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Abstract
Southern right whales (*Eubalaena australis*) in South Africa have been extensively studied since 1979 through annual photo-identification surveys. The resulting database revealed an increased rate of reproductive failure in the last decade. As reproductive success is mediated through body condition, this study aimed to assess the body condition and physiological indicators of stress of southern right whales on the South Africa breeding ground at present, and compare it to historical data and other populations. For this, aerial photographs of southern right whales were collected using an unmanned aerial vehicle in September 2019 from which body condition was assessed. Additionally, blubber biopsy samples were collected for glucocorticoid (GC) analysis and compared to the body condition estimates of individual whales. To assess temporal change, analogue aerial photographs taken in coastal South Africa in 1988 and 1989 were selected and digitized for body condition measurements, and compared to the 2019 data set. To determine population differences in body condition, the 2019 data set was compared to body condition data from breeding grounds in Argentina and Australia collected the same year. We found a positive relationship between body condition of lactating southern right whales of the South African population and their blubber GC levels, albeit in a small sample size. The temporal comparison revealed a 24% (SE=5.31) decrease in body condition between 2019 and 1988/1989. Furthermore, lactating females in South Africa were in significantly poorer condition compared to those in Australia and Argentina, at a magnitude of -8.1% (SE=3.07) and -7.1% (SE=3.31), respectively. The reduced maternal body condition in the South African population of southern right whales is of grave concern, as it is likely the main reason behind the reduced reproductive success that is decelerating population recovery.

Introduction
The South African population of southern right whales (*Eubalaena australis*) has been extensively monitored through annual aerial surveys since 1969 (Best, 1990). Since 1979, these surveys include a photo-identification component, allowing for the individual monitoring of reproductive females in the population. The resulting long-term sighting history dataset has enabled the estimation of vital population parameters, documenting population recovery post-whaling (Best et al., 2001; Brandão et al., 2018). However, despite the observed population increase over four decades (1979-2019), the past decade (2010-2019) is marked by noteworthy changes, including (a) a 50% to 80% decrease in coastal prevalence of unaccompanied adults, (b) an increase in

calving intervals from 3-year intervals to 4- and 5-year intervals and resulting mixture of calving cohorts (Vermeulen et al., 2020), (c) a suspected reduced residency time at the South African breeding ground (Vermeulen et al., 2020) and (d) a decrease in the estimated population growth rate from 7% to 6.5% per annum (Brandão et al., 2018). Together these changes suggest that 1) the migration and habitat use patterns in this population may be altered, and 2) the recovery rates of this population may be hindered by a decreased reproductive success.

Considering the high energetic cost of reproduction for southern right whale mothers (Gittleman & Thompson, 1988), particularly during lactation (Christiansen et al., 2018; Lockyer, 1981), reproductive success is mediated through foraging success (Forcada et al., 2005; Greene & Pershing, 2004; Seyboth et al., 2016). Studies in the southwest Atlantic have shown a direct link between a decreased calving success of southern right whales and a decreased krill (*Euphausia superba*) availability on their feeding grounds as a consequence of climate change (Leaper et al., 2006; Seyboth et al., 2016). Interestingly, coinciding with the observed decrease in reproductive success within the South African population, a recent study revealed a dramatic northward shift, and diversification, in foraging strategy of South Africa's southern right whales between the 1990s and 2010s likely related to changes in their preferred habitat or prey (van den Berg et al., 2021). In order to evaluate the effects of these behavioural changes on the foraging success of the population, and the possible link to the reduced reproductive success, an in-depth assessment of the physical body condition of the parous females is warranted. Certainly, understanding temporal changes in body condition, and how this may impact calving success and associated demographic processes is vital to predict a population´s resilience to external stressors.

The aim of this study was to assess the body condition and physiological indicators of stress of southern right whales on the South Africa breeding ground at present, and compare it to historical data and to other populations. More specifically, we aimed to answer the following questions:

1. Is there a relationship between body condition and blubber glucocorticoid concentrations in southern right whales in South Africa?
2. Has the body condition of southern right whales in South Africa declined over the last decades?
3. Is the average body condition of southern right whales in South Africa poorer compared to other populations in the southern hemisphere?

