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Declining reproductive success in the Gulf of St. Lawrence's humpback whales (*Megaptera novaeangliae*) reflects ecosystem shifts on their feeding grounds

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Abstract :

Climate change has resulted in physical and biological changes in the world's oceans. How the effects of these changes are buffered by top predator populations, and therefore how much plasticity lies at the highest trophic levels, are largely unknown. Here endocrine profiling, longitudinal observations of known individuals over 15 years between 2004 and 2018, and environmental data are combined to examine how the reproductive success of a top marine predator is being affected by ecosystem change. The Gulf of St. Lawrence, Canada, is a major summer feeding ground for humpback whales (*Megaptera novaeangliae*) in the North Atlantic. Blubber biopsy samples (n = 185) of female humpback whales were used to investigate variation in pregnancy rates through the quantification of progesterone. Annual pregnancy rates showed considerable variability, with no overall change detected over the study. However, a total of 457 photo-identified adult female sightings records with/without calves were collated, and showed that annual calving rates declined significantly. The probability of observing cow-calf pairs was related to favourable environmental conditions in the previous year; measured by herring spawning stock biomass, *Calanus* spp. abundance, overall copepod abundance and phytoplankton bloom magnitude. Approximately 39% of identified pregnancies were unsuccessful over the 15 years, and the average annual pregnancy rate was higher than the average annual calving rate at ~37% and ~23% respectively. Together, these data suggest that the declines in reproductive success could be, at least in part, the result of females being unable to accumulate the energy reserves necessary to maintain pregnancy and/or meet the energetic demands of lactation in years of poorer prey availability rather than solely an inability to become pregnant. The decline in calving rates over a period of major environmental variability may suggest that this population has limited resilience to such ecosystem change.

Keywords : biopsy, calving rates, endocrine profiling, environmental change, marine mammals, photo-identification, pregnancy rates

48 Widespread, major changes have been documented in the marine environment over the last
49 decades as a result of anthropogenically driven climate change. These include changes in
50 atmospheric / ocean circulation patterns, ocean acidification, ocean warming, loss of sea ice
51 and greater stratification of the water column (Hoegh-Guldberg and Bruno 2010). Rapid
52 physical oceanographic changes are triggering huge shifts and reorganizations in regional
53 ecosystems and biogeochemical cycles, especially at high latitudes (Ducklow, et al. 2007;
54 Moline, et al. 2008). Polar and sub-polar regions are particularly vulnerable environments, and
55 the most accelerated changes have been documented in these areas (Moritz, et al. 2002;
56 Schofield, et al. 2010; Wassmann, et al. 2011). These changes come with associated risks of
57 fundamental and irreversible ecological transformation. There is therefore an urgent need for

58 more information to improve the ability to predict the future resilience of high latitude
59 ecosystems as climate change progresses (Schofield, et al. 2010). Long term monitoring efforts
60 are thus vital to assess ecological responses and ecosystem variability as currently, how these
61 large scale changes will affect the highest trophic levels in terms of marine predator abundance
62 and distribution is largely unknown.

63 Marine mammals, as long-lived, conspicuous and wide ranging top marine predators, can be
64 used as sentinel species to assess ecosystem health as they integrate environmental conditions
65 from the bottom through to the top of the food chain (Moore and Huntington 2008; Hazen, et
66 al. 2019). Ultimately, the most fundamental consideration to understand how these species are
67 responding to environmental variability at a population level is to understand the extent to
68 which their reproductive success, and by inference their fitness, is being affected. Monitoring
69 the reproductive success of these animals can therefore provide vital information on ecosystem
70 productivity and environmental quality, and is imperative to predict population-level impacts
71 over meaningful timescales for these apex predators.

72 Knowledge of population vital rate parameters, specifically pregnancy rates and calving rates,
73 provides information on both population health and potential for growth. Historically, the
74 pregnancy status of cetaceans was assessed by examining carcasses taken in commercial whale
75 hunts. More recently, endocrinological profiling techniques have shown that blubber
76 progesterone concentrations are indicative of pregnancy in both mysticetes (Mansour, et al.
77 2002; Kellar, et al. 2013) and odontocetes (Kellar, et al. 2006; Perez, et al. 2011; Trego, et al.
78 2013), as confirmed by the physical examination of stranded carcasses and recording the
79 number of corpora as well as corpus lutea and foetus size, if present. Progesterone is produced
80 by the corpus luteum, and is the primary regulator of oestrous cycling and pregnancy in
81 mammals (Pineda 2003). Its lipophilic properties, as with other steroids, mean that increases
82 in circulating concentrations are measurable in the blubber. The collection and analysis of
83 blubber biopsy samples is therefore a readily obtainable and non-lethal way of assigning
84 pregnancy status in free-ranging cetaceans, and has been applied to bottlenose dolphins
85 (*Tursiops truncatus*), long-finned pilot whales (*Globicephala melas*) (Perez, et al. 2011), and
86 humpback whales (*Megaptera novaeangliae*) (Clark, et al. 2016; Pallin, Robbins, et al. 2018;
87 Pallin, Baker, et al. 2018). From these studies, it appears that there may be species-specific
88 and/or blubber processing method-specific progesterone concentrations indicative of
89 pregnancy. These methods therefore require pregnancy validation checks for each new
90 investigation.

91 To apply these methods, long-term time-series data are invaluable for identifying changes in
92 population pregnancy rates and reproductive success. The population dynamics of baleen
93 whales in the Gulf of St. Lawrence, Québec, Canada, have been studied since the 1980s by the
94 Mingan Island Cetacean Study (MICS), and therefore represent a unique study to investigate
95 reproductive success and how this has been affected by environmental variability over time.
96 The Gulf of St. Lawrence (GSL) is a major feeding grounds for baleen whales, and other top
97 marine predators in the North Atlantic, and like many other sub-polar regions, has experienced
98 major ecosystem changes in the last decades. These include both physical (e.g. water
99 temperature, sea-ice volume) and biological changes (e.g. plankton production) (Long, et al.
100 2016; Blais, et al. 2019). The MICS has conducted annual cetacean surveys of parts of the GSL
101 during the summer feeding season for over 40 years, which has resulted in a well-established
102 catalogue of photo-identified individuals of the three largest baleen whale species present,
103 including humpback whales, and, importantly, an extensive archive of tissue samples.

104 The retrospective analysis of these long-term data sets has shown that the migratory timing of
105 humpback whales into the GSL has shifted earlier by approximately 4 weeks between 1984
106 and 2010 (Ramp, et al. 2015). This difference was strongly related to earlier ice break-up as a
107 result of rising sea surface temperature (Ramp, et al. 2015). These observed changes in
108 phenology in response to ocean warming are an example of the behavioural plasticity of this
109 species (Ramp, et al. 2015), but whether the observed rate of change in timing is maintained,
110 and how this change will affect population status and trends is unknown. In addition, stable
111 isotope analysis of skin samples has also enabled an investigation of dietary changes in four
112 species of baleen whales in the GSL (Gavrilchuck, et al. 2014) following the collapse of the
113 several commercial groundfish populations in the early 1990s (Hutchings and Myers 1995;
114 Myers, et al. 1996). Specifically, there has been an increase in feeding on higher trophic-level
115 prey, such as small, pelagic, schooling fish, particularly for humpback whales (Gavrilchuck, et
116 al. 2014). This work provides evidence for differential resource use following ecosystem
117 change in the GSL, further demonstrating the behavioural plasticity of this species. However,
118 how changes in diet may affect vital rates are unknown. The next step in understanding how
119 population vital rates may be affected by such environmental variability is to apply newly
120 developed hormone profiling methods to investigate variation in reproductive success in this
121 population over time.

122 A unique dataset of archived blubber biopsy samples together with detailed, individual
123 sightings histories of female humpback whales was collected by the MICS between 2004 and

124 2018. These data were used to investigate firstly, changes in calving rates (calculated here using
125 the number of calves that survive long enough to be observed in the following feeding season,
126 rather than the *birth rate* directly measured on the breeding grounds), and pregnancy rates over
127 the 15 year study period. Secondly, the relationships between these vital rates and
128 environmental covariate data collated by the Department of Fisheries and Oceans, Canada
129 (DFO) were investigated. Thus, by combining long-term survey efforts of photo-identified
130 individuals, endocrine profiling and environmental data, the population level impacts of, or
131 resilience to ecosystem change in the GSL humpback whales can be assessed.

