Assessing the nutritional state of southern right whales (*Eubalaena australis*) through measurements of body volume and blubber glucocorticoids levels, and investigating the relation to reproductive success

by

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Submitted in partial fulfilment of the requirements for the degree Master of Science (Zoology)

in the

Department of Zoology and Entomology Faculty of Natural and Agricultural Sciences

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DECLARATION

I, Terriann Thavar, declare that this dissertation, which I hereby submit for the degree Master of Science (Zoology) at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

ETHICS STATEMENT

I, Terriann Thavar, declare that the research described in this work has observed the ethical standards required in terms of the University of Pretoria's Code of Ethics for Researchers and the Policy guidelines for responsible research and received approval from the University of Pretoria Animal Ethics Committee.

DISCLAIMER

Chapters 2 and 3 in this MSc dissertation were structured according to the target journal or scientific commission to which they were submitted for publication. Therefore, it is inevitable for some overlap and repetition between chapters to exist

Terriann Thavar August 2021

SUMMARY

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Keywords:	Southern right whale, reproductive success, aerial photogrammetry,
	body condition, stress, foraging success, climate change

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 dissertation 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 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 the body condition of lactating southern right whales of the South African population and their blubber glucocorticoid 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 (F=3.639, p=0.031, R²=0.083) 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 great concern, as it is indicative of the reduced reproductive success that is decelerating population growth.

RESEARCH OUTPUTS

Technical reports:

• **CHAPTER 2** was submitted and presented as a scientific committee report to the International Whaling Commission:

Thavar, T., Christiansen, F., Ganswindt, A., Sironi, M., Uhart, M., Bejder, L., Vermeulen, E. 2021. Southern right whale (*Eubalaena australis*) body condition and glucocorticoid levels at the South Africa breeding ground. Report SC/68C/SH/08 presented to the 68C IWC scientific committee (Southern Hemisphere Subcommittee), Cambridge, UK.

LIST OF ABBREVIATIONS

MRI	Mammal Research Institute
SRW	Southern right whale
IWC	International Whaling Commission
SST	Sea surface temperature
PF	Polar Front
STC	Subtropical Convergence
STF	Subtropical Front
SAF	Subantarctic Front
NARW	North Atlantic right whale
BC	Body condition
BV	Body volume
UAV	Unmanned Aerial Vehicle
НРА	Hypothalamic-pituitary-adrenal
GC	Glucocorticoid
BCI	Body condition index
AIC	Akaike's Information Criterion
IUCN	International Union for conservation of nature
SORP	Southern Ocean Research Partnership

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Terriann Thavar August 2021

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CHAPTER 1

Literature review

1.1 THE SOUTHERN RIGHT WHALE

The southern right whale (*Eubalaena australis*; SRW; Figure 1.1) is a baleen whale species only found in the Southern Hemisphere, between 20 and 64 degrees South (Mate et al., 2011). As with most baleen whales, they undertake an annual migration from their summer feeding grounds in sub-Antarctic waters, to their winter mating and calving grounds in coastal areas in higher latitudes, such as the coast of southern Africa (Cummings, 1985; Reeves & Kenny 2003).

SRW females give birth to a calf every three years on average (Cooke et al., 2001). After a gestation period of about 12 months, the female will give birth to a single calf that will stay with her, suckling for approximately one year (Brandão et al., 2011). Subsequently, she has a year of rest in order to recover and prepare for her next pregnancy. The calving interval is thus typically 3 years (Best, 2007; Brandão et al., 2011). They usually give birth to their first calf when they are eight years old (Best, 2007).

SRWs are characterised by the white patches (callosities) on their head, mostly in areas where hair follicles are present. These callosities are inhabited by tiny crustaceans called cyamids or whale lice, which gives the callosity a typical whitish colouration (Payne et al., 1983). The pattern these callosities form on the whale's head is unique for each animal and is used by researchers to identify each whale individually.



Figure 1.1. Aerial photograph of a southern right whale (Copyright MRI Whale Unit)

1.2 THE "RIGHT" WHALE

1.2.1 The plight of the southern right whale

The SRW is a species whose history over the last few centuries is defined by severe overexploitation through whaling, as the global population was decimated in the late 18th and early 19th centuries. They were considered the "right" whale to hunt, as they had a predictable presence in coastal waters, had a high oil yield, are generally slow and float when killed, allowing whalers to kill various individuals to only then collect them and bring them ashore (Best, 2007). Due to this 'ease' in whaling of the species, it is believed that roughly 125,000 – 151,000 individuals have been taken between 1770 and 1850 (Best, 2007), reducing the global population from an estimated 60,000 - 100,000 animals prior to exploitation (in around 1770) to approximately 300 animals by 1920 (IWC, 2013). The estimated catch history trajectory for the global population shows a steep decline in the global population between 1830 and 1850, followed by slow but further declines until their protection (IWC, 2013).

Due to this near-extinction, the SRW was among the first species to be internationally protected under the International Convention for the Regulation of Whaling of the International Whaling Commission (IWC) in 1935 (Best et al., 2001). Since then, the global population has been increasing at approximately 7% per year, a rate close to the maximum biologically possible for the species, although illegal catches of over 3,000 whales by the Soviet Union in the 1960s hampered the recovery by an estimated further 20 years (IWC, 2013). By 2009, the global population was estimated at 13,600 individuals, or about 20% of the pre-exploitation levels, of which South Africa has arguably the largest breeding stock, comprising some 32% of the global population (IWC, 2013).

1.2.2 Whaling in South Africa

Coastal whaling activities in South Africa occurred mainly between 1792 and 1975, with the SRW being the main target species especially in the early days (Best and Ross, 1986). It is believed that approximately 1,580 SRWs were taken in coastal South Africa before 1912, mainly at St Helena Bay, Table Bay, Robben Island, False Bay, Mossel Bay, Plettenberg Bay and Algoa Bay, with approximately 28 floating and/or land whaling stations erected along the South African coast by 1913 when modern whaling techniques were implemented (Best and

Ross, 1986). Particularly the Western Cape of South Africa (e.g. Saldhana Bay, St. Helena Bay) showed good potential for shore-based whaling since the late 1700s (Best, 2006).

British, French and Americans were all involved in occupying whaling vessels responsible for exploiting a minimum estimated total of 1,484 southern right whales in St. Helena Bay alone (Richards and Du Pasquier, 1989). Strikingly between 1918 and 1930, only 18 SRWs were killed in South Africa (Best, 1970). Although this may be a serious underestimate, the South African SRW population was so depleted by the early 1900s, that very few individuals could be caught regardless of the highly efficient whaling techniques (Best, 1970). Despite their international protection granted in 1935, a failure to incorporate the legal protection into South African legislation and a lack of national inspections and monitoring, meant that it was only from 1958 onwards that further SRW catches became unlikely (Best and Ross, 1986). Furthermore, the initial recovery of the population was abruptly halted in the 1950s – 1960s, where illegal Soviet whaling in the Southern Hemisphere resulted in at least 3,368 more SRWs killed (Tormosov et al., 1998).

1.2.3 Monitoring the South African right whale population recovery

Despite the severe overexploitation of SRWs, their legal protection eventually allowed most SRW breeding populations in the Southern Hemisphere, including the South African one, to begin their recovery. This recovery of the South African population has been monitored closely through annual aerial surveys since 1969 (Best, 1990a). Since 1979, these surveys started to include a photo-identification component, through which all encountered individual female whales would be photo-identified through their callosity pattern on their heads (Best, 1990b). This method has allowed the monitoring of reproductive histories of individual females and the modelling of key demographic parameters, such as female survival rates and calving intervals (Best et al., 2001; Brandão et al., 2018).

Aerial counts of SRW cow-calf pairs along South Africa's southern coast between 1971 and 2006 revealed an annual population growth rate of 6.9% (Brandão et al., 2010). However, in more recent years, some dramatic changes to the calving intervals and prevalence of SRWs have been seen, despite survey effort and monitoring techniques remaining consistent (Vermeulen et al., 2019). These changes include (Figure 1.2): 1) extreme fluctuations in the number of cow-calf pairs since 2015; 2) a decrease in the prevalence of unaccompanied adults

since 2009 (males and non-calving females); 3) an increase in calving intervals from 3-years to 4- and 5-year intervals (Vermeulen et al., 2019; Figure 1.3), implying calving failure (Knowlton et al., 1994); 4) a decrease in the population growth rate from 7.1% per annum in 2001 (Best et al., 2001) to 6.5% per annum in 2017 (Brandão et al., 2018); and 5) a suggested shift in the peak calf presence from October to August (Vermeulen et al., 2020), possibly related to earlier calving or a reduced residency time spent by cow-calf pairs on the South African coastline.

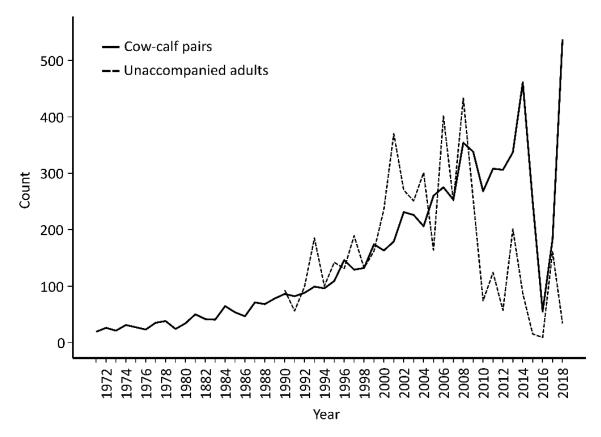


Figure 1.2. Aerial counts of southern right whale cow-calf pairs and unaccompanied adults (males and noncalving females) along South Africa's southern coast from 1971 – 2018 (MRI unpublished data).

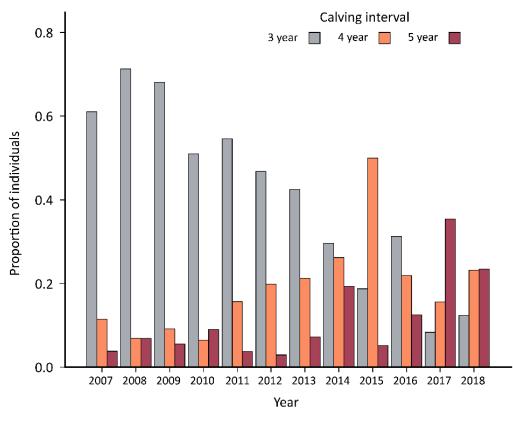


Figure 1.3. Calving interval of southern right whale females for the period of 2007 – 2018 (MRI unpublished data).

Together, these changes suggest that a fundamental shift in the population may be in progress, indicating that the continuation of the long-term monitoring of SRWs within the calving grounds of the southern Cape is more vital than ever before.

1.3 THE CAPITAL BREEDER

SRWs are capital breeders. This means that they rely on "stored capital" (aka energy reserves), obtained prior to breeding, to sustain the metabolic cost of reproduction (Lockyer, 2007). This is behaviourally reflected in foraging one time of the year while calving another time. This is opposed to "income breeders" which forage and reproduce concurrently (Stephens et al., 2009).

1.3.1 Migration and Foraging

In general, SRWs are distributed in the Southern Hemisphere between 20°S and 50°-55°S (Best, 2007). Like most baleen whales, they perform annual migrations from their summer feeding grounds in sub-Antarctic waters to their winter breeding grounds in higher latitudes, such as the coastal waters of South America, South Africa, Australia and New Zealand (Best, 2007). In general, the main reason why animals migrate remains unresolved, and there are various hypotheses on why such behaviour may have evolved, with most including an increased chance of offspring survival either through reduced predation (Corkeron and Connor, 1999), energetic benefits for the mother (Brodie, 1975) and/or increased sea surface temperature (SST; Norris, 1967).

SRWs are able to migrate over thousands of kilometres, with ranges of the South African population registered up to 8,200km based on satellite telemetry data (Mate et al., 2011). Other long-range distances recorded involve a SRW identified in Gough Island and coastal South Africa, and another individual identified in Peninsula Valdés, Argentina, and Tristan da Cunha, comprising some 3,000km and 4,500km respectively (Best et al., 1993). Within these vast migrations, SRWs are known to be highly philopatric, which means that they return to the same foraging and breeding area each year (e.g. Carroll et al., 2015). Such site-fidelity inevitably leads to a global population structure around the winter breeding areas (Patenaude et al., 2007). The related migratory routes are culturally transmitted from mother to offspring (Carroll et al., 2015; Patenaude et al., 2007; Valenzuela et al., 2009, 2008). Such maternal-directed learning of migratory behaviour in SRWs could exacerbate the species' limited ability to respond to changes in prey distribution and availability (e.g. Valenzuela et al., 2009).

