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

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39   • Baleen analysis of hormones and stable isotopes is a powerful tool to enable a comprehensive
40   and retrospective assessment of stress, reproduction, and nutritional status of the gray whale.
41   • Gray whale baleen holds an endocrine and isotopic record of the last 1.3 years of the individual
42   prior to death.
43   • Quantification of baleen glucocorticoid content enables discrimination between chronic illness
44   and acute stress as cause of death.
45   • Fluctuations in baleen $\delta^{15}N$ correspond to the expected migration phenology in gray whales.

ABSTRACT

47   Individual-level assessments of wild animal health, vital rates, and foraging ecology are critical for

48   understanding population-wide impacts of exposure to stressors. Large whales face multiple stressors,

49   including, but not limited to, ocean noise, pollution, and ship strikes. Because baleen is a continuously

50   growing keratinized structure, serial extraction, and quantification of hormones and stable isotopes

51   along the length of baleen provide a historical record of whale physiology and foraging ecology.

52   Furthermore, baleen analysis enables the investigation of dead specimens, even decades later, allowing

53   comparisons between historic and modern populations. Here, we examined baleen of five sub-adult

54   gray whales and observed distinct patterns of oscillations in $\delta^{15}N$ values along the length of their baleen

55   plates which enabled estimation of baleen growth rates and differentiation of isotopic niche widths of

56   the whales during winter and summer foraging. In contrast, no clear patterns were apparent in $\delta^{13}C$

57   values. Prolonged elevation of cortisol in four individuals before death indicate that chronic stress may

58   have impacted their health and survival. Triiodothyronine (T3) increased over months in the whales with

59   unknown causes of death, simultaneous with elevations in cortisol, but both hormones remained stable

60   in the one case of acute death attributed to killer whale predation. This parallel elevation of cortisol and

61   T3 challenges the classic understanding of their interaction and might relate to increased energetic

62   demands during exposure to stressors. Reproductive hormone profiles in subadults did not show cyclical

63   trends, suggesting they had not yet reached sexual maturity. This study highlights the potential of

64   baleen analysis to retrospectively assess gray whales' physiological status, exposure to stressors,

65   reproductive status, and foraging ecology in the months or years leading up to their death, which can be

66   a useful tool for conservation diagnostics to mitigate unusual mortality events.

KEY WORDS:

1. INTRODUCTION

Individual-level assessments of changes in health, vital rates, and foraging ecology of wild animals in response to disturbance events are key for identifying potential impacts on the broader population (Pirotta et al., 2022), as well as gaining insights needed for effective, targeted conservation strategies. Large whales 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., 2022a; Rolland et al., 2012), contaminant, plastic, heavy metal and chemical pollution (Lowe et al., 2022; Reckendorf, 2023; Torres et al., 2023), ship strikes, harmful algal blooms (D'Agostino et al., 2022), entanglement in fishing gear (Clapham, 2016; S. Derville et al., 2023), marine heatwaves (Suryan et al., 2021), and prey shifts (Solène Derville et al., 2023; Pallin et al., 2023; Thomas et al., 2016). Conservation efforts to mitigate threats to whale populations are hindered by challenges of monitoring and repeated sampling due to whales' large size, mobility, and their remote marine habitats (Hunt et al., 2013) and thus constrain assessment of natural and anthropogenic impacts on individual health, vital rates, and foraging ecology.

Recently, the use of innovative analytical methods for non-plasma sample types that can be collected from live or dead whales has increased our ability to disentangle different aspects of the complex foraging ecology and physiology of large whales (Fleming et al., 2018; Hunt et al., 2013; Teixeira et al., 2022). Baleen, for example, is a unique structure that forms the filter-feeding apparatus in mysticete whales and is perhaps the best biological tissue for acquiring longitudinal ecological and physiological data, with sufficient temporal resolution to examine seasonal patterns (Caraveo-Patiño et al., 2007; Fernández Ajó et al., 2020, 2018; Hunt et al., 2018). Like other keratinized epidermal tissues (e.g., claws, hair, and spines, whiskers), baleen is a continuously growing structure that extends from a well-vascularized dermal zone. During growth, baleen incorporates the isotopic ratios and endocrine signature of the circulating plasma. The slow growth rate of baleen allows for simultaneous incorporation of the whale's endocrine and stable isotope (SI) history spanning the time of baleen growth. 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., 2007a; Lowe et al., 2021b, 2021a) versus a decade or more in species with longer baleen (e.g., bowheads, *Balaena mysticetus*) (Hunt et al., 2022, 2017a, 2014; Lysiak et al., 2018). Consequently, paired quantification of hormones and SI values along the longitudinal axis of the baleen plate provides a historical record of the individuals' physiology and insights into their foraging ecology. Notably, baleen is routinely recovered

106   during necropsies, and its inherent strength, durability, and minimal storage requirements (these

107   samples can be preserved dry at room temperature) ensure the preservation of the analytes of interest

108   within the keratin matrix. As a result, detection of hormones and SI's remains feasible in dried samples

109   for decades (Fernández Ajó et al., 2018; Hunt et al., 2017b). These remarkable properties of baleen not

110   only capture multi-year timeframes, enabling the determination of the individuals' seasonal endocrine

111   and foraging patterns, but also facilitates comparisons between historic and modern populations of

112   whales (Fernández Ajó et al., 2020, 2018; Hunt et al., 2018, 2014).

113   Eastern North Pacific (ENP) gray whales migrate between their wintering grounds along the Baja

114   California, Mexico, coastline, and their summer foraging grounds in the Bering, Chukchi, and Beaufort

115   Seas. The ENP population has experienced at least two recorded Unusual Mortality Events (UMEs), in

116   1999-2000 and from 2019 to the present, during which an unusually high number of gray whales were

117   found dead along the Pacific coast from northern Mexico to the Alaskan Arctic, USA. Several factors

118   have been considered as possible causes for the high number of gray whale strandings, including

119   variation in Arctic prey availability and the duration of their feeding season caused by the timing of sea

120   ice formation and breakup (Stewart et al., 2023), starvation, anthropogenically derived toxicants,

121   biotoxins, infectious diseases, parasites, fisheries interactions, and ship strikes (Eguchi et al., 2023;

122   Gulland et al., 2005). In the current UME, dead whales are frequently emaciated, indicating nutritional

123   limitation as a causal factor of death (Christiansen et al., 2021). While poor condition of many of the

124   stranded whales supports the idea that starvation could be a significant contributing factor in these

125   mortalities, the underlying causes of starvation during these events are unknown, and it is also unclear

126   whether the whales' decline in body condition was rapid or gradual.

127   In this study, we analyzed patterns across time of stable isotopes and five hormones within five

128   baleen plates recovered postmortem from five subadult gray whales (4 males, 1 female) that stranded

129   during the 2019-present UME. Our goal is twofold: first, to retrospectively examine the hormone and

130   isotopic profiles in gray whales prior to mortality; and second, to assess potential factors contributing to

131   mortality and the onset timing of chronic illness leading to death. Our isotopic analysis includes the

132   longitudinal profiles of bulk carbon and nitrogen stable isotope ratios in baleen, as they are well-

133   established markers of seasonal diet and foraging grounds in large whales (Best and Schell, 1996;

134   Busquets-Vass et al., 2017; Matthews and Ferguson, 2015). Stable isotopes incorporated into baleen are

135   acquired from an animal's diet, with different prey having characteristic ratios of $^{13}C/^{12}C$ and $^{15}N/^{14}N$,

136   expressed as $\delta^{13}C$ and $\delta^{15}N$, respectively. Predictable enrichment of both $\delta^{13}C$ and $\delta^{15}N$ occurs at each

137   trophic level (Kelly, 2000), and additional latitudinal variation in prey isotope ratios often results in

138   annual oscillations in δ^{13}C and δ^{15}N across the length of whale baleen, reflecting the whales' annual

139   migrations between summering and wintering grounds. For example, δ^{15}N values are typically lower

140   when whales consume zooplankton at their summering grounds and are higher when whales are

141   sustained primarily by their own blubber reserves and/or feed on isotopically distinct food on their

142   wintering grounds (Lysiak, 2009). Recent studies have combined stable isotopes with baleen steroid

143   hormone analysis to establish a timeline of tissue growth, allowing interpretation of hormone

144   concentrations over time (e.g., (Hunt et al., 2017a, 2016b, 2014)).

145   Our hormonal analysis quantifies two adrenal glucocorticoid steroids, cortisol and

146   corticosterone, as well as the thyroid hormone triiodothyronine (T3), and two gonadal steroids,

147   progesterone and testosterone. Increased secretion of glucocorticoids from the hypothalamic-pituitary-

148   adrenal (HPA) axis signifies the activation of the vertebrate stress response (Romero and Wingfield,

149   2016). The hypothalamic-pituitary-thyroid axis (HPT) regulates the synthesis and secretion of thyroxine

150   (T4), which subsequently undergoes enzymatic conversion to the more active form, T3. Both T3 and T4

151   modulate basal metabolic rate, growth and development, and thermogenesis, along with other

152   permissive actions (Romero and Wingfield, 2016). Because T3 is generally recognized as the most

153   biologically active thyroid hormone, it has been considered a more relevant biomarker than other forms

154   of the thyroid hormones (Eales, 1988; Flamant et al., 2017). T3 is examined here as a biomarker of

155   nutritional state, i.e., a proxy of foraging success, given its role in regulating metabolic rate in mammals,

156   as reviewed in (Behringer et al., 2018). The two gonadal steroids, progesterone and testosterone, are

157   assessed here as markers of reproductive status. The analysis of reproductive hormones within baleen

158   has proven valuable for assessing pregnancy and inter-calving cycles in females, and testosterone cycles

159   in males, in multiple baleen whale species (Hunt et al., 2022, 2018, 2016b; Lowe et al., 2021b; Lysiak et

160   al., 2023). Here, we examine the reproductive steroids to assess sexual maturity; all our specimens are

161   from subadults, but subadult whales may initiate gonadal secretion of reproductive hormones well in

162   advance of full reproductive competence, and stress is known to delay sexual maturity in many

163   mammals (Dettmer and Chusyd, 2023; Hunt et al., 2022). Further, the individual baselines for each

164   hormone and each individual whale are assessed to monitor individual variability in response to

165   potential stressors.

166   Through the integration of SI and endocrine methodologies, we demonstrate that baleen

167   analysis provides a holistic narrative detailing the health and trophic ecology of individual whales across

168 time, effectively filling knowledge gaps between individual physiology and population impacts.

169 Ultimately, these biomarker techniques can make significant contributions to management and

170 conservation efforts by informing the complex physiological dynamics that underlie whale mortality.

171 **2. MATERIALS & METHODS**

172 **2.1 SAMPLE COLLECTION**

173 From April 2019 to August 2021, a baleen plate from each of five stranded gray whales (n = 4

174 males and n = 1 female) was collected by the Oregon Marine Mammal Stranding Network (OMMSN,

175 NMFS MMPA/ESA permit No. 18786-06) along the Oregon, USA coast, between Whaleshead Beach in

176 Brookings (42.15°N, -124.35°W) and Cape Mears (45.54°N, -123.96°W). All males had complete baleen

177 plates (i.e., including the most recent growth within the gum), while the female's baleen plate was

178 missing the most recently grown baleen at the root of the baleen plate (i.e., the baleen was cut at the

179 gumline when recovered at necropsy). All specimens were removed from the right side of the rostrum

180 and the center of the rack, where the longest baleen plates are located. The whale's total length (TL,

181 measured as snout-to-fluke-notch), presence of scars, general body condition, and presumed cause of

182 death were also recorded (Table 1). All individuals were classed as "subadults" based on the size

183 categories (i.e., female TL 9–11.7 m, male TL 9–11.1 m; (Rice and Wolman, 1971)), i.e., at least 24

184 months old but not yet sexually mature.

185 **2.2 PREPARATION OF BALEEN PLATES FOR HORMONE EXTRACTION AND QUANTIFICATION**

186 To remove any soft tissues adhered to the base of the baleen plates (proximal end near the gum

187 line with the newest baleen), we rehydrated and softened the tissues by submerging the baleen plates

188 in fresh water, and subsequently scraped the soft tissues off with a metal scraper or scalpel. We then

189 freeze-dried the baleen plates under vacuum (LabConco FreeZone 6L system with Stoppering Tray Dryer,

190 Kansas City, MO, USA), until the pressure reading of the lyophilizer stabilized for at least 12 h, indicating

191 that the samples were dry. Dried, cleaned plates were then stored at room temperature in individual

192 sealed plastic bags, each with a 50 g silica gel desiccant pack (Arbor Assays, Ann Arbor, MI, USA).