132

133

134 **Methods**

135 **1. Blubber Biopsy Sample Collection and Sexing**

136 A long-term dataset of 185 remotely obtained, shallow, blubber biopsy samples were collected
137 from 114 individually photo-identified, female humpback whales between 2004 and 2017. The
138 sex of individuals had been previously determined genetically using standard polymerase chain
139 reaction (PCR) methods targeting sex-specific gene regions in skin samples (Palsbøll et al.
140 1992; Bérubé & Palsbøll 1996). Sampling occurred during the summer feeding season from
141 May to October in the St. Lawrence Estuary, the Jacques Cartier Passage and Gaspé Peninsula
142 regions of the Gulf of St. Lawrence, Québec, Canada (Fig. 1). Biopsies were collected from
143 rigid-hulled, inflatable boats using a crossbow and arrow system with hollow-tipped, stainless-
144 steel biopsy darts (40 mm in length and 8 mm in diameter) (Borobia, et al. 1995; Gavrilchuck,
145 et al. 2014) from the dorsal and flank areas of individuals. Samples were stored either in
146 aluminium foil, plastic vials, or glass vials on ice immediately after collection, and were
147 subsequently frozen at -20°C before analysis. All samples were collected under annual permits
148 issued to the MICS by the Department of Fisheries and Oceans, Canada (DFO).

149 **2. Life-history Data**

150 Annual, boat-based surveys of baleen whale populations in the GSL conducted by the MICS
151 since 1982 have generated a catalogue of 476 photo-identified humpback whales (at the end of
152 the 2018 summer field season). Individuals were photo-identified using natural markings, with
153 particular emphasis on the pigmentation on the ventral face of the fluke and the size and shape
154 of the dorsal fin (Katona and Whitehead 1981). Life-history data were obtained for the sampled

155 individuals using this photo-identification catalogue and the MICS sightings database. Detailed
156 sightings histories of the individuals were recorded throughout a feeding season where they
157 were sighted multiple times in the study areas (Fig. 1) between May and October, and also
158 between years.

159 In order to assign females into age-specific, reproductive classes, information on age at sexual
160 maturity from other studies was assessed. Age at sexual maturity appears to vary between
161 populations, and possibly also over time as populations increased post-whaling. Based on
162 Australian whaling records, females were thought to reach maturity at between 4 and 5 years
163 of age (Chittleborough 1959; Chittleborough 1965). In the Gulf of Maine, long-term sighting
164 histories of individually identified females first observed as calves and then re-sighted every
165 year thereafter were used to determine an age at first calving of between 5 and 7 years, with a
166 mean of 5.9 ($n = 12$) (Clapham 1992). Later, for the same population of the Gulf of Maine
167 whales, age at first calving was estimated at 8.78 years ($n = 25$), thus showing either, an
168 apparent increase between the 1970s - 1980s and the 2000s, or, that the early estimate was
169 biased towards younger mothers, as in 1990s only very young mothers could have been
170 included due to the short length of the study (Robbins 2007). Similarly, using sightings
171 histories from photo-identified females in south east Alaska, the mean age at first calving was
172 11.8 years with a range of between 8 and 16 years ($n = 11$) (Gabriele, et al. 2007). Finally, for
173 the GSL population, based on a small sample size of 4 females sighted annually since birth, the
174 mean age at first reproduction was 12 years (range between 9 and 16 years) (MICS,
175 unpublished data). Thus, with ages ranging from between 4 and 12 years, for the purposes of
176 this study, individuals were considered as adults when they were sighted for the first time with
177 a calf, or when they were known for at least 8 years. Individuals were considered as juveniles
178 when they were known for less than 8 years or if they were first sighted as a calf, and their
179 exact age was therefore known. Lactating females were defined as large females that were
180 accompanied consistently by a smaller individual (approximately 1/3 of its size) during an
181 entire feeding season (Ramp, et al. 2010). Calves were considered as these smaller individuals
182 that were closely associated with the adult during an entire season. Finally, *confirmed* pregnant
183 females were defined as those that were seen in the subsequent year with a calf.

184 3. Gulf of St. Lawrence Environmental Data

185 Biological variables were used to describe variations in environmental conditions during the
186 summer feeding season in the GSL (Table 1). With a lack of specific data on a number of

187 potentially important humpback whale prey species, including sand lance (*Ammodytidae spp.*)
188 and krill, for example, these variables are a combination of indices that describe broad changes
189 in productivity as well as the abundance of some known prey species. These variables are
190 therefore used to investigate broad-scale, environmental variation on the humpback whales'
191 feeding grounds. Phytoplankton bloom magnitude, zooplankton biomass and both copepod
192 and *Calanus* spp. abundance data were collated from the DFO's Atlantic Zone Monitoring
193 Program (AZMP) (Therriault, et al. 1998). The AZMP uses satellite data and carries out regular
194 surveys along standard sections and at various stations in order to describe physical and
195 biological oceanographic conditions in the Canadian Atlantic region between June and
196 November. Phytoplankton bloom magnitude (integral of the chlorophyll *a* concentration under
197 the Gaussian curve) was thus derived for each year in the northwestern, northeastern and
198 southern GSL subregions to cover the different parts of the GSL (see details for the calculation
199 in Blais et al., 2019). In the same regions, zooplankton samples were collected during surveys
200 using a zooplankton net (202 µm) vertically towed from the bottom to the surface. Abundance
201 of the different copepod species was integrated over the water column (see Mitchell, et al. 2002
202 for the detailed AZMP protocol). Mackerel and herring (spring and fall spawning components)
203 annual spawning stock biomass estimates were extracted from DFO stock assessment reports
204 for the entire GSL (summed over the 4R, 4T and 4S areas for herring (DFO 2018a, b, 2019))
205 (Fig. 1). Specifically, annual spawning stock biomass estimates are generated as the product of
206 abundance, weight and the proportion of mature individuals (for the beginning of the year)
207 summed over all ages (for details see DFO 2018a, b, 2019). Finally, relative changes in capelin
208 abundance were also included as the number of individuals caught in standard tows during
209 DFO ecosystem surveys conducted annually, in August, in the northern GSL (including the
210 lower Estuary) (Bourdages et al. 2020).

211 Humpback whales are known to feed through the entire GSL region from the Estuary, the
212 Laurentian Channel, the Jacques Cartier Passage, and up to the Strait of Belle Isle (Stevick,
213 et al. 2006) (Fig. 1). Individual whales are sighted throughout a feeding season moving between
214 the Jacques Cartier Passage, the Gaspé Peninsula and the Estuary (Ramp, et al. 2010). For these
215 reasons, where different biological covariate values were available covering the various
216 subregions of the GSL (Blais, et al. 2019), these were averaged to produce a single value that
217 covers the whole area as a measure of the overall environmental conditions experienced by the
218 humpback whales on their summer feeding grounds in a year.

219 **4. Blubber Progesterone Extraction and Quantification**

220 **4.1 Progesterone Extraction**

221 The blubber steroid extraction protocol developed by Kellar and colleagues (2006), and
222 previously used to extract progesterone (Kellar, et al. 2006; Trego, et al. 2013) and cortisol
223 (Kershaw and Hall 2016; Kershaw, et al. 2017), was used to extract progesterone from the
224 blubber biopsy samples. The epidermis was removed from the biopsy, and either the whole
225 blubber sample was used for hormone extraction when the tissue mass was $\leq 0.2\text{g}$, or where
226 the tissue mass was great enough, longitudinal sub-samples (0.15 - 0.2g) were taken while the
227 tissue was still frozen. Briefly, the tissue was accurately weighed, and the extraction protocol
228 involved tissue homogenisation in ethanol followed by tissue debris removal in a series of
229 solvent rinses (ethanol : acetone, diethyl ether, and acetonitrile) with recovery of the
230 supernatant each time. The resulting residues are washed (using hexane) to remove any
231 remaining lipid, and the final extract dried down for resuspension and assaying. The final
232 residue is centrifuged briefly and then re-suspended in 500 μl phosphate buffered saline (PBS)
233 (pH 7.5) before assaying (Kershaw, et al. 2017).