Globally, SRWs are best studied at their winter calving grounds with the most important ones located in the coastal waters of Argentina, Brazil, South Africa, Australia and New Zealand. Additionally, there are a few other, smaller, calving grounds in the coastal waters of Chile/Peru, Namibia, Mozambique, Madagascar, Tristan da Cunha and Gough Island (IWC, 2012a). However, data on the location of the summer foraging grounds of these populations remain poorly understood. In general, SRWs of the southwest Atlantic are believed to mainly use the Patagonian Shelf, the waters near the Falkland Islands and South Georgia, as well as the Polar Front (PF) as summer foraging grounds (IWC, 2012a), although some foraging may also be happening on their calving ground (Vighi et al., 2014). The SRW population calving off the coasts of Australia and New Zealand likely forage between the Subtropical Convergence (STC) and 65°S (Bannister et al., 1999; Charlton, 2017; IWC, 2012a), although more recent data indicate foraging near Antarctic waters as well as the Subtropical Front (STF) (Mackay et al., 2020). The summer habitat of South African SRWs is difficult to pinpoint, due to a general lack of data. Based on illegal soviet catches (Tormosov et al., 1998) and the Townsend (1935) charts of open boat whaling catches, it is believed that South Africa's SRWs feed in three main areas (Figure 1.4) including 1) a broad band of the south-eastern Atlantic Ocean associated with the STC from Tristan da Cunha to the Cape (Townsend, 1935); 2) an area around the Crozet Islands in the southern Indian Ocean; 3) a diffuse area of the Southern Ocean south of 52°S between 013°W and 016°E (Tormosov et al., 1998); and finally, 4) a localized area of the South African west coast (Mate et al., 2011).

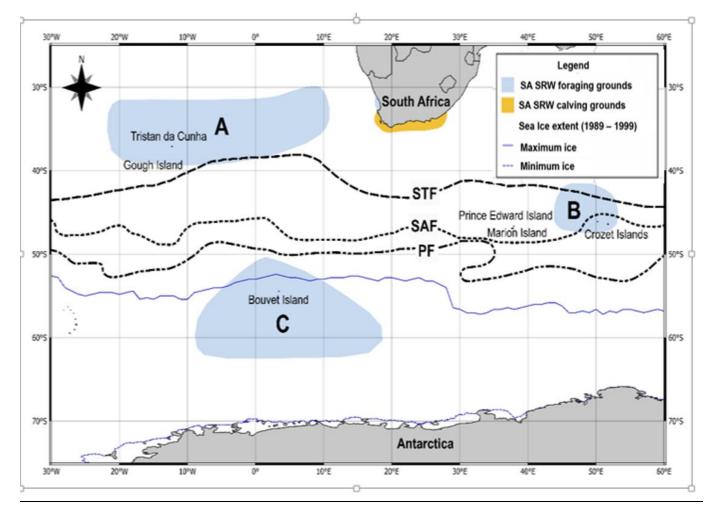


Figure 1.4. Map of the South Atlantic, Southwest Indian, and Southern Ocean, with blue shading indicating the summer feeding grounds (illustrated via A, B and C, and the South African west coast) of the South African population of SRW, as suggested by Best (2007); yellow shading: the South African SRW winter calving ground;

positions of the Subtropical Front (STF), Subantarctic Front (SAF), Polar Front (PF) (Orsi and Harris, 2019); and the minimum and maximum sea ice extents for the period of 1989 – 1999 (Smith and Jacka, 2003). Figure taken with permission from van den Berg (2020).

Similarly to the accuracy associated with the locations of South African SRW feeding grounds, little is known about the feeding habits and diet of the population (Best, 2007). The stomach contents of 249 illegal SRW catches in the 1960s provide the most substantial data for diet assessment (Tormosov et al., 1998). These data suggest that when feeding south of 50°S, krill form the most important component of SRW diet; when feeding north of 40°S, copepods become the main dietary source; and between 40° and 50°S, a combination of krill, copepods, and other 'small crustacea' are fed upon (Tormosov et al., 1998). More recently, van den Berg et al. (2021) used stable isotope analyses of South African SRW skin biopsy samples to reveal that during the 1990s, the population fed on prey isotopically similar to krill found at South Georgia, while more recently, the population seems to have shifted northwards, feeding on prey isotopically consistent with the waters of the STC, PF and Marion Island. Importantly, these findings do not represent absolute locations of feeding, but rather estimations of feeding across certain broad latitudinal bands (van den Berg et al., 2021).

1.3.2 Foraging in a changing climate

The physical properties of the global ocean are changing rapidly under various anthropogenic impacts (Bindoff et al., 2019) causing complex and multifaceted ecological change, and leading to disruptions in Southern Ocean food webs (Constable et al., 2014). Critically, Antarctic krill (*Euphausia superba*), the dominant mid-trophic level species in Southern Ocean food webs (Atkinson et al., 2004; Reid and Croxall, 2001) have shown radical decreases in density (Atkinson et al., 2004) and are contracting southwards (Atkinson et al., 2019). Rising SST and sea ice loss in the Southern Ocean are known to have a strong impact on Antarctic krill recruitment and survival on multiple levels (Flores et al., 2012). Trathan et al. (2006) showed that in the waters surrounding South Georgia, an increase in SST resulted in a decline in krill abundance, suggesting that Antarctic krill are highly vulnerable to changes in temperature. As the dominant mid-trophic level species in Southern Ocean food webs, such decreases in Antarctic krill abundance are likely to have detrimental impacts on various populations of top predators in the Southern Ocean (Barbosa et al., 2012; Forcada et al., 2005; Forcada and

Trathan, 2009; Fraser and Hofmann, 2003). In the context of this dissertation, such changes are particularly concerning for SRWs as they feed on large amounts of krill, and as previously established, are capital breeders that rely heavily on seasonal energy reserves to facilitate successful reproduction (Lockyer, 2007a) and migration (Best and Schell, 1996).

1.3.3 Foraging and reproductive success

SRWs typically produce their first calf when they are 8 years old and have a 12 – 13 month gestation period (Best, 2007). Females have a calving cycle that typically lasts three years; with a year for gestation, a year for lactation and weaning of the calf, and the third year to allow recovery and accumulation of fat reserves to support the next pregnancy (Best et al., 2001; Knowlton et al., 1994; Payne, 1986). Calving intervals of 4- and 5-years are usually believed to be associated with calving failure (Knowlton et al., 1994). In general, 4-year intervals are usually the result of the female taking an extra year of rest (and thus failing to fall pregnant) or a loss of a foetus early in gestation (Knowlton et al., 1994). Five-year intervals are usually interpreted as the result of the loss of a calf late in gestation or shortly after birth (in which case, the female had a normal 3-year interval which went undocumented, plus a 2-year interval) (Knowlton et al., 1994). In other SRW populations, such an increase in calving interval has led to a decreased population growth rate (Marón et al., 2015).

As mentioned previously, SRWs are extreme capital breeders, which means that they rely on stored energy for reproduction (Jönsson, 1997). As a result, they need to build sufficient energy reserves and body condition (BC) while at their high latitude summer feeding grounds, on which they will then depend during the various stages of reproduction (Lockyer, 2007) and likely even migration (Best and Schell, 1996). The dramatic reliance on stored energy is illustrated by Christiansen et al. (2018), who revealed that SRW mothers lose up to 25% of their body volume (BV) during the first few months of lactation. The importance of sufficient body fat reserves in driving successful reproduction in SRWs is similar to various other cetaceans and mammals in general (Lockyer, 1986; Thomas, 1990). In fact, several studies have already revealed that a decrease in krill abundance correlates with a decrease in reproductive performance in several species of marine mammals and seabirds (e.g. Constable et al., 2014; Forcada and Trathan, 2009). Greene et al. (2003) for example, revealed that the calving rates of North Atlantic right whales (*Eubalaena glacialis*; NARW) were correlated at

various lags to the abundance of copepods (*Calanus finmarchicus*). Further, Ford et al. (2010) showed that a reduced prey abundance correlated with reduced fecundity in killer whales (Orcinus orca); and finally, Williams et al. (2013) demonstrated a decreased rate of pregnancy in fin whales (Balaenoptera physalus) due to limited prey availability. More pertinent to this dissertation, strong correlations have also been found between the breeding success of SRWs in Argentina and Brazil with krill abundance in their summer foraging ground off South Georgia (Leaper et al., 2006; Seyboth et al., 2016). Interestingly, the krill abundance in South Georgia has also been linked to SST anomalies and large-scale global climate (Leaper et al., 2006; Seyboth et al., 2016; Trathan et al., 2003). Reproduction is clearly highly energetically costly for lactating females with a capital breeding strategy, as they do not only need to metabolically sustain themselves, but also their calves during the initial months of calf growth (Christiansen et al., 2018). It is therefore clear that adequate nutrition and thus foraging success is critical for SRWs to maintain their 3-year calving intervals and ultimately, ensure their continued recovery. Together, these above mentioned studies demonstrate that physical changes in the Southern Ocean are leading to cascading effects on food webs, disrupting the availability of prey, and having strong impacts on the reproductive success of species that are recovering from whaling, such as the SRW. These findings also suggest that a holistic understanding of the various facets of both physical and biological change occurring in the Southern Ocean is required, to reliably assess the impacts of anthropogenic climate change on top predators in the Southern Ocean.

1.4 MEASURING BODY CONDITION

1.4.1 External morphometrics

The BC of an individual is defined as the "*relative size of its energy reserves*" (Gosler, 1996), and it can be used as a critical metric to measure an individual's fitness related to several important life events, such as migration and reproduction (Green, 2001). Although a vast range of BC indices exists, most are related to morphological characteristics including body mass, volume, girth, and width, and are often expressed in relation to or as a ratio or proportion of body length (Labocha et al., 2014). Morphometry is thus the numerical expression of form and encompasses a technique that measures the size and shape of an organism and the relationship between the two (allometry) (Stower et al., 1960). However,

one of the main issues using morphometrics as an index of BC, is that the body of an animal is multivariate; many components vary and complicate the interpretation of BC indices thus making it difficult to detect changes in a specific aspect of an animal's body. Some assumptions thus have to be made. In regards to cetaceans, they are assumed to be comprised of an elliptical shape (Christiansen et al., 2018), and the body girth or width can be used as a proxy for energy reserves and thus BC (Best and Ruther, 1992; Lockyer, 1986). In turn, the relationship between body girth and body length has been extensively used to predict BV (e.g. Christiansen et al., 2016). Assuming the elliptical shape, BV for cetaceans is most commonly evaluated by the use of a truncated cone model (Figure 1.5), in which direct measurements of body girth and length is measured as a series of frustums (Christiansen et al., 2018).

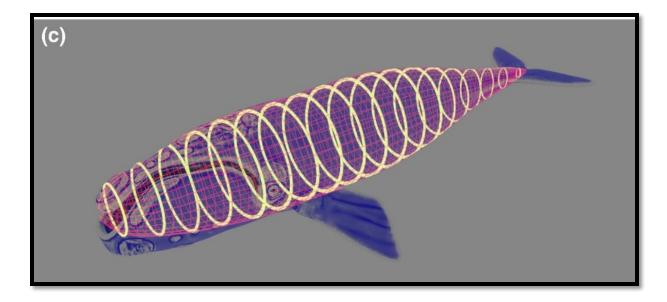


Figure 1.5. A 3D model of a southern right whale showing the truncated cone models for the measurements of body girth to estimate body volume (image used with permission from Christiansen et al., 2019).

1.4.2 Aerial photogrammetry

The technique of using aerial photogrammetry in large whales (done using traditional equipment such as aeroplanes and/or helicopters) to assess BC using morphometric indices began in the 1970s with SRWs (Whitehead and Payne, 1978). The technique was performed on a small population of SRWs off the coast of Argentina and provided an assessment of frequencies between lengths of different reproductive classes, as well as growth rates for newborn calves. Results showed that calves seen at Península Valdés are about 5.5m at birth

and grow about 35mm each day for the first weeks of life. About a decade later, Best and Ruther (1992) conducted a study applying the same technique to the South African population of SRW. This study showed that adult cows measured from 12.37 – 15.54m in length with primiparous females being smaller than multiparous females. The study further showed that the calves of primiparous females are significantly smaller than those of multiparous females (Best and Ruther, 1992). Over the years, aerial photogrammetry to study marine mammals has evolved using the same technique except that it is now fused with modern models and technology such as Unmanned Aerial Vehicles (UAVs).

1.4.3 Unmanned Aerial Vehicles

Measuring the size, mass and morphology of whales is a huge challenge, as they spend limited time on the surface making observation methods difficult. Additionally, their large body size and weight make direct measurements usually impossible. In response, UAVs are becoming increasingly popular in marine mammal science and conservation (e.g. Christiansen et al., 2020, 2016; Christiansen et al., 2018; Johnston, 2019), as they are able to perform various remote sensing tasks at minimal cost and with reduced risk for animals and humans involved. In particular, drones can aid significantly in overcoming most of the practical challenges related to aerial photogrammetry, as they are user-friendly and easy to launch, they obtain high-resolution imagery for photogrammetric work processes, and they are also very cost-effective in comparison with larger UAVs. One of the main concerns related to using UAVs is whether or not these devices cause a disturbance to the wildlife. Studies done on large whales (e.g. Christiansen et al., 2016b; Domínguez-Sánchez et al., 2018) revealed that the whales show no signs of stress when the UAVs are in close proximity and this also applies to the effect on acoustic disturbance because UAV sounds are not transmitted efficiently from the air to the water (Christiansen et al., 2016b).