193 We collected 20-50 mg of powder from sampling points spaced every 1 cm along the labial edge

194 of the plate, using a hand-held electric rotary grinder (Dremel® model 395 type 5) fitted with a tungsten

195 carbide ball-tip, with each sample collected from a <1.5 cm transverse groove across the posterior face

196 of the plate. The proximal-most point on the base of the baleen plate was designated as the 0 cm point.

197 Sampling started 1 cm from the base and continued every 1 cm to the tip (distal end) until the baleen

198   became too thin to collect the minimum required sample mass for hormone extraction (20 mg); thus,

199   we typically excluded the distal-most two centimeters of each plate (i.e., the oldest growth). To avoid

200   cross contamination, during sampling we shielded other regions of the plate with adhesive tape, and

201   between samples the entire baleen plate, sampling equipment, and fume hood were cleaned with

202   compressed air, and the work surface and all equipment were also cleaned with 70% ethanol. Powder

203   samples were weighed to the nearest 0.1 mg on an Ohaus Explorer Pro EP214C analytical balance

204   (Ohaus, Pine Brook, NJ, USA), with a nearby workstation ionizer (SPI No. 94000, SPIwestek.com) placed

205   next to the scale to minimize any effects of static electric charge. Weighed samples were placed in 16 ×

206   100 mm borosilicate glass tubes and securely capped until hormone extraction, which took place within

207   72 hours of drilling. In total, 110 powder samples were produced, with each whale's plate producing

208   between 12-27 samples.

209   **Table 1.** Biological information for individual gray whales, *Eschrichtius robustus*, collected along the
210   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

211
212   **Cause of death: Unk =** undetermined, **Orca =** evidence of Killer whale, *Orcinus orca,* predation as acute cause of death; **Sex =** Female (F), Male
213   (M); **TL =** Total Length from snout to fluke notch in cm**; Total samples =** number of subsamples obtained from each baleen plate; **BGR =** baleen
214   growth rate estimated in days per cm (from stable isotope analysis); **GS =** Growth span, estimated timespan represented by the entire baleen
215   plate, in days, derived from baleen growth rate and total length of plate; * only the erupted portion of the baleen was collected during the
216   necropsy.

217   **2.3. STABLE ISOTOPE ANALYSES (SIA)**

218   We weighed approximately 1 mg of baleen powder from each sampling location (i.e., every 1 cm

219   along the longitudinal axis on each baleen plate) directly into tin capsules. Bulk $\delta^{13}C$ and $\delta^{15}N$ were

220   measured using a Thermo FlashSmart elemental analyzer coupled to a Thermo Finnigan Delta Plus XP

221   continuous-flow isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany). Results are

222   expressed in parts per thousand (‰) and delta notation (δ) using the equation: $\delta_{sample} = [R_{sample}/R_{standard} -$

223    $1] * 1000$, where R_{sample} and $R_{standard}$ are the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratios of the sample and standard,

224   respectively (Peterson and Fry, 1987). The isotopic reference materials used were supplied by the

225   International Atomic Energy Agency (IAEA-N-1, $\delta^{15}N$ = 0.4 ± 0.2‰; IAEA-CH-7, $\delta^{13}C$ = −32.1 ± 0.05‰;

226   IAEA-CH-3, δ^{13}C = −24.7 ± 0.04‰) and the United States Geological Survey (USGS25,

227   δ^{13}C = −34.58 ± 0.06‰, δ^{15}N = −0.94 ± 0.16‰; USGS40, δ^{13}C = −26.3 ± 0.04‰, δ^{15}N = −4.5 ± 0.1‰;

228   USGS41, δ^{13}C = +37.6 ± 0.04‰, δ^{15}N = 47.6 ± 0.2‰;). Internal standards were included with all samples

229   as quality controls; all error data are SD (purified methionine, Alfa Aesar, δ^{13}C = −34.5 ± 0.06‰,

230   δ^{15}N = −0.9 ± 0.1‰; homogenized Chinook salmon muscle, NOAA Auke Bay Laboratories,

231   δ^{13}C = −19.2 ± 0.05‰, δ^{15}N = 15.5 ± 0.1‰). The analytical precision based on the standard deviation of

232   the standard laboratory replicas was <0.1‰ for both δ^{13}C and δ^{15}N. To ensure that our samples did not

233   contain any ^{13}C-depleted lipids, we also measured the C:N ratio of each sub-sample; all of which were

234   within the range expected for pure protein (2.7-3.5) ((Ambrose, 1990); see Supplementary Material,

235   Table S2).

236   **2.4. BALEEN GROWTH RATES AND TIMELINES**

237   To assign an estimated season of growth to each part of the baleen plate, we inspected the δ^{15}N

238   data for evidence of seasonal changes. Specifically, based on the patterns seen in other baleen whales

239   (Best and Schell, 1996; Lysiak et al., 2018; Matthews and Ferguson, 2015), we assumed that the areas of

240   baleen with lower δ^{15}N were grown during summer when whales are most actively foraging, while the

241   regions of baleen with higher δ^{15}N were assumed to have grown during winter. Similarly, points with

242   intermediate δ^{15}N values (i.e., between summer δ^{15}N troughs and winter δ^{15}N peaks) were assumed to

243   represent spring and fall migrations. However, because gray whale baleen is relatively short and hence

244   expected to only capture a single full annual cycle, these potential timelines may be imprecise. Thus, we

245   also compared each whale's δ^{15}N data to published estimates of baleen growth rate (BGR) for gray

246   whales, which vary from 3.2 mm/week (Sumich, 2001) to 4.7 mm /week (Caraveo-Patiño et al., 2007a).

247   Therefore, for each plate we calculated two potential timelines, counting cm from the base of the

248   baleen plate, using the two published BGR estimates, i.e., assuming the proximal-most point on the

249   plate was grown near the day the whale was found dead, with all other points on the plate then

250   assigned an estimated date of growth based on that BGR (either 3.2 or 4.7 mm/week). These two

251   timelines bracket a range of potential plausible BGRs. The two BGR-derived timelines were then

252   compared to the δ^{15}N timeline for that whale, i.e., to verify that our δ^{15}N interpretations involve a

253   plausible BGR for this species.

254   **2.5. HORMONE EXTRACTION AND QUANTIFICATION**

255   We extracted hormones from pre-weighed baleen powder samples using 1.6 mL of absolute

256   methanol per 20 mg powder, i.e., keeping a constant ratio of 80:1 mL of solvent to g of sample. This

257   solvent:sample ratio yields good detectability with low variation (inter-sample coefficient of variation <

258   10%; (Fernández Ajó et al., 2022). The solvent:sample mixture was vortexed 2 h at room temperature

259   (Large Capacity Mixer, Glas-Col, Terre Haute, IN, USA; speed set on 40) and centrifuged for 1 min at 4025

260   g. The supernatant from each tube was transferred to individual 13×100 mm borosilicate tubes and

261   dried at 45°C for a minimum of 4 h in a sample evaporator (SpeedVac 121P, Thermo Fisher Scientific,

262   Waltham, MA, USA) under vacuum. We reconstituted the dried samples in 0.50 mL of assay buffer (X065

263   buffer; Arbor Assays, Ann Arbor, MI, USA), sonicated for 5 min, vortexed for 5 min, and transferred the

264   sample to 1.5 mL vapor proof O-ring-capped cryovials. We stored the tubes overnight at −80°C and

265   decanted the extract into a new cryovial the following day. This was considered the "1:1" (full-strength,

266   neat) extract and was stored at -80C until assay.

267   We used commercial enzyme immunoassay (EIA) kits to quantify immunoreactive

268   corticosterone, cortisol, progesterone, testosterone, and T3 in baleen extracts (Arbor Assays kits:

269   corticosterone #K014, cortisol #K003, progesterone #K025, testosterone #K032, and T3 #K056, Ann

270   Arbor, MI, USA). These five kits have previously been validated for gray whale baleen extracts (Hunt et

271   al., 2017b). We assayed all samples at a 1:2 dilution, which in this species produces acceptable

272   detectability and percent-bounds while also allowing assay of multiple hormones from a single 500ul

273   extract. Final data are expressed as ng of hormone per g of dried baleen powder. All assays adhered to

274   standard QA/QC criteria, which included a full standard curve, NSB (non-specific binding), zero dose

275   ("blank"), and an independent control in every EIA microplate. All samples, standards, controls, NSBs,

276   and zeros were assayed in duplicate. Any sample that exhibited a coefficient of variation exceeding 10%

277   between duplicates was re-analyzed. For antibody cross-reactivities, assay sensitivities, and other

278   methodological details, see Hunt et al. (2017a) and the manufacturer's protocols

279   (www.arborassays.com).

280   We evaluated the complete longitudinal profiles for both glucocorticoids, cortisol and

281   corticosterone, in only two individuals (Er_1 and Er_4) to determine the dominant (most abundant)

282   glucocorticoid and to compare the longitudinal profiles of the two hormones. As cortisol was at higher

283   concentration than corticosterone for these two whales, corticosterone was not assayed for the other

284   baleen specimens (see Results). We assayed all other hormones (progesterone, testosterone, T3) for all

285   samples from all whales.

286   **2.6. STATISTICAL ANALYSIS**

287   **2.6.1. HORMONES**

288   All hormone data were log-transformed for data visualization and analyses due to non-normal

289   distribution. We estimated hormone baselines for each gray whale using an iterative process that

290   excludes all data points greater than the mean + two standard deviations until no points exceed this

291   maximum value, following methods from Brown et al., 1988. To test for differences in concentrations of

292   reproductive hormones between sexes, we fit a linear mixed-effects model with random intercepts

293   using the *lme4* R package. All statistical analyses were computed using R (R Development Core Team

294   2023).

295   **2.6.2. STABLE ISOTOPE ANALYSIS**

296   We gauged $\delta^{13}C$ and $\delta^{15}N$ fluctuations in baleen plates with a generalized additive model (GAM),

297   fitting a semi-parametric regression with smoothing by cross-validation. We used an ANOVA analysis to

298   test differences in the $\delta^{13}C$ and $\delta^{15}N$ values between the phenological phases (wintering vs. summer

299   foraging) utilizing the *aov* function from the stats R package. We then compared the isotopic niche width

300   of each individual gray whale per phenological phase by generating bivariate ellipses in SIBER (Stable

301   Isotope Bayesian Ellipses in R (Jackson et al., 2011), which employs Markov-Chain Monte Carlo (MCMC)

302   simulations to construct parameters of ellipses based on sampling points. We estimated the standard

303   ellipse area corrected for small sample sizes (SEA$_C$, expressed as ‰, which represents the mean core

304   area of each individual's isotopic niche (Jackson et al., 2011; Layman et al., 2007)). We also calculated

305   the Bayesian standard ellipse area (SEAB) to obtain unbiased estimates of the isotopic niche widths

306   (Jackson et al., 2011).To test for significant differences, we ran 20,000 MCMC iterations and constructed

307   95% credible intervals around the mean of each whale. Results are reported as mean ± standard

308   deviation (SD) unless otherwise stated. All statistical analyses were computed using R (R Development

309   Core Team 2023).

310   **3. RESULTS**

311   **3.1 STUDY ANIMALS, BODY EXAMINATION AND CAUSE OF DEATH.**

312   All five individuals were in fair to good body condition at necropsy, i.e., no evidence of

313   emaciation. Further, there was no indication that direct human interaction was the cause of death.

314   Notably, all individuals had "rake" mark scars, indicative of physical interactions with killer whales,

315   *Orcinus orca* (Corsi et al., 2022). These scars were primarily observed on the distal end of the fluke and

316   pectoral fins. One individual, HMSC21-08-16-Er (Er 5), presented evidence of acute mortality caused by

317   interactions with killer whales, showing multiple deep and recent "rake" marks on various body parts,

318   particularly the head and flippers, along with extensive and severe hemorrhaging on the top of the

319   head. Cause of death could not be determined for the other four whales.