234 **4.2 Progesterone Quantification**

235 A commercially available ELISA (DRG International Inc. Progesterone ELISA EIA-1561) was
236 used for the quantification of progesterone. This ELISA has been successfully used for the
237 quantification of progesterone in harbour seal (*Phoca vitulina*) blubber biopsies to determine
238 pregnancy status (Hall, et al. 2020). The concentrations were measured according to the ELISA
239 kit instructions with a standard curve ranging between 0 and 40ng/ml with a sensitivity of
240 0.045ng/ml. Progesterone concentrations were determined using a 4 parameter log-logistic
241 model based on the standard curve. All samples were assayed in duplicate and the mean
242 hormone concentration reported in ng/g wet weight of the sample. Extracts of varying
243 concentrations were used to calculate inter-assay (n = 22) and intra-assay (n = 12) CVs. The
244 mean inter-assay CV was 14.7%, and the mean intra-assay CV was 5.2%, and were therefore
245 below the acceptable limits of variation (mean percentage CVs of <20% and <10% were set as
246 the acceptable limits for inter-assay and intra-assay variability respectively (Andreasson, et al.
247 2015).

248 **4.3 Quality Assurance and Quality Control**

249 Quality assurance and quality control tests were performed to validate the use of this
250 progesterone ELISA for the first time with humpback whale blubber extracts. Parallelism

251 assays with the standard curve were carried out using diluted sample extracts to confirm that
252 the antigen binding characteristics allow the reliable determination of hormone concentrations.
253 Matrix effects tests confirmed that PBS was a compatible sample diluent using spiked samples
254 (Supplementary Fig. S1; for detailed quality assurance and quality control methods for blubber
255 steroid extraction see Kershaw, et al. 2017).

256 **5. Statistical Analysis**

257 **5.1 Blubber Progesterone Concentrations as an Indicator of Pregnancy Status**

258 In the full sample set of 185 females, there were 14 confirmed pregnant individuals (based on
259 sightings records the following year). A mixture of immature females (known to be less than 8
260 years old as they were first sighted as calves) and calves ($n = 31$) were used as non-pregnant
261 controls. The progesterone concentrations measured in these 45 control samples, as well as the
262 concentrations measured in the other ‘unknown’ samples ($n = 140$) where reproductive status
263 was to be determined, are shown in Fig. 2. Using these two datasets of confirmed pregnant and
264 immature females we used a logistic regression model (binomial family with a “logit” link
265 function) to model the probability of a female being pregnant as a function of measured blubber
266 progesterone concentrations (Kellar et al., 2017; Pallin, Robbins et al. 2018). Progesterone
267 concentrations were log transformed and used as the predictor variable. The model generated
268 probabilistic estimates of pregnancy for each of the 45 females input into the model ranging
269 from 0 (not pregnant) to 1 (pregnant) (Supplementary Fig. S2). We randomly resampled the
270 progesterone concentration data (with replacement) and fitted the model 500 times to estimate
271 uncertainty (Supplementary Fig. S2), and used the best fitting model to assign probabilistic
272 estimates of pregnancy for the other 140 females of unknown pregnancy status. Each
273 probability estimate was associated with a standard error (SE), and when the upper and lower
274 SE around the estimate was between 100% and $\geq 99.0\%$, the female was classified as pregnant
275 (see Supplementary Table S1 with probability estimates \pm SE for unknown females).

276 **5.2 Population Calving Rates and Pregnancy Rates**

277 Annual calving rates were calculated as the number of calves sighted as a proportion of the
278 total number of adult females (≥ 8 years old) sighted in that year (2004 – 2018) (Table 2).
279 Similarly, pregnancy rates were calculated as the number of pregnant females biopsied as a
280 proportion of the total number of adult females (≥ 8 years old) biopsied in that year (2004 –
281 2017) (Table 2). The small sample sizes of adult females biopsied in some years (e.g. 2 adults

282 compared to 18 juveniles and calves in 2007) (Table 2), introduces variability into the
283 pregnancy rates estimates, with some years thus producing more robust estimates than others.

284 **Generalised Linear Models:** As calving rates and pregnancy rates are both proportions, and
285 are thus not normally distributed, binomial Generalised Linear Models (GLMs) (R version
286 3.6.2, 2019) with a “logit” link function were used to investigate, firstly, the relationship
287 between calving rates and pregnancy rates over time, and secondly, the relationship between
288 pregnancy rate, and the calving rate in the following year. Quasi-binomial GLMs were used to
289 take into account over dispersion in the calving rate data.

290 Given the small sample sizes and the variability in the pregnancy and calving rates, it was
291 important to consider these data in the context of their power to detect significant relationships.
292 To estimate power for generalised linear models, the *pwr.f2.test* function in the *pwr* package
293 uses the number of coefficients in the model, the number of error degrees of freedom and the
294 effect size based on the proportion of variance explained by the model for regression power
295 calculations (Kutner, et al. 2005). Setting the significance level at 0.05, the power to detect a
296 relationship in the calving rate regression over time was 80.0%, while the power to detect a
297 relationship in the pregnancy rate regression over time was much lower at just 9.30%. Thus,
298 these data are sufficient to measure trends in the calving rates for this population, but the
299 variability in pregnancy rate estimates makes the detection of significant trends over this 15
300 year period unlikely. Using the same *pwr* package, the sample size required to detect a
301 significant relationship in the pregnancy rate data was estimated by setting power to 0.8, and
302 significance level to 0.05. It was estimated that, given the high variability in the data, a sample
303 size of 234 pregnancy rate estimates would be required to detect a significant trend in
304 pregnancy rate over time. For more robust pregnancy rate estimates, larger sample sizes of
305 biopsied adult females are required every year (Table 2).

306 **5.3 Population Calving Rates and Environmental Covariates**

307 **Generalised Linear Mixed Effects Model:** The relationships between the probability of
308 individual females being sighted with a calf (cow - calf pairs) and the environmental covariates
309 and pregnancy rate in the previous year were investigated.

310 Specifically, a total of 457 photo-identified adult female sightings records were collated
311 between 2004 and 2018 (Table 2). Every year, each female was recorded as sighted without a
312 calf (0 or absent) or with a calf (1 or present), resulting in a total of 106 cow - calf pair sightings
313 over the study period. These sightings were of 83 different females. As these observations are

314 thus of the same females sighted over multiple years, a Generalised Linear Mixed Effects
315 Model (GLMM) was used to take into account the repeated records of the same individuals.
316 GLMMs incorporate random effects such that they encompass variation among individuals
317 when multiple responses are measured per individual. A GLMM (*glmer* function in *lme4*
318 package) with a binomial distribution and a “logit” link function, and with female ID as a
319 random effect, was used to investigate the effects of the environmental covariates on the
320 probability of calf presence in the *following* year. The explanatory environmental variables
321 investigated are listed in Table 1. To ensure that the environmental variables are not correlated,
322 and thus to avoid multicollinearity in the final model, variables were selected for inclusion
323 based on variance inflation factors (VIFs). Starting with the full, global model, the VIF for each
324 of the six covariates in the model was calculated (*car* package in R). The covariate with the
325 highest VIF value was removed from the model in a stepwise fashion until the VIF values for
326 all covariates included in the model were below three (Zuur, et al. 2010). VIF calculation
327 showed that zooplankton biomass and mackerel spawning stock biomass were highly
328 correlated with a number of other covariates (Supplementary Fig. S3), and were thus removed
329 from the analyses.

330 A global model was generated including the five remaining environmental covariates in the
331 previous year (phytoplankton bloom magnitude, copepod abundance, *Calanus* abundance,
332 herring spawning stock biomass and capelin abundance) and pregnancy rate. Backwards model
333 selection using the *dredge* function was used to identify the variables that best explain the
334 variation in the calf presence/absence data, and thus to include in the final model based on the
335 smallest Akaike Information Criterion (AIC). Here, backwards model selection showed that
336 four models were of equal fit to the data as the AICs were within two units of each other. This
337 subset of four models was then used for model averaging (*model.avg* function in the *MuMIn*
338 package, R). This averages the regression coefficients across multiple models in order to
339 capture the overall effects of the different variables (Banner and Higgs, 2017), and is useful
340 when there is little to differentiate between a set of models. The model-averaged coefficients
341 were calculated based on full averages, which sets the coefficients of absent variables to zero,
342 rather than excluding them from the average (conditional averages). The full average ensures
343 that the presence of variables does not bias the model-averaged estimate away from zero.
344 Interpretations of the averaged model coefficients were used to assess the effects of each
345 variable. *Predict* plots, in the in the *ggeffects* package, were produced to visualize the effect of
346 each explanatory variable while all others were held constant at their mean. The residual plots,

347 the distribution of scaled residuals and the response against fitted values were checked for the
348 final, averaged model. An alternative modelling approach, using Generalized Additive Mixed
349 Models (GAMMs) was also trialled, but there was no evidence for non-linear smooths (all
350 effective degrees of freedom were estimated at 1), so the GLMM results are presented here.

