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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

Introduction

Widespread, major changes have been documented in the marine environment over the last decades as a result of anthropogenically driven climate change. These include changes in atmospheric / ocean circulation patterns, ocean acidification, ocean warming, loss of sea ice and greater stratification of the water column (Hoegh-Guldberg and Bruno 2010). Rapid physical oceanographic changes are triggering huge shifts and reorganizations in regional ecosystems and biogeochemical cycles, especially at high latitudes (Ducklow, et al. 2007; Moline, et al. 2008). Polar and sub-polar regions are particularly vulnerable environments, and the most accelerated changes have been documented in these areas (Moritz, et al. 2002; Schofield, et al. 2010; Wassmann, et al. 2011). These changes come with associated risks of fundamental and irreversible ecological transformation. There is therefore an urgent need for more information to improve the ability to predict the future resilience of high latitude ecosystems as climate change progresses (Schofield, et al. 2010). Long term monitoring efforts are thus vital to assess ecological responses and ecosystem variability as currently, how these large scale changes will affect the highest trophic levels in terms of marine predator abundance and distribution is largely unknown.

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

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

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

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

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

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

Methods

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1. Blubber Biopsy Sample Collection and Sexing

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

2. Life-history Data

Annual, boat-based surveys of baleen whale populations in the GSL conducted by the MICS since 1982 have generated a catalogue of 476 photo-identified humpback whales (at the end of the 2018 summer field season). Individuals were photo-identified using natural markings, with particular emphasis on the pigmentation on the ventral face of the fluke and the size and shape of the dorsal fin (Katona and Whitehead 1981). Life-history data were obtained for the sampled individuals using this photo-identification catalogue and the MICS sightings database. Detailed sightings histories of the individuals were recorded throughout a feeding season where they were sighted multiple times in the study areas (Fig. 1) between May and October, and also between years.

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

3. Gulf of St. Lawrence Environmental Data

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

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

4. Blubber Progesterone Extraction and Quantification

4.1 Progesterone Extraction

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

4.2 Progesterone Quantification

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

4.3 Quality Assurance and Quality Control

Quality assurance and quality control tests were performed to validate the use of this progesterone ELISA for the first time with humpback whale blubber extracts. Parallelism assays with the standard curve were carried out using diluted sample extracts to confirm that the antigen binding characteristics allow the reliable determination of hormone concentrations. Matrix effects tests confirmed that PBS was a compatible sample diluent using spiked samples (Supplementary Fig. S1; for detailed quality assurance and quality control methods for blubber steroid extraction see Kershaw, et al. 2017).

5. Statistical Analysis

5.1 Blubber Progesterone Concentrations as an Indicator of Pregnancy Status

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

5.2 Population Calving Rates and Pregnancy Rates

Annual calving rates were calculated as the number of calves sighted as a proportion of the total number of adult females (≥ 8 years old) sighted in that year (2004 – 2018) (Table 2). Similarly, pregnancy rates were calculated as the number of pregnant females biopsied as a proportion of the total number of adult females (≥ 8 years old) biopsied in that year (2004 – 2017) (Table 2). The small sample sizes of adult females biopsied in some years (e.g. 2 adults compared to 18 juveniles and calves in 2007) (Table 2), introduces variability into the pregnancy rates estimates, with some years thus producing more robust estimates than others.

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

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

5.3 Population Calving Rates and Environmental Covariates

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

Specifically, a total of 457 photo-identified adult female sightings records were collated between 2004 and 2018 (Table 2). Every year, each female was recorded as sighted without a calf (0 or absent) or with a calf (1 or present), resulting in a total of 106 cow - calf pair sightings over the study period. These sightings were of 83 different females. As these observations are thus of the same females sighted over multiple years, a Generalised Linear Mixed Effects Model (GLMM) was used to take into account the repeated records of the same individuals. GLMMs incorporate random effects such that they encompass variation among individuals when multiple responses are measured per individual. A GLMM (*glmer* function in *lme4* package) with a binomial distribution and a "logit" link function, and with female ID as a random effect, was used to investigate the effects of the environmental covariates on the probability of calf presence in the *following* year. The explanatory environmental variables investigated are listed in Table 1. To ensure that the environmental variables are not correlated, and thus to avoid multicollinearity in the final model, variables were selected for inclusion based on variance inflation factors (VIFs). Starting with the full, global model, the VIF for each of the six covariates in the model was calculated (*car* package in R). The covariate with the highest VIF value was removed from the model in a stepwise fashion until the VIF values for all covariates included in the model were below three (Zuur, et al. 2010). VIF calculation showed that zooplankton biomass and mackerel spawning stock biomass were highly correlated with a number of other covariates (Supplementary Fig. S3), and were thus removed from the analyses.