320   **3.2 ISOTOPIC PATTERNS AND BALEEN GROWTH RATES (BGR)**

321   All baleen plates oscillated in δ^{15}N values along their growth axis consistent with expected

322   migration phenology (Figure 1). The growth rate that best fit the expected oscillations with the

323   migration phenology was 3.2 mm/week for all whales except Er_5, for which the best estimate was 4.7

324   mm/week (Figure 1). Excluding Er_1, from which the baleen plate was incomplete (i.e., the proximal-

325   most portion within the gum was missing) we estimate that the baleen of these subadult gray whales

326   recorded around 1.3 years of individual hormone and SI data (n = 4; 471.5 ± 68.73 days; Mean ± SD,

327   Table 1).

328   Mean δ^{13}C values were similar among individuals: during wintering, values ranged from −15.2‰

329   ± 0.1‰ for Er_5, to -16.3‰ ± 0.1‰ for Er_2; during summer foraging period values ranged from -15.0

330   ‰ ± 0.4‰ for Er_5 to -16.3‰ ± 0.4‰ for Er_2 (Table 2). Mean δ^{13}C values for all individuals were -

331   15.0‰ ± 0.4‰ and -15.2‰ ± 0.1‰ for the summer and wintering period, respectively (Figure 2) with no

332   significant differences between these two periods ($F_{(1, 88)}$ = 1.73, p = 0.19). In contrast, mean δ^{15}N values

333   varied among individuals, ranging from 13.0‰ ± 0.3‰ for Er_2 to 15.1‰ ± 0.2‰ for Er_5 during the

334   wintering period to 11.9‰ ± 0.4‰ for the Er_5, to 13.1‰ ± 0.4‰ for the Er_3 during the summer

335   foraging period. The mean δ^{15}N values for all individuals was 12‰ ± 0.4‰ and 15.1‰ ± 0.2‰ for the

336   summer foraging and wintering periods, respectively (Figure 2), with a significant difference between

337   periods ($F_{(1, 88)}$ = 132, p < 0.001).

338
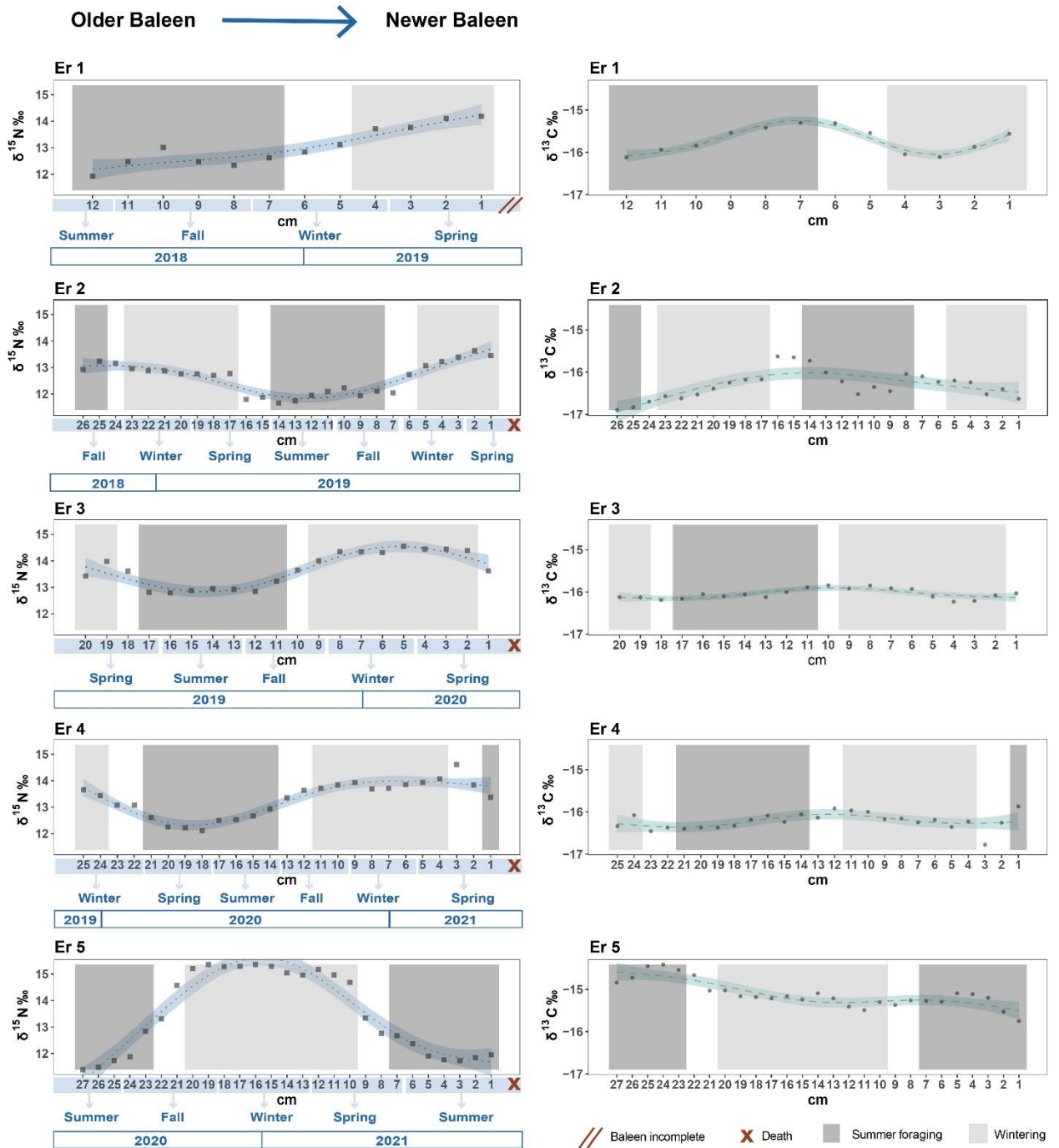
Figure 1: Longitudinal profiles of and δ¹⁵N (left) δ¹³C (right) in baleen plates of stranded subadult gray whales collected along the Oregon Coast. X-axes show sample location along the baleen, in cm from base of the baleen plate (i.e., newest baleen = 1 cm), with 1 cm (newest baleen) at far right, i.e., time runs from left to right. Y-axes show δ¹³C or δ¹⁵N 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 period) and light grey (putative wintering period) shading, estimated based on δ¹⁵N fluctuations. Squares and closed circles depict actual values of δ¹⁵N and δ¹³C, respectively; the dotted and dashed lines depict the fit of the GAM models, with the blue

347   (δ^{15}N) and green (δ^{13}C) fringe illustrating the 95% confidence intervals. Only the erupted portion of the
348   baleen plate from Er_1 (top) was available, i.e., the proximal-most portion of the base of the plate was
349   missing, denoted with two parallel red lines on the x-axis.

350

351
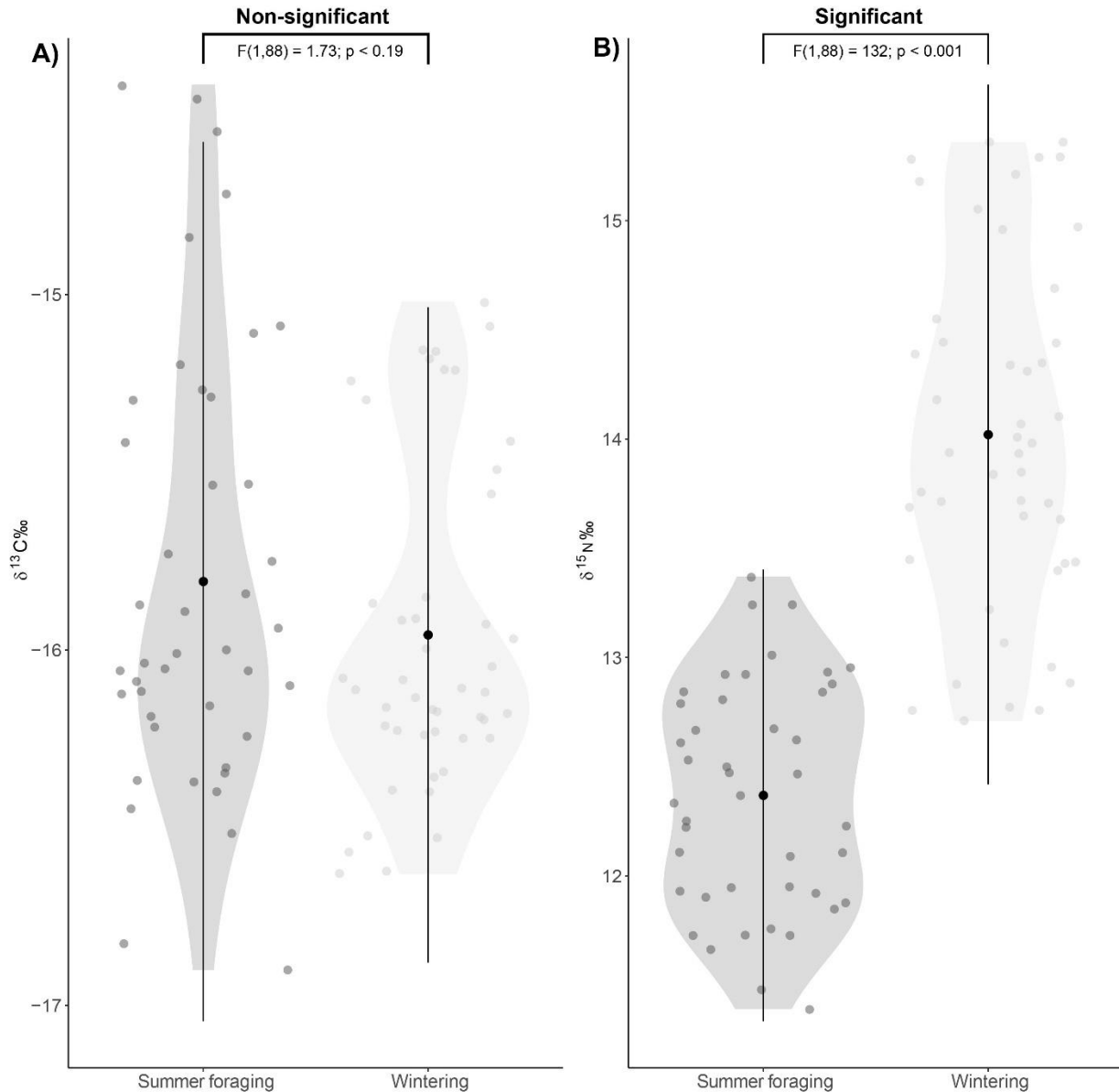
Figure 2. Violin plots for A) δ^{13}C and B) δ^{15}N values by phenology phase (i.e., summer foraging in dark
grey and wintering in light gray). Circles depict actual δ^{13}C and δ^{15}N values. The black dot represents the
mean, and whiskers indicate the standard deviation; statistically significant differences between groups
are shown at the top with F and p values from ANOVA.

356   The smallest isotopic niche areas for the gray whales were observed during the wintering

357   period, consistent with winter fasting (Figure 3). During winter, Er_4 and Er_5 presented the smallest

358   standard ellipse areas (SEA$_C$: 0.07‰^{2} / SEA$_B$: 0.06‰^{2} and SEA$_C$: 0.09‰^{2} / SEA$_B$: 0.07‰^{2}, respectively) and

359 Er_2 and Er_3 presented the largest standard ellipse areas (SEAc: 0.18‰^{2} / SEA$_B$: 0.16‰^{2} and SEAc:

360 0.16‰^{2}/ SEAc: 0.12‰^{2}, respectively). The largest standard ellipse areas of δ^{13}C in summer is consistent

361 with foraging on varied benthic prey in summer with different isotopic compositions (Burnham and

362 Duffus, 2016a; Nelson et al., 2008a; Newell and Cowles, 2006). During summer foraging, Er_3 and

363 Er_4 had the smallest standard ellipse areas (SEAc: 0.03‰2/ SEA$_B$: 0.02‰^{2} and SEAc: 0.13‰^{2}/ SEA$_B$:

364 0.12‰^{2}, respectively) while Er_5 and Er_1 had the largest standard ellipse areas (SEAc: 0.65‰^{2} / SEA$_B$:

365 0.54‰^{2} and SEAc: 0.43‰^{2}/ SEA$_B$: 0.30‰^{2}) (Figure 3; Table 2).

366 **Table 2.** Mean δ^{13}C and δ^{15}N values ± SD for each of the five gray whale baleen plates sampled by season
367 (summer foraging *vs*. wintering). Standard ellipse area corrected for small sample sizes (SEA$_C$, expressed
368 as ‰^{2}), which represents the mean core area of each individual's isotopic niche, and Bayesian standard
369 ellipse areas (SEA$_B$) with credible intervals (CI).