Materials and methods

Data collection

Southern right whale body condition data and blubber biopsy samples were collected in Walker Bay and St Sebastian Bay, South Africa, between the 3rd and 15th of September 2019, from a small (7m) research vessel. Sampling was only conducted during calm weather conditions (Beaufort <3).

A Phantom 4 Pro (diameter = 350 mm, weight = 1388 g, 1'' CMOS sensor, 20MP, 5472x3078 pixels, 8.8mm focal length) multirotor unmanned aerial vehicle (UAV) was used to take aerial photographs of southern right whales at altitudes ranging from 15 to 25m. Photographs were taken of the dorsal side of the whales as they surfaced to breathe (Christiansen et al., 2016). Following the protocol of (Christiansen et al., 2018), each photograph was quality graded based on camera focus, body posture (degree of rolling and arching) and body shape visibility (ability to see the rostrum, fluke notch and the body contour) and only photographs of adequate quality (see Christiansen et al., 2018) were kept for the analyses.

Biopsy samples were collected from the outer blubber layer of a sub-set of the whales using a Barnet Panzer 5 (150lb string) crossbow with flotation darts with 8 mm diameter and 25 mm length cutting heads. During sampling, the research vessel approached the whales from the rear and side and biopsies were taken from the mid-dorsal surface of the whales at distances ranging from 20 to 50m. The darts were positively buoyant and were collected by hand from the water after sampling. The samples were stored in a cooler on ice and relocated to a freezer for storage at -80°C as soon as possible upon return to land at the end of the day (Ranging between 1 and 6 hours after sample collection, depending on timing of sample collection in the field day).

Individual southern right whales were identified from the unmanned aerial vehicle aerial photographs using the unique callosity pattern on their heads (Payne et al., 1983). Whales were classified into calves (a smaller animal accompanied by a larger animal > 1.5x its body length), juveniles (relative head length < 23% of the body length), non-lactating adults (relative head length > 23% of the body length) and lactating females (a larger animal accompanied by a smaller animal < 2/3 its body length) (Christiansen et al., 2020).

Body condition index

From the aerial photographs, the body length and width (at 5% increments along the body axis) of each whale were measured, in pixels, and standardized against the body length (fixing the length to 1). For each width measurement, the corresponding height (dorso-ventral distance) was calculated, using the known height-width ratio of southern right whales given by Christiansen et al., (2019). Since the inbuilt barometric altimeter of the UAV did not provide accurate altitude measurements, only relative length and width measurements (standardized against a body length of 1) were used. The body volume (BV) of each whale was then estimated using the elliptical volume approach by Christiansen et al., (2019) and the body condition index (BCI) was calculated using the formula of Christiansen et al., (2018) modified for relative measurements:

$$BCI = \frac{BV_{Obs} - \mu(BV)}{\mu(BV)} \qquad (1)$$

where BV_{Obs} is the observed body volume, standardized against a body length of 1, and $\mu(BV)$ is the mean body volume, standardized against a body length of 1, of the sample population. A positive BCI means that an animal is in better condition than the average of the sample population and a negative BCI indicate that the animal is in poorer than average condition. Because all measurements were standardized against a body length of 1, our BCI could be estimated without knowing the absolute length of the animals.