1.5 GLUCOCORTICOIDS

Natural selection is based on an animal's ability to adapt or change to its environment over time (Gregory, 2009). In response, animals develop behavioural and physiological strategies to cope with the changes and associated stressors (Reeder and Kramer, 2005; Wingfield and Romero, 2001). Stress can be defined as a stimulus that disrupts an animal's homeostasis

(Seyle 1936). One of the primary physiological responses to stress is the activation of the hypothalamic-pituitary-adrenal (HPA) axis, which helps restore homeostasis. The HPA axis is made up of the adrenal cortex, the anterior pituitary gland and the hypothalamic paraventricular nucleus (Sapolsky 1992). When an animal is exposed to stress, the secretion of glucocorticoids (GC) from the adrenal cortex, which are involved in the mammalian energy balance, are released which can last from minutes to hours depending on the severity of the stressor (Sheriff et al., 2011). Acute situations may result in strong bursts of GCs over short periods of time. However, prolonged periods of elevated GCs related to chronic stress, along with the recurring activation of the HPA axis, may contribute to the reduced fitness of an individual (Boonstra et al., 1998; Sapolsky, 2002). Hence, both magnitude and duration are important factors to consider when assessing GC levels. Monitoring GC levels can thus be a promising way to assess perceived stress, including, in the context of this dissertation, nutritional stress.

Since the 1930s, blood samples have been the most common method in assessing GC levels in mammals (Selye 1936). Although measuring glucocorticoids from blood samples is advantageous in that 1) it is a direct product of the adrenal cortex for intricate analysis (Sapolsky 1992) and 2) it allows a comprehensive assessment of health status as samples provide additional data on other physiological attributes such as fatty acid composition (energy resources) and reproductive hormones (Boonstra et al., 1998; Clinchy et al., 2004; Trumble et al., 2018). However the collection of blood samples are invasive and more importantly, GC levels obtained from blood samples can be a biased representation due to the direct stress inflicted from capture methods (Sheriff et al., 2011). Fortunately, a few alternative non- invasive ways to assess GC concentrations in mammals are recently being applied by scientists through the use of hair, saliva, faecal samples, blow samples as well as the blubber found in marine mammals (Champagne et al., 2018; Kershaw et al., 2013; Kershaw and Hall, 2016; Narayan, 2013; Sheriff et al., 2011; Thompson et al., 2014).

GC are easily diffused from the capillaries into the lipid layer of the adipose tissue (Deslypere et al., 1985) and take longer to break down in blubber than in blood or skin. Therefore GC levels in adipose tissue change less rapidly than in blood (Deslypere et al., 1985). Furthermore, since GC concentrations in the outermost blubber layer of marine mammals such as the

harbour porpoise (*Phocoena phocoena*), are found to be representative of concentrations through the full blubber depth, and are comparable across the entire blubber layer regardless of the area of the body (Kershaw et al., 2017), remote biopsy sampling can be used to obtain representative samples of free-ranging marine mammals. When combined with other biomarkers of health such as body condition index (BCI), blubber GC concentrations can be used to assess potential changes in the overall health of cetacean populations (Kellar et al., 2015), a valuable tool for understanding their response to a rapidly changing ocean (Trana et al., 2016).

To date, the assessment of GC levels in free-ranging marine mammals has focused mainly on pinnipeds (e.g. Guinet et al., 2004; Kershaw and Hall, 2016). For example, Beaulieu-McCoy et al. (2017) found that blubber GC concentrations decreased inversely with the proportion of blubber lipid content in California sea lions (Zalophus californianus). A study done by Kershaw and Hall (2016) on harbour seals (Phoca vitulina) found that blubber GC levels were shown to be not significantly affected by the duration of restrain but varied significantly by sex and by season, with higher concentrations during natural fasting periods of their life cycle, particularly during the moult. These results suggest that GC play a key role in increased fat metabolism during highly energetically demanding periods, and that blubber concentrations have the potential to be used as physiological state indicators in phocid seals (Kershaw and Hall, 2016). Within cetacean research, Kellar et al. (2015) assessed GC levels in short-beaked common dolphins (Delphinus delphis) and found that prolonged periods of GC activation can lead to serious consequences on reproduction and survival. Graham et al. (2021) revealed that in the blubber of NARWs, GC levels were significantly higher in individuals that died from anthropogenic causes compared to live, healthy individuals, further indicating the usability of the methodology to provide a relative measure for perceived stress. Mingramm et al. (2020) showed similar findings for humpback whales (*Megaptera novaeangliae*) and additionally indicated a seasonal variation in blubber GC levels possibly related to increased levels of stress associated with the breeding season. Despite small variations, these studies indicate that quantifying blubber GC levels is a useful way to get information on prolonged perceived stress in baleen whales. When combined with other biomarkers of health such as body condition index (BCI), blubber GC concentrations can be used to assess potential changes in the overall health of cetacean populations (Kellar et al., 2015), a valuable tool for understanding their response to a rapidly changing ocean (Trana et al., 2016).

1.6 PROJECT RATIONALE

The South African population of SRWs has been extensively monitored for the past 41 years by means of annual photo-identification surveys. As outlined in the literature review above, recent trends in the population revealed anomalous counts of unaccompanied adults and cow-calf pairs, and, most relevant to this dissertation, an increase in calving intervals from three-year cycles, to four- and five-year cycles suggesting reproductive failure (Brandão et al., 2018; Vermeulen et al., 2018).

As capital breeders, SRW reproductive success, and thus population dynamics, are known to be mediated through foraging success (e.g. Forcada et al., 2005; Greene and Pershing, 2004; Seyboth et al., 2016; Ward et al., 2009). Indeed, for females, BC (and thus foraging success) is an essential prerequisite for ovulation, maintaining gestation and foetal development, and the quality of lactation and thus offspring survival (Lockyer, 1981). Studies in the southwest Atlantic have shown a direct link between a decreased calving success of SRWs and a decreased krill availability in feeding grounds as a consequence of climate change (Leaper et al., 2006; Seyboth et al., 2016). This may be of concern since more and more studies clearly show a decreased krill availability in the Southern Ocean with rising SST, sea ice loss, and displacement of the frontal systems of the Antarctic Circumpolar Current due to climate change (e.g. Loeb et al., 2009; Murphy et al., 2007; Trathan et al., 2006). In the context of the South African population of SRWs, van den Berg et al. (2021) revealed a drastic shift in foraging strategy over the past decades likely driven by large-scale ecological changes occurring in SRW feeding grounds in the Southern Ocean. Considering the simultaneous decreased reproductive success observed in this population and the apparent direct relationship between foraging success, BC and reproduction, the authors suggested that the observed changes may not be sufficient to adapt to a changing ocean (van den Berg et al., 2021). Given the above, an indepth assessment of the BC of SRWs in the population is warranted.

Therefore, the first data chapter (Chapter 2) of this dissertation assesses the BC of South Africa's SRWs and puts it in a temporal perspective by comparing data with a previous photogrammetry study conducted by the MRI in the late 1980s (Best and Ruther, 1992).

Additionally, to put things into a global perspective, the contemporary BC of South Africa's SRWs is compared to that of SRWs of the Argentinean and Australian population. In order to improve our understanding regarding the physiological responses of SRWs to possible nutritional stress, Chapter 3 outlines the quantification of GCs in blubber biopsy samples of individual SRWs and assesses these in relation to their respective BC. Lastly, Chapter 4 summarizes and discusses the overall synopsis of the dissertation.

1.7 PROJECT AIMS AND RESEARCH QUESTIONS

1.7.1 Aim of the dissertation

The aim of this dissertation is to assess the BC of SRW's on the South African breeding ground at present, and compare it to historical data and other SRW populations. Additionally, BC measurements are assessed in relation to blubber GC concentrations as a physiological indicator of nutritional stress.

1.7.2 Objectives of the dissertation

- Calculate the BCI of South Africa's SRWs.
- Compare the BCI of South Africa's SRWs to photogrammetry data collected in the country in the late 1980s (Best and Ruther, 1992).
- Compare the BCI of South Africa's SRWs to that of SRWs in populations of Argentina and Australia.
- Quantify GC concentrations in the blubber of South African SRWs.
- Assess blubber GC concentrations in relation to BCI of South African SRWs.

CHAPTER 2

Body condition of southern right whales (*Eubalaena australis*) on the South African breeding ground

*This data chapter is based on a published report presented as a scientific committee report to the IWC.

Thavar, T., Christiansen, F., Ganswindt, A., Sironi, M., Uhart, M., Bejder, L., Vermeulen, E. 2021. Southern right whale (*Eubalaena australis*) body condition and glucocorticoid levels at the South Africa breeding ground. Report SC/68C/SH/08 presented to the 68C IWC scientific committee (Southern Hemisphere Subcommittee), Cambridge, UK

2.1 ABSTRACT

The population of South African SRWs has recently experienced a reduction in their calving success. As capital breeders, they depend on stored energy to support their seasonal reproduction. It is therefore assumed that a reduced success of summer foraging and consequently BC lies at the basis of the observed reduction in calving success. To assess this hypothesis, this study used aerial photogrammetry and 3D volumetrics to study the BC of the South African population of SRWs, and investigated this 1) prior to, and during the reduction in calving success; and 2) in comparison to two neighbouring populations. To assess the temporal variation of BC in South African SRW, BC metrics were calculated using two sources of photographic data, each taken during a distinct period in the reproductive success of the South African population: 1) previously collected analogue aerial photographs, collected in a helicopter-based photogrammetry study in the late 1980s, a period of high calving output; and 2) aerial photographs collected in 2019 using an UAV, a period of low calving output. To place these data in a global context, 2019 South African SRW BC was compared to the BC datasets for SRWs breeding off Australia and Argentina. Results of the study revealed a 24% reduction in the BC of lactating South African SRWs in the past two decades. In addition, the global comparison revealed that South African lactating female SRWs were in a significantly poorer condition (F=3.639, p=0.031, R²=0.083) compared to lactating females from Australia and Argentina. This study indicates a distinct reduction in the BC of South African SRWs during a period of reduced calving success, likely linked to reduced prey availability and broad-scale ecological changes occurring in their feeding grounds in the Southern Ocean. These findings, exacerbated by the global comparison, raise serious concern for the South African population of SRWs under a rapidly changing ocean.

2.2 INTRODUCTION

The South African population of SRWs has been extensively monitored through annual aerial surveys since 1969 (Best, 1990a). Since 1979, these surveys have included a photo-identification component, allowing for the individual monitoring of reproductive females in the population (Best, 1990b). The resulting long-term sighting history dataset, which has enabled the estimation of vital population demographic parameters, revealed an annual

population growth rate of 6.9%, between 1971 and 2006 (Brandão et al., 2010). However, the last several years have been characterized by some noteworthy changes; including a) a sharp decrease in the coastal prevalence of unaccompanied adults (i.e. juveniles; males and non-calving females) since 2009; b) an increase in calving intervals from 3-year intervals to 4- and 5-year intervals, implying either additional resting years between successive calving events or calving failure (Vermeulen et al., 2020); c) a suspected reduced residency time at the South African calving ground (Vermeulen et al., 2020); and d) a decrease in the estimated population growth rate from approximately 7% to 6.5% per annum (Brandão et al., 2018). Ultimately, these changes suggest that the population's migration and habitat use patterns may be changing, and secondly, the population's recovery rate may be hindered by a reduced reproductive success.

The role of adequate BC in mammal reproduction is well established (Thomas et al., 1990). Also in baleen whales, female BC influences fertility (Miller et al., 2011), foetal growth (Christiansen et al., 2014) and the BC of calves (Christiansen et al., 2016a). SRWs build their energy reserves and attain their required BC in their sub-Antarctic feeding grounds during summer, and they depend on these reserves to sustain themselves during winter at lower latitude calving grounds (Christiansen et al., 2018). SRW females have extremely high energetic costs associated with reproduction (Gittleman and Thompson, 1988), and thus, it is vital for females to maximise their feeding success, to ensure adequate body fat reserves are acquired prior to conception. This link between reproductive success and feeding success for SRWs has been demonstrated in the southwest Atlantic, with a direct link found between decreased calving success of SRWs in Brazil, and reduced prey availability (Seyboth et al., 2016). In the context of the recent reduction in reproductive success.

Interestingly, coinciding with this reduction in reproductive success, a recent study revealed a dramatic northward shift, and diversification, in the foraging strategy of South Africa's SRWs, likely related to changes in their preferred habitat or prey (van den Berg et al., 2021). Van den Berg et al. (2021) suggests that while this shift is positive, suggestive of potential adaptive capacity in a rapidly changing ocean, the shift is likely still suboptimal due to the concurrent reduction in calving success. In order to evaluate the effects of this foraging strategy shift on

the foraging success of the population, and its link to the reduced reproductive success, an indepth assessment of the physical BC of parous South African SRW females is warranted. Certainly, understanding the possible temporal changes in BC, and how this may impact calving success and associated demographic processes is vital to predicting the population's resilience to a changing environment.