370

ID	Summer foraging					Wintering				
	δ^{13}C (‰)	δ^{15}N (‰)	SEA$_C$ (‰2)	SEA$_b$ (‰2)	CI	δ^{13}C (‰)	δ^{15}N (‰)	SEA$_C$ (‰2)	SEA$_b$ (‰2)	CI
Er 1	-15.7 ± 0.3	12.5 ± 0.2	0.43	0.3	(CI: 0.23-0.42)	-15.9 ± 0.2	13.9 ± 0.2	0.12	0.11	(CI: 0.07-0.16)
Er 2	-16.3 ± 0.3	12.2 ± 0.5	0.37	0.38	(CI: 0.30-0.49)	-16.4 ± 0.1	13.0 ± 0.3	0.18	0.16	(CI: 0.13-0.19)
Er 3	-16.0 ± 0.1	13.2 ± 0.4	0.03	0.02	(CI: 0.02-0.04)	-16.1 ± 0.1	14.1 ± 0.4	0.16	0.12	(CI: 0.10-0.16)
Er 4	-16.0 ± 0.1	13.1 ± 0.4	0.13	0.12	(CI: 0.10-0.17)	-16.3 ± 0.1	13.4 ± 0.5	0.07	0.06	(CI: 0.05-0.08)
Er 5	-15.0 ± 0.1	12.0 ± 0.4	0.65	0.54	(CI: 0.46-0.70)	-15.2 ± 0.4	15.1 ± 0.2	0.09	0.07	(CI: 0.06-0.09)

371
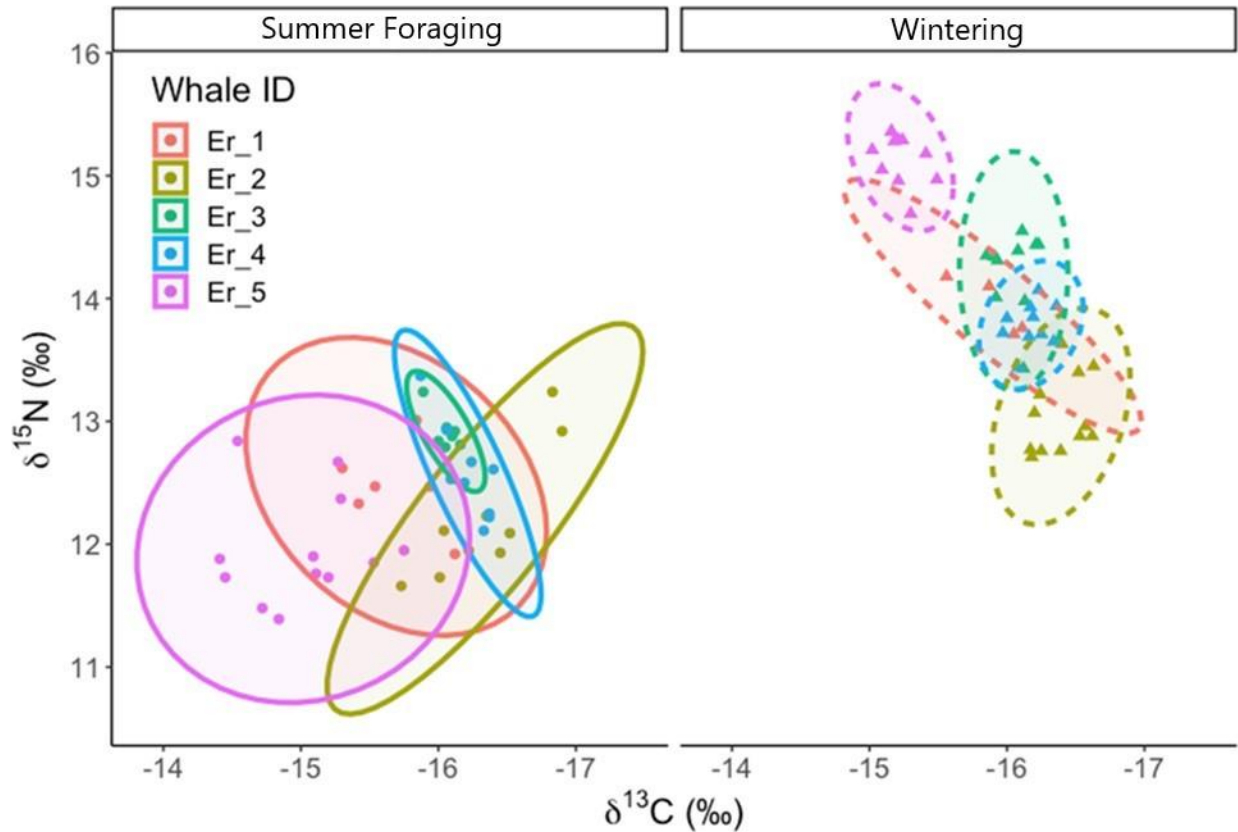
Figure 3. $\delta^{13}C$ and $\delta^{15}N$ biplot illustrating the isotopic niche width of five subadult gray whales that stranded along the Oregon Coast, divided by season (Summer foraging *vs*. wintering). Points within each ellipse represent sub-samples from each sampled baleen plate, and ellipses represent the estimated standard ellipse area corrected for small sample sizes (SEA$_C$, expressed as ‰^{2}).

3.4. 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; Figure 4). The longitudinal profiles of the two hormones generally mirrored each other, with cortisol consistently showing a slightly higher apparent concentration compared to corticosterone at every sampling point along the baleen longitudinal axis (Figure 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 ± 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 (Figure 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

389   the elevation in cortisol to the time of death was estimated to be 284.37 days (13 cm) for Er_2, 240.62

390   days (11 cm) for Er_3, and 262.50 days (12 cm) for Er_4. On average, this elapsed time was 262.5 days

391   (approximately 0.72 years).

392   **Table 3.** Individual baselines of gray whale baleen immunoreactive hormone concentrations (expressed
393   in ng of immunoreactive hormone per g of baleen powder (ng/g)). Baselines are estimated via an
394   iterative process that excluded all data points greater than the mean + 2SD until no points exceeded this
395   maximum value (following Brown *et al.,* 1988). Cortisol = immunoreactive baleen cortisol; Progesterone
396   = immunoreactive baleen progesterone; Testosterone = immunoreactive baleen testosterone; T3 =
397   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

398

Older Baleen → Newer Baleen

Er 1

Er 2

Er 3

Er 4

Er 5
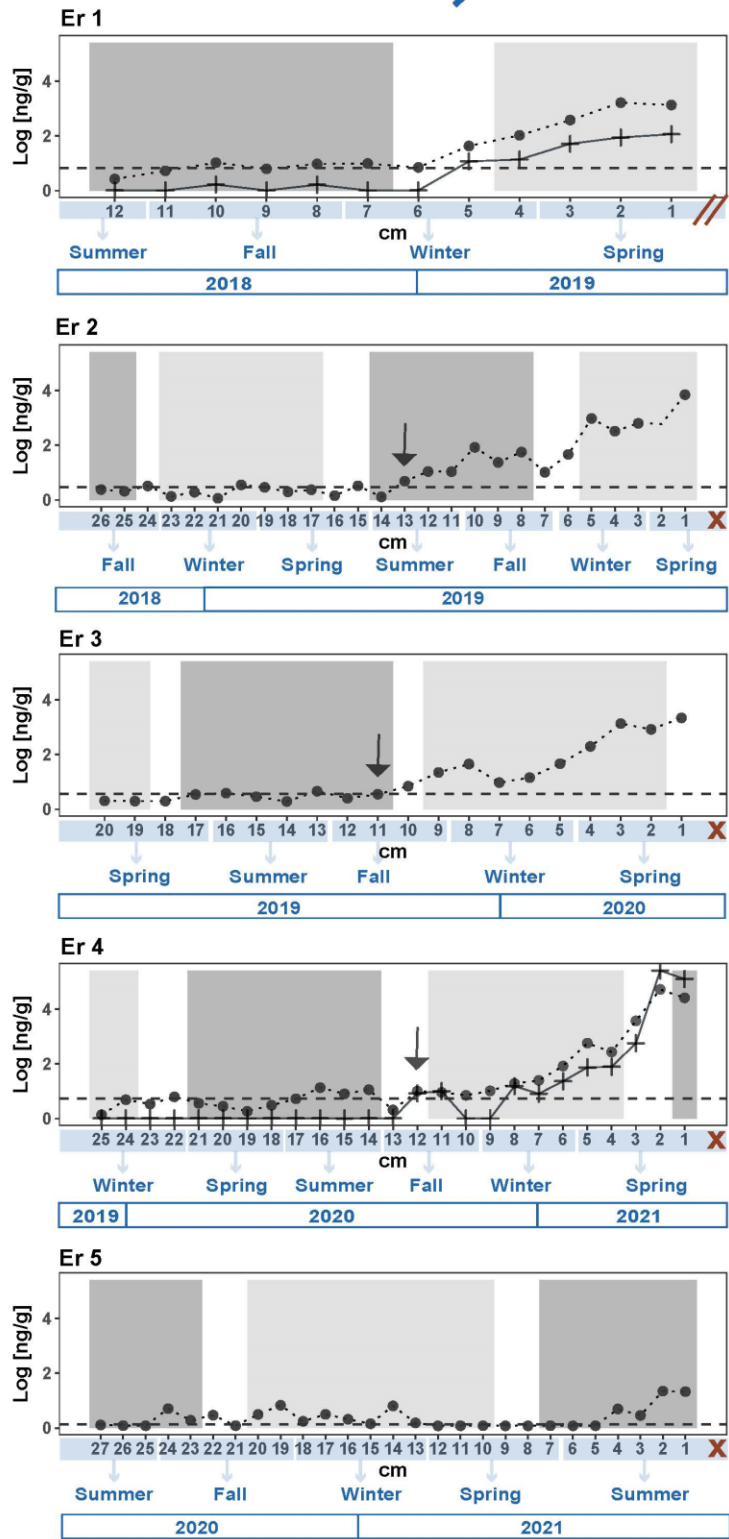
Legend:
// Baleen incomplete   X Death   + Log_Corticosterone   • Log_Cortisol
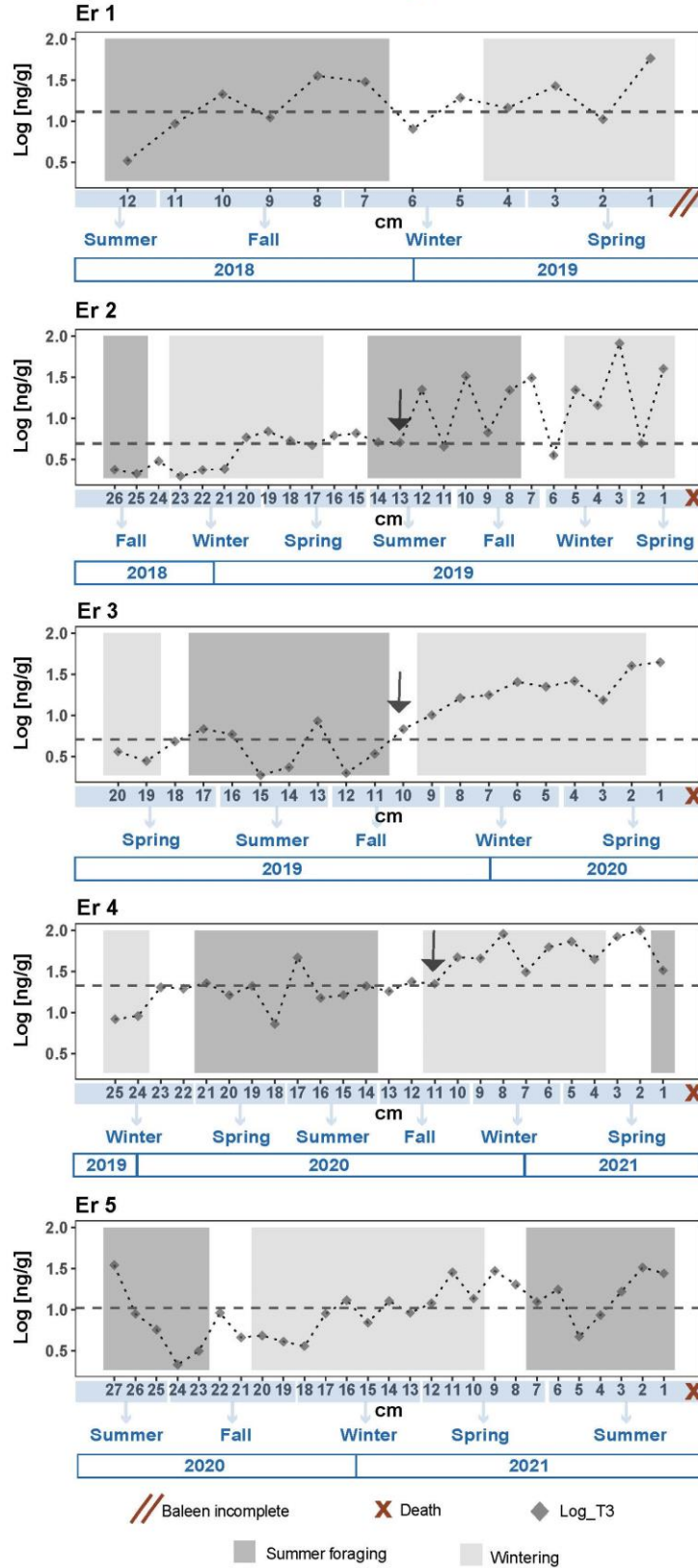
Summer foraging   Wintering