351 Results

352 1. Identifying Pregnant Females

353 Blubber progesterone concentrations measured for each reproductive class confirmed by field
354 observations are shown in Fig. 3. Pregnant females had significantly higher blubber
355 progesterone concentrations than the other reproductive classes (ANOVA; $df = 2$, $F = 8.40$, p
356 < 0.001), that were not significantly different from each other. An additional 20 females were
357 identified as pregnant based on measured blubber progesterone concentrations. Over the study
358 period, 9 females were sampled twice in one season. These were 4 non-pregnant adult females,
359 2 pregnant females, 2 juveniles and a calf. Progesterone concentrations extracted in the
360 duplicate samples from each of these females classified them into the same reproductive class
361 each time, thus giving confidence in the consistency of this method and these results.

362 2. Pregnancy Rates and Calving Rates Over Time

363 Calving rates ranged between 5% in 2014 to 52% in 2007, with an annual average over the 15
364 year period of $23.4 \pm 12.4\%$. These represent the number of calves that survive long enough to
365 be observed in the following feeding season, rather than the *birth rate* directly measured on the
366 breeding grounds. The generalised linear regression model showed that there has been a
367 significant decline in annual calving rates over the 15 year study period (2004 – 2018), from
368 $\sim 40\%$ to $\sim 20\%$ (quasi-binomial GLM: slope = -0.1 , $p = 0.01$) (Fig. 4a). However, there was no
369 parallel trend in pregnancy rates as these showed considerable inter-annual variability ranging
370 from 0 to 100% (binomial GLM: slope = -0.04 , $p = 0.45$) (Fig. 4a), and averaged at $36.7 \pm$
371 27.9% over the study period. The small sample size of biopsied and pregnant females in some
372 years likely introduced variability in the pregnancy rate estimates, which led to a lack of power
373 to detect a trend in these data. Equally, there was a non-significant correlation between
374 pregnancy rate and calving rate observed in the following year (quasi-binomial GLM; slope =
375 0.29 , $p = 0.72$) (Fig 4b). Again, the variability introduced by the small sample size resulting in
376 fluctuating pregnancy rates could explain the inability to detect a significant correlation. This
377 is exemplified in the years where no pregnancies were detected (2005, 2011, 2013) but calves

378 were sighted in the following year, clearly demonstrating that too few adult females were
379 biopsied in those years for robust pregnancy rate estimates.

380 The lack of a significant correlation may also suggest that the number of pregnant females is
381 not necessarily a good predictor of the number of females successfully returning to their
382 feeding grounds with a calf the following year. It was not possible to calculate the total number
383 of annual reproductive successes as not all females that were biopsied when pregnant were re-
384 sighted in the study area the following year to confirm the presence / absence of a calf.
385 However, over the full study period, of the pregnant females that were re-sighted in the
386 following year, 39.4% had no calf ($n = 13$), suggesting reproductive failure in these individuals
387 (blubber progesterone concentrations ranging between 5.93 - 68.97 ng/g). There were no
388 significant differences in the blubber progesterone concentrations measured in the pregnant
389 females that were not re-sighted (15.6 ± 9.67 ng/g), resighted without a calf (22.00 ± 18.22
390 ng/g) and those that were resighted with a calf (19.41 ± 27.82 ng/g) (ANOVA; $df = 2$, $F = 0.22$,
391 $p = 0.8$) (Supplementary Fig. S3).

392 **3. Probability of Cow-Calf Pairs and Environmental Variables**

393 Using the sightings records of 83 photo-identified females between 2004 and 2018, the shortest
394 inter-calf interval observed for this population was two years. Results from the model
395 averaging of the four best-fitting GLMMs showed that all five environmental covariates were
396 retained as important predictors of the probability of females being sighted with a calf in the
397 following year, but pregnancy rate was not. Specifically, the probability of females being
398 sighted with a calf was positively correlated with four of the environmental covariates in the
399 previous year, although herring spawning stock biomass alone was individually statistically
400 significant ($p = 0.001$) and had the strongest effect on increasing this probability up to ~40%
401 in the “best” years when biomass was highest (Fig. 5d).

402 Over the 15 years, the average herring spawning stock biomass over the whole of the GSL has
403 decreased dramatically (linear regression model: $p = 0.0002$, Adjusted $R^2 = 0.6$) (Fig. 6d).
404 *Calanus spp.* abundance has also shown an overall decline over the same period (Fig. 6c), but
405 not to the same extent as this was only significant at the 10% level (linear regression model: p
406 $= 0.07$, Adjusted $R^2 = 0.2$). Annual phytoplankton bloom magnitude, copepod abundance and
407 capelin abundance and have remained highly variable (Fig. 6a, b and e).

408 **Discussion**

409

410 **Decline in Calving Rates**

411 There was a significant decline in observed calving rates between 2004 and 2018. It is possible
412 that some females gave birth but were not observed at the study site as they moved to other,
413 more profitable feeding grounds. However, as there was no decline in the total number of adult
414 females sighted ever year, this suggests that a significant proportion of the population has not
415 been missed. The calving rate estimates are thus not overly affected by individuals not returning
416 to the study area. The small sample size of biopsied females in some years resulted in
417 significant variability in the pregnancy rate estimates, and led to a lack of power to detect any
418 trends over the same time period. This could also have resulted in the lack of relationship
419 detected between the probability of sighting cow-calf pairs and the pregnancy rate in the
420 previous year. These results highlight the need for large, annual samples sizes of known adult
421 females in order to calculate consistently robust estimates. In the 10 years where there were
422 large enough sample sizes to calculate more accurate estimates (where the number of adult
423 females biopsied was more than 25% of the total number of females biopsied), pregnancy rates
424 ranged from 25% to 63%. These rates are similar to those previously published for other
425 humpback whale populations (Pallin, Baker, et al. 2018; Clark, et al. 2016).

426

427 **Estimating Reproductive Failure**

428 Over the full study period, the average annual pregnancy rate was higher than the average
429 annual calving rate at ~37% and ~23% respectively. Reproductive failure, defined as identified
430 pregnancies failing to produce a calf, or failing to produce a calf that survives long enough to
431 be observed in the following feeding season, was estimated here, over the full study period, to
432 be approximately 39%. Confirming reproductive failure is dependent on correctly identifying
433 pregnancy. Similarly to another study in humpback whales (Pallin, Robbins, et al. 2018), there
434 was considerable variation in the blubber progesterone concentrations in confirmed pregnant
435 females. It is possible that the variation in progesterone concentrations in these females,
436 combined with the small difference between some non-pregnant and pregnant females, led to
437 females with “intermediate” concentrations being misclassified here. These intermediate
438 progesterone concentrations could be the result of pseudopregnancies (Atkinson, et al. 1999;
439 Robeck, et al. 2009), and while this has been documented in captive cetaceans, the rate at which
440 this anomaly occurs in free-ranging animals is unknown, and therefore cannot be controlled for
441 here. However, even if a small number of potentially misclassified females are removed from
442 the estimate, the rate of reproductive failure here remains much higher than the reproductive
443 failure estimated at 13.3% for another North Atlantic feeding ground population in the Gulf of

444 Maine (Pallin, Robbins, et al. 2018). It therefore appears that this declining trend in calving
445 rates may not be solely the result of the inability of females to become pregnant. The declining
446 calving rates could therefore result from combinations of failures to maintain pregnancies to
447 term, foetal losses, and calf mortality on the breeding grounds or during the northward
448 migration back to the GSL.

