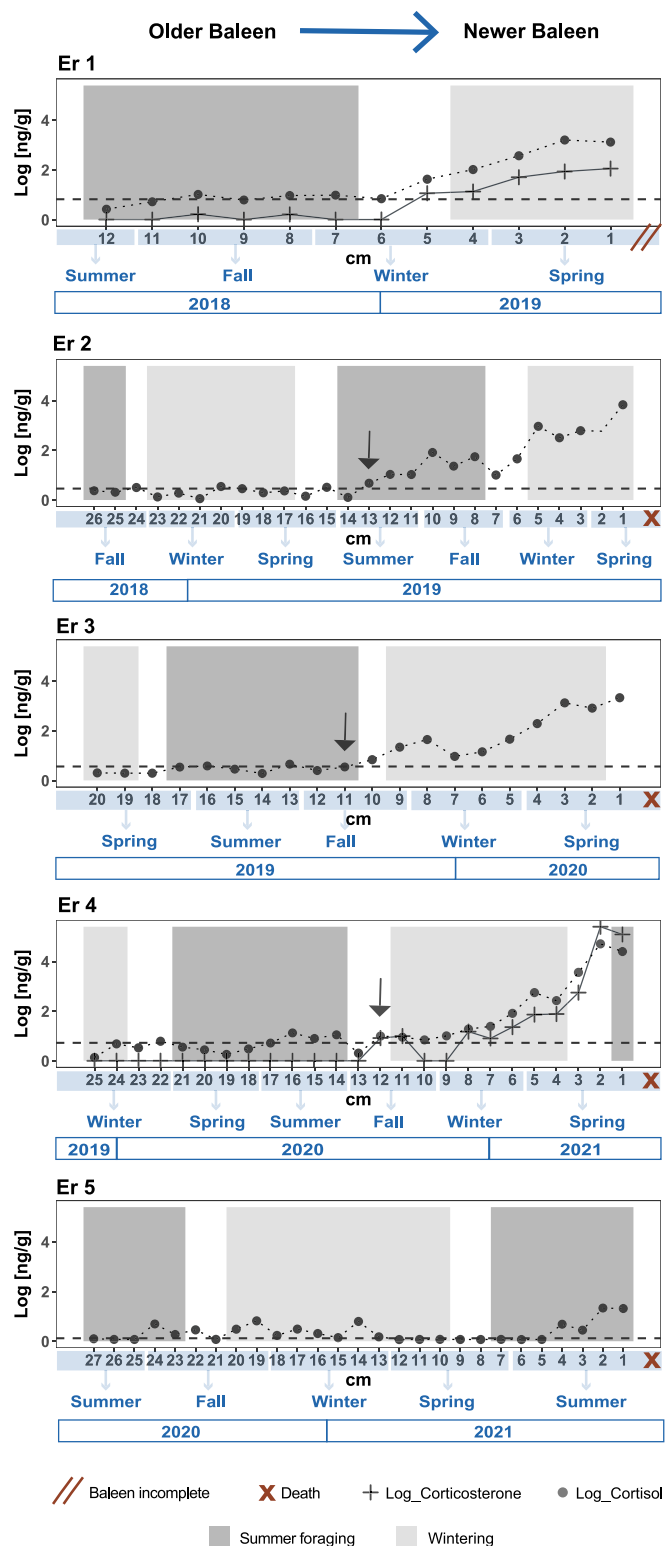
2.6.1. Hormones

All hormone data were log-transformed for data visualization and analyses due to non-normal distribution. We estimated hormone base-lines for each gray whale using an iterative process that excludes all data points greater than the mean + two standard deviations until no points exceed this maximum value, following methods from (Brown et al., 1988). To test for differences in concentrations of reproductive hormones between sexes, we fit a linear mixed-effects model with random intercepts using the lme4 R package. To assess the linear relationships between the hormone variables, we performed a Pearson's correlation (Supplementary material Fig. S2). Statistical analyses were computed using R (R Development Core Team 2023).

2.6.2. Stable isotope analysis

We gauged $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ fluctuations in baleen plates with a generalized additive model (GAM) utilizing the Restricted Maximum Likelihood method (method = 'REML'), and fitting a semi-parametric regression with smoothing by cross-validation, the smoothing process

was tailored to each ID, allowing the smoothing parameter to vary independently for different whales, using the "mgcv" R package. We estimated the phenology phase of each individual whale at each sampling point inferred from both the $\delta^{15}\text{N}$ values and the date assigned to each point (see Supp. Material Table S2) and used an ANOVA analysis to test differences in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between the phenological phases (wintering vs. summer foraging) with the "aov" function from the stats R package. To visualize the isotopic values for each individual at each phenology phase, we utilized ggplot (Fig. 3) to generate a biplot of both isotopes and ellipses at 95 %. We then calculated the niche region size of each phenology phase per individual and the pairwise niche overlap between phenology phases using the method developed by Swanson et al. (2015), available as the R package 'nicheROVER' (Lysy et al., 2014), which uses a Bayesian framework to quantify probabilistic metrics in niche space (Swanson et al., 2015). For each Bayesian model, we used 10,000 Markov chain Monte Carlo (MCMC) iterations with the default burn-in of 100 to characterize the posterior distributions for isotope values of each group (mean μ and variance-covariance matrix Σ) using an uninformative Normal-Inverse-Wishart prior (Lysy et al., 2014). We defined the niche size as the niche region for each group (e.g., summer foraging in individual Er1; wintering in individual Er1) with a 95 % probability of finding that group, and this was estimated by calculating a point estimate of the mean niche size across posterior sample of mean μ and covariance Σ (Swanson et al., 2015) and reported as $\%^{2}$. We reported the uncertainty in niche overlap as the posterior distribution of the overlap percentage, and we then calculated the Bayesian 95 % credible intervals for each pairwise comparison. To calculate overlap, the alpha value was set as 0.95, as this provides the 95 % probability region of the two-dimensional isotopic niche (Swanson et al., 2015). Results are reported as mean $\pm$ standard deviation (SD) unless otherwise stated. All statistical analyses were computed using R (R Development Core Team 2023).



(caption on next column)

Fig. 4. Longitudinal profiles of immunoreactive hormone concentrations of corticosterone (Corticosterone; black crosses and dashed line) and cortisol (Cortisol; black circles and dotted line) across the length of baleen plates from five stranded gray whales. The dashed horizontal line indicates the log-transformed baseline for baleen cortisol. X-axes show the location of each sample, in cm from base of the baleen plate (i.e., newest baleen = 1 cm) with y-axes showing concentration of hormone (log-transformed ng of immunoreactive hormone per g of dried baleen powder). Migration phenology is derived from $\delta^{15}\text{N}$ data and the estimated BGR; dark gray indicates summer foraging and light grey indicates wintering (see Fig. 1), and season of growth at each point on the plate was estimated from time of death (noted with a red X on the x-axis). Only the erupted portion of the baleen plate from Er_1 (top) was available, indicated with two parallel red lines on the x-axis. Blue arrows denote the onset of cortisol elevation prior to death. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3. Results

3.1. Study animals, body examination and cause of death

All five individuals were in fair to good body condition at necropsy, i.e., no evidence of emaciation. Further, there was no indication of direct human interaction as the cause of death. Notably, all individuals had “rake” mark scars, an indicative of physical interactions with killer whales, *Orcinus orca* (Corsi et al., 2022). These scars were primarily observed on the distal end of the fluke and pectoral fins. One individual, HMSC21-08-16-Er (Er 5), presented evidence of acute mortality caused by interactions with killer whales, showing multiple deep and recent “rake” marks on various body parts, particularly the head and flippers, along with extensive and severe hemorrhaging on the top of the head. Cause of death could not be determined for the other four whales.

3.2. Isotopic patterns and baleen growth rates (BGR)

All baleen plates oscillated in $\delta^{15}\text{N}$ values along their growth axis consistent with expected migration phenology (Fig. 1). The growth rate that best fit the expected oscillations with the migration phenology was 3.2 mm/week for all whales except Er_5, for which the best estimate was 4.7 mm/week (Fig. 1). Excluding Er_1, from which the baleen plate was incomplete (i.e., the proximal-most portion within the gum was missing) we estimate that the baleen of these subadult gray whales recorded around 1.3 years of individual hormone and isotope data ($n = 4$; 471.5 ± 68.73 days; Mean $\pm$ SD, Table 1).