399

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

411 **3.5. TRIIODOTHYRONINE (T3)**

412 Immunoreactive T3 was detectable along the full length of all baleen plates (Figure 5; electronic

413 supplementary material, Table S1). The baseline concentration of T3 ranged from 1.66 ± 1.37 to 3.46 ±

414 1.44 ng/g (mean ± SD; Table 3). Similar to the glucocorticoids, the three individuals with an unknown

415 cause of death that also had a full-length baleen plate (Er_2, Er_3, and Er_4) all had elevated T3

416 preceding death (Figure 5). The onset of the elevation in T3 prior to death was nearly coincident with

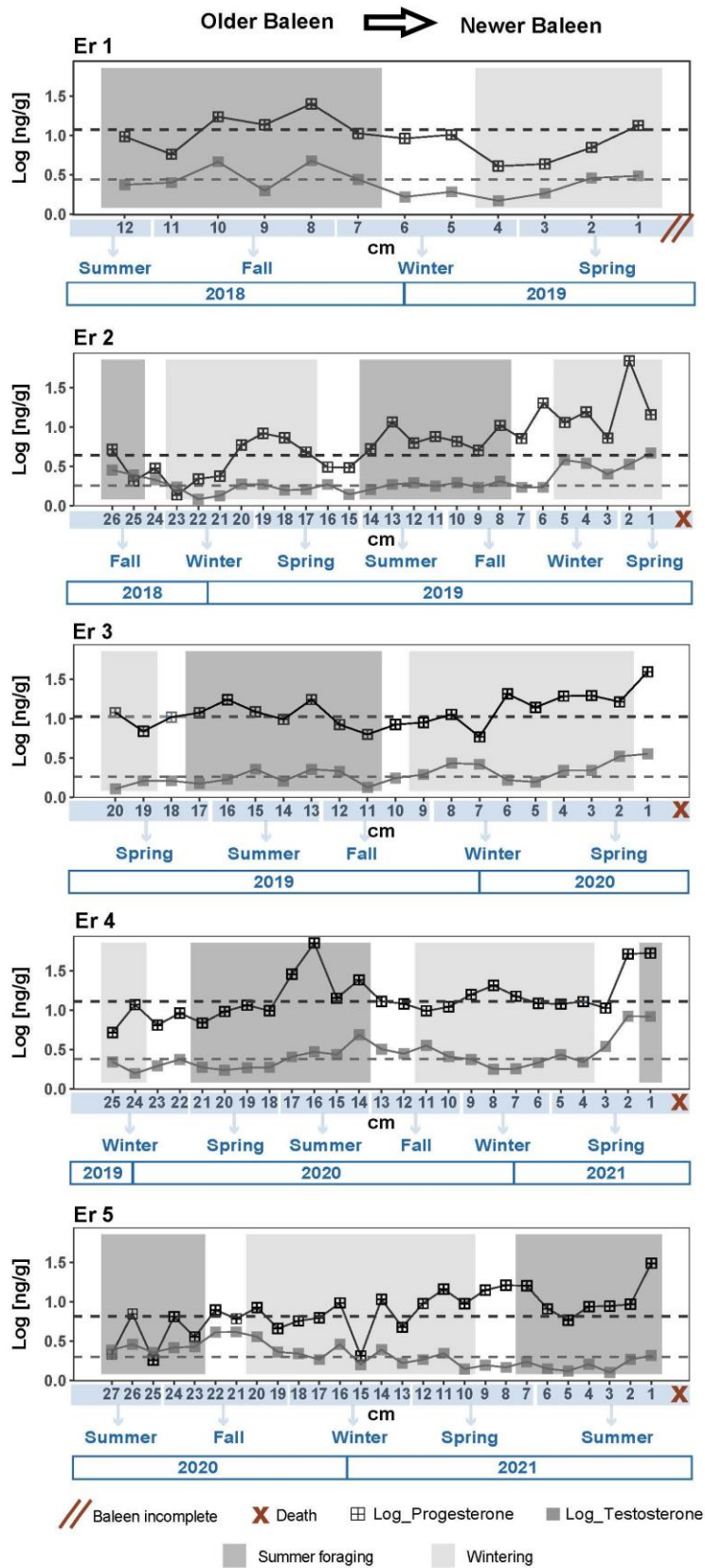417 the timing of elevated cortisol (see Results 3.4).

Older Baleen → Newer Baleen

Er 1

Er 2

Er 3

Er 4

Er 5

// Baleen incomplete   X Death   ◆ Log_T3

■ Summer foraging   ▨ Wintering

418

419   **Figure 5.** Longitudinal profiles of immunoreactive hormone concentrations of triiodothyronine (Log_T3;
420   grey rhomboids and dotted line) across the length of baleen plates from five stranded gray whales. The
421   dashed horizontal line indicates the log-transformed baseline for baleen T3. X-axes show the location of
422   each sample, in cm from base of the baleen plate (i.e., newest baleen = 1 cm) with y-axes showing
423   concentration of hormone (log-transformed ng of immunoreactive hormone per g of dried baleen
424   powder). Migration phenology is derived from δ^{15}N data; dark gray indicates summer foraging and light
425   grey indicates wintering (see Figure 1), and season of growth at each point on the plate was estimated
426   from time of death (noted with a red X on the x-axis). Only the erupted portion of the baleen plate from
427   Er_1 (top) was available, indicated with two parallel red lines on the x-axis. Blue arrows denote the
428   onset of T3 elevation prior to death.

429   **3.6. REPRODUCTIVE HORMONES (PROGESTERONE AND TESTOSTERONE)**

430   Both progesterone and testosterone were detectable along the full length of the baleen plates

431   (Figure 6; electronic supplementary material, Table S1). The baseline concentration for progesterone in

432   all individuals fell within the range of 1.39 ± 1.02 to 2.32 ± 1.08 ng/g (mean ± SD; Table 3), and for

433   testosterone the baseline concentration ranged from 0.35 ± 0.17 to 0.56 ± 0.34 ng/g (mean ± SD; Table

434   3). No significant differences between the two sexes were found in the apparent immunoreactive

435   progesterone and testosterone (p = 0.9999).

Older Baleen ➡ Newer Baleen

Er 1

Er 2

Er 3

Er 4

Er 5

Baleen incomplete ✗ Death ⊞ Log_Progesterone ▪ Log_Testosterone

Summer foraging   Wintering

436

437   **Figure 6:** Longitudinal profiles of immunoreactive hormone concentrations of progesterone
438   (Progesterone; black cross-square) and testosterone (Testosterone; grey solid squares) across the length
439   of baleen plates from five stranded gray whales. The dashed horizontal lines indicate the log-
440   transformed baseline for baleen progesterone (black) and testosterone (light grey). X-axes show the
441   location of each sample, in cm from base of the baleen plate (i.e., newest baleen = 1 cm) with y-axes
442   showing concentration of hormone (log-transformed ng of immunoreactive hormone per g of dried
443   baleen powder). Migration phenology is derived from δ^{15}N data; dark gray indicates summer foraging
444   and light grey indicates wintering (see Figure 1), and season of growth at each point on the plate was
445   estimated from time of death (noted with a red X on the x-axis). Only the erupted portion of the baleen
446   plate from Er_1 (top) was available, indicated with two parallel red lines on the x-axis. Blue arrows
447   denote the onset of T3 elevations prior to death.

448   **4. DISCUSSION AND CONCLUSION**

449   In this study, we analyzed the SI and hormone profiles in baleen of five sub-adult gray whales,

450   which enabled us to estimate baleen growth rates, distinguish isotopic niche widths during summer and

451   winter periods, and document different endocrinology patterns leading up to death between the whale

452   that died acutely (killer whale attack) and those whales of unknown cause of death. The SI assessment

453   revealed distinct oscillation patterns in δ^{15}N values along the baleen plates. The hormone analysis is the

454   first to provide longitudinal profiles of the adrenal, thyroidal, and gonadal axes obtained from the

455   baleen of gray whales in the months leading up to the whales' deaths. Our work demonstrates

456   feasibility of this retrospective approach for gaining insight into gray whale health, physiology, and cause

457   of death.

458   While spatial and temporal variability of stable isotopes in gray whale habitats is still not fully

459   understood (Graham and Bury, 2019; Riekenberg et al., 2021; Trueman and St John Glew, 2019), distinct

460   oscillations in δ^{15}N values along the length of subadult gray whale baleen plates allowed us to 1) infer

461   baleen growth rates (which generally agreed with prior estimates (Caraveo-Patiño et al., 2007b; Sumich,

462   2001); and 2) differentiate standard ellipse areas of individuals during summer foraging and wintering

463   locations. The overlap among standard isotopic niche width of the five gray whales during summer

464   suggests either a similar diet across individuals or a diet based on prey species with similar isotopic

465   values. The difference in niche widths between seasons were mostly driven by the low δ^{15}N values

466   during summer, likely attributed to the benthic foraging of gray whales on lower trophic level organisms

467   including amphipods (Burnham and Duffus, 2016b), mysids (Newell and Cowles, 2006), crab larvae

468   (Nelson et al., 2008b), shrimps and herring roe (Darling et al., 1998).

469   The larger standard ellipse areas during summer may reflect a diet of a wide range of prey

470   sources in different areas, whereas the smaller standard ellipse areas during winter - mostly driven by

471   high δ^{15}N values - are likely related to the fasting period of gray whales during the winter, given that the

472   fasting in mammals is usually associated with increased δ^{15}N values due to catabolic or anabolic

473   processes (Fuller et al., 2005; Lee et al., 2012). In accordance with previous reports (Caraveo-Patiño et

474   al., 2007b), there was no distinguishable annual δ^{13}C pattern in the gray whale baleen plates. In whale

475   species that migrate between ocean-basins with large gradients in plankton δ^{13}C, δ^{13}C patterns along the

476   baleen reflect seasonal cycles of movements and diet between summer/winter grounds. In bowhead

477   whales, for example, annual oscillations in δ^{13}C values are common, presumably resulting from feeding

478   along the whale's annual migratory route that reflects the contrasting geographic isotope values in

479   zooplankton prey found in the Bering (^{13}C-enriched) and Beaufort Seas (^{13}C-depleted) (Saupe et al.,

480   1989; Schell et al., 1989). To better understand the isotopic patterns observed in gray whales and how

481   they reflect the energetic pathways of their summer/winter grounds, future studies may use different

482   analytical approaches including: the use of *isoscapes* (i.e., stable isotope mapping; (Graham and Bury,

483   2019; Trueman and St John Glew, 2019); the combination of bulk and compound-specific amino acid

484   analysis to disentangle the relative contributions of trophic and baseline variation in δ^{13}C and δ^{15}N

485   values (e.g., Riekenberg et al., 2021); and/or integrating isotopic data from prey sources in isotopic

486   mixing models to assess the proportional contribution of each prey sources in their diet.

487   In the glucocorticoid (GC) analyses, we found that both cortisol and corticosterone were

488   detectable along the full length of baleen from two individuals (Er_1 & Er_4), but cortisol was

489   consistently more abundant than corticosterone. Furthermore, corticosterone exhibited similar patterns

490   to cortisol, i.e., corticosterone seemingly did not provide additional information. This pattern generally

491   aligns with traditional assumptions of "cortisol dominance" in mysticetes (primarily based on rare

492   plasma samples from hunted specimens) as well as assumptions that only the more abundant GC need

493   be analyzed, but contrasts with recent findings of more corticosterone than cortisol in baleen of other

494   mysticetes (Fernández Ajó et al., 2018; Hunt et al., 2017a; Lowe et al., 2021a). Most mammals produce

495   both glucocorticoids, and some data indicate the two hormones can respond differently to exogenous

496   stressors, depending on the type and duration of the stressor (Koren et al., 2012). Given our small

497   sample size, we encourage future research on both GCs to further investigate whether they might show

498   species-specific differences or individual differences in glucocorticoid dominance or might provide

499   differing information for acute vs. chronic stressors.