A global model was generated including the five remaining environmental covariates in the previous year (phytoplankton bloom magnitude, copepod abundance, *Calanus* abundance, herring spawning stock biomass and capelin abundance) and pregnancy rate. Backwards model selection using the *dredge* function was used to identify the variables that best explain the variation in the calf presence/absence data, and thus to include in the final model based on the smallest Akaike Information Criterion (AIC). Here, backwards model selection showed that four models were of equal fit to the data as the AICs were within two units of each other. This subset of four models was then used for model averaging (*model.avg* function in the *MuMIn* package, R). This averages the regression coefficients across multiple models in order to capture the overall effects of the different variables (Banner and Higgs, 2017), and is useful when there is little to differentiate between a set of models. The model-averaged coefficients were calculated based on full averages, which sets the coefficients of absent variables to zero, rather than excluding them from the average (conditional averages). The full average ensures that the presence of variables does not bias the model-averaged estimate away from zero. Interpretations of the averaged model coefficients were used to assess the effects of each variable. *Predict* plots, in the in the *ggeffects* package, were produced to visualize the effect of each explanatory variable while all others were held constant at their mean. The residual plots, the distribution of scaled residuals and the response against fitted values were checked for the final, averaged model. An alternative modelling approach, using Generalized Additive Mixed Models (GAMMs) was also trialled, but there was no evidence for non-linear smooths (all effective degrees of freedom were estimated at 1), so the GLMM results are presented here.

Results

1. Identifying Pregnant Females

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

2. Pregnancy Rates and Calving Rates Over Time

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

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

3. Probability of Cow-Calf Pairs and Environmental Variables

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

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

Discussion

Decline in Calving Rates

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

Estimating Reproductive Failure

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

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

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

Calving Success Varies with Environmental Conditions

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

In mammals, spontaneous abortions, premature births, still births, and neonatal mortality occur under harsh environmental conditions (Wasser and Barash 1983; Sibly, et al. 2002; Stockley 2003). For example, in marine mammals specifically, significant fetal mortality occurs in late gestation in New Zealand Fur Seals (*Arctocephalus forsteri*), and reproductive failure during gestation appeared to be the most significant factor in determining reproductive success in the early 2000s (McKenzie, et al. 2005). A combination of ultrasound measurements and endocrine profiling revealed high rates of pregnancy failure in the Gulf of Mexico bottlenose dolphin populations following the Deep Water Horizon oil spill (Kellar, et al. 2017). Rates of pregnancy failure were higher than in other populations in areas not impacted by the spill (Kellar, et al. 2017). Pregnancy failure has also been documented in the Southern Resident killer whale (*Orcinus orca*) population whereby up to 69% of all detectable pregnancies were unsuccessful between 2008 and 2014 (Wasser, et al. 2017). The reduced availability of their main prey, Chinook salmon (*Oncorhynchus tshawytscha*) was identified as a significant cause of late pregnancy failure, including unobserved perinatal loss (Wasser, et al. 2017). In fact, half of these failures occurred at later stages of reproduction, and were significantly associated with nutritional stress. It was concluded that pregnancy failure is a major constraint on killer whale population growth, triggered by insufficient prey resources (Wasser, et al. 2017).

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

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

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

Environmental Variability and Implications for Population Growth

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

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

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

Conclusions and Future Directions

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

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Data Sharing and Accessibility

The data that support the findings of this study are openly available in the University of St Andrews Research Portal, Pure, at https://doi.org/10.5194/essd-10-1807-2018, Pure ID: 271446508.