449

450 High calf mortality rates, as with the young of other marine mammals, could be due to
451 predation, anthropogenic associated mortality including entanglements and vessel strike, or
452 starvation (Stroud and Roffe 1979; Kirkwood, et al. 1997; Mazzuca, et al. 1998; Bogomolni,
453 et al. 2010; Pitman, et al. 2015; Groch, et al. 2018). Calf mortality rates were estimated at 0.182
454 (95% CI = 0.023, 0.518) for a Pacific population, although this was based on a very small
455 sample size of mothers sighted on the breeding grounds in Hawaii with calves, and then re-
456 sighted on their feeding grounds in Alaska with/without the calf (Gabriele, et al. 2001). It is
457 currently not possible to estimate specific calf mortality rates for this population as there have
458 not been sightings of known females with calves on their Caribbean breeding grounds that were
459 subsequently sighted in the same year upon their return to the GSL feeding grounds.

460

461 **Calving Success Varies with Environmental Conditions**

462 Here we provide evidence for links between the probability of sighting cow-calf pairs and
463 environmental conditions on their feeding grounds in the previous year. Specifically, the
464 probability of females being sighted with a calf was related to the availability of herring, *Calanus*
465 *spp.*, copepods and the overall phytoplankton bloom magnitude in the GSL in the previous
466 year. In poor years of environmental productivity and thus prey abundance, females may have
467 been unable to accumulate the energy reserves necessary for the successful completion of
468 pregnancy and / or lactation as a result of the high energetic costs of these life-history stages.
469 Failure to maintain pregnancy and / or failing to transfer adequate energy reserves to their
470 calves to ensure their survival during lactation could therefore have contributed to the observed
471 calving rate declines in this population.

472

473 In mammals, spontaneous abortions, premature births, still births, and neonatal mortality occur
474 under harsh environmental conditions (Wasser and Barash 1983; Sibly, et al. 2002; Stockley
475 2003). For example, in marine mammals specifically, significant fetal mortality occurs in late
476 gestation in New Zealand Fur Seals (*Arctocephalus forsteri*), and reproductive failure during
477 gestation appeared to be the most significant factor in determining reproductive success in the

478 early 2000s (McKenzie, et al. 2005). A combination of ultrasound measurements and endocrine
479 profiling revealed high rates of pregnancy failure in the Gulf of Mexico bottlenose dolphin
480 populations following the Deep Water Horizon oil spill (Kellar, et al. 2017). Rates of
481 pregnancy failure were higher than in other populations in areas not impacted by the spill
482 (Kellar, et al. 2017). Pregnancy failure has also been documented in the Southern Resident
483 killer whale (*Orcinus orca*) population whereby up to 69% of all detectable pregnancies were
484 unsuccessful between 2008 and 2014 (Wasser, et al. 2017). The reduced availability of their
485 main prey, Chinook salmon (*Oncorhynchus tshawytscha*) was identified as a significant cause
486 of late pregnancy failure, including unobserved perinatal loss (Wasser, et al. 2017). In fact, half
487 of these failures occurred at later stages of reproduction, and were significantly associated with
488 nutritional stress. It was concluded that pregnancy failure is a major constraint on killer whale
489 population growth, triggered by insufficient prey resources (Wasser, et al. 2017).

490

491 For migratory baleen whales, including humpback whales, reproductive success is dependent
492 on the abundance of important prey resources on their high-latitude summer feeding grounds.
493 As capital breeders, they feed intensively on these high-latitude prey resources, then depend
494 entirely on stored energy reserves to fuel the rest of their seasonal cycle; migration to low-
495 latitude breeding grounds, the breeding season itself, and migration back to summer feeding
496 grounds (Baker, et al. 1986). In addition, for female baleen whales specifically, each stage of
497 reproduction requires the adequate availability of stored energy reserves. Firstly, an increase
498 in stored energy reserves is required prior to pregnancy (Lockyer 1986; Miller, et al. 2012).
499 Then, accumulated energy reserves support foetal growth and development during pregnancy,
500 which is estimated to cost them up to 25% of their energy stores (Lockyer 1981). Finally,
501 energy stores are mobilized to meet the high energetic demands of lactation, estimated to cost
502 a further 20-35% of their remaining reserves (Miller, et al. 2012; Christiansen, et al. 2016).
503 Longitudinal studies on the endangered North Atlantic Right Whale (*Eubalaena glacialis*),
504 have hypothesised that the observed lengthened calving intervals suggest that abortion and
505 neonatal losses may be contributing to lower reproductive success in this species (Browning,
506 et al. 2010).

507

508 Other studies on baleen whale reproductive rates have also shown responses to prey availability
509 and oceanic conditions; specifically with changes in sea-ice conditions affecting temporal
510 access to preferred feeding grounds (Perryman, et al. 2002; Moore and Huntington 2008),
511 changes in the availability of their preferred copepod prey (Meyer-Gutbrod, et al. 2015; Meyer-

512 Gutbrod and Greene 2018) and large scale climate anomalies (Cartwright, et al. 2019). Here,
513 similarly to previous reports in other cetacean populations (Kellar, et al. 2017; Wasser, et al.
514 2017), it appears that one of the drivers of the relationship between variation in environmental
515 conditions and calving success could be the effects on foetal loss / calf survival, rather than on
516 pregnancy rates alone. Such longitudinal studies of well-known individuals with detailed
517 sightings histories are thus imperative to allow reproductive success to be investigated over
518 time, and thus the drivers of apparently low calving rates to be established.

519

520 **Environmental Variability and Implications for Population Growth**

521 Widespread physical and biological oceanographic condition changes have been documented
522 in the GSL as part of the Atlantic Zone Monitoring Program (AZMP), implemented in 1998
523 (Therriault et al. 1998). A strong warming event began in 2010, and it is estimated that on
524 average, GSL water will become warmer between now and 2060 (Long, et al. 2016). Oxygen
525 concentration anomalies have been measured, with the lowest ever concentration recorded in
526 2018 (Blais, et al. 2019). Changes in nitrate patterns through the water column associated with
527 intrusions of warm, salty water have occurred since 2012 (Blais, et al. 2019). These changes,
528 together with changes in the timing of sea ice dynamics, then have knock on effects for the
529 whole ecosystem. For example, satellite observations show that the spring phytoplankton
530 bloom starts earlier, lasts longer, and shows an above-normal magnitude across most regions
531 of the GSL (Blais, et al. 2019). Further, overall zooplankton biomass remains below normal
532 across the GSL and, large calanoid abundance has declined, largely as a result of decreases in
533 *Calanus hyperboreus* and *Calanus finmarchicus* abundance in certain areas (Blais, et al. 2019).
534 These, and other copepod species, are prey for humpback whales (Borobia, et al. 1995;
535 Gavrilchuk, et al. 2014), and predicted declines in these prey species from ocean warming and
536 increasing interspecific competition between baleen whale species are predicted to contribute
537 to declining populations of Atlantic humpback whales (Tulloch, et al. 2019). Small calanoid
538 abundances have not shown the same declining trend in the GSL, but the abundances of warm-
539 water-associated copepods have increased in some areas (Blais, et al. 2019). There therefore
540 appears to be a shift in community structure at these lower trophic levels which have impacts
541 up the food chain resulting in decreases in herring recruitment and abundance (Brosset, et al.
542 2019). Herring are known to be a hugely important prey species for humpback whales in both
543 the North Pacific and the North Atlantic (Gavrilchuk, et al. 2014; Jourdain and Vongraven
544 2017; Richard, et al. 2017; Moran, et al. 2018). Forage fish, specifically capelin and herring
545 together, are estimated to make up 44% of their diet in the GSL (Gavrilchuk, et al. 2014). Here,

546 we demonstrate that their reproductive success is correlated with the annual abundance of
547 herring.

548

549 Responses in the distribution and demography of baleen whales to climate change have been
550 documented (Leaper, et al. 2006; MacLeod 2009; Simard, et al. 2019), and long-term studies
551 are invaluable for such responses to be detected. Thus, while this population of humpback
552 whales has shown some behavioural plasticity to cope with environmental changes
553 (Gavrilchuck, et al. 2014; Ramp, et al. 2015), the declining calving rates measured here could
554 be evidence that this population does not show as much resilience to change as previously
555 thought, and population trends could be affected. Equally, for long lived animals, such as
556 humpback whales that may be adapted to dealing with decadal-scale shifts in oceanographic
557 regimes (Fleming et al. 2015), decreases in reproductive output over the timescales presented
558 here may also be a sign of plasticity as they reduce current reproductive investment in favour
559 of future survival. Thus, it is possible that these animals reduce reproductive effort in
560 unfavourable conditions, which could manifest as fewer females carrying pregnancies to term,
561 and then increase it when conditions later improve. Continued monitoring of this study
562 population will help to determine if this decline in reproductive success continues, and thus
563 distinguish between a shorter-term trade-off in reproductive investment and longer-term
564 reproductive failure.