Blubber glucocorticoid extraction and quantification

Glucocorticoids (GC) were extracted from blubber samples following the method of Kellar et al., (2013) with modifications. Approximately 0.07 to 0.15 g of blubber was weighed out, and after adding 1 ml Ethanol (100%) homogenised for 2 h using a Qiagen® TissueLyser II at a speed of 30 m/s. After that, 0.5 ml of the homogenate was transferred into a 5 ml Borosilicate glass tube and 2 ml Ethanol/Acetone (4:1) was added. The resulting solution was vortexed for 5 min at 1500 rpm, and centrifuged for 10 minutes at 4000 rpm before the supernatant was transferred into another 5 ml Borosilicate glass tube and evaporated. Two millilitres of Diethyl Ether were added to the evaporated sample, before being vortexed for 5 min at 1500 rpm and centrifuged for 10 min at 4000 rpm. The supernatant was again transferred into another 5 mL Borosilicate glass tube and evaporated. One millilitre of Acetonitrile was added to the sample and the solution vortexed for 5 min at 1500 rpm. Then, 1 ml of Hexane was added, the solution again vortexed for 5 min at 1500 rpm, and centrifuged for 10 min at 4000 rpm. After that, the lower Acetonitrile layer of the solution was aspirated into another 5 mL Borosilicate glass tube. This process was repeated with another 1 ml Hexane. Finally, the solution was evaporated, the residue centrifuged for 5 min at 4000 rpm and stored at -20°C until assayed. Extract residues were reconstituted in 250 to 500 ml assay buffer (depending on original sample weight). Subsequently, extracts were measured for glucocorticoid metabolite concentrations, using a Cortisol assay (Palme & Möstl, 1997), utilizing a Cortisol-3-CMO:BSA antibody and a Cortisol-3-CMO-DADOO-biotin label. Assay characteristics including antibody cross-reactivities are provide by Palme & Möstl, (1997). Intra- and inter-assay coefficients of variation (CV), determined by repeated measurements of high and low quality controls was 4.42% and 6.24% (intra-assay CV) and 11.57% and 12.51% (inter-assay CV), respectively. The sensitivity of the Cortisol EIA used is 83.3 pg/g blubber weight. Serial dilutions of blubber extracts gave displacement curves that were parallel to the respective standard curve with the relative variation of the slope of the trend lines being 5%). Assay procedures were conducted in the Endocrine Research Laboratory, University of Pretoria, South Africa, and followed published protocols (see Ganswindt et al., 2002).

Body condition versus blubber GC levels

Linear models were developed in R 4.0.3 (R Core Team, 2020) to determine the relationship between blubber GC level and body condition. We also investigated the effect of Day of year, location (Walker Bay versus St Sebastian Bay), reproductive class, calf relative length (as % of maternal body length) as well as interactions between the different covariates, on GC levels.

Temporal body condition comparisons

To investigate a possible temporal change in the body condition of southern right whales we compared our 2019 body condition data set ("late" period) with aerial photographs taken over De Hoop Nature Reserve, South Africa, between July and November 1988 and 1989 ("early" period) by Best & Rüther, (1992) for photogrammetry purposes. The early photographs were taken from a helicopter using a vertically mounted Hasselblad ELM camera with a 250mm lens (for details see Best & Rüther, 1992). All good quality photographs were scanned professionally for conversion to digital images. The same picture grading protocol was applied to this data set and the body length and width of all the whales were measured to estimate the body volume and body condition of the individuals. Linear models were then used to compare the body condition of different reproductive classes between the early (1988/89) and late (2019) sampling period. For lactating females, calf relative body length (% of maternal length) was included as a covariate since maternal body condition is known to decline as the calf grows in length (Christiansen et al., 2018, 2020).

Interpopulation body condition comparisons

To investigate if southern right whales in South Africa are in poorer body condition than other southern hemisphere populations, we compared the 2019 data set with body condition measurements collected in Australia (Head of Bight) and Argentina (Península Valdés) in 2019.

For details about the study sites and sampling procedures, see Christiansen et al., (2018) for Australia and Christiansen et al., (2019) for Argentina. Since the Australian and Argentinian data sets both contained repeated measurements of the same individuals over the breeding season, a single measurement was extracted randomly for each individual to avoid pseudo-replication. Further, each data set was restricted to the same sampling period (3^{rd} and 15^{th} of September 2019) as South Africa, to avoid seasonal sampling biases. After filtering the data, the same picture grading protocol and measurements were applied to all three populations and the body condition of different reproductive classes were compared using linear models. For lactating females, calf relative body length (% of maternal length) was again included as a covariate in the model.