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Table 1. Annual biological data collated and/or collected by the Department of Fisheries 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
	Calanus Abundance (ind / m^2)	Average of large *Calanus* species abundance in June and October (*C. fimarchicus*, *C. glacialis*, *C. hyperboreus*) over the South, NE and NW GSL.	Atlantic Zone Monitoring Program

Fish	Herring SSB.	Herring (*Clupea harengus*) spawning stock biomass in the entire GSL	DFO stock assessment
	Mackerel SSB.	Mackerel (*Scomber scombrus*) 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 (*Mallotus villosus*) abundance in August in the northern GSL.	DFO ecosystem survey

Table 2. Summary of the annual sightings and biopsy information used to calculate 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**

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

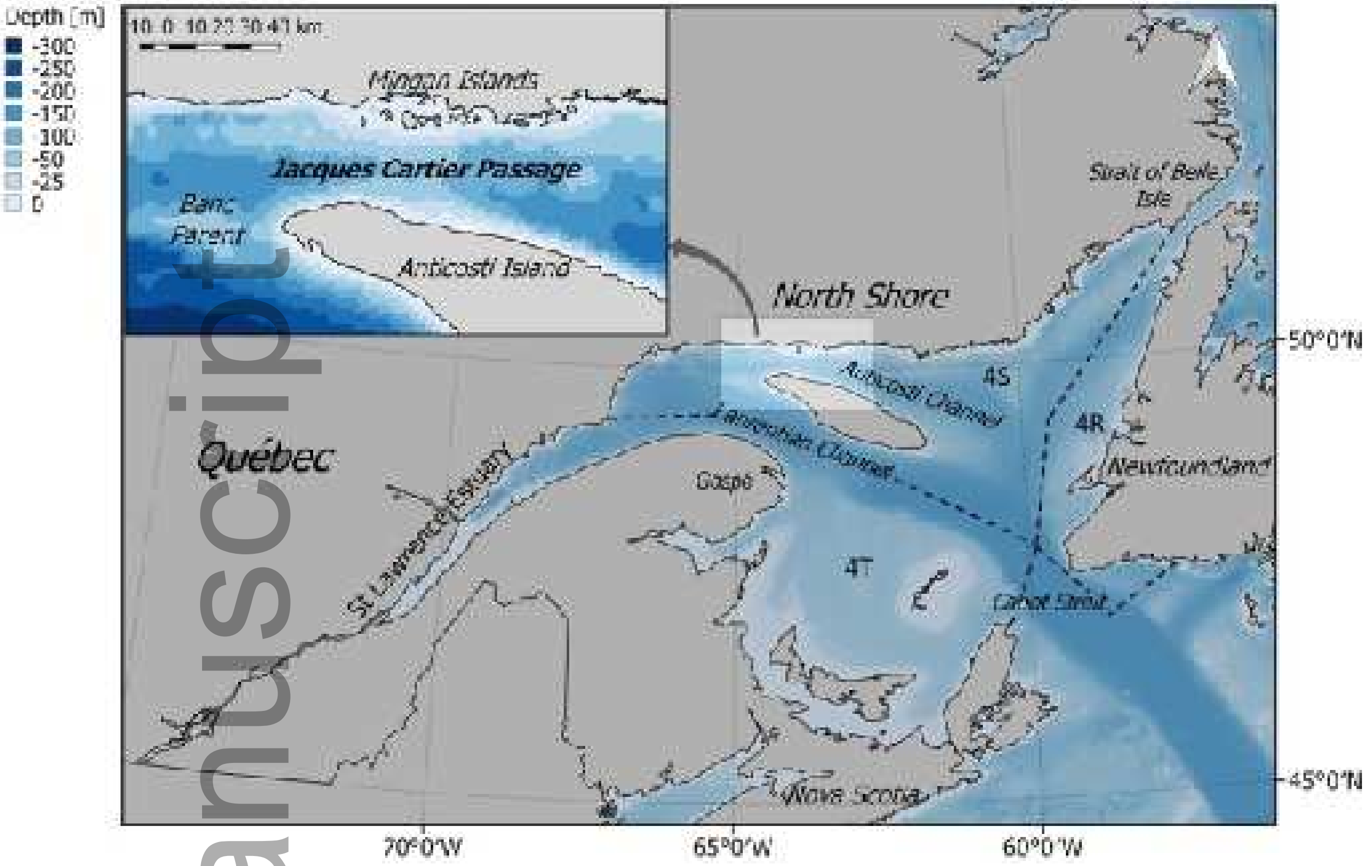
Fig. 2. Progesterone concentrations in 185 female blubber biopsy samples coloured by known or unknown reproductive status. The concentrations measured in the confirmed pregnant (black) and not pregnant (light green) samples were used in a logistic regression model to determine the probability that a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