500   Cortisol profiles for the four individuals with unknown cause of death demonstrated a long-term

501   elevation in cortisol that began an estimated 8 months before death. In contrast, the individual known

502   to have died acutely due to killer whale predation (Er_5) had cortisol concentrations that approximate

503   baseline levels across the span of the baleen, suggesting that this individual was in good health before

504   its acute death. These data suggest that the other four individuals experienced a prolonged period of

505   stress. Generally, individual perception of a stressor activates the HPA-axis, leading to an increase in

506   circulating GC levels. Short-term elevations in GCs are thought to aid animals in coping with the stressor

507   (McEwen and Wingfield, 2010; Romero et al., 2009; Romero and Wingfield, 2016), but if the

508   perturbation is severe and/or chronic, the individual deviates from its current life-history stage and

509   enters an "emergency life-history stage", during which all activities not essential for immediate survival

510   are suppressed (Romero and Wingfield, 2016; Wingfield, 2005; Wingfield et al., 1998). Consequently,

511   chronic elevation of GCs can itself have negative effects on long-term health, through

512   immunosuppression, reduced growth, and inhibition of reproduction (Buck et al., 2007; Dhabhar, 2009,

513   2000; Dhabhar et al., 2012; Kitaysky et al., 1999). Therefore, although the immediate cause of death

514   remains unknown for these four individuals, it is conceivable that the prolonged elevation of cortisol

515   may eventually have directly impacted health and survival, i.e., in addition to any direct negative effects

516   of the stressor itself (Romero and Wingfield, 2016). The presence of "rake marks" attributed to killer

517   whale interactions could provide evidence for increased vulnerability of these individuals. It is plausible

518   that whales undergoing chronic illness might become more susceptible to predation and other threats.

519   T3, like cortisol, tended to show a gradual, months-long increase in the four whales of unknown

520   cause of death (albeit with high individual variability), but remained relatively stable in the whale with

521   an acute cause of death. The simultaneous elevation of both cortisol and T3 was unexpected, as the HPA

522   axis is classically thought to inhibit the HPT axis (Behringer et al., 2018). In fact, elevated GCs in

523   mammals often directly downregulate the HPT axis, resulting in decreases in circulating T3 (Charmandari

524   et al., 2005). However, this downregulation can be temporary (Nicoloff et al., 1970). Further, emerging

525   data indicate that in marine species, T3 may elevate simultaneously with the GCs during those stressors

526   that require increased energetic output, such as swimming while entangled in fishing gear (Hunt et al.,

527   2016a; Lemos et al., 2020; Lysiak et al., 2018). In mammals, T3 can also elevate during thermoregulatory

528   challenges, as elevated T3 directly raises metabolic rate, which elevates body temperature (Behringer et

529   al., 2018; Williams et al., 2019). Indeed, (Lemos et al., 2022b) found that gray whales in poor body

530   condition exhibited higher thyroid hormone concentrations in feces, compared to whales in good body

531   condition, suggesting a possible thermoregulatory influence on T3. In other words, poor body condition

532   in cetaceans entails thinning of the insulative blubber layer and might therefore require a compensatory

533   elevation in metabolic rate and thus an elevation in T3. Similarly, fecal thyroid hormones may reflect

534   changes in food availability (Ayres et al., 2012; Wasser et al., 2017), while psychological/perceptual

535   stressors, i.e., vessel traffic or harassment, are thought to have little impact on T3 levels in whales (Ayres

536   et al., 2012; Fernández Ajó et al., 2020). In our study, all five of our study whales died during the current

537   UME, which generally has been linked to poor nutrition and emaciation (Christiansen et al., 2021).

538   Though it is tempting to ascribe the gradual elevation in T3 seen here to the poor body condition

539   reported in gray whales during the UME generally, the necropsy reports of these five individuals did not

540   describe severe emaciation. However, it is possible that these whales were in relatively lower body

541   condition with respect to the population mean. Overall, we speculate that whales in poor body

542   condition may elevate T3 in response to thermoregulatory demands. This hypothesis could be tested

543   with further comparisons of baleen from stranded whales in poor vs. good body condition, ideally with

544   measurements of body condition, e.g., blubber thickness or body area index derived from drone-based

545   photogrammetry (Bierlich et al., 2021; Burnett et al., 2019). Finally, T3 also commonly varies across life

546   history stages (Wilsterman et al., 2015), and thus studies of T3 patterns in baleen of juveniles as

547   compared to adults may be informative.

548   We also quantified reproductive hormones across the baleen's entire length in four subadult

549   males and one subadult female. To our knowledge, these are the first longitudinal profiles of

550   reproductive hormones from gray whales across a full calendar year. As expected for this reproductive

551   age class (subadults), we did not observe temporal patterns, cyclical trends, or elevated hormone

552   concentrations, suggesting none of the subadults had yet reached sexual maturity. Nevertheless, our

553   results add to knowledge about expected baselines of reproductive hormones in subadults and may thus

554   inform future efforts to identify onset of sexual maturity. Our results also underscore the potential to

555   capture at least one year of information from adult gray whale baleen, as there have been uncertainties

556   about the feasibility of capturing complete pregnancies or multiple pregnancies within gray whale

557   baleen (max baleen length ~30 cm), or whether seasonal testosterone cyclicity in males could be

558   discerned. Our subadult baleen specimens captured an estimated timeframe of 1.3 years. Further, adult

559   baleen generally captures a longer timespan than subadult baleen (since subadults not only might have

560   shorter baleen but also tend to have faster baleen growth rate); thus, these results suggest that adult

561   gray whale baleen may capture a sufficient timeframe to examine at least one if not two prior

562   reproductive cycles.

563   The ENP gray whale population has rebounded from a dramatic decline attributed to whaling

564   from less than 4,000 by 1900 to a peak abundance estimated at 26,916 individuals (Stewart and Weller,

565   2021; Swartz et al., 2006). However, the ENP gray whale population has exhibited significant

566   fluctuations, marked by two Unusual Mortality Events (UMEs) that curtailed population size,

567   underscoring the susceptibility of gray whales to oceanic conditions, resource availability, and other

568   influences (Torres et al., 2022). The occurrence of recurrent UMEs with often-unknown causes in the

569   ENP gray whale population highlights the necessity for innovative methodologies to investigate and

570   better understand the causes of death and physiological response of individuals to fluctuations in the

571   environment. Despite the characteristic shorter length of gray whale baleen compared to other

572   mysticete species, and thus the relatively brief period of longitudinal data that can be inferred, even

573   subadult gray whale baleen captures a >1 year timespan, and adult baleen specimens may capture a

574   longer timeframe. In sum, baleen analysis in gray whales allows assessment of physiological status of at

575   least the past year and may enable inferences as to the cause of death (acute *vs*. chronic, nutritional vs.

576   non-nutritional stress). Overall, baleen analysis has emerged as powerful tool that enables a

577   comprehensive and retrospective assessment of gray whale hormonal profiles, stress responses,

578   reproductive status, and foraging ecology in the months or years leading up to their death.

579   **5. REFERENCES**

580   Ambrose, S.H., 1990. Preparation and characterization of bone and tooth collagen for isotopic analysis. J
581   Archaeol Sci 17, 431–451. https://doi.org/10.1016/0305-4403(90)90007-R

582   Ayres, K.L., Booth, R.K., Hempelmann, J.A., Koski, K.L., Emmons, C.K., Baird, R.W., Balcomb-Bartok, K.,
583   Hanson, M.B., Ford, M.J., Wasser, S.K., 2012. Distinguishing the impacts of inadequate prey and
584   vessel traffic on an endangered killer whale (*Orcinus orca*) population. PLoS One 7.
585   https://doi.org/10.1371/journal.pone.0036842

586   Behringer, V., Deimel, C., Hohmann, G., Negrey, J., Schaebs, F.S., Deschner, T., 2018. Applications for
587   non-invasive thyroid hormone measurements in mammalian ecology, growth, and maintenance.
588   Horm Behav 105, 66–85. https://doi.org/10.1016/j.yhbeh.2018.07.011

589   Best PB, Schell DM. 1996. Stable isotopes in southern right whale (*Eubalaena australis*) baleen as
590   indicators of seasonal movements, feeding and growth. Marine Biology 124:483-94.

591   Bierlich, K.C., Hewitt, J., Bird, C.N., Schick, R.S., Friedlaender, A., Torres, L.G., Dale, J., Goldbogen, J.,
592   Read, A.J., Calambokidis, J., Johnston, D.W., 2021. Comparing uncertainty associated with 1-, 2-,
593   and 3d aerial photogrammetry-based body condition measurements of baleen whales. Front Mar
594   Sci 8. https://doi.org/10.3389/fmars.2021.749943

595   Brown, J.L., Goodrowe, K.L., Simmons, L.G., Armstrong, D.L., Wildt, D.E., 1988. Evaluation of the

596   pituitary-gonadal response to GnRH, and adrenal status, in the leopard (*Panthera pardus*

597   *japonensis*) and tiger (*Panthera tigris*). Reproduction 82, 227–236.

598   https://doi.org/10.1530/jrf.0.0820227

599   Buck, C.L., O'Reilly, K.M., Kildaw, S.D., 2007. Interannual variability of Black-legged Kittiwake productivity

600   is reflected in baseline plasma corticosterone. Gen Comp Endocrinol 150.

601   https://doi.org/10.1016/j.ygcen.2006.10.011

602   Burnett, J.D., Lemos, L., Barlow, D., Wing, M.G., Chandler, T., Torres, L.G., 2019. Estimating

603   morphometric attributes of baleen whales with photogrammetry from small UASs: A case study

604   with blue and gray whales. Mar Mamm Sci 35. https://doi.org/10.1111/mms.12527

605   Burnham, R.E., Duffus, D.A., 2016a. Gray Whale (*Eschrichtius robustus*) predation and the demise of

606   amphipod prey reserves in Clayoquot Sound, British Columbia. Aquat Mamm 42, 123–126.

607   https://doi.org/10.1578/AM.42.2.2016.123

608   Busquets-Vass, G., Newsome, S.D., Calambokidis, J., Serra-Valente, G., Jacobsen, J.K., Aguíñiga-García, S.,

609   Gendron, D., 2017. Estimating blue whale skin isotopic incorporation rates and baleen growth

610   rates: Implications for assessing diet and movement patterns in mysticetes. PLoS One 12.

611   https://doi.org/10.1371/journal.pone.0177880

612   Caraveo-Patiño, J., Hobson, K.A., Soto, L.A., 2007b. Feeding ecology of gray whales inferred from stable-

613   carbon and nitrogen isotopic analysis of baleen plates. Hydrobiologia 586, 17–25.

614   https://doi.org/10.1007/s10750-006-0477-5

615   Charmandari, E., Tsigos, C., Chrousos, G., 2005. Endocrinology of the stress response. Annu Rev Physiol

616   67, 259–284. https://doi.org/10.1146/annurev.physiol.67.040403.120816

617   Christiansen, F., Rodríguez-González, F., Martínez-Aguilar, S., Urbán, J., Swartz, S., Warick, H., Vivier, F.,

618   Bejder, L., 2021. Poor body condition associated with an unusual mortality event in gray whales.

619   Mar Ecol Prog Ser 658, 237–252. https://doi.org/10.3354/meps13585

620   Clapham, P., 2016. Managing Leviathan: conservation challenges for the great whales in a post-whaling

621   world. Oceanography 29, 214–225. https://doi.org/10.5670/oceanog.2016.70

622   Corsi, E., Calambokidis, J., Flynn, K.R., Steiger, G.H., 2022. Killer whale predatory scarring on mysticetes:

623   A comparison of rake marks among blue, humpback, and gray whales in the eastern North Pacific.

624   Mar Mamm Sci 38, 223–234. https://doi.org/10.1111/mms.12863

625   D'Agostino, V.C., Fernández Ajó, A., Degrati, M., Krock, B., Hunt, K.E., Uhart, M.M., Buck, C.L., 2022.

626   Potential endocrine correlation with exposure to domoic acid in Southern Right Whale (*Eubalaena*

627   *australis*) at the Península Valdés breeding ground. Oecologia 198, 21–34.

628   https://doi.org/10.1007/s00442-021-05078-4

629   Darling, J.D., Keogh, K.E., Steeves, T.E., 1998. GRAY WHALE (*Eschrichtius robustus*) habitat utilization and

630   prey species off Vancouver island, B. C. Mar Mamm Sci 14, 692–720.