Mean $\delta^{13}\text{C}$ values were similar among individuals, ranging from -15.0‰ to -16.3‰ during the summer foraging and from -15.2‰ to -16.3‰ during the wintering phenology phase (Table 2). Mean $\delta^{13}\text{C}$ values for all subadults individuals during the summer foraging and wintering period were $-15.0\text{‰} \pm 0.4\text{‰}$ and $-15.2\text{‰} \pm 0.1\text{‰}$, respectively (Fig. 2), with no significant differences between these periods ($F_{(1, 88)} = 1.73$, $p = 0.19$). In contrast, mean $\delta^{15}\text{N}$ values for all varied among individuals, ranging from 11.9‰ to 12.9‰ during the summer foraging and from 13.0‰ to 15.1‰ during the wintering phenology. Mean $\delta^{15}\text{N}$ values during the summer foraging and wintering period for all individuals was $12\text{‰} \pm 0.4\text{‰}$ and $15.1\text{‰} \pm 0.2\text{‰}$, respectively (Fig. 2), with a significant difference between these periods ($F_{(1, 88)} = 132$, $p < 0.001$).

The smallest niche sizes for the subadult gray whales were observed during the wintering phenology (Fig. 3 and Table 2). Er_1, Er_2 and Er_5 showed similar isotopic patterns, in which the niche sizes increased from the wintering to the summer foraging phenology phases. Er_3 and Er_4 had unique isotopic patterns: while Er_3 had a smaller niche size during summer foraging when compared to its niche size in the wintering phenology, Er_4 had the smallest niche sizes when compared to all the other individuals, with similar niche sizes for both summer foraging and wintering phenology phases (Fig. 3, Table 2). The isotopic niche overlap

Table 3

Individual baselines of gray whale baleen immunoreactive hormone concentrations (expressed in ng of immunoreactive hormone per g of baleen powder (ng/g)). Baselines are estimated via an iterative process that excluded all data points greater than the mean + 2SD until no points exceeded this maximum value (following Brown et al., 1988). Cortisol = immunoreactive baleen cortisol; Progesterone = immunoreactive baleen progesterone; Testosterone = immunoreactive baleen testosterone; T3 = immunoreactive baleen triiodothyronine.

ID	Hormone Baselines (ng/g) +/- Standard Deviation			
	Cortisol	Progesterone	Testosterone	T3
Er 1	6.46 +/- 8.29	1.73 +/- 0.64	0.51 +/- 0.25	2.51 +/- 1.15
Er 2	5.19 +/- 9.78	1.39 +/- 1.02	0.38 +/- 0.21	1.66 +/- 1.37
Er 3	4.86 +/- 7.85	2.04 +/- 0.66	0.35 +/- 0.17	1.77 +/- 1.21
Er 4	11.20 +/- 26.66	2.32 +/- 1.08	0.56 +/- 0.34	3.46 +/- 1.44
Er 5	0.55 +/- 0.75	1.46 +/- 0.68	0.39 +/- 0.21	1.88 +/- 0.95

between summer foraging and wintering phenology phases within individuals was also variable: in Er_1, 63 % of the summer foraging ellipsoid overlapped the wintering ellipsoid, and 1 % of the wintering ellipsoid overlapped the summer foraging ellipsoid. In Er_2, 50 % of the summer foraging ellipsoid overlapped the wintering ellipsoid, and 31 % of the wintering ellipsoid overlapped the summer foraging ellipsoid. In Er_3, Er_4 and Er_5 there was no overlap between the summer foraging and wintering ellipsoids (Fig. 3 and Figure S2).

3.3. Baleen glucocorticoids (cortisol and corticosterone)

Both glucocorticoids (cortisol & corticosterone) were detectable along the full length of the two plates for which both hormones were assayed (i.e., Er_1 & Er_4; Fig. 4). The longitudinal profiles of the two hormones exhibit a significant positive correlation (Pearson's correlation coefficient of $r = 0.947$, $t_{(35)} = 17.464$, $p < 2 \times 10^{-16}$), with cortisol consistently showing a slightly higher apparent concentration compared to corticosterone at every sampling point along the baleen longitudinal axis (Fig. 4; electronic supplementary material, Table S1). Therefore, only cortisol was analyzed for the other three whales. The baseline concentration for cortisol in all individuals fell within the range of 0.55 ± 0.75 to 11.20 ± 26.66 ng/g (mean $\pm$ SD; Table 3). Among all individuals, except for Er_5 (the individual presumed to have died acutely due to killer whale predation), there were pronounced elevations in the apparent concentration of cortisol preceding death (Fig. 4). For the three individuals with unknown cause of death that had complete baleen length (i.e., excluding both Er_1, missing part of the baleen, and Er_5, known cause of death), the time elapsed from the onset of the elevation in cortisol to the time of death was estimated to be 284.37 days (13 cm) for Er_2, 240.62 days (11 cm) for Er_3, and 262.50 days (12 cm) for Er_4. On average, this elapsed time was 262.5 days (approximately 0.72 years).

3.4. Triiodothyronine (T3)

Immunoreactive T3 was detectable along the full length of all baleen plates (Fig. 5; electronic supplementary material, Table S1). The baseline concentration of T3 ranged from 1.66 ± 1.37 to 3.46 ± 1.44 ng/g (mean $\pm$ SD; Table 3). Similar to the glucocorticoids, the three individuals with an unknown cause of death that also had a full-length baleen plate (Er_2, Er_3, and Er_4) all had elevated T3 preceding death (Fig. 5). The onset of the elevation in T3 prior to death was nearly coincident with the timing of elevated cortisol (see Results 3.4).

3.5. Reproductive hormones (progesterone and testosterone)

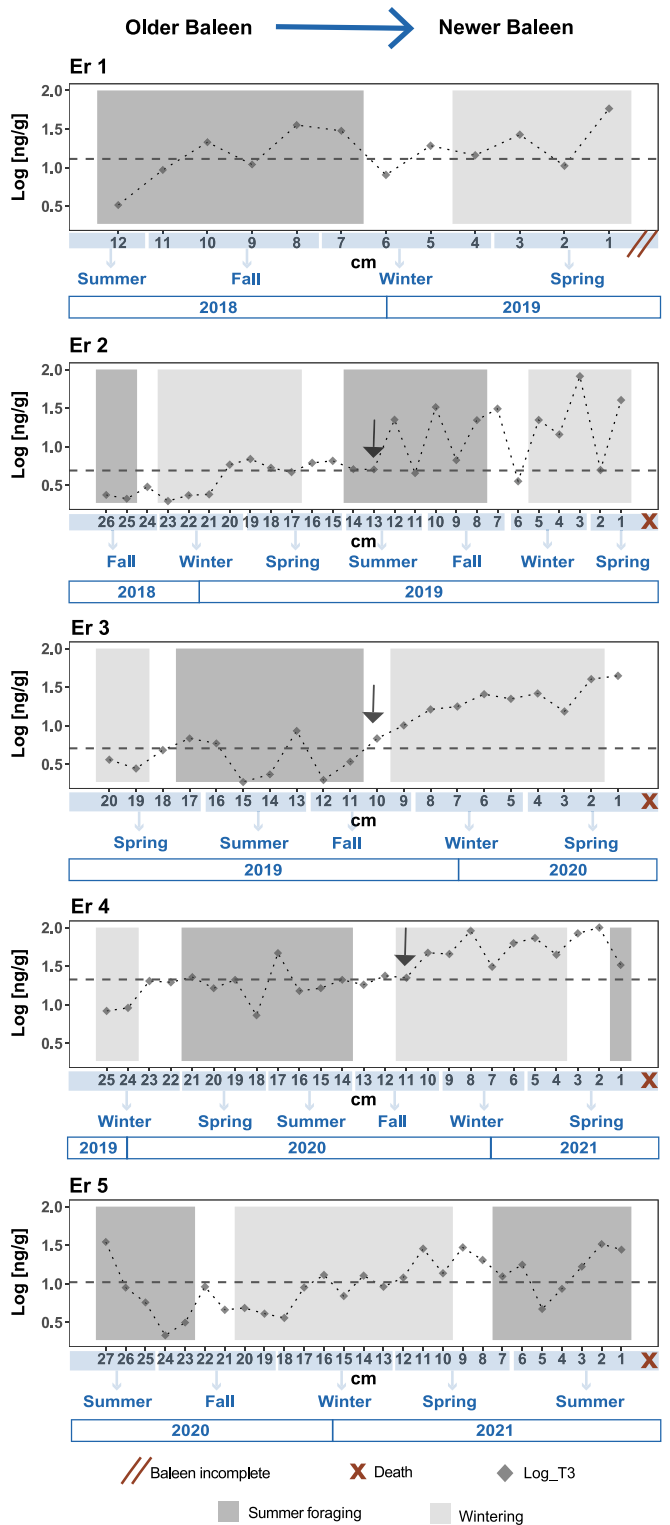
Both progesterone and testosterone were detectable along the full length of the baleen plates (Fig. 6; electronic supplementary material, Table S1). The baseline concentration for progesterone in all individuals fell within the range of 1.39 ± 1.02 to 2.32 ± 1.08 ng/g (mean $\pm$ SD;

Table 3), and for testosterone the baseline concentration ranged from 0.35 ± 0.17 to 0.56 ± 0.34 ng/g (mean $\pm$ SD; Table 3). No significant differences between the two sexes were found in the apparent immunoreactive progesterone and testosterone ($p = 0.9999$).