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

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

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

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



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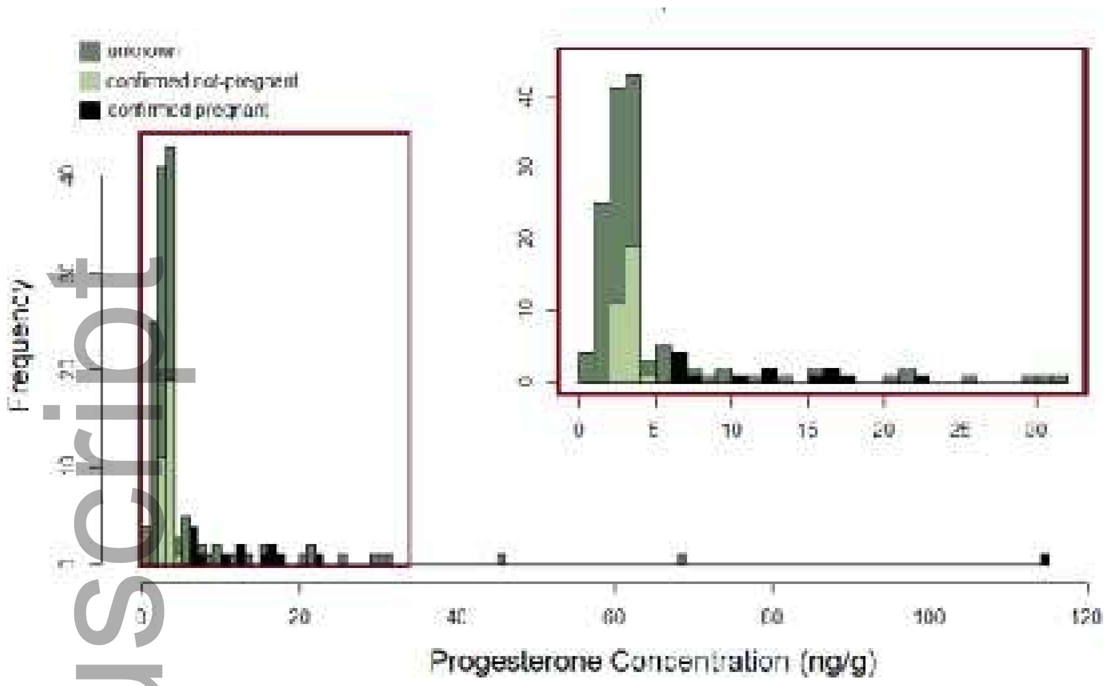

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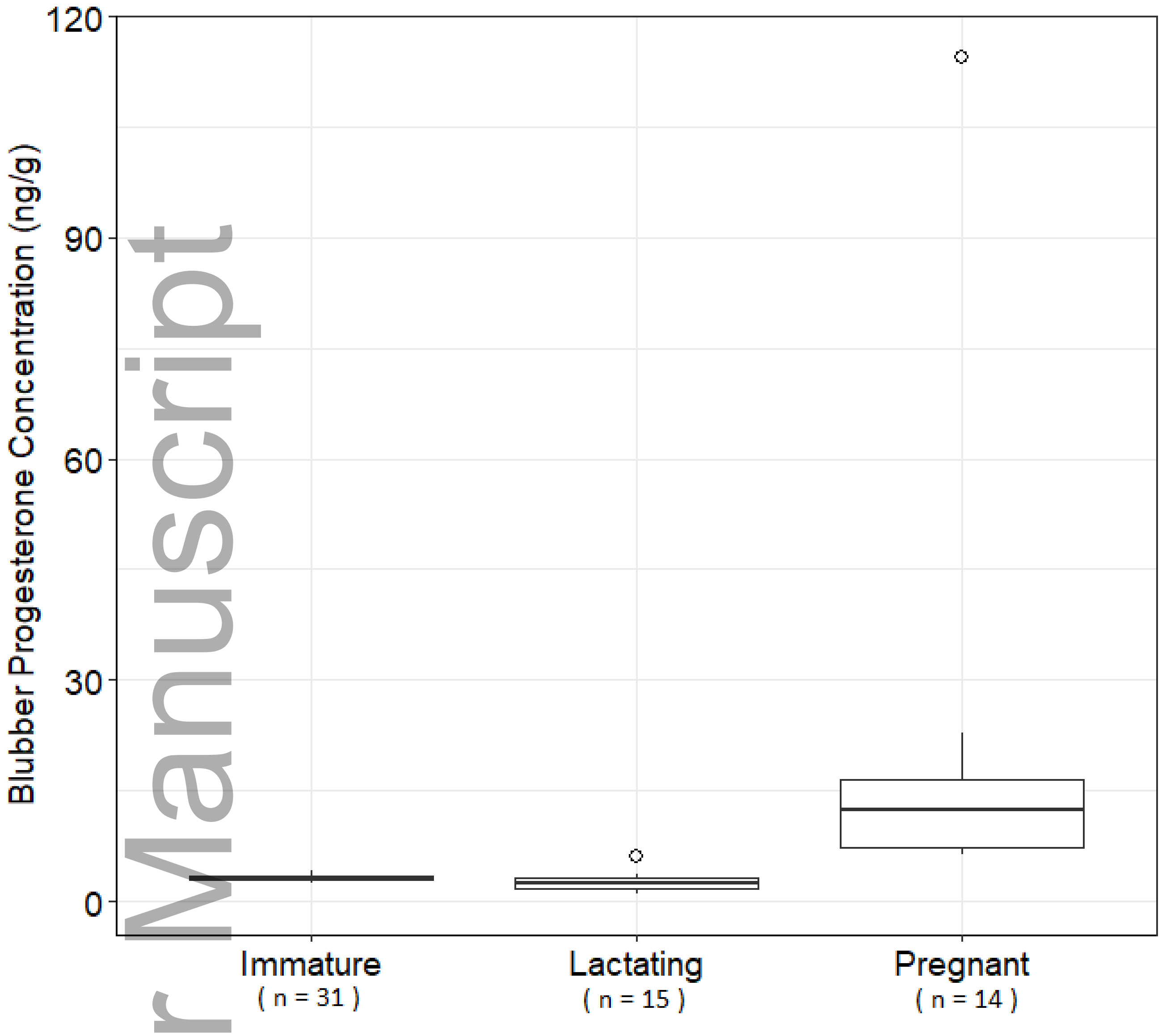