565 A review of data on life-history parameters influencing rates of population growth in humpback
566 whales, including survival, age at first parturition and calving rate was carried out to establish
567 possible growth rate estimates for humpback whale populations in general; estimates varied
568 between a mean of 7.3% per year (95% CI = 3.5–10.5% per year) and mean of 8.6% per year
569 (95% CI = 5.0–11.4% per year) (Zerbini, et al. 2010). A meta-analysis of published humpback
570 whale population growth rates suggests that, globally, they are increasing, but growth rates in
571 the Northern Hemisphere are predicted to be slower than those in the Southern Hemisphere
572 (Wedekin et al. 2017). To date, there are no published growth rates for the GSL population
573 specifically, but the most recent estimate from another population in the North West Atlantic
574 is an annual increase of 9.4% (SE = 0.01) in Greenland (Heide-Jorgsen et al. 2012). Moving
575 forward, estimating population growth rates for the GSL population would help to establish
576 how the observed decline in calving rates are affecting population growth and viability.

577 **Conclusions and Future Directions**

578 Our results show decadal variation in population pregnancy rates and calving rates,
579 highlighting the importance of longitudinal studies and linking endocrine measures with
580 observational data. Humpback whales are recognized as a sentinel species of ecosystem health
581 (Bengtson Nash, et al. 2018), so changes in a key vital rate, such as reproductive success,
582 provide a detectable signal of the population level impact of environmental change. Here, we
583 improve our understanding of the environmental factors affecting the reproductive biology of
584 humpback whales on their feeding grounds, by showing the relationship between prey species
585 availability and subsequent calving success. This understanding will assist monitoring,
586 management and conservation efforts as continued changes in the GSL, and other sub-polar
587 regions experience ecosystem shifts as a result of climate change. Maximising the use of
588 interdisciplinary and long-term datasets, like these, is imperative to provide insight into the
589 functioning and challenges facing ecosystems in warming oceans.

590

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602

603 **Data Sharing and Accessibility**

604

605 The data that support the findings of this study are openly available in the University of St
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607 271446508.

608

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875 **Table 1. Annual biological data collated and/or collected by the Department of Fisheries**
 876 **and Oceans, Canada, between 2003 and 2017 in the Gulf of St Lawrence, Canada.**

Biological Data	Covariate	Description	Source
Phytoplankton	Phytoplankton Boom Magnitude (mg Chl / m ³)	Quantifies both the duration and the amplitude of the bloom, averaged over the entire GSL.	Satellite
Zooplankton	Zooplankton Biomass (dry weight g/m ²)	Average zooplankton biomass in June and in October, over the South, NE and NW GSL.	Atlantic Zone Monitoring Program
	Copepod Abundance (ind / m ²)	Average of the abundance of all copepod species measured in June and in October, over the South, NE and NW GSL.	Atlantic Zone Monitoring Program
	<i>Calanus</i> Abundance (ind / m ²)	Average of large <i>Calanus</i> species abundance in June and October (<i>C. fimarchicus</i> , <i>C. glacialis</i> , <i>C. hyperboreus</i>) over the South, NE and NW GSL.	Atlantic Zone Monitoring Program

Fish	Herring SSB.	Herring (<i>Clupea harengus</i>) spawning stock biomass in the entire GSL	DFO stock assessment
	Mackerel SSB.	Mackerel (<i>Scomber scombrus</i>) spawning stock biomass of the entire Canadian spawning contingent which spawns mainly in the southern GSL.	DFO stock assessment
	Capelin Abundance (ind / standard tow)	Average capelin (<i>Mallotus villosus</i>) abundance in August in the northern GSL.	DFO ecosystem survey

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885 **Table 2. Summary of the annual sightings and biopsy information used to calculate**
886 **pregnancy rates and calving rates.**

Year	Adult Females Sighted	Calves Sighted	Females Biopsied	Adult Females Biopsied	Pregnancies Detected
2004	26	4	5	4	1
2005	34	17	8	1	0
2006	28	6	13	5	2
2007	27	14	18	2	1
2008	35	11	6	2	2
2009	30	6	18	8	6

2010	29	9	14	6	3
2011	31	6	10	1	0
2012	23	4	21	11	3
2013	18	4	9	6	0
2014	19	1	NA	NA	NA
2015	27	4	15	11	4
2016	43	4	20	16	6
2017	51	10	18	14	5
2018	36	6	NA	NA	NA
Total	457	106	175	87	33

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889 **Fig. 1. Map of the Gulf of St. Lawrence, Québec, Canada, with the main study area of the**
 890 **Mingan Island Cetacean Study in the Jacques Cartier Passage indicated in the top left**
 891 **insert.** Between 2004 and 2018 most sightings data and biopsy sample collection took place in
 892 the Jacques Cartier Passage. Some surveys took place, and a number of samples were also
 893 collected in the Estuary and off the Gaspé Peninsula. The three North Atlantic Fisheries
 894 Organisation management divisions where biological environmental data were collected and
 895 collated by DFO and used here are indicated (4R, 4S, 4T). Figure adapted with permission
 896 from Schleimer, et al. 2019.

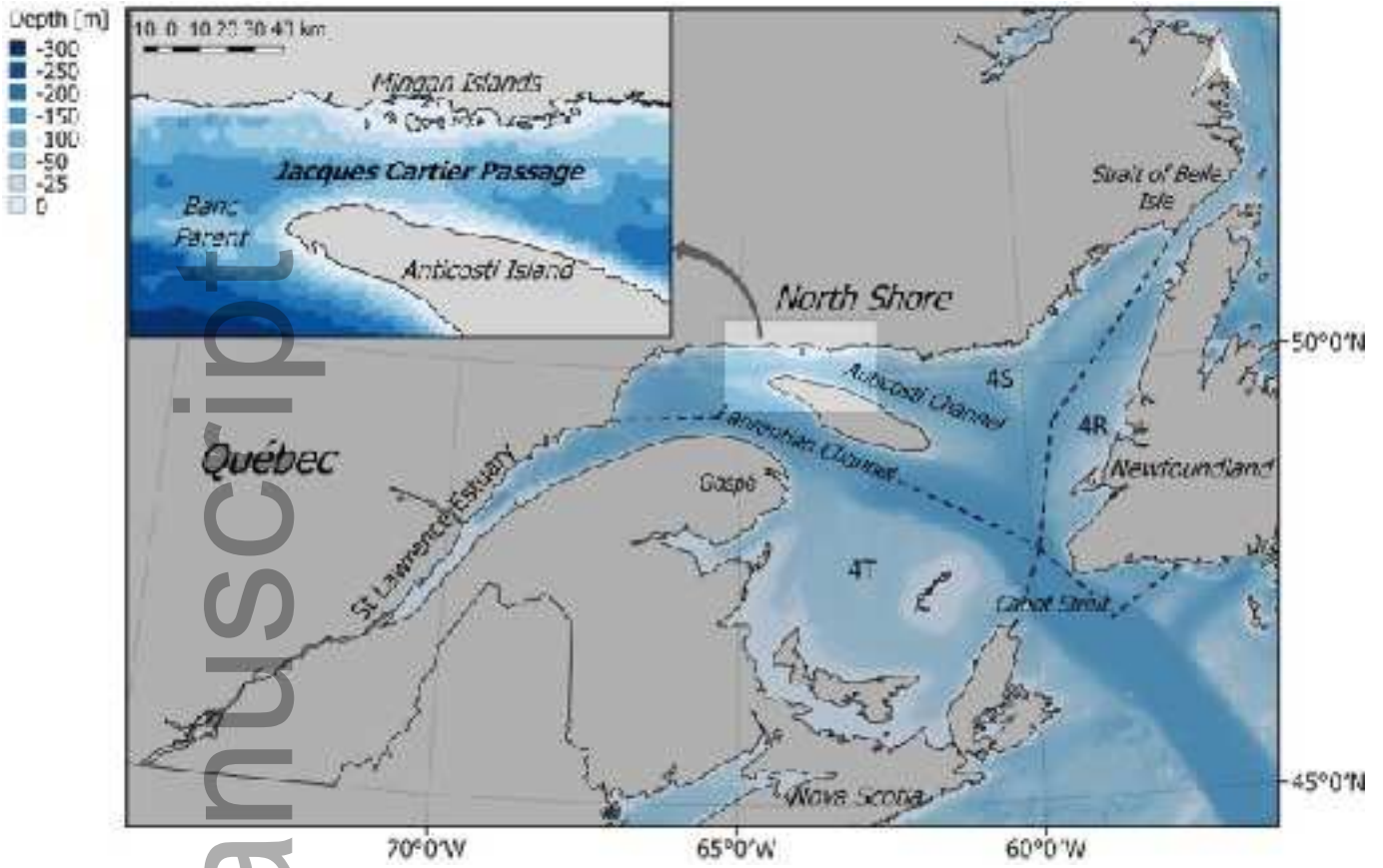
897 **Fig. 2. Progesterone concentrations in 185 female blubber biopsy samples coloured by**
 898 **known or unknown reproductive status.** The concentrations measured in the confirmed
 899 pregnant (black) and not pregnant (light green) samples were used in a logistic regression
 900 model to determine the probability that a female was pregnant in order to assign a pregnancy
 901 status to the ‘unknown’ individuals (dark green) based on their measured progesterone
 902 concentrations.