4. Discussion and conclusion

In this study, we analyzed the isotope and hormone profiles in the baleen of five subadult gray whales. Our isotopic analysis allowed us to infer baleen growth rates, which generally agreed with prior estimates for the species (Caraveo-Patiño et al., 2007; Sumich, 2001). The hormone analysis provided distinct endocrinology patterns between the whale that died acutely (killer whale attack) and those whales of unknown cause of death. Providing the first longitudinal profiles of the adrenal, thyroidal, and gonadal axes obtained from the baleen of gray whales in the months leading up to their deaths. Our results demonstrate the feasibility of this integrated retrospective approach for gaining insights into the foraging ecology, endocrinology, and discriminating endocrine patterns between chronic illness versus acute cause of death in gray whales.

Gray whales are assumed to be capital breeders, relying largely on stored energy reserves acquired during the summer season to cover the costs of migration and reproduction (Jönsson and Jonsson, 1997). The fluctuations in $\delta^{15}\text{N}$ values observed along the subadult gray whale baleen plates agrees with the anticipated patterns for capital breeders: i) lower values during the summer foraging phase, that are likely attributed to the benthic foraging on lower trophic level organisms (Burnham and Duffus, 2016; Newell and Cowles, 2006; Nelson et al., 2008; Darling et al., 1998); and ii) higher values during the wintering phase when whales are primarily catabolizing their own tissues to fuel metabolism (Fuller et al., 2005; Lee et al., 2012). While these $\delta^{15}\text{N}$ patterns allowed us to deduce baleen growth rates that were consistent with previous estimates (Caraveo-Patiño et al., 2007; Sumich, 2001), there were no regular oscillations in $\delta^{13}\text{C}$ within the sampled gray whale baleen. The variable $\delta^{15}\text{N}$ values between the different individuals during the wintering phenology phase (see table 2) may suggest some degree of plasticity in foraging strategies of individual gray whales (Caraveo-Patiño et al., 2007; Durham & Duffus 2018; Gelippi et al., 2022). Er_5, for example, had the largest range of $\delta^{15}\text{N}$ values, with the highest peak during the wintering phase and the lowest values in the summer foraging. In addition, this individual had a clear segregation in the isotopic niches between phenology phases (Fig. 3). In contrast, Er_2 presented the lowest $\delta^{15}\text{N}$ values for the wintering phase compared to all individuals, with overlapping isotopic niches between phenology phases (Fig. 3), suggesting that Er_2 was potentially foraging in the wintering lagoons and/or across their migratory corridors. Such foraging strategy has been previously described for gray whales via molecular analysis (Caraveo-Patiño et al., 2007, 2009; Gelippi et al., 2022) and direct observations (Caraveo-Patiño et al., 2007). If we consider the isotopic composition of potential prey sources for gray whales across their presumed migratory range (e.g., Alaska, Oregon, California, and Baja California), assuming a trophic enrichment factor ($\Delta^{15}\text{N} = 2 \text{ ‰}$), as established by Caraveo-Patiño & Soto (2005) for gray whale baleen plates; the individual Er_2 will have $\delta^{13}\text{C}$ values that closely resembles the isotopic composition of *Erichthonius brasiliensis* in Baja California ($\sim -18.3 \text{ ‰}$, Caraveo-Patiño and Soto, 2005). Nevertheless, our low sample size and the fact that our dataset is composed only of subadult individuals demand caution in interpretation. To better understand the isotopic patterns observed in gray whales and how they reflect the energetic pathways of their summer/winter grounds, we recommend that future studies complement the bulk isotopic analysis with different analytical approaches including: the use of *isoscapes* (i.e., stable isotope mapping (e.g., Forbes et al., 2023); the use of compound-specific amino acid analysis to disentangle the relative contributions of trophic and baseline variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (e.g., Riekenberg et al., 2021); and/or integrating isotopic information of prey sources in Bayesian



(caption on next column)

Fig. 5. Longitudinal profiles of immunoreactive hormone concentrations of triiodothyronine (Log_T3; grey rhomboids and dotted line) across the length of baleen plates from five stranded gray whales. The dashed horizontal line indicates the log-transformed baseline for baleen T3. X-axes show the location of each sample, in cm from base of the baleen plate (i.e., newest baleen = 1 cm) with y-axes showing concentration of hormone (log-transformed ng of immunoreactive hormone per g of dried baleen powder). Migration phenology is derived from $\delta^{15}\text{N}$ data and the estimated BGR; dark gray indicates summer foraging and light grey indicates wintering (see Fig. 1), and season of growth at each point on the plate was estimated from time of death (noted with a red X on the x-axis). Only the erupted portion of the baleen plate from Er.1 (top) was available, indicated with two parallel red lines on the x-axis. Blue arrows denote the onset of T3 elevation prior to death. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

mixing models to assess the proportional contribution of each prey source in their diet (e.g., Gelippi et al., 2022).

In the glucocorticoid (GC) analyses, we found that both cortisol and corticosterone were detectable along the full length of the baleen from two individuals (Er_1 & Er_4), but cortisol was consistently more abundant than corticosterone. Furthermore, corticosterone exhibited similar patterns to cortisol, i.e., corticosterone seemingly did not provide additional information. This pattern generally aligns with traditional assumptions of “cortisol dominance” in mysticetes (primarily based on rare plasma samples from hunted specimens) as well as assumptions that only the more abundant GC need be analyzed, but contrasts with recent findings of more corticosterone than cortisol in baleen of other mysticetes (Fernández Ajó et al., 2018; Hunt et al., 2017a; Lowe et al., 2021a). Most mammals produce both glucocorticoids, and some data indicate the two hormones can respond differently to exogenous stressors, depending on the type and duration of the stressor (Koren et al., 2012). Given our small sample size, we encourage future research on both GCs to further investigate whether they might show species-specific differences or individual differences in glucocorticoid dominance or might provide differing information for acute vs. chronic stressors.

Cortisol profiles for the four individuals with unknown cause of death demonstrated a long-term elevation in cortisol that began an estimated 8 months before death. In contrast, the individual known to have died acutely due to killer whale predation (Er_5) had cortisol concentrations that approximate baseline levels across the span of the baleen, suggesting that this individual was in good health before its acute death. These data suggest that the other four individuals experienced a prolonged period of stress. Generally, individual perception of a stressor activates the HPA-axis, leading to an increase in circulating GC levels. Short-term elevations in GCs are thought to aid animals in coping with the stressor (McEwen and Wingfield, 2010; Romero et al., 2009; Romero and Wingfield, 2016), but if the perturbation is severe and/or chronic, the individual deviates from its current life-history stage and enters an “emergency life-history stage”, during which all activities not essential for immediate survival are suppressed (Romero and Wingfield, 2016; Wingfield, 2005; Wingfield et al., 1998). Consequently, chronic elevation of GCs can itself have negative effects on long-term health, through immunosuppression, reduced growth, and inhibition of reproduction (Buck et al., 2007; Dhabhar, 2009; Dhabhar et al., 2012; Kitaysky et al., 1999). Therefore, although the immediate cause of death remains unknown for these four individuals, it is conceivable that the prolonged elevation of cortisol may eventually have directly impacted health and survival, i.e., in addition to any direct negative effects of the stressor itself (Romero and Wingfield, 2016). The presence of “rake marks” attributed to killer whale interactions could provide evidence for increased vulnerability of these individuals. It is plausible that whales undergoing chronic illness might become more susceptible to predation and other threats.