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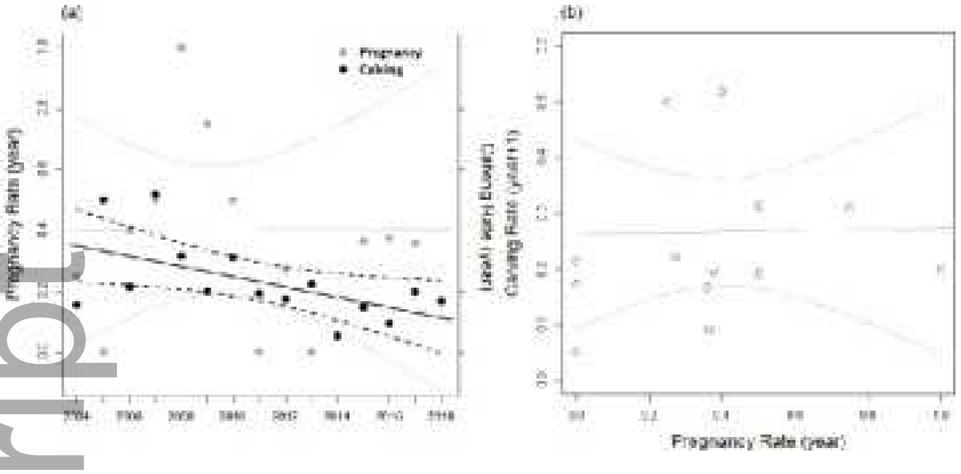