631   https://doi.org/10.1111/j.1748-7692.1998.tb00757.x

632   Derville, S., Buell, T.V., Corbett, K.C., Hayslip, C., Torres, L.G., 2023. Exposure of whales to entanglement

633   risk in Dungeness crab fishing gear in Oregon, USA, reveals distinctive spatio-temporal and climatic

634   patterns. Biol Conserv 281, 109989. https://doi.org/10.1016/j.biocon.2023.109989

635   Derville, Solène, Torres, L.G., Newsome, S.D., Somes, C.J., Valenzuela, L.O., Vander Zanden, H.B., Baker,

636   C.S., Bérubé, M., Busquets-Vass, G., Carlyon, K., Childerhouse, S.J., Constantine, R., Dunshea, G.,

637   Flores, P.A.C., Goldsworthy, S.D., Graham, B., Groch, K., Gröcke, D.R., Harcourt, R., Hindell, M.A.,

638   Hulva, P., Jackson, J.A., Kennedy, A.S., Lundquist, D., Mackay, A.I., Neveceralova, P., Oliveira, L.,

639   Ott, P.H., Palsbøll, P.J., Patenaude, N.J., Rowntree, V., Sironi, M., Vermeuelen, E., Watson, M.,

640   Zerbini, A.N., Carroll, E.L., 2023. Long-term stability in the circumpolar foraging range of a Southern

641   Ocean predator between the eras of whaling and rapid climate change. Proceedings of the

642   National Academy of Sciences 120. https://doi.org/10.1073/pnas.2214035120

643   Dettmer, A.M., Chusyd, D.E., 2023. Early life adversities and lifelong health outcomes: A review of the

644   literature on large, social, long-lived nonhuman mammals. Neurosci Biobehav Rev 152, 105297.

645   https://doi.org/10.1016/j.neubiorev.2023.105297

646   Dhabhar, F.S., 2009. Enhancing versus suppressive effects of stress on immune function: implications for

647   immunoprotection and immunopathology. Neuroimmunomodulation 16, 300–317.

648   https://doi.org/10.1159/000216188

649   Dhabhar, F.S., 2000. Acute stress enhances while chronic stress suppresses skin immunity: the role of

650   stress hormones and leukocyte trafficking. Ann N Y Acad Sci 917, 876–893.

651   https://doi.org/10.1111/j.1749-6632.2000.tb05454.x

652 Dhabhar, F.S., Malarkey, W.B., Neri, E., McEwen, B.S., 2012. Stress-induced redistribution of immune

653   cells—From barracks to boulevards to battlefields: A tale of three hormones – Curt Richter Award

654   Winner. Psychoneuroendocrinology 37, 1345–1368.

655   https://doi.org/10.1016/j.psyneuen.2012.05.008

656 Eales, J.G., 1988. The influence of nutritional state on thyroid function in various vertebrates. Am Zool

657   28, 351–362. https://doi.org/10.1093/icb/28.2.351

658 Eguchi, T., Lang, A.R., Weller, D.W., 2023. NOAA Technical Memorandum NMFS Eastern north pacific

659   gray whale calf production 1994-2023. https://doi.org/10.25923/e9at-x936

660 Fernández Ajó, A., Hunt, K.E., Dillon, D., Uhart, M., Sironi, M., Rowntree, V., Loren Buck, C., 2022.

661   Optimizing hormone extraction protocols for whale baleen: Tackling questions of solvent:sample

662   ratio and variation. Gen Comp Endocrinol 315, 113828.

663   https://doi.org/10.1016/j.ygcen.2021.113828

664 Fernández Ajó, A.A., Hunt, K.E., Giese, A.C., Sironi, M., Uhart, M., Rowntree, V.J., Marón, C.F., Dillon, D.,

665   DiMartino, M., Buck, C.L., 2020. Retrospective analysis of the lifetime endocrine response of

666   southern right whale calves to gull wounding and harassment: A baleen hormone approach. Gen

667   Comp Endocrinol 296, 113536. https://doi.org/10.1016/j.ygcen.2020.113536

668 Fernández Ajó, A.A., Hunt, K.E., Uhart, M., Rowntree, V., Sironi, M., Marón, C.F., Di Martino, M., Buck,

669   C.L., 2018. Lifetime glucocorticoid profiles in baleen of right whale calves: potential relationships to

670   chronic stress of repeated wounding by Kelp Gulls. Conserv Physiol 6, 1–12.

671   https://doi.org/10.1093/conphys/coy045

672 Flamant, F., Cheng, S.Y., Hollenberg, A.N., Moeller, L.C., Samarut, J., Wondisford, F.E., Yen, P.M.,

673   Refetoff, S., 2017. Thyroid hormone signaling pathways: Time for a more precise nomenclature.

674   Endocrinology. https://doi.org/10.1210/en.2017-00250

675 Fleming, A.H., Kellar, N.M., Allen, C.D., Kurle, C.M., 2018. The utility of combining stable isotope and

676   hormone analyses for marine megafauna research. Front Mar Sci 5.

677   https://doi.org/10.3389/fmars.2018.00338

678 Fuller, B.T., Fuller, J.L., Sage, N.E., Harris, D.A., O'Connell, T.C., Hedges, R.E.M., 2005. Nitrogen balance

679   and $\delta 15N$: Why you're not what you eat during nutritional stress. Rapid Communications in Mass

680   Spectrometry 19. https://doi.org/10.1002/rcm.2090

681 Graham, B., Bury, S., 2019. Marine Isoscapes for trophic and animal movement studies in the southwest
682   Pacific Ocean, New Zealand Aquatic Environment and Biodiversity Report No. 2018.

683 Gulland, F., Pérez-Cortés, H., Urbán, J.R., Rojas-Bracho, L., Ylitalo, G., Weir, J., Norman, S., Muto, M.,
684   Rugh, D., Kreuder, C., Rowles, T., 2005. Eastern North Pacific gray whale (*Eschrichtius robustus*)
685   unusual mortality event, 1999-2000. U.S. Department of Commerce. NOAA Technical
686   Memorandum. NMFS-AFSC-150. 33 pp.

687 Hunt, K.E., Buck, C.L., Ferguson, S.H., Fernández Ajo, A., Heide-Jørgensen, M.P., Matthews, C.J.D., 2022.
688   Male bowhead whale reproductive histories inferred from baleen testosterone and stable
689   isotopes. Integrative Organismal Biology 4. https://doi.org/10.1093/iob/obac014

690 Hunt, K.E., Innis, C.J., Merigo, C., Rolland, R.M., 2016a. Endocrine responses to diverse stressors of
691   capture, entanglement and stranding in leatherback turtles (*Dermochelys coriacea*). Conserv
692   Physiol 4, cow022. https://doi.org/10.1093/conphys/cow022

693 Hunt, K.E., Lysiak, N.S., Moore, M., Rolland, R.M., 2017a. Multi-year longitudinal profiles of cortisol and
694   corticosterone recovered from baleen of North Atlantic right whales (Eubalaena glacialis). Gen
695   Comp Endocrinol 254, 50–59. https://doi.org/10.1016/j.ygcen.2017.09.009

696 Hunt, K.E., Lysiak, N.S., Moore, M.J., Rolland, R.M., 2016b. Longitudinal progesterone profiles in baleen
697   from female North Atlantic right whales (*Eubalaena glacialis*) match known calving history.
698   Conserv Physiol 4, cow014. https://doi.org/10.1093/conphys/cow014

699 Hunt, K.E., Lysiak, N.S., Robbins, J., Moore, M.J., Seton, R.E., Torres, L., Loren Buck, C., Buck, C.L., 2017b.
700   Multiple steroid and thyroid hormones detected in baleen from eight whale species. Conserv
701   Physiol 5. https://doi.org/10.1093/conphys/cox061

702 Hunt, K.E., Lysiak, N.S.J., Matthews, C.J.D., Lowe, C., Fernández Ajó, A., Dillon, D., Willing, C., Heide-
703   Jørgensen, M.P., Ferguson, S.H., Moore, M.J., Buck, C.L., 2018. Multi-year patterns in testosterone,
704   cortisol and corticosterone in baleen from adult males of three whale species. Conserv Physiol 6,
705   1–16. https://doi.org/10.1093/conphys/coy049

706 Hunt, K.E., Moore, M.J., Rolland, R.M., Kellar, N.M., Hall, A.J., Kershaw, J., Raverty, S.A., Davis, C.E.,
707   Yeates, L.C., Fauquier, D.A., Rowles, T.K., Kraus, S.D., 2013. Overcoming the challenges of studying
708   conservation physiology in large whales: a review of available methods. Conserv Physiol 1, cot006–
709   cot006. https://doi.org/10.1093/conphys/cot006

710   Hunt, K.E., Stimmelmayr, R., George, C., Hanns, C., Suydam, R., Brower, H., Rolland, R.M., 2014. Baleen

711   hormones: a novel tool for retrospective assessment of stress and reproduction in bowhead

712   whales (*Balaena mysticetus*). Conserv Physiol 2, cou030–cou030.

713   https://doi.org/10.1093/conphys/cou030

714   Jackson, A.L., Inger, R., Parnell, A.C., Bearhop, S., 2011. Comparing isotopic niche widths among and

715   within communities: SIBER - Stable Isotope Bayesian Ellipses in R. Journal of Animal Ecology 80,

716   595–602. https://doi.org/10.1111/j.1365-2656.2011.01806.x

717   Kelly, J.F., 2000. Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic

718   ecology. Can J Zool 78, 1–27. https://doi.org/10.1139/z99-165

719   Kitaysky, A.S., Wingfield, J.C., Piatt, J.F., 1999. Dynamics of food availability, body condition and

720   physiological stress response in breeding Black-legged Kittiwakes. Funct Ecol 13, 577–584.

721   https://doi.org/10.1046/j.1365-2435.1999.00352.x

722   Koren, L., Whiteside, D., Fahlman, Å., Ruckstuhl, K., Kutz, S., Checkley, S., Dumond, M., Wynne-Edwards,

723   K., 2012. Cortisol and corticosterone independence in cortisol-dominant wildlife. Gen Comp

724   Endocrinol 177. https://doi.org/10.1016/j.ygcen.2012.02.020

725   Layman, C.A., Arrington, D.A., Montaña, C.G., Post, D.M., 2007. Can stable isotope ratios provide for

726   community-wide measures of trophic structure? Ecology 88, 42–48. https://doi.org/10.1890/0012-

727   9658(2007)88[42:CSIRPF]2.0.CO;2

728   Lee, T.N., Buck, C.L., Barnes, B.M., O'Brien, D.M., 2012. A test of alternate models for increased tissue

729   nitrogen isotope ratios during fasting in hibernating arctic ground squirrels. Journal of

730   Experimental Biology. https://doi.org/10.1242/jeb.068528

731   Lemos, L.S., Haxel, J.H., Olsen, A., Burnett, J.D., Smith, A., Chandler, T.E., Nieukirk, S.L., Larson, S.E., Hunt,

732   K.E., Torres, L.G., 2022a. Effects of vessel traffic and ocean noise on gray whale stress hormones.

733   Sci Rep 12. https://doi.org/10.1038/s41598-022-14510-5

734   Lemos, L.S., Olsen, A., Smith, A., Burnett, J.D., Chandler, T.E., Larson, S., Hunt, K.E., Torres, L.G., 2022b.

735   Stressed and slim or relaxed and chubby? A simultaneous assessment of gray whale body condition

736   and hormone variability. Mar Mamm Sci 38, 801–811. https://doi.org/10.1111/mms.12877

737 Lemos, L.S., Olsen, A., Smith, A., Chandler, T.E., Larson, S., Hunt, K., Torres, L.G., 2020. Assessment of

738   fecal steroid and thyroid hormone metabolites in eastern North Pacific gray whales. Conserv

739   Physiol 8. https://doi.org/10.1093/conphys/coaa110

740 Lowe, C.L., Hunt, K.E., Robbins, J., Seton, R.E., Rogers, M., Gabriele, C.M., Neilson, J.L., Landry, S.,

741   Teerlink, S.S., Buck, C.L., 2021a. Patterns of cortisol and corticosterone concentrations in

742   humpback whale (*Megaptera novaeangliae*) baleen are associated with different causes of death.