T3, like cortisol, tended to show a gradual, months-long increase in

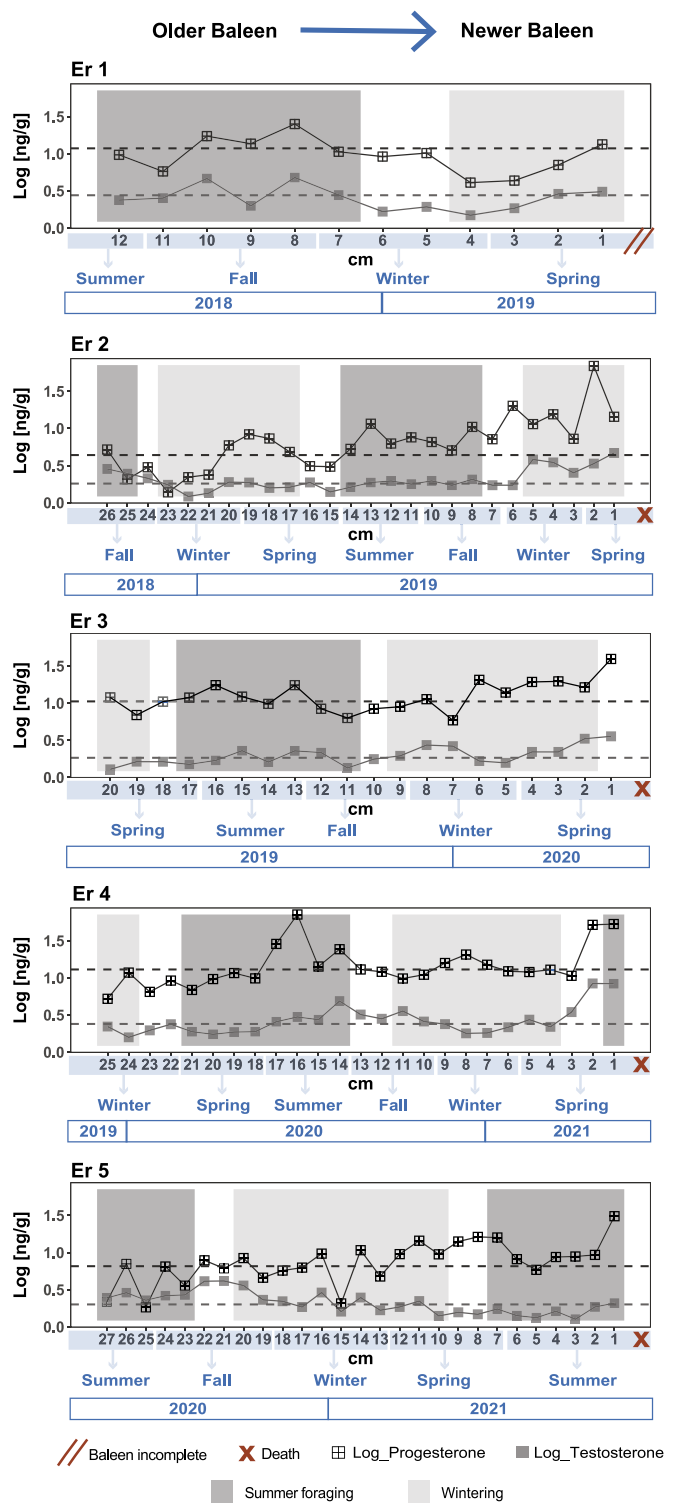


Fig. 6. Longitudinal profiles of immunoreactive hormone concentrations of progesterone (Progesterone; black cross-square) and testosterone (Testosterone; grey solid squares) across the length of baleen plates from five stranded sub-adult gray whales. The dashed horizontal lines indicate the log-transformed baseline for baleen progesterone (black) and testosterone (light grey). X-axes show the location of each sample, in cm from base of the baleen plate (i.e., newest baleen = 1 cm) with y-axes showing concentration of hormone (log-transformed ng of immunoreactive hormone per g of dried baleen powder). Migration phenology is derived from $\delta^{15}\text{N}$ data and the estimated BGR; dark gray indicates summer foraging and light grey indicates wintering (see Fig. 1), and season of growth at each point on the plate was estimated from time of death (noted with a red X on the x-axis). Only the erupted portion of the baleen plate from Er_1 (top) was available, indicated with two parallel red lines on the x-axis. Blue arrows denote the onset of T3 elevations prior to death. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the four whales of unknown cause of death (albeit with high individual variability), but remained relatively stable in the whale with an acute cause of death. The simultaneous elevation of both cortisol and T3 was unexpected, as the HPA axis is classically thought to inhibit the HPT axis (Behringer et al., 2018). In fact, elevated GCs in mammals often directly downregulate the HPT axis, resulting in decreases in circulating T3 (Charmandari et al., 2005). However, this downregulation can be temporary (Nicoloff et al., 1970). Further, emerging data indicate that in marine species, T3 may elevate simultaneously with the GCs during those stressors that require increased energetic output, such as swimming while entangled in fishing gear (Lysiak et al., 2018). In mammals, T3 can also elevate during thermoregulatory challenges, as elevated T3 directly raises metabolic rate, which elevates body temperature (Behringer et al., 2018; Williams et al., 2019). Indeed, Lemos et al., 2022b found that gray whales in poor body condition exhibited higher thyroid hormone concentrations in feces, compared to whales in good body condition, suggesting a possible thermoregulatory influence on T3. In other words, poor body condition in cetaceans entails thinning of the insulative blubber layer and might therefore require a compensatory elevation in metabolic rate and thus an elevation in T3. Similarly, fecal thyroid hormones may reflect changes in food availability (Ayres et al., 2012). In our study, all five of our study whales died during the current UME, which generally has been linked to poor nutrition and emaciation (Christiansen et al., 2021). Though it is tempting to ascribe the gradual elevation in T3 seen here to the poor body condition reported in gray whales during the UME generally, the necropsy reports of these five individuals did not describe severe emaciation. However, it is possible that these whales were in relatively lower body condition with respect to the population mean. Overall, we speculate that whales in poor body condition may elevate T3 in response to thermoregulatory demands. This hypothesis could be tested with further comparisons of baleen from stranded whales in poor vs. good body condition, ideally with measurements of body condition, e.g., blubber thickness or body area index derived from drone-based photogrammetry (Bierlich et al., 2021; Burnett et al., 2019). Finally, T3 also commonly varies across life history stages (Wilsterman et al., 2015), and thus studies of T3 patterns in the baleen of juveniles as compared to adults may be informative.

We also quantified reproductive hormones across the baleen's entire length in four subadult males and one subadult female. To our knowledge, these are the first longitudinal profiles of reproductive hormones from gray whales across a full calendar year. As expected for this reproductive age class (subadults), we did not observe temporal patterns, cyclical trends, or elevated hormone concentrations, suggesting none of the subadults had yet reached sexual maturity. Nevertheless, our results add to knowledge about expected baselines of reproductive hormones in subadults and may thus inform future efforts to identify onset of sexual maturity. Our results also underscore the potential to capture at least one year of information from adult gray whale baleen, as there have been uncertainties about the feasibility of capturing complete

pregnancies or multiple pregnancies within gray whale baleen (max baleen length ~ 30 cm), or whether seasonal testosterone cyclicity in males could be discerned. Our subadult baleen specimens captured an estimated timeframe of 1.3 years. Further, adult baleen generally captures a longer timespan than subadult baleen (since subadults not only might have shorter baleen but also tend to have faster baleen growth rate); thus, these results suggest that adult gray whale baleen may capture a sufficient time frame to examine at least one if not two prior reproductive cycles.

The ENP gray whale population has rebounded from a dramatic decline attributed to whaling from less than 4,000 by 1900 to a peak abundance estimated at 26,916 individuals (Stewart and Weller, 2021; Swartz et al., 2006). However, the ENP gray whale population has exhibited significant fluctuations, marked by two Unusual Mortality Events (UMEs) that curtailed population size, underscoring the susceptibility of gray whales to oceanic conditions, resource availability, and other influences (Stewart et al., 2023; Torres et al., 2022). The occurrence of recurrent UMEs with often-unknown causes in the ENP gray whale population highlights the necessity for innovative methodologies to investigate and better understand the causes of death and physiological response of individuals to fluctuations in the environment. Despite the characteristic shorter length of gray whale baleen compared to other mysticete species, and thus the relatively brief period of longitudinal data that can be inferred, even subadult gray whale baleen captures a > 1 year timespan, and adult baleen specimens may capture a longer timeframe. In sum, baleen analysis in gray whales allows assessment of physiological status of at least the past year and may enable inferences as to the cause of death (acute vs. chronic, nutritional vs. non-nutritional stress). Overall, baleen analysis emerges as a powerful tool that enables a comprehensive and retrospective assessment of gray whale hormonal and isotopic profiles, including the assessments of the stress response, reproductive status, and foraging ecology in the months or years leading up to their death.