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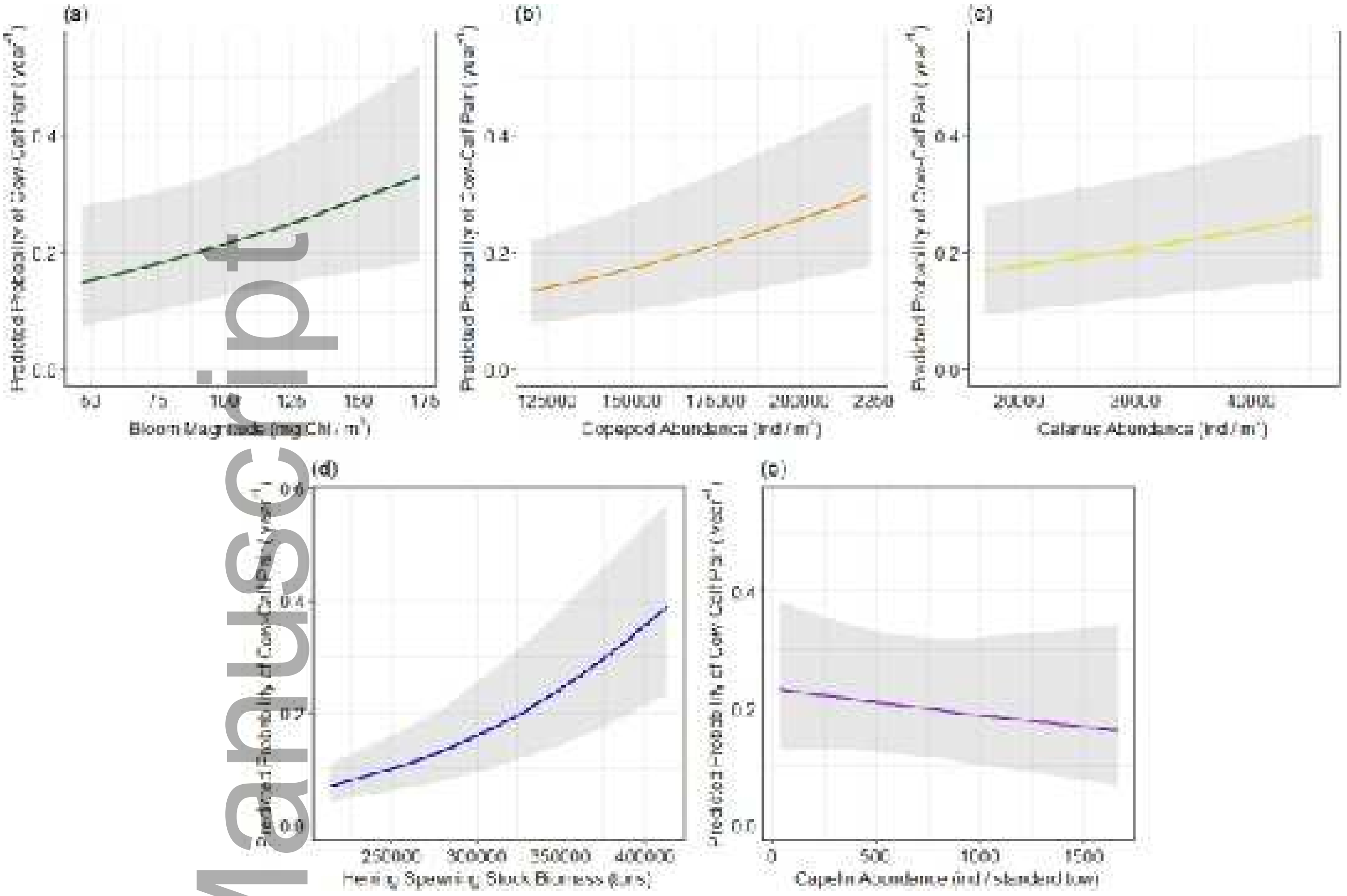

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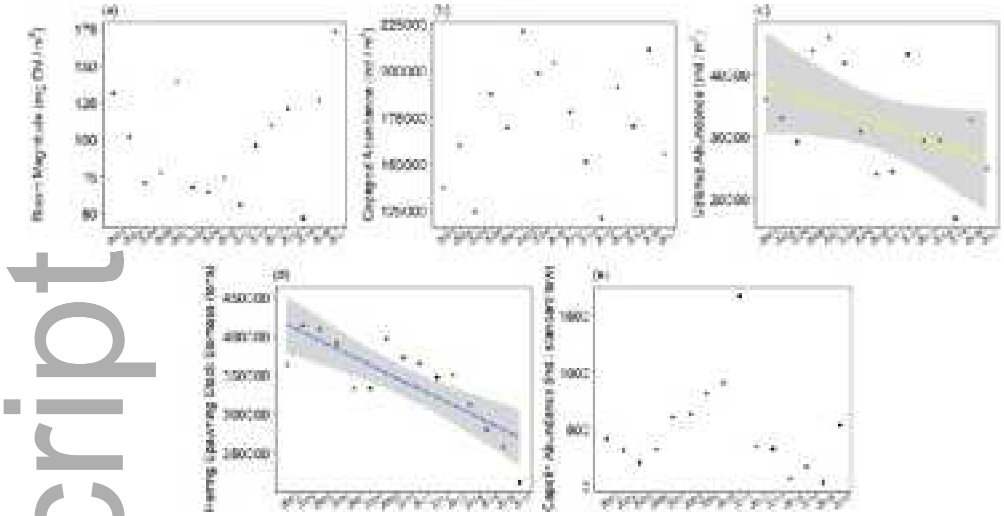

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