903 **Fig. 3. Median blubber progesterone concentrations by reproductive status confirmed**
904 **from field observations.** The 25th and 75th percentiles are indicated within the boxes, and the
905 whiskers show values up to 1.5 times the inter-quartile range. Outlier data points beyond the
906 end of the whiskers are plotted individually.

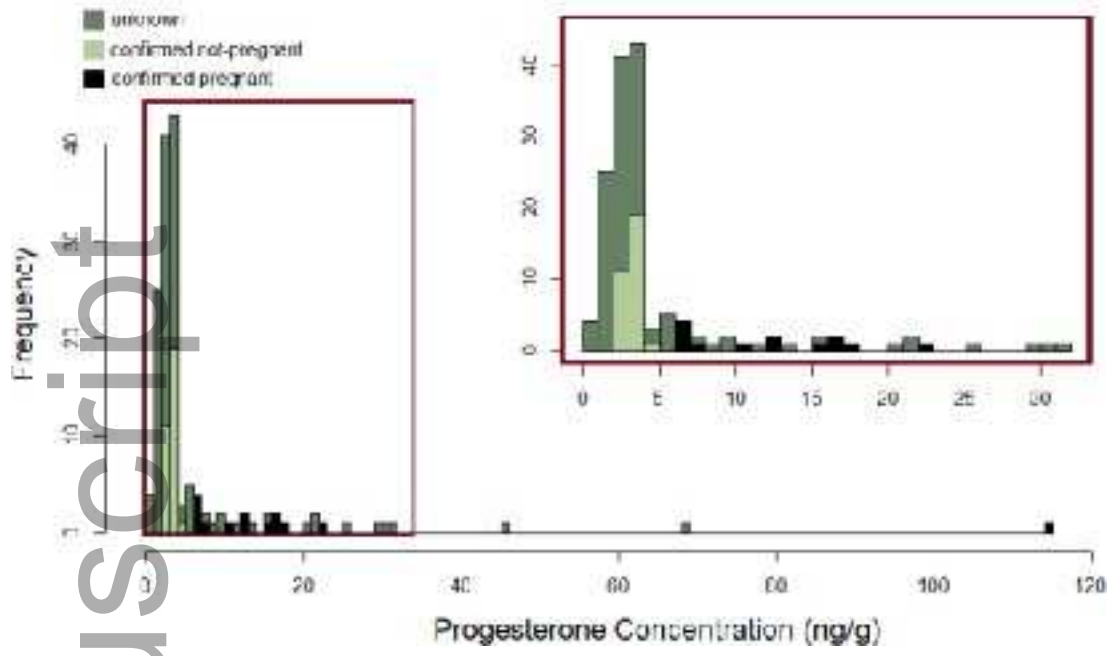
907 **Fig. 4. a) Annual calving rates declined over the 15 year study period, but there was no**
908 **significant decrease detected in annual pregnancy rates.** N.B. A pregnancy rate estimate
909 was not available for 2014. **b) No significant correlation was detected between annual**
910 **pregnancy and calving rates in the following year.** Solid lines indicate the generalised linear
911 model trends, while dashed lines indicate the 95% confidence intervals.

912 **Fig. 5. Predict plots from the best fitting, averaged calf presence / absence GLMM**
913 **following backwards model selection.** The predicted probability of cow-calf pairs are shown
914 on the y-axis, all on the same scale to visualise the relative effects of each covariate. **a), b), c)**
915 The probability of cow-calf pairs is positively correlated with phytoplankton bloom magnitude,
916 copepod abundance and *Calanus* abundance in the previous year. **d)** The probability of cow-
917 calf pairs is significantly correlated with herring spawning stock biomass in the previous year
918 ($p = 0.001$). **e)** The probability of cow-calf pairs is not positively correlated with capelin
919 abundance in the previous year.

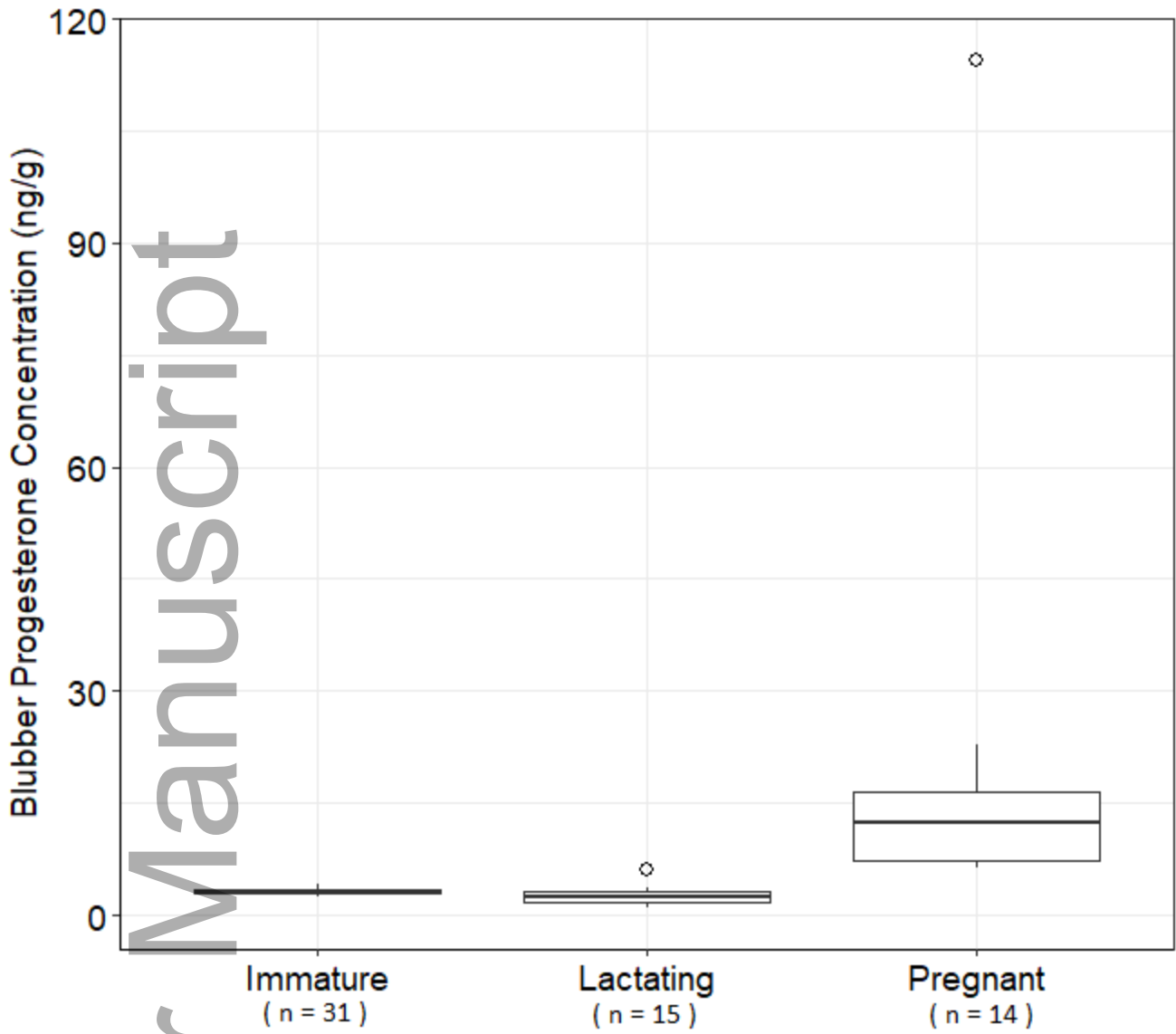
920 **Fig. 6. Averaged annual environmental covariates over the whole of the Gulf of St**
921 **Lawrence.** **a)** Overall, annual phytoplankton bloom magnitude was highly variable between
922 2003 and 2017 **b)** Annual copepod abundance was also highly variable over the study period.
923 **c)** *Calanus spp.* abundance showed an overall decline, significant at the 10% level. **d)**
924 Significant declines in annual herring spawning stock biomass occurred over the study period.
925 **e)** Capelin abundance was highly variable over the study period with a peak in 2010.