CRedit authorship contribution statement

Alejandro Fernández Ajó: Writing – review & editing, Writing – original draft, Visualization, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Clarissa Teixeira:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Daniela M.D. de Mello:** . **Danielle Dillon:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation. **James M. Rice:** . **C. Loren Buck:** Writing – review & editing, Writing – original draft, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Kathleen E. Hunt:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Matthew C. Rogers:** . **Leigh G. Torres:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Electronic [supplementary material](#) is available online via figshare

Acknowledgments

We are grateful to all personnel and volunteers who assisted with stranding response, necropsy, and collection of specimens.

Funding

This project was supported by the Office of Naval Research Marine Mammals and Biology Program (no. N00014-20-1-2760), the Oregon State University Marine Mammal Institute, and The John H. Prescott Marine Mammal Rescue Assistance Grant Program provided essential funding for stranding response and tissue collection.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ygcen.2024.114492>.

References

- Ambrose, S.H., 1990. Preparation and characterization of bone and tooth collagen for isotopic analysis. *J Archaeol Sci* 17, 431–451. [https://doi.org/10.1016/0305-4403\(90\)90007-R](https://doi.org/10.1016/0305-4403(90)90007-R).
- Ayres, K.L., Booth, R.K., Hempelmann, J.A., Koski, K.L., Emmons, C.K., Baird, R.W., Balcomb-Bartok, K., Hanson, M.B., Ford, M.J., Wasser, S.K., 2012. Distinguishing the impacts of inadequate prey and vessel traffic on an endangered killer whale (*Orcinus orca*) population. *PLoS One* 7. <https://doi.org/10.1371/journal.pone.0036842>.
- Behringer, V., Deimel, C., Hohmann, G., Negrey, J., Schaebs, F.S., Deschner, T., 2018. Applications for non-invasive thyroid hormone measurements in mammalian ecology, growth, and maintenance. *Horm Behav* 105, 66–85. <https://doi.org/10.1016/j.yhbeh.2018.07.011>.
- Ben-David, M., Newsome, S.D., Whiteman, J.P., 2012. Lipid and amino acid composition influence incorporation and discrimination of ¹³C and ¹⁵N in mink. *J Mammal* 93, 399–412. <https://doi.org/10.1644/11-MAMM-S-168.1>.
- Best, P.B., Schell, D.M., 1996. Stable isotopes in southern right whale (*Eubalaena australis*) baleen as indicators of seasonal movements, feeding and growth. *Mar Biol* 124. <https://doi.org/10.1007/BF00351030>.
- Bierlich, K.C., Schick, R.S., Hewitt, J., Dale, J., Goldbogen, J.A., Friedlaender, A.S., Johnston, D.W., 2021. Bayesian approach for predicting photogrammetric uncertainty in morphometric measurements derived from drones. *Mar Ecol Prog Ser* 673, 193–210. <https://doi.org/10.3354/meps13814>.
- Borrell, A., Abad-Oliva, N., Gómez-Campos, E., Giménez, J., Aguilar, A., 2012. Discrimination of stable isotopes in fin whale tissues and application to diet assessment in cetaceans. *Rapid Communications in Mass Spectrometry* 26, 1596–1602. <https://doi.org/10.1002/rcm.6267>.
- Brower, A.A., Ferguson, M.C., Schonberg, S. V., Jewett, S.C., Clarke, J.T., 2017. Gray whale distribution relative to benthic invertebrate biomass and abundance: Northeastern Chukchi Sea 2009–2012. *Deep Sea Res 2 Top Stud Oceanogr* 144. <https://doi.org/10.1016/j.dsr2.2016.12.007>.
- Brown, J.L., Goodrowe, K.L., Simmons, L.G., Armstrong, D.L., Wildt, D.E., 1988. Evaluation of the pituitary-gonadal response to GnRH, and adrenal status, in the leopard (*Panthera pardus japonensis*) and tiger (*Panthera tigris*). *Reproduction* 82, 227–236. <https://doi.org/10.1530/jrf.0.0820227>.
- Buck, C.L., O'Reilly, K.M., Kildaw, S.D., 2007. Interannual variability of black-legged kittiwake productivity is reflected in baseline plasma corticosterone. *Gen Comp Endocrinol* 150. <https://doi.org/10.1016/j.ygcen.2006.10.011>.
- Burnett, J.D., Lemos, L., Barlow, D., Wing, M.G., Chandler, T., Torres, L.G., 2019. Estimating morphometric attributes of baleen whales with photogrammetry from small UASs: a case study with blue and gray whales. *Mar Mamm Sci* 35. <https://doi.org/10.1111/mms.12527>.
- Burnham, R.E., Duffus, D.A., 2016. Gray Whale (*Eschrichtius robustus*) Predation and the Demise of Amphipod Prey Reserves in Clayoquot Sound, British Columbia. *Aquat. Mamm.* 42 (2), 123–126. <https://doi.org/10.1578/AM.42.2.2016.123>.
- Busquets-Vass, G., Newsome, S.D., Calambokidis, J., Serra-Valente, G., Jacobsen, J.K., Aguñiga-García, S., Gendron, D., 2017. Estimating blue whale skin isotopic incorporation rates and baleen growth rates: implications for assessing diet and movement patterns in mysticetes. *PLoS One* 12. <https://doi.org/10.1371/journal.pone.0177880>.
- Calambokidis, J., Darling, J.D., Deecke, V., Gearin, P., Goshko, M., Megill, W., Tombach, C.M., Goley, D., Toropova, C., Gisborne, B., 2002. Abundance, range and movements of a feeding aggregation of gray whales (*Eschrichtius robustus*) from California to southeastern Alaska in 1998. *Journal of Cetacean Research and Management* 4.
- Calambokidis, J., Pérez, A., Laake, J., 2019. Updated analysis of abundance and population structure of seasonal gray whales in the Pacific northwest, 1996–2017. Final Report to NOAA, Final report to the National Marine Fisheries Service.
- Caraveo-Patiño, J., Hobson, K.A., Soto, L.A., 2007. Feeding ecology of gray whales inferred from stable-carbon and nitrogen isotopic analysis of baleen plates. *Hydrobiologia* 586, 17–25. <https://doi.org/10.1007/s10750-006-0477-5>.
- Caraveo-Patiño, J., Soto, L.A., 2005. Stable carbon isotope ratios for the gray whale (*Eschrichtius robustus*) in the breeding grounds of Baja California Sur, Mexico. *Hydrobiologia* 539. <https://doi.org/10.1007/s10750-004-3370-0>.
- Charmandari, E., Tsigos, C., Chrousos, G., 2005. Endocrinology of the stress response. *Annu Rev Physiol* 67, 259–284. <https://doi.org/10.1146/annurev.physiol.67.040403.120816>.
- Christiansen, F., Rodríguez-González, F., Martínez-Aguilar, S., Urbán, J., Swartz, S., Warick, H., Vivier, F., Bejder, L., 2021. Poor body condition associated with an