This study, therefore, aims to assess the BC of South African SRWs in relation to their reproductive classes, including lactating females, unaccompanied adults (males and non-calving females), juveniles and calves. Additionally, it is aimed to assess a temporal variation in the context of their reduced reproductive success; given the strong links between feeding success, BC, and reproductive success, we expect overall poorer BC in parous SRW females in recent years. Interestingly, the dramatic fluctuations in SRW calving success noted on South Africa's coastline are not unique to South Africa; similar fluctuations have recently been seen in other SRW calving grounds in Argentina (Crespo et al., 2019) and Australia (Charlton et al., 2018). Based on this global trend, this study additionally aims to compare the BC of South African lactating SRWs to those found off the Argentinean and Australian breeding grounds. Considering the more pronounced decreased coastal prevalence and increased calving intervals in the South African population compared to the Argentinian and Australian populations (e.g. Charlton et al., 2018; Vermeulen et al., 2020), we expect slightly poorer BC in the South African population.

2.3 METHODS

2.3.1 Study population

The South African population of SRWs migrates annually from their summer feeding grounds in high latitudes to their winter breeding grounds along the South African coast, with the main concentration area situated around the southern Cape coast. This population was estimated at some 6,100 individuals (Brandão et al., 2018), comprising the largest part of the global SRW population (IWC, 2013), and has been studied extensively since the late 1960s (Best, 1981).

2.3.2 Data collection

In this study, photographs used to assess the BC of South Africa's SRWs are composed of a mixture of aerial photographs collected in South Africa in 1988 and 1989 (Best and Rüther, 1992) and UAV photographs collected in 2019 in South Africa, as well as in Australia and Argentina for comparative purposes.

Recently collected photographs

Body morphometric data of SRWs were collected in Walker Bay, Hermanus and San Sebastian Bay, Witsand, South Africa, on 7 successful field trips conducted between the 3rd and 15th of September 2019, from a small (7m) research vessel. Sampling was only conducted during calm weather conditions (Beaufort <3). These data from 2019 are hereafter referred to as the "late period".

A Phantom 4 Pro (diameter = 350 mm, weight = 1388 g, sensor size = 13.2mm, 20MP, 5472x3078 pixels, 8.8mm focal length) multirotor UAV was used to take aerial photographs of SRWs at altitudes ranging from 15 to 25m. Once above a whale, the UAV would hover and take photographs with the camera facing straight down (zenithally) until an optimal photo could be obtained, defined as a whale lying flat at the surface with the dorsal side up and a straight body axis (i.e. non-arching) (see Christiansen et al., 2016a). The UAV operator was able to adjust the position of the UAV through a live video link connected to the remote controller screen.

Previously collected photographs

Previously collected (hereafter referred to as the "early period") photographs were taken in De Hoop Nature Reserve, South Africa, in July – November 1988 and 1989 by the MRI staff based at the University of Pretoria, South Africa (see Best and Rüther, 1992; Miller et al.,2012). The photographs were taken from a helicopter using a Hasselblad ELM camera with a 250mm lens (6 x 6 format frame, actual image size = 56 x 56mm) (Best and Rüther, 1992). The camera was mounted in a gimbal system with a maximum allowable tilt from the vertical of 10 degrees in any one direction from the vertical. Before each photographic flight, the lens was fixed with a tape for photography at heights ranging from (76.2m- 121.9m). Original slides held at the MRI were visually inspected for optimal body posture and sufficient quality. The

selected photos were then scanned for conversion to digital images, and further treated identically as digital photographs obtained in the "late period" to ensure comparability of methods and therefore BC measurements.

Data collection in other breeding grounds

Raw data on the 2019 BC of SRWs in Argentina (Península Valdés) and Australia (Head of Bight) used in this dissertation were collected applying the same methodology and kindly provided by Fredrik Christiansen from the studies Christiansen et al. (2018) and (2019). For details of the study sites and sampling procedures, see Christiansen et al. (2019) for data collected in Argentina and Christiansen et al. (2018) for data collected in Australia. 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 (3rd and 15th of September 2019) as South Africa, to avoid seasonal sampling biases.

2.3.3 Picture grading and image selection

First of all, photo-identification, based on the natural marking of the whale's callosities (Payne et al., 1983), was used to ensure no individual whale was assessed twice within the South African dataset, avoiding pseudo-replication. Following the protocol of Christiansen et al. (2018), the best photograph of each individual whale was then selected and quality graded based on camera focus, body posture (horizontal roll, body arch and vertical pitch) and body shape visibility (ability to see the rostrum, fluke notch and the body contour). Each photograph was given a score from 1 (good) to 3 (bad) for each of these attributes (see Appendix Table A1.1). The photographs which has at least one score of 3 were excluded for further analyses. Additionally, images with a score of 2 for two of the three categories under body posture were excluded from further analyses.

2.3.4 Body Condition Index

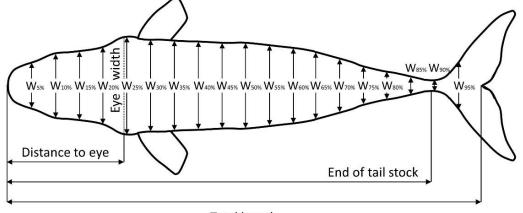
After image selection, the selected images were used for photogrammetry purposes. Using a custom-written script in R (download available from Christiansen et al., 2016a), measurements were made (in pixels) of the total body length (defined as the tip of the rostrum

to fluke notch) and width at 5% increments perpendicular to the body axis (Figure 2.1) for each individual whale.

Subsequently, individuals were assigned an age class based on their size and/or proportion of head length to total body length, with calves being smaller animals accompanied by a larger animal (a calf being < 2/3 the body length of the accompanying adult), juveniles having a relative head length <23% of the body length (based on body measurements conducted in R, see below), and adults being individuals with a relative head length >23% of the body length (Christiansen et al., 2020). Of these adults, lactating females were identified by the presence of an associated calf.



В



Total length

Figure 2.1. (A) Example of a good quality aerial photograph of a southern right whale to take morphometric measurements. (B) Sketch indicating the locations of measurement points. Figure taken with permission from Christiansen et al. (2018).

For each width measurement, the corresponding height (dorso-ventral distance) was calculated, using the known height-width ratio of SRWs given by Christiansen et al. (2019),

which ranges between 0.847 and 0.953 in the region between the tip of the rostrum down to 35% of the body length, close to one between 40% and 50°% of the body length and between 1.09 and 1.93 for the area posterior to 50% of the body length. Since the inbuilt barometric altimeter of the UAV did not provide accurate altitude measurements, only relative length and width measurements (standardised against a body length of 1) were used. The BV of each whale was then estimated using the elliptical volume approach by Christiansen et al. (2019). Subsequently, the BCI was calculated using the formula of Christiansen et al. (2018) modified for relative measurements:

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

where BV_{Obs} is the observed BV, standardized against a body length of 1, and $\mu(BV)$ is the mean BV of the sample population, standardized against a body length of 1. 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. Since all measurements were standardized against a body length of 1, the BCI could be estimated without knowing the absolute length of the animals.

2.3.5 Statistical analyses

BCI values were plotted in R 1.4.1717 (R Core Team, 2020) to visually inspect the data for any trends or outliers. Several linear models were created to assess the effect of *year*, *sampling period* and *relative calf length* on the BCI for the temporal comparison, and the single and combined effects of *location* and *relative calf length* on the BCI of lactating females for the interpopulation comparison. The best-fitting model was selected based on Akaike's Information Criterion (AIC; Akaike, 1998). ANOVA statistics were then used to compare the BCI between different demographic and reproductive classes, the early and late sampling period, and between the different breeding grounds. For lactating females, calf relative body length (% of maternal length) was included as a covariate in the models of all analyses, since maternal BC is known to decline as the calf grows in length (Christiansen et al., 2018). Model validation tests were run to ensure that the model assumptions were met for each model. These included scatter plots of model residuals against fitted model values (to evaluate homogenous residuals), frequency histograms of model

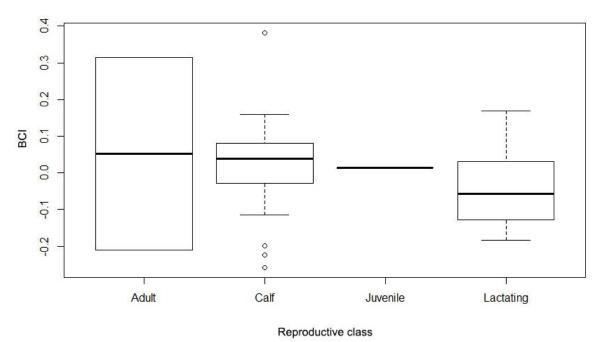
residuals (to examine the normality of residuals) and calculating leverage scores and Cook's distance (to identify influential points and outliers, respectively).

All data were collected under UP ethics permit number NAS206/2019 as well as the permit of the Department of Forestry, Fisheries and the Environment and Civil Aviation Authority.

2.4 RESULTS

2.4.1 Contemporary BC

In total, 3,959 UAV images were obtained during fieldwork in 2019. From these, the BC of 100 individual right whales were determined, including 7 non-lactating adults, 45 calves, 3 juveniles and 45 lactating females. After data selection based on photo quality, 46 were selected to be included in analyses, including 26 were calves, 1 juvenile, 2 non-lactating adults 17 lactating females. Using the different height-width ratios, the BV was calculated for all individuals (see Appendix Table A1.2). The mean BV was 0.02 (SD = 2.17e-03) which was used in further calculation of the BCI. Comparing the BCI of all individuals depending on their demographic and reproductive classes (Figure 2.2), non-lactating adults displayed the highest mean BCI followed by calves at -3% (SE = 9.65) and the one juvenile at -3.9% (SE = 16.1). Lactating females displayed the lowest BCI at a magnitude of -9.3% (SE = 9.8). However, there was no significant difference in BCI among these different age and reproductive classes (F = 0.9036, p = 0.45, $R^2 = 0.060$).



CHAPTER 2: Southern right whale body condition

Figure 2.2. Mean body condition index of South African southern right whales according to their age and

reproductive class (Adults (non-lactating): n = 2; Calf: n=26; Juveniles: n=1; Lactating (females): n=17).

2.4.2 Temporal variability

A total of 236 original diapositives from the early period were selected and professionally scanned. Of these, 188 images were good enough to proceed to picture grading and BC measurements. After image selection based on picture quality, the BC measures of 83 SRWs remained for analysis purposes, including 20 lactating females and 68 calves. The mean BV of all the measured SRWs (early and late period combined) was 0.017 (SE = 2.69e-03), used in further calculation of BCI. Due to the low sample size of juveniles and adults of the "late period", the temporal change in the BCI of only lactating females was assessed (see Table A1.3) for BCI values of lactating females in the "early period"). Based on the AIC values, the model which included only *sampling period* was selected as the best fitting model, as it was within 2.5 AIC units of the more complex models including additionally relative calf length (Occam's razor) (Table 2.1).

Table 2.1. Linear models used to assess the effect of various predictive variables on the response variable (BC = body condition), and the resulting AIC values used for model selection.

Model	AIC
BC~1	-11.13
BC~period	-26.20
BC~year	-24.43

BC~rel.calf.length	-13.51
BC~rel.calf.length+period	-28.47
BC~rel.calf.length*period	-26.49

Comparing subsequently the BCI of lactating females between both sampling periods, a significant difference was found (F = 20.408, p < 0.001, R² = 0.362), with the BCI of lactating females in the "late period" being on average 24% (SE=5.31) lower than in the "early period" (Figure 2.3 A). Model selection indicated calf body length had no effect on maternal BC, indicating the results are not confounded by calf length and therefore duration of lactation (Figure 2.3 B). 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). Model validation plots are provided in the appendix (Figure A1.1).

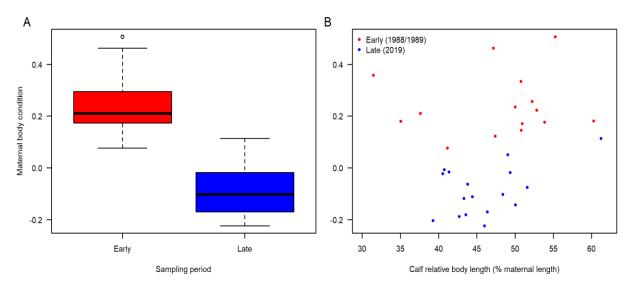


Figure 2.3. (A) Comparison of southern right whale maternal body condition measured in 1988/89 (early sampling period, n=15 lactating females) and 2019 (late sampling period, n=17 lactating females). (B) Maternal body condition as a function of calf relative body length (% maternal length). The colour of the data points indicates the sampling period (see key).