Model validation

Model validation tests included scatter plots of model residuals against fitted model values (to evaluate homogenous residuals), frequency histograms of model residuals (to examine normality of residuals) and calculating leverage scores and Cook's distance (to identify influential points and outliers, respectively).

Results

We collected data on 7 out of 13 days in the field between the 3^{rd} and 15^{th} of September 2019. In total we obtained body condition measurements for 100 individual southern right whales, of which 46 were of adequate quality to be included in the analyses. Of these, 26 were calves, 1 juvenile, 2 adults and 17 lactating females.

Body condition versus blubber GC levels

A total of 15 blubber samples were obtained from measured individuals, including 11 lactating females, 3 juveniles and 1 unaccompanied adult. Including all individuals and photo qualities, there was no clear relationship between blubber GC concentration and body condition index (Fig. 1A). However, when restricting the data to lactating females and good quality measurements only (n=7), we found a significant positive relationship ($F_{1,5}$=8.993, p-value=0.030, R^2=0.643) between blubber GC concentrations, with GC concentrations decreasing at a rate of 0.271 (SE=0.091) ng/g blubber for every 10% decrease in maternal body condition (Fig. 1B).

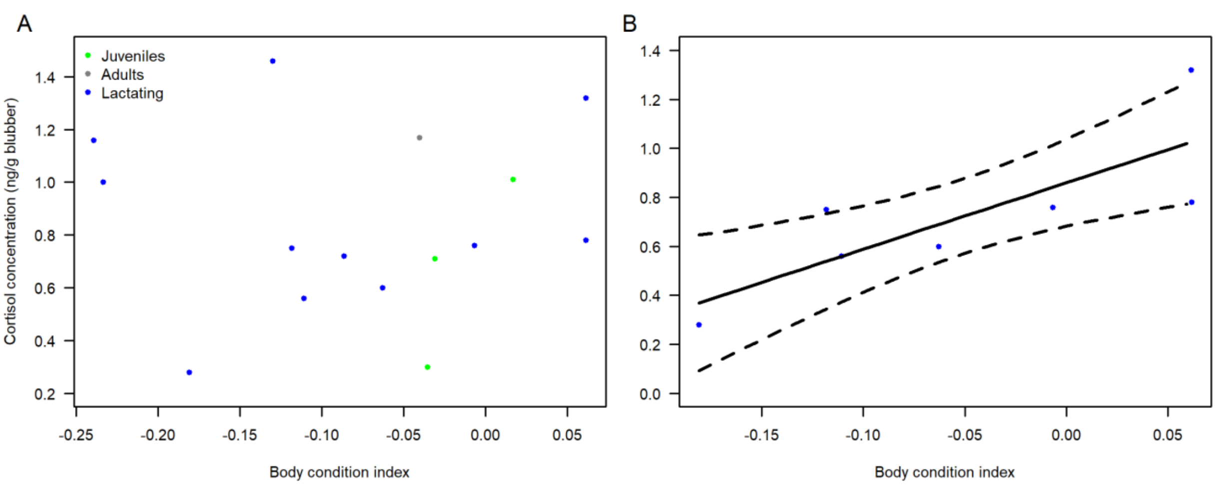

Figure 1. *(A) Southern right whale blubber glucocorticoid concentrations as a function of body condition. The colour of the data points indicate the reproductive class (see key) of the sampled individuals (n=3*

juveniles, 1 adult, 11 lactating females). (B) Maternal blubber glucocorticoid concentration against body condition (n=7 females) with the solid black line representing the fitted values of the linear model and the dashed lines representing 95% confidence intervals.