- unusual mortality event in gray whales. *Mar Ecol Prog Ser* 658, 237–252. <https://doi.org/10.3354/meps13585>.
- Clapham, P., 2016. Managing leviathan: conservation challenges for the great whales in a post-whaling world. *Oceanography* 29, 214–225. <https://doi.org/10.5670/oceanog.2016.70>.
- D'Agostino, V.C., Fernández Ajó, A., Degrat, M., Krock, B., Hunt, K.E., Uhart, M.M., Buck, C.L., 2022. Potential endocrine correlation with exposure to domoic acid in southern right whale (*Eubalaena australis*) at the península Valdés breeding ground. *Oecologia* 198, 21–34. <https://doi.org/10.1007/s00442-021-05078-4>.
- Darling, J.D., Keogh, K.E., Steeves, T.E., 1998. Gray whale (*Eschrichtius robustus*) habitat utilization and prey species off Vancouver Island, B.C. *Mar Mamm Sci* 14, 692–720. <https://doi.org/10.1111/j.1748-7692.1998.tb00757.x>.
- Derville, S., Buell, T.V., Corbett, K.C., Hayslip, C., Torres, L.G., 2023a. Exposure of whales to entanglement risk in Dungeness crab fishing gear in Oregon, USA, reveals distinctive spatio-temporal and climatic patterns. *Biol Conserv* 281, 109989. <https://doi.org/10.1016/j.biocon.2023.109989>.
- Derville, S., Torres, L.G., Newsome, S.D., Simes, C.J., Valenzuela, L.O., Vander Zanden, H.B., Baker, C.S., Bérubé, M., Busquets-Vass, G., Carlyon, K., Childerhouse, S.J., Constantine, R., Dunshea, G., Flores, P.A.C., Goldsworthy, S.D., Graham, B., Groch, K., Gröcke, D.R., Harcourt, R., Hindell, M.A., Hulva, P., Jackson, J.A., Kennedy, A.S., Lundquist, D., Mackay, A.I., Neveceralova, P., Oliveira, L., Ott, P.H., Palsbøll, P.J., Patenaude, N.J., Rowntree, V., Sironi, M., Vermeulen, E., Watson, M., Zerbin, A.N., Carroll, E.L., 2023b. Long-term stability in the circumpolar foraging range of a Southern Ocean predator between the eras of whaling and rapid climate change. In: *Proceedings of the National Academy of Sciences* 120. <https://doi.org/10.1073/pnas.2214035120>.
- Dettmer, A.M., Chusyd, D.E., 2023. Early life adversities and lifelong health outcomes: a review of the literature on large, social, long-lived nonhuman mammals. *Neurosci Biobehav Rev* 152, 105297. <https://doi.org/10.1016/j.neubiorev.2023.105297>.
- Dhabhar, F.S., 2009. Enhancing versus suppressive effects of stress on immune function: implications for immunoprotection and immunopathology. *Neuroimmunomodulation* 16, 300–317. <https://doi.org/10.1159/000216188>.
- Dhabhar, F.S., Malarkey, W.B., Neri, E., McEwen, B.S., 2012. Stress-induced redistribution of immune cells—From barracks to battlefields: a tale of three hormones – Curt richter award winner. *Psychoneuroendocrinology* 37, 1345–1368. <https://doi.org/10.1016/j.psyneuen.2012.05.008>.
- Dunham, J.S., Duffus, D.A., 2001. Foraging patterns of gray whales in central clayoquot sound, British Columbia. Canada. *Mar Ecol Prog Ser* 223. <https://doi.org/10.3354/meps223299>.
- Dunham, J.S., Duffus, D.A., 2002. Diet of gray whales (*Eschrichtius robustus*) in clayoquot sound, British Columbia. Canada. *Mar Mamm Sci* 18. <https://doi.org/10.1111/j.1748-7692.2002.tb01046.x>.
- Eales, J.G., 1988. The influence of nutritional state on thyroid function in various vertebrates. *Am Zool* 28, 351–362. <https://doi.org/10.1093/icb/28.2.351>.
- Eguchi, T., Lang, A.R., Weller, D.W., 2023. NOAA Technical Memorandum NMFS EASTERN NORTH PACIFIC GRAY WHALE CALF PRODUCTION 1994–2023. <https://doi.org/10.25923/e9at-x936>.
- Eisenmann, P., Fry, B., Holyoake, C., Coughran, D., Nicol, S., Bengtson Nash, S., 2016. Isotopic evidence of a wide spectrum of feeding strategies in southern hemisphere humpback whale baleen records. *PLoS One* 11. <https://doi.org/10.1371/journal.pone.0156698>.
- Fernández Ajó, A.A., Hunt, K.E., Uhart, M., Rowntree, V., Sironi, M., Marón, C.F., Di Martino, M., Buck, C.L., 2018. Lifetime glucocorticoid profiles in baleen of right whale calves: potential relationships to chronic stress of repeated wounding by kelp gulls. *Conserv Physiol* 6, 1–12. <https://doi.org/10.1093/conphys/coy045>.
- Fernández Ajó, A.A., Hunt, K.E., Giese, A.C., Sironi, M., Uhart, M., Rowntree, V.J., Marón, C.F., Dillon, D., DiMartino, M., Buck, C.L., 2020. Retrospective analysis of the lifetime endocrine response of southern right whale calves to gull wounding and harassment: a baleen hormone approach. *Gen Comp Endocrinol* 296, 113536. <https://doi.org/10.1016/j.ygcen.2020.113536>.
- Fernández Ajó, A., Hunt, K.E., Dillon, D., Uhart, M., Sironi, M., Rowntree, V., Loren Buck, C., 2022. Optimizing hormone extraction protocols for whale baleen: tackling questions of solvent:sample ratio and variation. *Gen Comp Endocrinol* 315, 113828. <https://doi.org/10.1016/j.ygcen.2021.113828>.
- Flamant, F., Cheng, S.Y., Hollenberg, A.N., Moeller, L.C., Samarut, J., Wondisford, F.E., Yen, P.M., Refetoff, S., 2017. Thyroid hormone signaling pathways: time for a more precise nomenclature. *Endocrinology*. <https://doi.org/10.1210/en.2017-00250>.
- Fleming, A.H., Kellar, N.M., Allen, C.D., Kurl, C.M., 2018. The utility of combining stable isotope and hormone analyses for marine megafauna research. *Front Mar Sci* 5. <https://doi.org/10.3389/fmars.2018.00338>.
- Forbes, R., Nakamoto, B., Lysiak, N., Wimmer, T., Hayden, B., 2023. Stable isotope analysis of baleen from North Atlantic right whales *Eubalaena glacialis* reflects distribution shift to the Gulf of St. Lawrence. *Mar Ecol Prog Ser* 722. <https://doi.org/10.3354/meps14428>.
- Fry, B., 1988. Food web structure on Georges Bank from stable C, N, and S isotopic compositions. *Limnol Oceanogr*. <https://doi.org/10.4319/lo.1988.33.5.1182>.
- Gabriele, C., Taylor, L., Burek Huntington, K., Buck, C.L., Hunt, K., Lefebvre, K., Lockyer, C., Lowe, C., Moran, J., Murphy, A., Rogers, M., Trumble, S., Raverty, S., 2021. Humpback whale #441 (Festus): life, death, necropsy, and research findings. *Fort Collins, Colorado* <https://doi.org/10.36967/nrr-2285345>.
- Gelipini, M., Caraveo-Patiño, J., Gauger, M.F.W., Popp, B.N., Panigada, S., Marcín-Medina, R., 2022. Isotopic composition of the eastern gray whale epidermis indicates contribution of prey outside Arctic feeding grounds. *Sci Rep* 12, 7055. <https://doi.org/10.1038/s41598-022-10780-1>.