2.4.3 Interpopulation BC comparisons

After image selection, measurements of 86 SRWs 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) were used for analyses. These respective raw data are not accessible via

this dissertation, due to their unpublished nature and provision from another study (pers. comm. Vermeulen). Due to the small sample sizes for juveniles and unaccompanied adults, further analysis was restricted to lactating females only. Based on the AIC values, the model that included only *location* was selected as the best-fitting model (Table 2.2).

Table 2.2. Linear models used to assess the effect of various predictive variables on the response variable (BC = body condition), and the resulting AIC values used for model selection.

Model	AIC		
BC~1	-128.57		
BC~location	-131.80		
BC~rel.calf.length	-129.37		
BC~rel.calf.length+location	-131.62		
BC~rel.calf.length*location	-129.13		

Results of the ANOVA showed a significant difference (F = 3.639, p = 0.031, R² = 0.083) in BCI 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), respectively (Figure 2.4 A). There was no difference in maternal BCI between Australia and Argentina. As in the previous analyses, calf body length did not have a significant effect on maternal BC in any of the three populations (Figure 2.4 B). Model validation plots are provided in Appendix 1 (Figure A1.2).

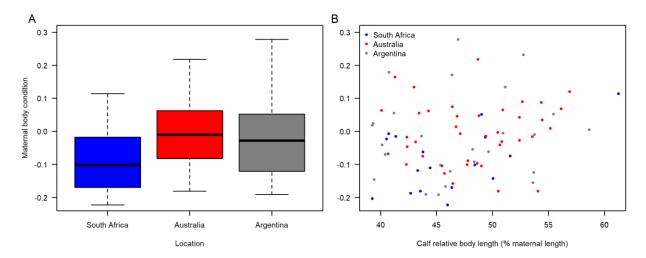


Figure 2.4. (A) Comparison of lactating female body condition between South Africa (n=17), Australia (n=40) and Argentina (n=26) in 2019. (B) Maternal body condition as a function of calf relative body length (% maternal length). The colour of the data points indicates the sampling location (see key).

2.5 DISCUSSION

This study used 3D volumetrics and photogrammetry to assess the temporal variation in SRW BC in the South African breeding ground and placed these findings in a temporal and global context. Results show a distinct decrease in the BC of South African SRWs between the late 1980s and 2019, indicative of a dramatic reduction in foraging success of South African SRWs in recent years. Additionally, this study also finds that in 2019, South African SRWs are in a significantly poorer condition than SRWs in Australia and Argentina.

2.5.1 Poor BC and reduced calving success

The results of this study demonstrate a 24% reduction in the maternal BC of South African SRWs between the late 1980s and 2019. To demonstrate the magnitude of this reduction, lactating SRW females in Australia lose on average 25% of their initial BC over the three month breeding season (Christiansen et al., 2018). Body fat condition influences reproduction in most mammals (see Thomas, 1990 for a review), as it is crucial in all parts of reproduction including ovulation, gestation and lactation. This is also the case in marine mammals and various studies have shown such a direct relationship to exist. For example, Ford et al. (2010) indicated reduced prey abundance correlated with reduced fecundity in killer whales (Orcinus orca). Williams et al. (2013) indicated a decreased rate of pregnancy in North Atlantic fin whales (Balaenoptera physalus) related to prey limitation mediated through BC. Such a relationship has also been found for SRWs, in which the reproductive success was shown to be dependent on BC and therefore dependent on foraging success (Leaper et al., 2006; Seyboth et al., 2016). It is perhaps unsurprising then, that the poor BC of South African SRWs in 2019 falls within a period of drastically reduced calving success in the population. Among several observed recent changes on the South African coastline, the most notable anomalous trend has been an increase in female SRW calving intervals (Vermeulen et al., 2020). Most commonly, female SRWs have a three-year calving interval (Best, 2007). However, a recent increase in the occurrence of 4- and 5-year calving intervals have become the norm since 2014 (Brandão et al., 2018; Vermeulen et al., 2019). These calving intervals imply either additional resting years between successive calving events or a loss of a foetus (Knowlton et al., 1994). Therefore, the poor BC of South African SRW females in 2019 revealed in this study, provides a strong suggestion that suboptimal nutrition is likely the primary cause for this reduced calving

success observed in the population. Especially for a capital breeder like the SRW, which heavily relies on adequate nutrition and BC to facilitate successful reproduction (Lockyer, 2007), this finding raises grave concern for the reproductive success and continued recovery of the South African population of SRWs.

Interestingly, a study conducted by van den Berg et al. (2021) indicated a drastic northward shift in foraging location and a shift in diet over the same time period, and hypothesised this was related to an energetic need. Results of the present study suggest this shift relates to suboptimal foraging conditions, and thus confirms the hypothesis of van den Berg et al. (2021). Although the environmental changes that may be driving this change remain to be determined, they clearly result in either a decreased prey availability or a decreased quality of prey for the whales. Illegal Soviet whaling data revealed that euphausiids form the most important component of the SRW 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 the 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 BC in the South African SRW population is a major concern, as it is likely a major indication of the poor foraging success behind the reduced reproductive success that is decelerating population recovery.

2.5.2 SRW BC in the global context

Further results of this study reveal that the South African population of SRWs are in significantly poorer BC 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 Argentinian and Australian populations (Charlton et al., 2018; Charlton, 2017; Vermeulen et al., 2020). While the reasons for this discrepancy between the different breeding populations remain to be determined, they are likely influenced by the substantial spatial heterogeneity found in the impacts of environmental and climate change in the Southern Ocean (Rogers et al., 2020), affecting their prey availability. For example, Gutt et al. (2015) reveal dramatic longitudinal variation in observed SST and sea-ice changes, with

regions such as the Antarctic Peninsula demonstrating substantial sea-ice concentration decreases, while many other regions of Antarctica demonstrate substantial increases in sea-ice concentration. Such radical differences in sea-ice concentration trends are likely to strongly impact the regional abundances of Antarctic krill (Reiss et al., 2017). Nevertheless, the size, complexity, and relative difficulty of attaining long-term measurements in the Southern Ocean make it difficult to develop a full spatial understanding of oceanographic variation under climate change in the Southern Ocean (Hoegh-Guldberg and Bruno, 2010). Future studies assessing the changes occurring in oceanographic conditions in the Southern Ocean, known to be important in driving suitable SRW feeding habitat (see Carman et al., 2019), should help to alleviate this considerable knowledge gap.

Unlike its sister species, the North Atlantic (Endangered) and North Pacific (Critically Endangered) right whales, the SRW is classified as Least Concern according to the International Union for conservation of nature (IUCN) criteria, meaning that at the species level, the criteria to be classified as Threatened are not met (Thomas et al., 2016). To date, the recovery of the NARW has been the slowest with the population declining at a rate of 1% annually and the abundance for females being lower than males (Christiansen et al., 2020). Photogrammetry studies assessing body condition in NARW revealed that the NARWs were all in significantly poorer body condition compared to the SRWs (Christiansen., 2020). The poor fecundity and survival of the NARWs in comparison to the SRWs indicates the direct effect of anthropogenic activities (fish gear entanglements, ship strikes and noise) and poor foraging strategies on the right whale species (Christiansen et al., 2020; Miller et al., 2012). Although most subpopulations of SRWs (including South Africa, Argentina and Australia) are still recovering at fairly consistent rates, the latest global trends in SRW calving success, and the associated decrease in BC and shifting foraging strategies of the South Africa population do warrant some concern on the species-level. Identifying techniques to standardize data collection for reproductive health (BC, visual health assessments, foraging success, assessments of endocrine correlates, etc.) in the northern and southern populations, are strongly recommended, to identify potential early warning signs of increasing threats to the species as soon as possible.

2.5.3 Influence of Climate

As the global ocean changes in response to a host of anthropogenic drivers (Bindoff et al., 2019), top ocean predators will experience climate change through physiology or indirectly through alterations in food web dynamics (Stenseth et al., 2002). Impacts on food web dynamics are important for capital breeders such as SRWs, since they consume large amounts of zooplankton in their Southern Ocean feeding grounds. This is concerning given the evidence which suggests that several oceanographic shifts, including sea ice declines and ocean warming, alter the abundance and distribution of Antarctic krill (Flores et al., 2012), a key food source for SRWs when feeding south of 50°S (Tormosov et al., 1998). When foraging more north, there appears to be an increased intake of copepods (Tormosov et al., 1998), which are much smaller in size and biomass (Atkinson et al., 2001). However, some studies suggest the nutritional content (caloric value) in copepods has altered over time due to climate change and sea ice loss (Atkinson et al., 1999; Costa et al., 2006; Linder et al., 2010; McKinstry et al., 2013). As a result, the possibility that a shift in diet combined with a reduction in the nutritional value of SRW prey may have played a role in the reduction of South African SRW BC in recent years, cannot be ignored. This hypothesis has also been indirectly alluded to in a previous study investigating SRW calf mortality in Argentina (Maron et al., 2020). However, the question of whether decreases in the nutritional content of SRW prey are occurring, remains to be determined. Furthermore, foraging strategies within the South African population of SRWs would need to be investigated to determine if the whales are adopting a trade-off strategy by shorter migration routes to minimize energy expenditure.

2.6 CONCLUSION

Along with most baleen whales, SRWs depend heavily on the energy storage from summer foraging to facilitate successful reproduction (Lockyer, 1981a; Miller et al., 2011; Williams et al., 2013). An adequate BC is thus of utmost importance in maintaining the successful recovery of baleen whales from the impacts of historical whaling. This study revealed that the South African population of SRWs have reduced drastically in BC over the past two decades, and are in a significantly poorer BC compared to the SRW females that calve off Australia and

Argentina. The suboptimal BC in the South African population is concurrent with a reduction in the population's calving success. As a result, these findings support the hypotheses of van den Berg et al., (2021), who suggests that large-scale ecological change occurring in SRW feeding grounds in the Southern Ocean, have recently forced South African SRWs to shift to a suboptimal foraging strategy, likely the primary mechanism behind the sub-optimal BC and overall reduced calving success ultimately hampering population recovery. Although the underlying environmental and oceanographic changes on the South African SRW feeding grounds remain to be determined, they clearly resulted in a decreased prey availability and/or a decreased quality of prey for these whales.

<u>Caveats</u>

Considerations should be made to the limitations in the results of this chapter related to the technical methodologies used between the different comparisons of BCI estimates, for instance, an 8mm lens camera (used for the 2019 data collection) is considered to have a wider angle view compared to a 250mm lens (used in 1988/89 data collection) and a shorter focal length compared to the 25mm lens (used in the data collection for SRWs in Argentina and Australia). As a result image distortion could lead to a biased representation of the South African SRW BCI estimates for this study. To put this in perspective, the methods of Dawson et al., (2017), describes a system enabling accurate measurement of free ranging marine mammals using unmanned aerial vehicle (UAV) equipped with gimballed micro4/3 camera with a 25 mm lens. Photogrammetric calibration of the camera and lens during the image analysis accounted for distortion parameters and found that the lens exhibited moderate barrel distortion only (Dawson et al., 2017). If uncorrected, the combination of uncertainties on focal length and lens distortion introduced a 1% error in the size estimate (Dawson et al., 2017).

This is the same lens (25mm) used for the methodology performed for the SRW BCI estimates generated for the Argentina and Australia population in Chapter 2 of this study (Christiansen et al., 2020). While this may be a good representation of the BCI estimates represented for the SRW body condition in the Argentinian and Australian population, a similar technical approach should be applied to the photogrammetry data generated from the South African population of SRWs to account for distortions introduced by the camera lens after calibrations

to further define the accuracy of the SRW BCI estimates. Until the camera interior orientation parameters and lens distortion parameters are accurately known within comparisons, the results of this chapter should acknowledged as a mere indication of loss in body condition of the South African population of SRWs.

Moreover, failure to achieve altitude values for the calculations of absolute body condition estimates resulted in the use of relative body condition estimates for this study. As a result, BCI measurements were calculated from 5% to 85% almost representing the entire body length of the whale (Figure 2.1). This approach is slightly different from the methods of Christiansen et al., (2016a). As relatively little energy is stored in the head, fins and tail regions, only segments between the position of the eyes (~25% body length from the rostrum) (representing the metabolically active region of lactating females) down to the end of the tail stock (~85% body length from the rostrum) are typically used to estimate body volume. This could account for potential biases in the interpretation of the results and thus, restricting the measurements to metabolically active areas can aid in achieving a more sensitive BCI.

CHAPTER 3

Blubber glucocorticoid concentrations in relation to body condition in southern right whales

* This data chapter is based on a published report presented as a scientific committee report to the IWC.