Temporal body condition comparisons

From the 1988-1989 aerial photograph data set, we extracted measurements from 188 southern right whales (1988=89, 1989=99), of which 83 were of adequate quality to be included in the analyses, including 68 calves and 15 lactating females. Comparing the body condition of lactating females between both sampling periods, we found a significant difference ($F_{1,36}$=20.408, p-value<0.001, R^2=0.362), with the body condition of whales in 2019 being on average 24% (SE=5.31) lower than in 1988/89 (Fig. 2A). We found no effect of calf body length on maternal body condition, however it should be noted that the length range of calves in 2019 was much narrower (the majority were between 40-50% of maternal body length) compared to the 1989/99 data set (the majority were between 35-55% of maternal body length) (Fig. 2B).

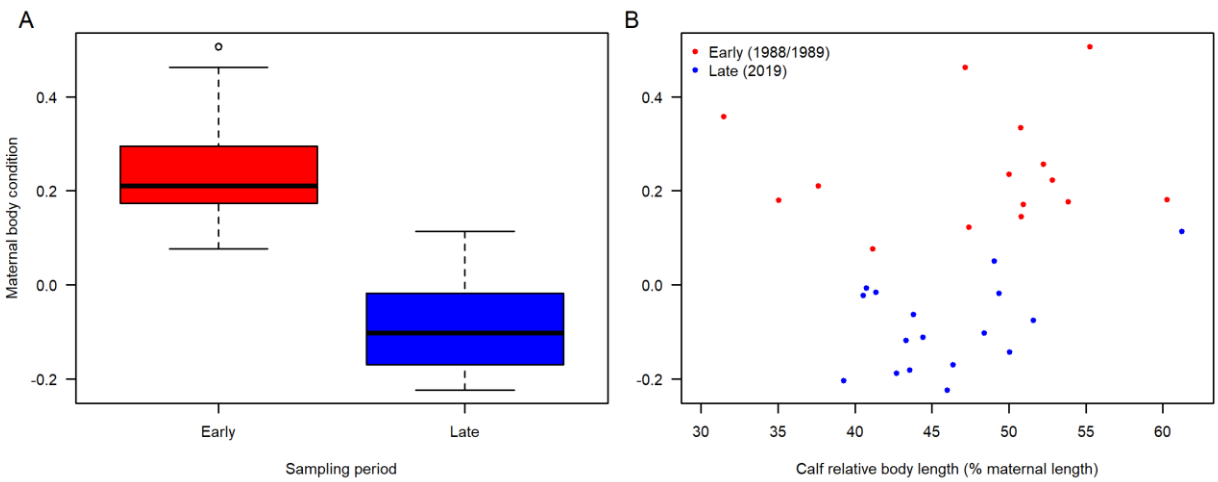

Figure 2. *(A) Comparison of southern right whale maternal body condition measured in 1988/89 (early sampling period, n=15 lactating females) and 2019 (late sampling period, n=17 lactating females). (B) Maternal body condition as a function of calf relative body length (% maternal length). The colour of the data points indicates the sampling period (see key).*

Interpopulation body condition comparisons

The filtered 2019 data set contained 86 animals from Australia (40 calves, 3 juveniles, 3 adults and 40 lactating females) and 64 from Argentina (26 calves, 8 juveniles, 4 adults and 26 lactating females). Due to the small sample sizes for juveniles and unaccompanied adults, we restricted the analysis to lactating females only. We found a significant difference ($F_{2,80}$=3.639, p-value=0.031, R^2=0.083) in body condition between locations, with lactating females in South Africa being in significantly poorer condition compared to Australia and Argentina, at a magnitude of -8.1% (SE=3.07) and -7.1% (SE=3.31) body condition, respectively (Fig. 3). There was no difference in maternal body condition between Australia and Argentina (Fig. 3). Calf body length did not have a significant effect on maternal body condition in any of the three populations.