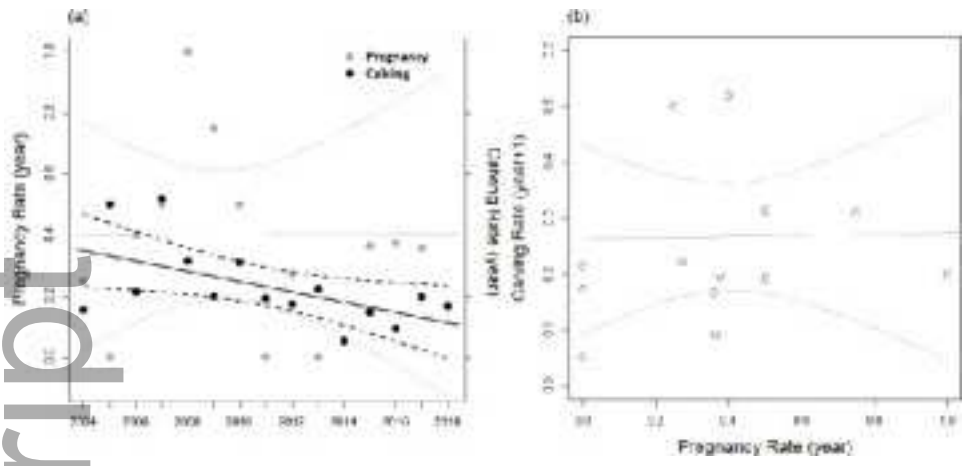
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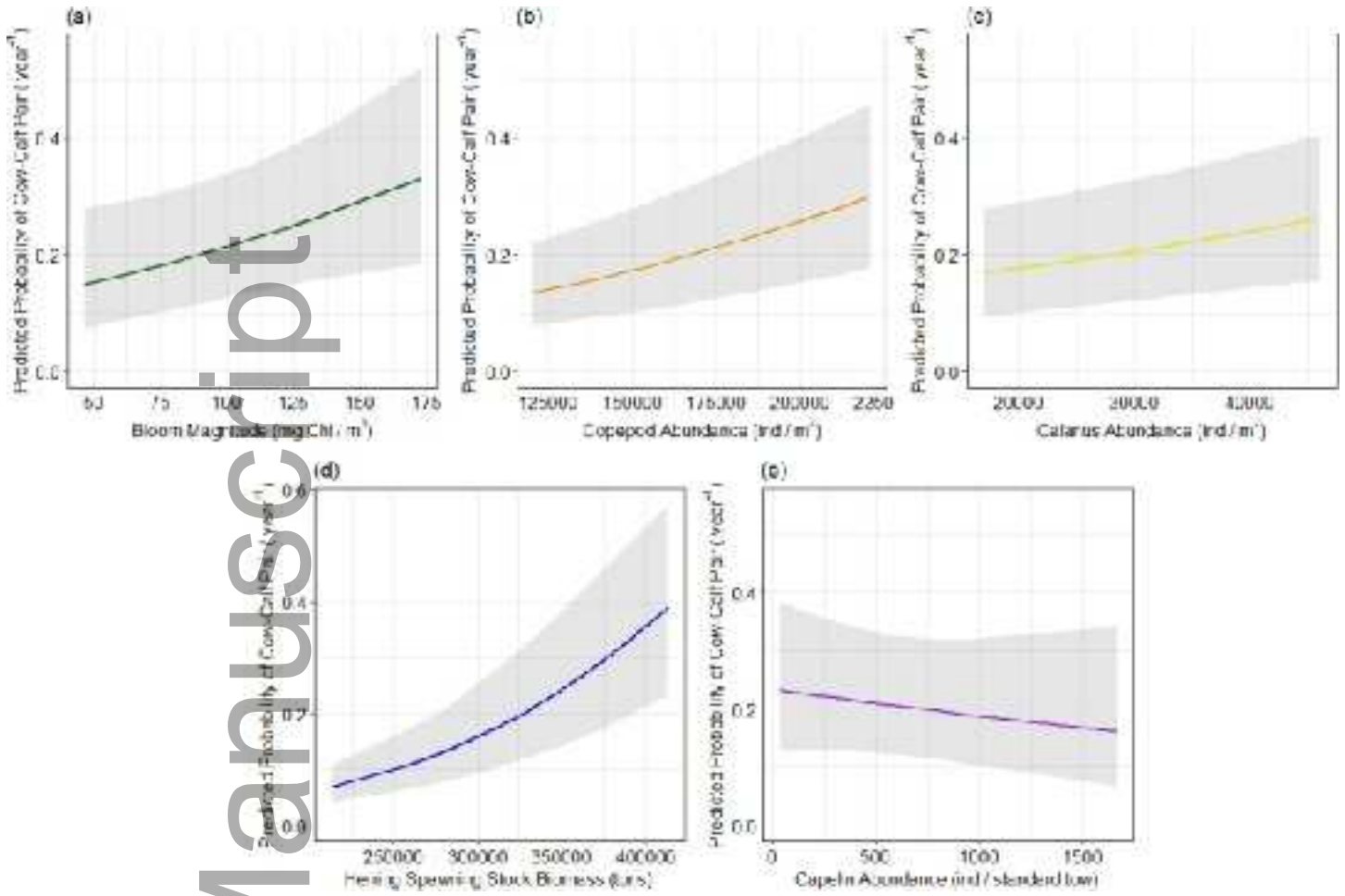
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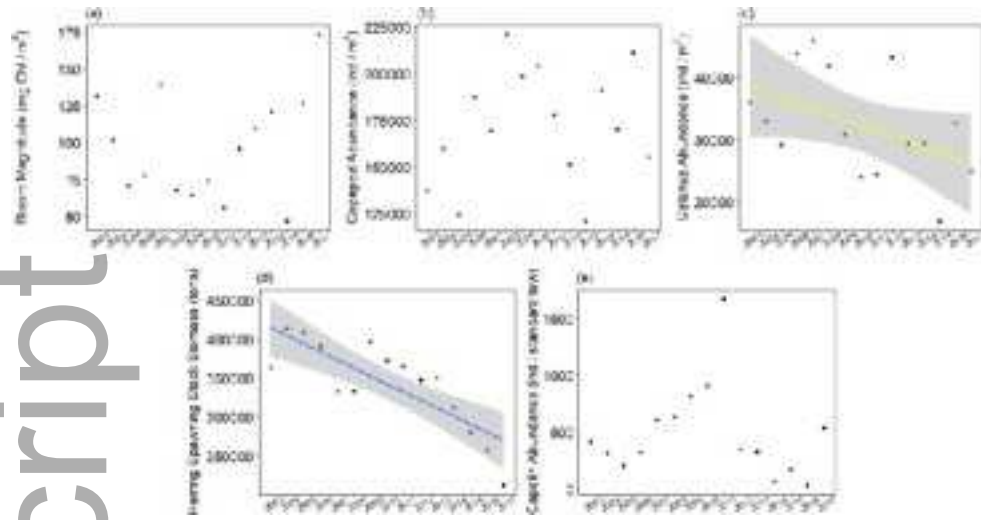
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