Thavar, T., Christiansen, F., Ganswindt, A., Sironi, M., Uhart, M., Bejder, L., Vermeulen, E. 2021. Southern right whale (*Eubalaena australis*) body condition and glucocorticoid levels at the South Africa breeding ground. Report presented to the 68C IWC scientific committee (Southern Hemisphere Subcommittee), Cambridge, UK

3.1 ABSTRACT

South African SRWs have been extensively studied since the late 1960s to monitor population recovery since the cessation of whaling. Data indicate perturbing changes in the population in the past decade, including a reduced calving success, a shift in their foraging strategies and a reduction of their physical BC. Considering these factors may relate to a decreased food availability in their Southern Ocean feeding grounds, this study aims to assess if SRWs are physiologically responding to such changes in the environment. For this, blubber samples were collected to quantify GC concentrations and respective hormone values correlated with the whales' BCI. Results of this study revealed a positive relationship between blubber GC concentrations and BCI of lactating female SRWs, an opposing trend to what was expected based on the involvement of GC in nutritional stress responses. Hypotheses are formulated on the influence of a low samples size and the assessed reproductive class on the obtained results, as well as the possible effect of a decreased BC on the quality of lactation. Nevertheless, sample size remains too low to draw robust conclusions from this study, and a substantial increase in sampling is recommended going forward to test the various hypotheses. Nonetheless, the presented data will serve as a baseline for future research.

3.2 INTRODUCTION

The physical properties of the global ocean are changing rapidly under various anthropogenic impacts (Bindoff et al., 2019) causing complex and multifaceted ecological change, and leading to disruptions in Southern Ocean food webs (Constable et al., 2014). Critically, Antarctic krill, the dominant mid-trophic level species in Southern Ocean food webs (Atkinson et al., 2004; Reid and Croxall, 2001) have shown radical decreases in density and are contracting southwards (Atkinson et al., 2019). Such changes are particularly concerning for SRWs, which feed on large amounts of krill during summer and store this energy to sustain their long-distance migrations to lower latitudes, for mating and calving during the rest of the year (Lockyer, 2007). As capital breeders that rely heavily on such seasonal energy reserves, the body condition of SRWs is thus deemed essential to facilitate successful reproduction and migration (Lockyer, 2007) and is a valuable proxy for general reproductive fitness (Christiansen et al., 2016a).

A critical component of SRW body condition is their blubber, a thick, subcutaneous layer containing deposits of fatty acids and lipids, serving a number of functions, including energy storage (Lockyer, 1981; Miller et al., 2011). Furthermore, adipose tissue in the blubber amasses high concentrations of steroid hormones as the breakdown or removal processes from the blubber are slow compared to those from blood (Kellar et al., 2015). In response to perceived stressors, physiological responses take place to restore homeostasis (Sapolsky, 2002), including the activation of the HPA axis (Whirledge and Cidlowski, 2010), which leads to increased levels of GCs (Palme, 2005). GCs are mainly involved in the mammalian energy balance and the regulation of glucose (Sapolsky et al., 2000). As a consequence, extended periods of nutritional stress in mammals are associated with increased GC levels (Champagne et al., 2017; Guinet et al., 2004; McCue, 2013; Wasser et al., 2017), which can affect reproductive success through the suppression of gonadotrophins (Sapolsky, 2002; Tilbrook et al., 2000). Monitoring GC levels can thus be a promising tool to assess physiological stress, including nutritional stress, in SRWs.

To date, several studies have provided insight for GC concentrations in the blubber of marine mammals, including humpback whales, NARWs, blue whales (Balaenoptera musculus), belugas (Delphinapterus leucas), narwals (Monodon monoceros) and harbour seals (Phoca vitulina) (Kellar et al., 2015; Kershaw and Hall, 2016; Mingramm et al., 2020; Trana et al., 2016; Atkinson et al., 2020; Watt et al., 2021; Graham et al., 2021), indicating that quantifying blubber GCs can be an effective technique to investigate the wellbeing of individual cetaceans in relation to, for example, environmental disturbances (e.g. Narayan, 2013). However, the utility of quantifying these hormones to assess health in cetaceans requires additional information, as baseline GC levels vary in different sample matrices and with respect to age, sex, season and reproductive state (Bennett et al., 2012; Graham et al., 2021; Hunt et al., 2006). Thus, measuring GC concentrations in blubber or any other matrix requires a careful validation of the assay finally utilized to ensure a reliable quantification of GCs or its metabolites (Kellar et al., 2015; Kershaw, 2018; Trumble et al., 2018). Nevertheless, providing a certain approach has been reliably established and the technique of blubber GC analysis has become prominent in monitoring respective steroid levels as an indicator of physiological stress in free-ranging marine mammals (e.g. Champagne et al., 2018; Galligan et al., 2020; Kellar et al., 2015). Furthermore, since GC concentrations in the outermost blubber layer seem representative of concentrations through the full blubber depth, and they are comparable across the entire blubber layer regardless of the area of the body (Kershaw et al., 2017), remote biopsy sampling can be used to obtain representative samples of free-ranging marine mammals. When combined with other biomarkers of health such as BCI, blubber GC concentrations can be used to assess potential changes in the overall health of cetacean populations (Kellar et al., 2015), a valuable tool for understanding their response to a rapidly changing ocean (Trana et al., 2016).

Chapter 2 of this dissertation indicated that the BC of lactating female SRWs has decreased 24% in the past two decades, and suggested this may be related to a decreased food availability in their Southern Ocean feeding grounds. Considering this may be an indication of nutritional stress in the population, this study aims to quantify GCs in South African SRWs blubber biopsy samples, as well as correlate these to their BCI. Understanding how SRWs are physiologically responding to changes in their foraging success is vital to forecast the population's resilience in a changing environment. Given the recent reduced calving success, and likely reduced foraging success, we hypothesize that South African SRWs will show higher GC concentrations in response to poor BCI.

3.3 METHODS

3.3.1 Study population

The South African population of SRWs migrates annually in winter from high latitude feeding grounds to their main breeding ground along the coast of the southern Cape, where they give birth and nurse their young. This population has been extensively studied since the late 1960s to monitor population recovery post-whaling (Best, 1981).

3.3.2 Data and sample collection

Boat-based surveys were carried out between 3rd and 15th of September 2019 in Walker Bay, Hermanus (34°26'S, 19°18'E) and San Sebastian Bay, Witsand (34°23'S 20°52'E). During such surveys, overhead images were obtained for 3D volumetric and photogrammetry purposes (see Chapter 2). Additionally, when possible, SRW blubber biopsy samples were obtained using a crossbow (Barnett Panzer 5, 150lb string) and darts with mounted sterilized small stainless steel biopsy tips. Prior to obtaining a biopsy sample, the callosity pattern of the whale was visually inspected to ensure the same whale would not be sampled twice over the study period. Subsequently, individuals were assigned an age class based on the size of the individual and/or the proportion of head length to total body length (see Chapter 2 methods for more detail), with calves being smaller animals accompanied by a larger animal (a calf being < 2/3 the body length of the accompanying adult), juveniles having a relative head length <23% of the body length (based on exact body measurements conducted in R, see below), and adults being individuals with a relative head length >23% of the body length (Christiansen et al., 2020). Of these adults, lactating females were identified by the presence of an associated calf.

In total, 15 blubber samples were obtained during fieldwork, including from 11 lactating females, 3 juveniles and 1 unaccompanied adult (of unknown gender).

All samples were collected under the ethics permit number NAS206/2019 as well as the permits of the Department of Forestry, Fisheries and the Environment.

3.3.3 Blubber glucocorticoid extraction

Steroids 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. Subsequently, 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 suspension 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 into another 5 ml Borosilicate Glass tube and evaporated into another 5 ml Borosilicate glass tube and evaporated for 5 min at 1500 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, 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.

3.3.4 Blubber glucocorticoid quantification

Steroid extract residues were reconstituted in 250 to 500 µl assay buffer (depending on original sample weight). Subsequently, reconstituted extracts were measured for GC concentrations, using a Cortisol assay (Palme and Möstl, 1997), utilizing a Cortisol-3-CMO:BSA antibody and a Cortisol-3-CMO-DADOO-biotin label. Assay characteristics including antibody cross-reactivities are provided by Palme and Möstl (1997). Intra- and inter-assay coefficients of variation (CV), determined by repeated measurements of high and low-quality controls were 4.42% and 6.24% (intra-assay CV) and 11.57% and 12.51% (inter-assay CV), respectively. The sensitivity of the Cortisol EIA was 83.3 pg/g blubber. 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).

3.3.5 Body condition and data analysis

SRW BCI was calculated based on the methods described in Chapter 2. Of the 15 individuals from which blubber samples were obtained, 7 were selected for further analyses based on the availability of good quality photogrammetry data (see Appendix Table A2.1). Several linear models were created in R 4.0.3 (R Core Team, 2020) to assess the single and combined effect of *Julian day*, *BC* and *location* (Witsand vs Walker Bay) on the blubber GC levels. The best-fitting model was selected based on Akaike's Information Criterion (AIC; Akaike, 1998). ANOVA statistics were then used to assess the effect of the predicting variable in the best fitting model. Model validation tests were run to ensure that the model assumptions were met for each model. These included scatter plots of model residuals against fitted model values (to evaluate homogenous residuals), frequency histograms of model residuals (to examine the normality of residuals) and calculating leverage scores and Cook's distance (to identify influential points and outliers, respectively).

3.4 RESULTS

Blubber GC concentrations ranged from 0.28 to 1.46 ng/g overall, with an average blubber GC concentration for the 11 lactating females of 0.83 ng/g (SD = 0.24 ng/g) and 0.61 ng/g (SD = 0.26 ng/g) for the 3 juveniles (Table 3.1).

For the seven females selected to compare blubber GC concentrations and BCI, mean blubber GC level were 0.72 ng/g (SD = 0.32 ng/g; range 0.28 to 1.32 ng/g; Table 3.1), and their BCI had an overall mean of -0.05 (SD = 0.09; see Chapter 2 and Table 3.1).

Table 3.1. Blubber GC concentrations for each obtained southern right whale blubber sample, including detailson date and location of the biopsy sample, as well as age and reproductive class. Body condition index (BCI)and relative calf length is given for those individuals selected for further analyses, based on good qualityphotogrammetry data.

Sample number	Date	Latitude	Longitude	Age class	Glucocorticoid concentration (ng/g blubber)	BCI	Relative calf length (proportion to maternal length)
1	2019/09/07	-34.42	19.29	Juvenile	0.3		
2	2019/09/07	-34.43	19.30	Lactating female	0.28	-0.18083	0.4356
3	2019/09/07	-34.43	19.30	Juvenile	0.71		
4	2019/09/07	-34.47	19.34	Lactating female	0.75	-0.11822	0.4323
5	2019/09/07	-34.51	19.35	Juvenile	1.01		
6	2019/09/09	-34.39	20.86	Adult	1.17		
7	2019/09/09	-34.39	20.88	Lactating female	1.16		
8	2019/09/09	-34.38	20.89	Lactating female	1.46		
9	2019/09/09	-34.38	20.89	Lactating female	0.76	-0.00677	0.4073
10	2019/09/10	-34.40	20.86	Lactating female	0.60	-0.06278	0.4379
11	2019/09/10	-34.37	20.89	Lactating female	1.32	0.06131	0.4311
12	2019/09/11	-34.40	20.85	Lactating female	0.72		
13	2019/09/11	-34.39	20.87	Lactating female	1.00		
14	2019/09/11	-34.37	20.90	Lactating female	0.78	0.06150	0.4856
15	2019/09/15	-34.39	20.88	Lactating female	0.56	-0.11083	0.4442

Based on the AIC values, the model which included only BC was selected as the best fitting model out of the six models tested (Table 3.2).

Table 3.2. Linear models used to assess the effect of various predictive variables on the response variable (BC = body condition), and the resulting AIC values used for model selection.

Model	AIC
Corticol.conc.ng.g.blubbe~1	6.90
Corticol.conc.ng.g.blubber~BC	3.04
Corticol.conc.ng.g.blubbe~day	7.15
Corticol.conc.ng.g.blubbe~location	6.87
Corticol.conc.ng.g.blubbe~BC+location	3.70
Corticol.conc.ng.g.blubbe~BC*location	4.79

A significant positive relationship (F = 8.993, p = 0.030, R² = 0.643) was found between blubber GC concentrations and BC, with blubber GC concentrations decreasing at a rate of 0.271 (SE = 0.091) ng/g blubber for every 10% decrease in maternal body condition (Figure 3.1). No clear relationship was detected between GC concentrations and relative calve lengths (F = 0.02, p = 0.901, R² = 0.0039).