- Gulland, F., Pérez-Cortés, H., Urbán, J.R., Rojas-Bracho, L., Ylitalo, G., Weir, J., Norman, S., Muto, M., Rugh, D., Kreuder, C., Rowles, T., 2005. Eastern North Pacific gray whale (*Eschrichtius robustus*) unusual mortality event, 1999–2000. U.S. Department of Commerce. NOAA Technical Memorandum NMFS-AFSC-150. 33 pp.
- Hildebrand, L., Bernard, K.S., Torres, L.G., 2021. Do gray whales count calories? comparing energetic values of gray whale prey across two different feeding grounds in the eastern North Pacific. *Front Mar Sci* 8, 1–13. <https://doi.org/10.3389/fmars.2021.683634>.
- Hunt, K.E., Moore, M.J., Rolland, R.M., Kellar, N.M., Hall, A.J., Kershaw, J., Raverty, S.A., Davis, C.E., Yeates, L.C., Fauquier, D.A., Rowles, T.K., Kraus, S.D., 2013. Overcoming the challenges of studying conservation physiology in large whales: a review of available methods. *Conserv Physiol* 1, cot006–cot006. <https://doi.org/10.1093/conphys/cot006>.
- Hunt, K.E., Stimmelmayer, 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–cou030. <https://doi.org/10.1093/conphys/cou030>.
- Hunt, K.E., Lysiak, N.S., Moore, M.J., Rolland, R.M., 2016. Longitudinal progesterone profiles in baleen from female North Atlantic right whales (*Eubalaena glacialis*) match known calving history. *Conserv Physiol* 4, cow014. <https://doi.org/10.1093/conphys/cow014>.
- Hunt, K.E., Lysiak, N.S., Moore, M., Rolland, R.M., 2017a. Multi-year longitudinal profiles of cortisol and corticosterone recovered from baleen of North Atlantic right whales (*Eubalaena glacialis*). *Gen Comp Endocrinol* 254, 50–59. <https://doi.org/10.1016/j.ygcen.2017.09.009>.
- Hunt, K.E., Lysiak, N.S., Robbins, J., Moore, M.J., Seton, R.E., Torres, L., Loren Buck, C., Buck, C.L., 2017b. Multiple steroid and thyroid hormones detected in baleen from eight whale species. *Conserv Physiol* 5. <https://doi.org/10.1093/conphys/cox061>.
- Hunt, K.E., Lysiak, N.S.J., Matthews, C.J.D., Lowe, C., Fernández Ajó, A., Dillon, D., Willing, C., Heide-Jørgensen, M.P., Ferguson, S.H., Moore, M.J., Buck, C.L., 2018. Multi-year patterns in testosterone, cortisol and corticosterone in baleen from adult males of three whale species. *Conserv Physiol* 6, 1–16. <https://doi.org/10.1093/conphys/coy049>.
- Hunt, K.E., Fernández Ajó, A., Lowe, C., Burgess, E.A., Buck, C.L., 2020. A tale of two whales. In: *Conservation Physiology*. Oxford University Press, pp. 205–226. <https://doi.org/10.1093/oso/9780198843610.003.0012>.
- Hunt, K.E., Buck, C.L., Ferguson, S.H., Fernández Ajó, A., Heide-Jørgensen, M.P., Matthews, C.J.D., 2022. Male bowhead whale reproductive histories inferred from baleen testosterone and stable isotopes. *Integrative Organismal Biology* 4. <https://doi.org/10.1093/iob/obac014>.
- Jönsson, K.I., Jönsson, K.I., 1997. Capital and income breeding as alternative tactics of resource use in reproduction. *Oikos* 78. <https://doi.org/10.2307/3545800>.
- Kitaysky, A.S., Wingfield, J.C., Piatt, J.F., 1999. Dynamics of food availability, body condition and physiological stress response in breeding black-legged kittiwakes. *Funct Ecol* 13, 577–584. <https://doi.org/10.1046/j.1365-2435.1999.00352.x>.
- Koren, L., Whiteside, D., Fahlman, A., Ruckstuhl, K., Kutz, S., Checkley, S., Dumond, M., Wynne-Edwards, K., 2012. Cortisol and corticosterone independence in cortisol-dominant wildlife. *Gen Comp Endocrinol* 177. <https://doi.org/10.1016/j.ygcen.2012.02.020>.
- Lemos, L.S., Haxel, J.H., Olsen, A., Burnett, J.D., Smith, A., Chandler, T.E., Nieuwkerk, S.L., Larson, S., Hunt, K.E., Torres, L.G., 2022a. Effects of vessel traffic and ocean noise on gray whale stress hormones. *Sci Rep* 12. <https://doi.org/10.1038/s41598-022-14510-5>.
- Lemos, L.S., Olsen, A., Smith, A., Burnett, J.D., Chandler, T.E., Larson, S., Hunt, K.E., Torres, L.G., 2022b. Stressed and slim or relaxed and chubby? a simultaneous assessment of gray whale body condition and hormone variability. *Mar Mamm Sci* 38, 801–811. <https://doi.org/10.1111/mms.12877>.
- Lowe, C.L., Hunt, K.E., Robbins, J., Seton, R.E., Rogers, M., Gabriele, C.M., Neilson, J.L., Landry, S., Teerlink, S.S., Buck, C.L., 2021a. Patterns of cortisol and corticosterone concentrations in humpback whale (*Megaptera novaeangliae*) baleen are associated with different causes of death. *Conserv Physiol* 9. <https://doi.org/10.1093/conphys/coab096>.
- Lowe, C.L., Hunt, K.E., Rogers, M.C., Neilson, J.L., Robbins, J., Gabriele, C.M., Teerlink, S.S., Seton, R., Buck, C.L., 2021b. Multi-year progesterone profiles during pregnancy in baleen of humpback whales (*Megaptera novaeangliae*). *Conserv Physiol* 9. <https://doi.org/10.1093/conphys/coab059>.
- Lowe, C.L., Jordan-Ward, R., Hunt, K.E., Rogers, M.C., Werth, A.J., Gabriele, C., Neilson, J., von Hippel, F.A., Buck, C.L., 2022. Case studies on longitudinal mercury content in humpback whale (*Megaptera novaeangliae*) baleen. *Heliyon* 8, e08681.
- Lysiak, N.S.J., Trumble, S.J., Knowlton, A.R., Moore, M.J., 2018. Characterizing the duration and severity of fishing gear entanglement on a North Atlantic right whale (*Eubalaena glacialis*) using stable isotopes, steroid and thyroid hormones in baleen. *Front Mar Sci* 5. <https://doi.org/10.3389/fmars.2018.00168>.
- Lysiak, N.S.J., Ferguson, S.H., Hornby, C.A., Heide-Jørgensen, M.P., Matthews, C.J.D., 2023. Prolonged baleen hormone cycles suggest atypical reproductive endocrinology of female bowhead whales. *R Soc Open Sci* 10. <https://doi.org/10.1098/rsos.230365>.
- Lysy, M., Stasko, A.D., Swanson, H.K., 2014. NicheROVER(Niche) Region and Niche Overlap Metrics for Multidimensional Ecological Niches (Version 1.0). <https://cran.r-project.org/web/packages/nicheROVER/index.html>.
- Martínez-Aguilar, S., Mariano-Meléndez, E., López-Paz, N., Castillo-Romero, F., Zaragoza-aguilar, G.A., Rivera-Rodríguez, J., Zaragoza-Aguilar, A., Swartz, S., Viloria-Gómora, L., Urbán, J.R., 2019. Gray whale (*Eschrichtius robustus*) stranding records in Mexico during the winter breeding season in 2019. Report of the International Whaling Commission. Document SC/68A/CMP/14.
- Mate, B.R., Urban-Ramirez, J., 2003. A note on the route and speed of a gray whale on its northern migration from Mexico to central California, tracked by satellite-monitored radio tag. *J Cetacean Res Manage* 5.