743   Conserv Physiol 9. https://doi.org/10.1093/conphys/coab096

744 Lowe, C.L., Hunt, K.E., Rogers, M.C., Neilson, J.L., Robbins, J., Gabriele, C.M., Teerlink, S.S., Seton, R.,

745   Buck, C.L., 2021b. Multi-year progesterone profiles during pregnancy in baleen of humpback

746   whales (*Megaptera novaeangliae*). Conserv Physiol 9. https://doi.org/10.1093/conphys/coab059

747 Lowe, C.L., Jordan-Ward, R., Hunt, K.E., Rogers, M.C., Werth, A.J., Gabriele, C., Neilson, J., von Hippel,

748   F.A., Buck, C.L., 2022. Case studies on longitudinal mercury content in humpback whale

749   (*Megaptera novaeangliae*) baleen. Heliyon 8, e08681.

750   https://doi.org/10.1016/j.heliyon.2021.e08681

751 Lysiak, N. 2009. Investigating the migration and foraging ecology of North Atlantic right whales with

752   stable isotope geochemistry of baleen and zooplankton. Ph.D. dissertation, Boston University,

753   Boston, MA, USA.

754 Lysiak, N.S.J., Ferguson, S.H., Hornby, C.A., Heide-Jørgensen, M.P., Matthews, C.J.D., 2023. Prolonged

755   baleen hormone cycles suggest atypical reproductive endocrinology of female bowhead whales. R

756   Soc Open Sci 10. https://doi.org/10.1098/rsos.230365

757 Lysiak, N.S.J., Trumble, S.J., Knowlton, A.R., Moore, M.J., 2018. Characterizing the duration and severity

758   of fishing gear entanglement on a North Atlantic Right Whale (*Eubalaena glacialis*) using stable

759   isotopes, steroid and thyroid hormones in Baleen. Front Mar Sci 5.

760   https://doi.org/10.3389/FMARS.2018.00168

761 Matthews, C.J.D., Ferguson, S.H., 2015. Seasonal foraging behaviour of eastern Canada-West Greenland

762   bowhead whales: An assessment of isotopic cycles along baleen. Mar Ecol Prog Ser 522, 269–286.

763   https://doi.org/10.3354/meps11145

764 McAninch, E.A., Bianco, A.C., 2014. Thyroid hormone signaling in energy homeostasis and energy

765   metabolism. Ann N Y Acad Sci 1311, 77–87. https://doi.org/10.1111/nyas.12374

766   McEwen, B.S., Wingfield, J.C., 2010. What is in a name? Integrating homeostasis, allostasis and stress.

767   Horm Behav 57, 105–111. https://doi.org/10.1016/j.yhbeh.2009.09.011

768   Mullur, R., Liu, Y.-Y., Brent, G.A., 2014. Thyroid Hormone Regulation of Metabolism. Physiol Rev 94, 355–

769   382. https://doi.org/10.1152/physrev.00030.2013

770   Nelson, T.A., Duffus, D.A., Robertson, C., Feyrer, L.J., 2008a. Spatial-temporal patterns in intra-annual

771   gray whale foraging: Characterizing interactions between predators and prey in Clayquot Sound,

772   British Columbia, Canada. Mar Mamm Sci 24, 356–370. https://doi.org/10.1111/j.1748-

773   7692.2008.00190.x

774   Newell, C.L., Cowles, T.J., 2006. Unusual gray whale *Eschrichtius robustus* feeding in the summer of 2005

775   off the central Oregon Coast. Geophys Res Lett 33, L22S11. https://doi.org/10.1029/2006GL027189

776   Nicoloff, J.T., Fisher, D.A., Appleman, M.D., 1970. The role of glucocorticoids in the regulation of thyroid

777   function in man. Journal of Clinical Investigation 49, 1922–1929.

778   https://doi.org/10.1172/JCI106411

779   Pallin, L.J., Kellar, N.M., Steel, D., Botero-Acosta, N., Baker, C.S., Conroy, J.A., Costa, D.P., Johnson, C.M.,

780   Johnston, D.W., Nichols, R.C., Nowacek, D.P., Read, A.J., Savenko, O., Schofield, O.M.,

781   Stammerjohn, S.E., Steinberg, D.K., Friedlaender, A.S., 2023. A surplus no more? Variation in krill

782   availability impacts reproductive rates of Antarctic baleen whales. Glob Chang Biol 29, 2108–2121.

783   https://doi.org/10.1111/gcb.16559

784   Peterson, B.J., Fry, B., 1987. Stable isotopes in ecosystem studies. Annu Rev Ecol Syst 18, 293–320.

785   https://doi.org/10.1146/annurev.es.18.110187.001453

786   Pirotta, E., Thomas, L., Costa, D.P., Hall, A.J., Harris, C.M., Harwood, J., Kraus, S.D., Miller, P.J.O., Moore,

787   M.J., Photopoulou, T., Rolland, R.M., Schwacke, L., Simmons, S.E., Southall, B.L., Tyack, P.L., 2022.

788   Understanding the combined effects of multiple stressors: A new perspective on a longstanding

789   challenge. Science of The Total Environment 821, 153322.

790   https://doi.org/10.1016/j.scitotenv.2022.153322

791   Reckendorf, A., S.U., P.E., & D.K., 2023. Marine Mammals. Springer International Publishing, Cham.

792   https://doi.org/10.1007/978-3-031-06836-2

793   Rice, D.W., Wolman, A.A., 1971. Life history and ecology of the gray whale (*Eschrichtius robustus*),

794   American Society of Mammalogists. American Society of Mammalogists, Stillwater, Oklahoma.

795 Riekenberg, P.M., Camalich, J., Svensson, E., IJsseldijk, L.L., Brasseur, S.M.J.M., Witbaard, R., Leopold,
796   M.F., Rebolledo, E.B., Middelburg, J.J., van der Meer, M.T.J., Sinninghe Damsté, J.S., Schouten, S.,
797   2021. Reconstructing the diet, trophic level and migration pattern of mysticete whales based on
798   baleen isotopic composition. R Soc Open Sci 8. https://doi.org/10.1098/rsos.210949

799 Rolland, R.M., Parks, S.E., Hunt, K.E., Castellote, M., Corkeron, P.J., Nowacek, D.P., Wasser, S.K., Kraus,
800   S.D., 2012. Evidence that ship noise increases stress in right whales. Proceedings of the Royal
801   Society B: Biological Sciences 279, 2363–2368. https://doi.org/10.1098/rspb.2011.2429

802 Romero, L.M., Dickens, M.J., Cyr, N.E., 2009. The Reactive Scope Model - a new model integrating
803   homeostasis, allostasis, and stress. Horm Behav 55, 375–89.
804   https://doi.org/10.1016/j.yhbeh.2008.12.009

805 Romero, M.L., Wingfield, J.C., 2016. Tempests, poxes, predators, and people: stress in wild animals and
806   how they cope Oxford University Press (Oxford, UK).

807 Saupe, S.M., Schell, D.M., Griffiths, W.B., 1989. Carbon-isotope ratio gradients in western arctic
808   zooplankton. Mar Biol 103, 427–432. https://doi.org/10.1007/BF00399574

809 Schell, D.M., Saupe, S.M., Haubenstock, N., 1989. Bowhead whale (*Balaena mysticetus*) growth and
810   feeding as estimated by d^{13}C techniques. Mar Biol 103, 433–443.
811   https://doi.org/10.1007/BF00399575

812 Stewart, J.D., Joyce, T.W., Durban, J.W., Calambokidis, J., Fauquier, D., Fearnbach, H., Grebmeier, J.M.,
813   Lynn, M., Manizza, M., Perryman, W.L., Tinker, M.T., Weller, D.W., 2023. Boom-bust cycles in gray
814   whales associated with dynamic and changing Arctic conditions. Science (1979) 382, 207–211.
815   https://doi.org/10.1126/science.adi1847

816 Stewart, J.D., Weller, D.W., 2021. Abundance of Eastern North Pacific gray whales 2019/2020. NOAA
817   Technical Memorandum NMFS-SWFSC-639.

818 Sumich, J.L., 2001. Growth of baleen of a rehabilitating gray whale calf. Aquat Mamm 27.3.

819 Suryan, R.M., Arimitsu, M.L., Coletti, H.A., Hopcroft, R.R., Lindeberg, M.R., Barbeaux, S.J., Batten, S.D.,
820   Burt, W.J., Bishop, M.A., Bodkin, J.L., Brenner, R., Campbell, R.W., Cushing, D.A., Danielson, S.L.,
821   Dorn, M.W., Drummond, B., Esler, D., Gelatt, T., Hanselman, D.H., Hatch, S.A., Haught, S.,
822   Holderied, K., Iken, K., Irons, D.B., Kettle, A.B., Kimmel, D.G., Konar, B., Kuletz, K.J., Laurel, B.J.,
823   Maniscalco, J.M., Matkin, C., McKinstry, C.A.E., Monson, D.H., Moran, J.R., Olsen, D., Palsson, W.A.,

824   Pegau, W.S., Piatt, J.F., Rogers, L.A., Rojek, N.A., Schaefer, A., Spies, I.B., Straley, J.M., Strom, S.L.,

825   Sweeney, K.L., Szymkowiak, M., Weitzman, B.P., Yasumiishi, E.M., Zador, S.G., 2021. Ecosystem

826   response persists after a prolonged marine heatwave. Sci Rep 11, 6235.

827   https://doi.org/10.1038/s41598-021-83818-5

828   Swartz, S.L., Taylor, B.L., Rugh, D.J., 2006. Gray whale Eschrichtius robustus population and stock

829   identity. Mamm Rev. https://doi.org/10.1111/j.1365-2907.2006.00082.x

830   Teixeira, C.R., Troina, G.C., Daura-Jorge, F.G., Simões-Lopes, P.C., Botta, S., 2022. A practical guide on

831   stable isotope analysis for cetacean research. Mar Mamm Sci. https://doi.org/10.1111/mms.12911

832   Thomas, P.O., Reeves, R.R., Brownell, R.L., 2016. Status of the world's baleen whales. Mar Mamm Sci 32,

833   682–734. https://doi.org/10.1111/mms.12281

834   Torres, L.G., Bird, C.N., Rodríguez-González, F., Christiansen, F., Bejder, L., Lemos, L., Urban R, J., Swartz,

835   S., Willoughby, A., Hewitt, J., Bierlich, KC., 2022. Range-wide comparison of gray whale body

836   condition reveals contrasting sub-population health characteristics and vulnerability to

837   environmental change. Front Mar Sci 9. https://doi.org/10.3389/fmars.2022.867258

838   Torres, L.G., Brander, S.M., Parker, J.I., Bloom, E.M., Norman, R., Van Brocklin, J.E., Lasdin, K.S.,

839   Hildebrand, L., 2023. Zoop to poop: assessment of microparticle loads in gray whale zooplankton

840   prey and fecal matter reveal high daily consumption rates. Front Mar Sci 10.

841   https://doi.org/10.3389/fmars.2023.1201078

842   Trueman, C.N., St John Glew, K., 2019. Isotopic tracking of marine animal movement, in: Tracking animal

843   migration with stable isotopes. https://doi.org/10.1016/B978-0-12-814723-8.00006-4

844   Wasser, S.K., Lundin, J.I., Ayres, K., Seely, E., Giles, D., Balcomb, K., Hempelmann, J., Parsons, K., Booth,

845   R., 2017. Population growth is limited by nutritional impacts on pregnancy success in endangered

846   Southern Resident killer whales (Orcinus orca). PLoS One 12, e0179824.

847   https://doi.org/10.1371/journal.pone.0179824

848   Williams, C.T., Chmura, H.E., Zhang, V., Dillon, D., Wilsterman, K., Barnes, B.M., Buck, C.L., 2019.

849   Environmental heterogeneity affects seasonal variation in thyroid hormone physiology of free-

850   living arctic ground squirrels (*Urocitellus parryii*). Can J Zool 97, 783–790.

851   https://doi.org/10.1139/cjz-2018-0302

852   Wilsterman, K., Buck, C.L., Barnes, B.M., Williams, C.T., 2015. Energy regulation in context: Free-living
853   female arctic ground squirrels modulate the relationship between thyroid hormones and activity
854   among life history stages. Horm Behav 75. https://doi.org/10.1016/j.yhbeh.2015.09.003

855   Wingfield, J.C., 2005. The concept of allostasis: coping with a capricious environment. J Mammal 86,
856   248–254. https://doi.org/10.1644/BHE-004.1

857   Wingfield, J.C., Maney, D.L., Breuner, C.W., Jacobs, J.D., Lynn, S., Ramenofsky, M., Richardson, R.D.,
858   1998. Ecological bases of hormone—behavior interactions: The "Emergency life history stage." Am
859   Zool 38, 191–206. https://doi.org/10.1093/icb/38.1.191

860