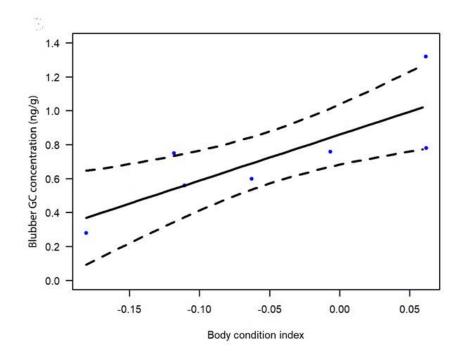
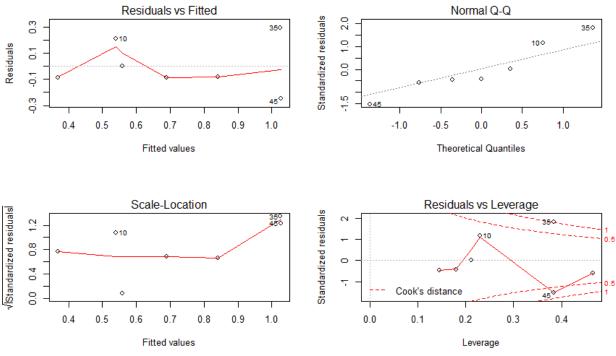


Figure 3.1. Blubber glucocorticoid concentration of 7 lactating SRW females against their body condition index (BCI), with the solid black line representing the fitted values of the linear model and the dashed lines representing 95% confidence intervals.



However, additionally to the small sample size, leverage scores and Cooke's distance (Figure 3.2) indicate the presence of at least 2 outliers with high influence on the model result.

Figure 3.2. Model validation plots comparing blubber GC levels as a function of BCI of lactating females (n=7), including a scatter plot 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).

3.5 DISCUSSION

This study is the first of its kind in South Africa and revealed a positive relationship between blubber GC concentrations and BC of lactating female SRWs. This is an intriguing trend opposite to what we would expect, as GC are known to be catabolic in nature, involved in lipid mobilization to supply an increase in metabolic demand (Peckett et al., 2011), often leading to a negative correlation between blubber GC concentrations and various measures of BC (Kershaw et al., 2017; Mingramm et al., 2020). In fact, various studies in mammals have shown that extended periods of nutritional stress (and thus likely decreased BC) are associated with increased GC levels (Champagne et al., 2017; Guinet et al., 2004; McCue, 2013; Wasser et al., 2017). This relates to the « Cortisol-Fitness Hypothesis » (Bonier et al., 2009) which suggests that high baseline GC levels are indicative of low fitness. However, although believed to be true over the general line, Bonier et al. (2009) also concludes that the relationship between GC levels and fitness is not always straightforward, as it may be impacted by other variables like e.g. reproductive class and age.

In this study, only data from lactating females have been used for analysing that relationship. Several studies have shown that GC concentrations increase with pregnancy and lactation in females (Bennett et al., 2012; Hunt et al., 2006; Kellar et al., 2015; Sheriff et al., 2009). In particular, lactation is known to be a costly part of the reproductive cycle (Lockyer, 2007), and would be related to higher GC concentrations, as seen in a number of species (e.g. Alekseeva et al., 2020; Guinet et al., 2004). At the same time, due to the high energetic cost, lactating females comparatively have poorer BC than other adult individuals (Christiansen et al., 2018), which could also be demonstrated in our study (see Chapter 2). This should thus result in elevated GC levels in poorer BC lactating females, but this effect might only become visible when other reproductive classes are included in the analysis. Unfortunately, the limited sample size in this study prevented us from including blubber GC concentrations and BCs of other reproductive classes, and so increased sampling would be required to address this issue further in future studies.

Interestingly, relative calf-length did not seem to be related to blubber GC concentrations of related lactating females, suggesting that the duration of lactation (and thus the time lag from late pregnancy and the birthing process) was not a confounding factor. This is similar to the finding of Kellar et al. (2015) who indicated that there is no apparent evidence that blubber GC concentrations vary with lactation state in free-ranging dolphins. However, the BCI of the sampled lactating females did not vary greatly, especially when removing 2 outliers (samples 2 and 12). Similarly, relative calf lengths all ranged in the 40% value, suggesting the duration of lactation would not have differed drastically among the females at the time of sampling. Such low variation in BC could hinder a visualization of a clear relationship with GC concentrations and therefore the formulation of a firm conclusion in this study. Additionally, the low sample size in itself will play a critical role considering the complex context-dependent effects of GC concentrations in relation to fitness (see Jaatinen et al., 2013).

It is also known that female SRWs in a comparatively poorer BC reduce their energetic investment in their calves to maintain their own survival probability (Christiansen et al., 2018).

Indeed, capital breeders which fast during lactation must ration on stored energy reserves according to their metabolic demands (Fowler et al., 2016), and may experience conflicting energetic demands when caring for an offspring. However, as iteroparous species, they will prioritize energy investment towards maximizing their own survival over that of their calves in order to maximize life-long reproductive success (Lockyer, 2007). As SRWs belong to one of the three groups of mammals that fast during lactation (alongside bears and phocids; Fowler et al., 2016), they must mobilize nutrients from energy reserves, a process involving the release of GC hormones. Furthermore, as fasting mammals, their high-quality milk is characterized by high lipid content (Oftedal, 1993). Interestingly, a study by Fowler et al. (2016) indicated GC concentrations being positively correlated to milk lipid content in elephant seals (a phocid), likely associated with the lipid mobilization from the adipose tissue of the mother. And so, as lactation is a distinct energetically expensive part of reproduction for such animals (Oftedal, 1993), it could therefore be argued that lactating females in a poor BC may be forced to reduce their quality of lactation, reducing lipid content in the milk and therefore mobilizing less nutrients from their energy reserves, possibly leading to decreased levels of GC compared to lactating females with a comparatively better BC. This would inevitably lead to a positive relationship between GC concentrations and BC, as observed in our study. However, the sample size presented here is low, and data preliminary, to confirm or reject such a hypothesis.

3.6 CONCLUSION

Overall, the relationship between BC and GC concentrations appears inherently complex (Jaatinen et al., 2013), and the data present in this study are too preliminary and low in sample size to formulate a clear conclusion for SRWs to contribute to the bigger picture. However, in a more general context, determining GC concentrations in blubber samples have shown the potential to aid in our understanding of how various sources of stressors can impact marine mammal physiology (Kellar et al., 2015; Trana et al., 2016). This potential should prove particularly valuable as broad-scale ecological changes in marine ecosystems are expected to impact marine mammals in a multitude of ways (Simmonds and Isaac, 2007). In the South African context, SRWs have recently demonstrated some concerning demographic changes, the most notable of which being a reduced calving success (Vermeulen et al., 2020). These

changes are associated with a change in the population's foraging strategy (van den Berg et al., 2021) and a reduced BC (Chapter 2 of this dissertation). It is therefore recommended to increase blubber sample size substantially alongside BC data to build on this concept. Nonetheless, the presented results will serve as a baseline for future research.

CHAPTER 4

General discussion and conclusion

4.1. GENERAL DISCUSSION AND CONCLUSION

The first data chapter (Chapter 2) of this dissertation used aerial photogrammetry to reveal that the still recovering population of South African SRW's have a distinctively reduced BC compared to individuals three decades ago. These results clearly expose the presumed reduction in foraging success of these whales in recent years, and are of great concern considering the dependence of these animals on body fat reserves to sustain their reproductive success. Additionally, results reveal that the BC of South African SRWs are significantly lower compared to SRWs from neighbouring populations, indicating that the prey available to South African SRWs seems reduced compared to other populations. This is perhaps not surprising considering the substantial regional differences in changes to the physical condition of the Southern Ocean as a result of climate change, propagated throughout the food web (Rogers et al., 2020).

The second data chapter (Chapter 3) of this dissertation took the research into the BC of South Africa's SRWs one step further and correlated it to the levels of GC in blubber biopsy samples. Results of this analysis showed a positive relationship between maternal BC and blubber GC levels. Considering the involvement of GC in mammalian energy balance and lipid mobilization, these results may be indicative of a reduced quality of lactation, and thus reduced energy transfer to the calf, in those females with low BCIs. Although the small sample size warrants careful interpretation, results strongly call for continued research. If the formulated hypothesis is accurate, climate change and related reduced prey availability may not only be affecting the females' ability to produce offspring but also reduce their ability to invest in the calf after birth, possibly affecting calf survival rates and hampering population recovery even further.

Since 2009, the proportion of females in the population with a normal 3-year reproductive cycle started to decrease to a point in 2015, when for the first time, the largest proportion of females in the population showed a 4-year cycle and the numbers of cow-calf pairs on the South African coast dropped substantially. Ultimately, a few years of suboptimal feeding conditions will precede a reduced reproductive success in a female (Greene et al., 2003). It can thus be hypothesised that the BC of South African SRWs may have gradually started to decrease in the past decade, leading eventually to a threshold about two reproductive cycles

later (equal to 6 years) where the reproductive success of a large part of the female population started to be affected. These findings, in combination with the result of van den Berg et al. (2021) who found a drastic shift in foraging strategy, are clearly indicative of large-scale environmental changes in SRW feeding grounds in the Southern Ocean affecting prey availability and/or quality at least in the past decade, ultimately hampering population recovery of this population.

In conclusion, this dissertation strongly suggests that the reproductive performance, including gestation and possibly lactation, of the South African population of SRWs is under threat due to an inability to uptake sufficient energy reserves during the foraging season. Ultimately, this is believed to be caused by anthropogenic climate change affecting prey availability and/or quality in their Southern Ocean feeding grounds.

4.2 IMPLICATIONS

4.2.1 The southern right whale as an indicator species

Anthropogenic climate change is known to be leading to a number of changes in physical oceanic properties (Bindoff et al., 2019), including in the Southern Ocean (see Rogers et al., 2020). However, understanding the impacts of climate and environmental change on marine ecosystems is still at a relatively early stage, and are complex to unravel (Constable et al., 2014). Therefore, identifying animals that are useful for monitoring such impacts and that allow for future predictions of responses of marine top predators, is deemed critical (Fleming et al., 2016).

Indicator species are those species that can be easily monitored and whose status reflect the environmental condition of their habitat (Bartell, 2006; Burger, 2006; Cairns and Pratt, 1993; Landres et al., 1988; Siddig et al., 2016). Due to their predictable seasonal presence and residency in continental coastal waters of the Southern Hemisphere, limiting logistic difficulties, the SRW can be deemed the "right" whale to study. As such, recovering populations of SRWs have been extensively studied on their main breeding areas, with long-term (at least 20 years+) databases existing in South Africa, South America and Australia (IWC, 2012). Results of this global long-term monitoring have indicated decreased reproductive success through extended calving intervals in all three of these breeding grounds (see Carroll

et al., 2015; Sueyro et al., 2018; Brandão et al., 2018). Additionally, several studies have indicated links between such a reduced reproductive success and prey availability driven by climatic variations in foraging areas (Leaper et al., 2006; Seyboth et al., 2016; van den Berg, 2020). So, due to the ease to study these whales in their breeding ground, the existing longterm databases and their dependence on an often inaccessible vast oceanic region of the Southern Ocean for feeding, these whales seem to be the "right sentinel" to assess, from continental coastal regions, the health condition of a wide region in the Southern Ocean. Because of this, the effect of environmental variability on the foraging success and ultimately the reproductive success of SRWs was selected as a priority research topic within the Southern Ocean Research Partnership (SORP) within the scientific committee of the IWC, titled "The right sentinel for climate change: linking foraging ground variability to population recovery in the southern right whale". This project aims to leverage the uniquely existing long-term global population monitoring data on SRWs, to investigate the effects of rapid anthropogenic climate change on environmental conditions and ultimately krill availability in the larger Southern Ocean. One of the four core objectives of this research topic is to integrate health indicators with long-term monitoring data through visual health assessments, photogrammetry, and physiological indicators of health. The findings of this dissertation fall in line with these objectives and play a crucial part in contributing sound and comprehensive data from the South African population and perspective.

4.2.2 Economic implications

Recovering SRW populations have a large positive socio-economic impact on the coastal regions to which they migrate. As such, due to their predictable seasonal presence and their coastal nature when on the breeding ground, they form the basis of large thriving whale-watching industries in various places of all main breeding areas including Argentina, Brazil, South Africa, Australia and New Zealand (Hoyt, 1995). Within South Africa, this activity attracted around five million tourists and generated a total revenue of > 61 million US dollars in 2008 (O'Connor et al., 2009). As in most countries, the South African whale-watching industry is primarily based on unaccompanied adult and juvenile SRWs (including males and non-calving females) along the southern Cape coast, as an approach to females with calves at a distance < 300m is restricted by permit conditions due to an increased sensitivity to

disturbance (e.g. Argüelles et al., 2016; Arias et al., 2018). However, the incessant low number of unaccompanied adults is of great concern for the country's whale-watching and other whale-based commercial activities, and inevitably leads to the repeated approach to cow-calf pairs in the desire to satisfy client expectations. Such accumulative effects of approaching vessels on cow-calf pairs could have energetic consequences for both cow and calf (Christiansen and Lusseau, 2014). Considering the already compromised energetic reserves of lactating females in the South African population, shown in this dissertation, the South African Boat-based Whale-watching Association should be precautious and ensure a limitation on unnecessary energy expenditure of cow-calf pairs in their most sensitive time, to ensure the best possible management of this valuable natural but currently fragile resource.