- Matthews, C.J.D., Ferguson, S.H., 2015. Seasonal foraging behaviour of eastern Canada-West Greenland bowhead whales: an assessment of isotopic cycles along baleen. *Mar Ecol Prog Ser* 522, 269–286. <https://doi.org/10.3354/meps11145>.
- McEwen, B.S., Wingfield, J.C., 2010. What is in a name? Integrating homeostasis, allostasis and stress. *Horm Behav* 57, 105–111. <https://doi.org/10.1016/j.yhbeh.2009.09.011>.
- Mitani, Y., Bando, T., Takai, N., Sakamoto, W., 2006. Patterns of stable carbon and nitrogen isotopes in the baleen of common minke whale *Balaenoptera acutorostrata* from the western North Pacific. *Fisheries Science* 72. <https://doi.org/10.1111/j.1444-2906.2006.01118.x>.
- Moore, S.E., Grebmeier, J.M., Davies, J.R., 2003. Gray whale distribution relative to forage habitat in the northern Bering Sea: current conditions and retrospective summary. *Can J Zool* 81. <https://doi.org/10.1139/z03-043>.
- Nelson, T.A., Duffus, D.A., Robertson, C., Feyrer, L.J., 2008. Spatial-temporal patterns in intra-annual gray whale foraging: Characterizing interactions between predators and prey in Clayquot Sound, British Columbia, Canada. *Mar. Mamm. Sci.* 24 (2), 356–370. <https://doi.org/10.1111/j.1748-7692.2008.00190.x>.
- Nerini, M., 2012. A Review of Gray Whale Feeding Ecology, in: *The Gray Whale: Eschrichtius Robustus*. <https://doi.org/10.1016/B978-0-08-092372-7.50024-8>.
- Newell, C.L., Cowles, T.J., 2006. Unusual gray whale *Eschrichtius robustus* feeding in the summer of 2005 off the central Oregon Coast. *Geophys. Res. Lett.* 33 (22), L22S11. <https://doi.org/10.1029/2006GL027189>.
- Nicoloff, J.T., Fisher, D.A., Appleman, M.D., 1970. The role of glucocorticoids in the regulation of thyroid function in man. *Journal of Clinical Investigation* 49, 1922–1929. <https://doi.org/10.1172/JCI106411>.
- Pallin, L.J., Kellar, N.M., Steel, D., Botero-Acosta, N., Baker, C.S., Conroy, J.A., Costa, D. P., Johnson, C.M., Johnston, D.W., Nichols, R.C., Nowacek, D.P., Read, A.J., Savenko, O., Schofield, O.M., Stammerjohn, S.E., Steinberg, D.K., Friedlaender, A.S., 2023. A surplus no more? variation in krill availability impacts reproductive rates of Antarctic baleen whales. *Glob Chang Biol* 29, 2108–2121. <https://doi.org/10.1111/gcb.16559>.
- Peterson, B.J., Fry, B., 1987. STABLE isotopes in ecosystem studies. *Annu Rev Ecol Syst* 18, 293–320. <https://doi.org/10.1146/annurev.es.18.110187.001453>.
- Pirotta, E., Thomas, L., Costa, D.P., Hall, A.J., Harris, C.M., Harwood, J., Kraus, S.D., Miller, P.J.O., Moore, M.J., Photopoulou, T., Rolland, R.M., Schwacke, L., Simmons, S.E., Southall, B.L., Tyack, P.L., 2022. Understanding the combined effects of multiple stressors: a new perspective on a longstanding challenge. *Science of the Total Environment* 821, 153322. <https://doi.org/10.1016/j.scitotenv.2022.153322>.
- Pomeroy, C., Matthews, C.J.D., Gobeil, C., Stern, G.A., Ferguson, S.H., Macdonald, R. W., 2018. Mercury and stable isotope cycles in baleen plates are consistent with year-round feeding in two bowhead whale (*Balaena mysticetus*) populations. *Polar Biol* 41, 1881–1893. <https://doi.org/10.1007/s00300-018-2329-y>.
- Post, D.M., 2002. Using stable isotopes to estimate trophic position: models, methods, and assumption. *Ecology* 83, 703–718.
- Reckendorf, A., S.U., P.E., & D.K., 2023. *Marine Mammals*. Springer International Publishing, Cham. <https://doi.org/10.1007/978-3-031-06836-2>.
- Rice, D.W., Wolman, A.A., 1971. Life history and ecology of the gray whale (*Eschrichtius robustus*). *American Society of Mammalogists, American Society of Mammalogists, Stillwater, Oklahoma*.
- Riekenberg, P.M., Camalich, J., Svensson, E., Ljsseldijk, L.L., Brasseur, S.M.J.M., Witbaard, R., Leopold, M.F., Rebolledo, E.B., Middelburg, J.J., van der Meer, M.T.J., Sinninghe Damsté, J.S., Schouten, S., 2021. Reconstructing the diet, trophic level and migration pattern of mysticete whales based on baleen isotopic composition. *R Soc Open Sci* 8. <https://doi.org/10.1098/rsos.210949>.
- Rita, D., Borrell, A., Vikingsson, G., Aguilar, A., 2019. Histological structure of baleen plates and its relevance to sampling for stable isotope studies. *Mammalian Biology* 99, 63–70. <https://doi.org/10.1016/j.mambio.2019.10.004>.
- 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. *Proceedings of the Royal Society B: Biological Sciences* 279, 2363–2368. <https://doi.org/10.1098/rspb.2011.2429>.
- Romero, L.M., Dickens, M.J., Cyr, N.E., 2009. The reactive scope model - a new model integrating homeostasis, allostasis, and stress. *Horm Behav* 55, 375–389. <https://doi.org/10.1016/j.yhbeh.2008.12.009>.
- Romero, M.L., Wingfield, J.C., 2016. *Oxford series in behavioral neuroendocrinology. Tempests, Poxes, Predators, and People: Stress in Wild Animals and How They Cope* 1–2.
- Sang, H.L., Schell, D.M., McDonald, T.L., Richardson, W.J., 2005. Regional and seasonal feeding by bowhead whales *Balaena mysticetus* as indicated by stable isotope ratios. *Mar Ecol Prog Ser* 285. <https://doi.org/10.3354/meps285271>.
- Schell, D.M., Saupe, S.M., Haubenstock, N., 1989. Bowhead whale (*Balaena mysticetus*) growth and feeding as estimated by ^{13}C techniques. *Mar Biol* 103, 433–443. <https://doi.org/10.1007/BF00399575>.
- Silva, M.A., Borrell, A., Prieto, R., Gauffier, P., Bérubé, M., Palsbøl, P.J., Colaço, A., 2019. Stable isotopes reveal winter feeding in different habitats in blue, fin and sei whales migrating through the Azores. *R Soc Open Sci* 6. <https://doi.org/10.1098/rsos.181800>.
- Stewart, J.D., Joyce, T.W., Durban, J.W., Calambokidis, J., Fauquier, D., Fearnbach, H., Grebmeier, J.M., Lynn, M., Manizza, M., Perryman, W.L., Tinker, M.T., Weller, D.W., 2023. Boom-bust cycles in gray whales associated with dynamic and changing Arctic conditions. *Science* 1979 (382), 207–211. <https://doi.org/10.1126/science.adl1847>.
- Stewart, J.D., Weller, D.W., 2021. *Abundance of eastern North Pacific gray whales 2019/2020*. NOAA Technical Memorandum. NMFS-SWFSC-639.
- Sumich, J.L., 2001. Growth of baleen of a rehabilitating gray whale calf. *Aquat. Mamm.* 27 (3).
- Suryan, R.M., Arimitsu, M.L., Coletti, H.A., Hopcroft, R.R., Lindeberg, M.R., Barbeaux, S. J., Batten, S.D., Burt, W.J., Bishop, M.A., Bodkin, J.L., Brenner, R., Campbell, R.W., Cushing, D.A., Danielson, S.L., Dorn, M.W., Drummond, B., Esler, D., Gelatt, T., Hanselman, D.H., Hatch, S.A., Haught, S., Holderied, K., Iken, K., Irons, D.B., Kettle, A.B., Kimmel, D.G., Konar, B., Kuletz, K.J., Laurel, B.J., Maniscalco, J.M., Matkin, C., McKinstry, C.A.E., Monson, D.H., Moran, J.R., Olsen, D., Palsson, W.A., Pegau, W.S., Piatt, J.F., Rogers, L.A., Rojek, N.A., Schaefer, A., Spies, I.B., Straley, J. M., Strom, S.L., Sweeney, K.L., Szymkowiak, M., Weitzman, B.P., Yasumiishi, E.M., Zador, S.G., 2021. Ecosystem response persists after a prolonged marine heatwave. *Sci Rep* 11, 6235. <https://doi.org/10.1038/s41598-021-83818-5>.
- Swanson, H.K., Lysy, M., Pöwer, M., Stasko, A.D., Johnson, J.D., Reist, J.D., 2015. A new probabilistic method for quantifying n-dimensional ecological niches and niche overlap. *Ecology* 96. <https://doi.org/10.1890/14-0235.1>.
- Swartz, S.L., Taylor, B.L., Rugh, D.J., 2006. Gray whale *Eschrichtius robustus* population and stock identity. *Mamm Rev.* <https://doi.org/10.1111/j.1365-2907.2006.00082.x>.
- Teixeira, C.R., Troina, G.C., Daura-Jorge, F.G., Simões-Lopes, P.C., Botta, S., 2022. A practical guide on stable isotope analysis for cetacean research. *Mar Mamm Sci.* <https://doi.org/10.1111/mms.12911>.
- Torres, L.G., Brander, S.M., Parker, J.I., Bloom, E.M., Norman, R., Van Brocklin, J.E., Lasdin, K.S., Hildebrand, L., 2023. Zoop to poop: assessment of microparticle loads in gray whale zooplankton prey and fecal matter reveal high daily consumption rates. *Front Mar Sci* 10. <https://doi.org/10.3389/fmars.2023.1201078>.
- Torres, L.G., Bird, C.N., Rodríguez-González, F., Christiansen, F., Beijder, L., Lemos, L., Urban R, J., Swartz, S., Willoughby, A., Hewitt, J., Bierlich, K.C., 2022. Range-Wide Comparison of Gray Whale Body Condition Reveals Contrasting Sub-Population Health Characteristics and Vulnerability to Environmental Change. *Front Mar Sci* 9. <https://doi.org/10.3389/fmars.2022.867258>.
- Urbán, R., 2020. Gray whale stranding records in Mexico during the 2020 winter breeding season. Unpublished paper SC/68B/CMP/13 presented to the IWC Scientific Committee, Cambridge.
- Williams, C.T., Chmura, H.E., Zhang, V., Dillon, D., Wilsterman, K., Barnes, B.M., Buck, C.L., 2019. Environmental heterogeneity affects seasonal variation in thyroid hormone physiology of free-living arctic ground squirrels (*Urocitellus parryi*). *Can J Zool* 97, 783–790. <https://doi.org/10.1139/cjz-2018-0302>.
- Wilsterman, K., Buck, C.L., Barnes, B.M., Williams, C.T., 2015. Energy regulation in context: free-living female arctic ground squirrels modulate the relationship between thyroid hormones and activity among life history stages. *Horm Behav* 75. <https://doi.org/10.1016/j.yhbeh.2015.09.003>.
- Wingfield, J.C., 2005. THE concept of allostasis: coping with a capricious environment. *J Mammal* 86, 248–254. <https://doi.org/10.1644/BHE-004.1>.
- Wingfield, J.C., Maney, D.L., Breuner, C.W., Jacobs, J.D., Lynn, S., Ramenofsky, M., Richardson, R.D., 1998. Ecological bases of hormone—Behavior interactions: the “emergency life history stage”. *Am Zool* 38, 191–206. <https://doi.org/10.1093/icb/38.1.191>.