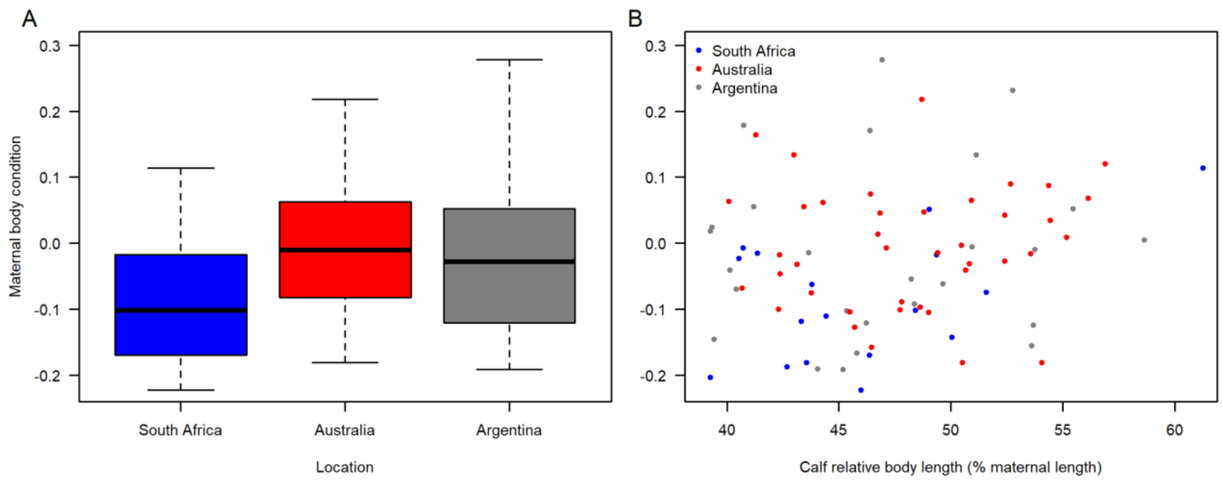

Figure 3. *(A) Comparison of lactating female body condition between South Africa (n=17), Australia (n=40) and Argentina (n=26) in 2019. (B) Maternal body condition as a function of calf relative body length (% maternal length). The colour of the data points indicates the sampling location (see key).*

Discussion

This is the first report on the southern right whale body condition work conducted in South Africa. Prior to completion, additional sensitivity analyses will need to be done to ensure high data standards. Nonetheless, this provides a first insight in the current status of the maternal body condition and preliminary GC levels of South Africa's lactating female southern right whales.

Our results indicate an increased level of blubber GC with increased body condition. This is opposite of what we would expect, and may be related to the low sample size.

The reported 24% decrease in maternal body condition between the late 1980s and 2019 is an alarming finding and indicates a drastic reduction in foraging success of the South Africa right whales. To put this into perspective, lactating southern right whale females in Australia lose on average 25% of their initial body condition over the three month breeding season (Christiansen et al., 2018). Our findings confirm the hypothesis of van den Berg et al., (2021) who suggested that the altered foraging strategy in the past two decades is related to an energetic need, albeit the alteration seems insufficient to fully cope with the changing ocean conditions. Although the underlying environmental and oceanographic changes on the South Africa's southern right whale foraging grounds remain to be determined, they clearly result in either a decreased prey availability or a decreased quality of prey for the whales and other krill consumers. Illegal Soviet whaling data revealed that euphausiids form the most important component of the southern right whale´s diet when feeding south of 50°S, while copepods were the dominant food source when feeding north of 40°S (Tormosov et al., 1998). The observed northward shift in foraging location of the South African population (van den Berg et al., 2021) could thus suggest increased foraging on copepods. However, further studies are needed to confirm this line of thought. Regardless, the reduced maternal body condition in the South African population is of grave concern, as it is likely the main reason behind the reduced reproductive success that is decelerating population recovery.

Additionally, the South African population of southern right whales is in a significant poorer body condition compared to the populations calving off Argentina and Australia, suggesting their foraging success has been reduced more drastically. This is in line with the more pronounced decreased coastal prevalence and increased calving intervals in the South African population compared to the Argentinean and Australian populations (C. Charlton et al., 2018; C. M. Charlton, 2017; Vermeulen et al., 2020). The reason/s for this interpopulation variation remains to be determined.

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