4.3 FUTURE RESEARCH

The South African population of SRWs comprises the largest part of the entire SRW species (IWC, 2012). The annual aerial photo-identification surveys focused on these SRWs calving along the southern Cape coast (Best, 1990b) is one of the longest continuous survey series on any marine mammal, showing abrupt changes in the population in the past decade. Considering the dependency on the Southern Ocean as a feeding ground of this capital breeder, this database provides a remarkable and unique opportunity to investigate the effects of environmental variability, including climate change, on prey availability for this krill-dependent species. Continued monitoring of this population by means of the annual aerial surveys is thus of critical importance. Although this is only the second photogrammetry study on the species in the country, the results of this dissertation form a sound basis to continue monitoring the nutritional condition of South Africa's SRW females in relation to their reproductive success. Similarly, as the first study to assess blubber GC levels in SRWs globally, this study can be a starting point for continued research on the topic.

There are various ways in which the research presented in this study can improve in the future. Although no temporal comparison in the BC of non-calving adults could be made in this study, it is likely that the BC in South African SRWs has influenced the migratory behaviour of the population as a whole, as demonstrated by the abnormal counts since 2009 of unaccompanied adults (including males and females not calving that year) (Vermeulen et al., 2020). The main drive for a whale to migrate is believed to be related to an increase in calf survival probabilities (Corkeron and Connor, 1999; Norris, 1967) or to energetic benefits to the mother (Brodie, 1975). In this concept, the motivation of non-calving individuals to make the full costly migration to the South African coast may be reduced in years of poor feeding. Therefore, an increased sample size of all reproductive and age classes will allow for a better ability to compare the BC among these groups on a temporal and global scale, and allow for the ability to eliminate confounding factors (such as having a dependent calf) when needed. Furthermore, repeated measurements throughout one season will allow for a more close follow up on the possible variation in BC of SRWs when fasting on their breeding ground, and more importantly on the BC loss of adult females and energy transfer to their calves throughout the first few weeks of lactation. Last but not least, due to the malfunction of the altimeter on the UAV, no absolute measurements could be obtained from the whales in this study. Future research must thus ensure that this problem is overcome (e.g. by attaching a range finder), so that absolute values can be assessed as well as relative ones for further calculations and interpopulation comparisons. Furthermore, it is recommended that researchers apply a standardized approach in the technical procedure of aerial photogrammetry to obtain BC measurements. For instance, given that the 25mm lens used for BC measurements (Christansen et al., 2016a; Christansen et al., 2020) has proved to generate high precision estimates for aerial photogrammetry relating specifically to the measurement of free ranging whales (Dawson et al., 2014), using this camera lens for future body measurements will ensure that the protocol of Christiansen et al. (2016a) is replicated in an accurate manner to achieve robust BCI measurements for the SRWs.

In terms of blubber GC concentrations, an increased sample size, including from different reproductive and age classes, is critical for any further assessment of the formulated hypotheses. Furthermore, the assessment of GCs in baleen plates of SRW collected over various years would allow for an assessment of perceived stress levels of a single individual on an interannual level (as a baleen plate provides data over various years of an individual's life), among individuals on an inter-decadal level (if baleen plates can be collected from animals stranded in different decades), and among different populations (if baleen plates can be collected from different populations). Furthermore, assessing these baleen GC concentrations in relation to stable isotope profiles will furthermore allow for an interpretation of these

results in relation to the whale's migratory behaviour. Ultimately, a population response to a reduced prey availability is driven by individual responses, and understanding these in relation to different influencing factors such as age, season as well as reproductive class and state, may help predict the effects on the overall population more accurately.

CHAPTER 5

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APPENDIX 1

This appendix accompanies Chapter 2 of this dissertation:

Body condition of southern right whales (*Eubalaena australis*) on the South African breeding ground

Attribute	Score 1 (good)	Score 2 (medium)	Score 3 (poor)
(A) Camera focus	The picture is sharp with the contour of the whale's body clearly visible.	The picture is blurry, but still clear enough to make out the contour of the whales body.	The picture is too blurry to make out the contour of the whales body.
(B) Body straightness	The body axis midline crosses the peduncle closer to the centre than to the edges.	The body axis midline crosses the peduncle closer to the edges than to the centre, but does not cross outside the peduncle.	The body axis midline crosses outside the peduncle of the animal.
(C) Body rolling	The blowhole of the whale is aligned with the midline body axis of the whale.	The blowhole of the whale deviates slightly (<1/3 of the eye width) from the midline body axis of the animal.	The blowhole of the whale deviates significantly (>1/3 of the eye width) from the midline body axis.
(D) Body arching	No visible arching of the body. The animal is lying vertically flat in the water.	The head or the peduncle/tail region of the animal is slightly lifted or dropped.	The head or the peduncle/tail region of the animal is significantly lifted or dropped, or both the head and the peduncle/tail region is slightly lifted or dropped.
(E) Body pitching	The body axis of the whale is not angled vertically.	The body axis of the whale is angled slightly in the vertical plane, either up or down.	The body axis of the whale is angled significantly in the vertical plane, either up or down.
(F) Length measurability	Both the tip of the rostrum and the notch of the tail fluke are clearly visible.	The tip of the rostrum or the notch of the tail fluke is unclear or partly obscured, but can still be approximated.	The tip of the rostrum and/or the notch of the tail fluke are not visible due to spray, water distortion, another animal or object, or is too far down in the water column.

Table A1.1. Definition of attributes used to score and select BC photographs from the early and late periodusing the Christiansen et al. (2016a) approach.

	1		
(G) Width measurability	The body contour of the	The body contour of the	The body contour of the
	whale is clearly visible.	whale is mostly visible,	whale is unclear and
		but some parts of the	obscured to such a
		body are unclear or	degree that extrapolation
		obscured. Missing parts of	of the body contour
		the contour can be	based on visible parts of
		reliably extrapolated from	the body is not possible.
		visible parts of the body.	

Table A1.2. Late period (2019) BC values for 46 southern right whale lactating females, including details on year and BC measurements in pixels. Body condition index (BCI) and relative calf length is given for those individuals selected for further analyses, based on good quality photogrammetry data using the Christiansen et al. (2016a) approach.

Number	Date	Reproductive class	Mother length (pixels)	Accomp anying calf length (pixels)	Volume (Pixels)	BCI	Relative calf length (proportion to maternal length)
1	9/3/2019	Juvenile			0.0130671	-0.247902401	
2	9/7/2019	Adult (non- lactating)			0.0167598	-0.035362708	
3	9/7/2019	Lactating female	3199.03	1393.47	0.0142324	-0.180828991	0.435591414
4	9/7/2019	Calf			0.0179003	0.030281533	
5	9/7/2019	Lactating female	3123.63	1353.25	0.0153202	-0.118222585	0.433229928
6	9/7/2019	Calf			0.017194	-0.010371392	
7	9/9/2019	Lactating female	2677.81	1381.03	0.0160792	-0.074536972	0.515731139
8	9/9/2019	Adult (non- lactating)			0.0217558	0.252188172	
9	9/9/2019	Calf			0.0171645	-0.012070706	
10	9/9/2019	Calf			0.0169951	-0.021821923	
11	9/9/2019	Calf			0.0149584	-0.139044512	
12	9/9/2019	Lactating female	2899.37	1174.86	0.0169789	-0.022751185	0.405212167
13	9/9/2019	Calf			0.0128521	-0.260275025	
14	9/9/2019	Lactating female	2947.76	1200.49	0.0172566	-0.006767665	0.407255
15	9/9/2019	Calf			0.0166223	-0.043278084	
16	9/10/2019	Lactating female	2689.38	1345.95	0.0148997	-0.142422377	0.500468509

17	9/10/2019	Lactating female	2992.4	1310.26	0.0162834	-0.062781287	0.437862585
18	9/10/2019	Calf			0.0175207	0.008433987	
19	9/10/2019	Lactating female	2918.8	1353.33	0.0144264	-0.169665691	0.463659723
20	9/10/2019	Calf			0.0183067	0.053669554	
21	9/10/2019	Lactating female	2574.28	1270.57	0.0170664	-0.017715137	0.493563249
22	9/10/2019	Calf			0.0172624	-0.006436952	
23	9/10/2019	Lactating female	3114.41	1527.1	0.018266	0.051328705	0.490333643
24	9/10/2019	Lactating female	2869.17	1224.49	0.0141233	-0.187112362	0.426774991
25	9/10/2019	Calf			0.0159255	-0.083379411	
26	9/10/2019	Lactating female	3018.13	1387.55	0.013499	-0.223043807	0.459738315
27	9/10/2019	Calf			0.0170329	-0.019644534	
28	9/10/2019	Calf			0.0176139	0.013798884	
29	9/10/2019	Calf			0.0190263	0.095091345	
30	9/11/2019	Calf			0.0178718	0.028640219	
31	9/11/2019	Calf			0.0161671	-0.069475003	
32	9/11/2019	Lactating female	2757.13	1140.34	0.0171065	-0.015405928	0.413596747
33	9/11/2019	Calf			0.0178592	0.027914409	
34	9/11/2019	Calf			0.0132657	-0.236471537	
35	9/11/2019	Lactating female	2767.93	1086.74	0.0138414	-0.203334991	0.39261831
36	9/11/2019	Calf			0.0160609	-0.075587913	
37	9/11/2019	Calf			0.014648	-0.15690901	
38	9/15/2019	Lactating female	2454.54	1187.96	0.0156094	-0.101577213	0.483984779
39	9/15/2019	Calf			0.0178949	0.02997223	
40	9/15/2019	Calf			0.0166294	-0.042868119	
41	9/15/2019	Lactating female	3017.4	1340.33	0.0154486	-0.110829825	0.444200305
42	9/15/2019	Calf			0.0186613	0.074082319	
43	9/15/2019	Calf			0.0191933	0.10470277	
44	9/15/2019	Calf			0.0122643	-0.294110929	
45	9/15/2019	Calf			0.0228743	0.31656584	
46	9/15/2019	Lactating female	2479.43	1518.34	0.0193521	0.113842294	0.612374618

Table A1.3. Early period (1988/1989) BC values for 15 southern right whale lactating females, including details on year and BC measurements in pixels. Body condition index (BCI) and relative calf length is given for those individuals selected for further analyses, based on good quality photogrammetry data using the Christiansen et al. (2016a) approach.

Number	Year	Reproductive class	Mother length (pixels)	Calflength Volume (pixels) (Pixels) BC		BCI	Relative calf length (proportion to maternal length)
1	1988	Lactating female	931.21	472.77	0.023177	0.333992	0.50769429
2	1988	Lactating female	854.1	402.86	0.025416	0.462858	0.471677789
3	1988	Lactating female	857.98	435.85	0.019893	0.144969	0.507995524
4	1988	Lactating female	839.22	438.44	0.021838	0.256932	0.522437501
5	1988	Lactating female	852.89	513.86	0.020526	0.181392	0.602492701
6	1988	Lactating female	862.69	464.47	0.020443	0.176627	0.538397339
7	1989	Lactating female	716.3	358.12	0.021463	0.235354	0.499958118
8	1989	Lactating female	775.01	318.77	0.018718	0.077318	0.411310822
9	1989	Lactating female	1010.77	318.15	0.023591	0.357837	0.314760034
10	1989	Lactating female	951.4	333.42	0.020508	0.180398	0.350451966
11	1989	Lactating female	937.94	352.86	0.021035	0.210682	0.376207433
12	1989	Lactating female	892.03	454.22	0.020344	0.170943	0.509198121
13	1989	Lactating female	1137.12	538.85	0.019506	0.122706	0.47387259
14	1989	Lactating female	972.87	537.41	0.026173	0.506434	0.552396518
15	1989	Lactating female	996.94	526.45	0.021247	0.222909	0.528065882

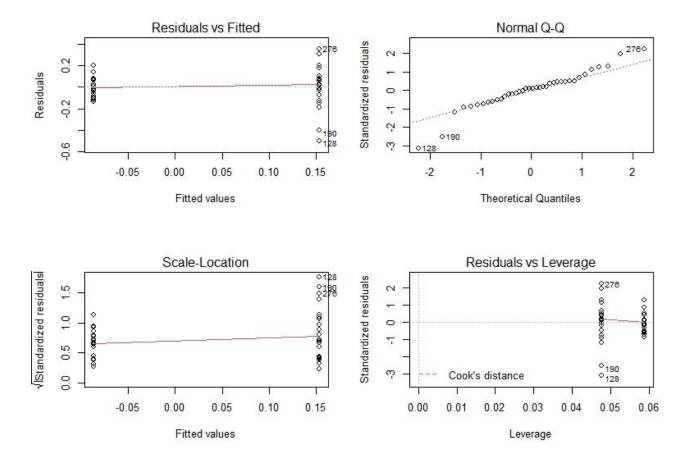


Figure A1.1. Model validation plots of the temporal comparison of BCI in 1988/89 (early sampling period, n=15 lactating females) and 2019 (late sampling period, n=17 lactating females), including a scatter plot of model residuals against fitted model values (to evaluate homogenous residuals), frequency histograms of model residuals (to examine normality of residuals) and calculating leverage scores and Cook's distance (to identify influential points and outliers, respectively.

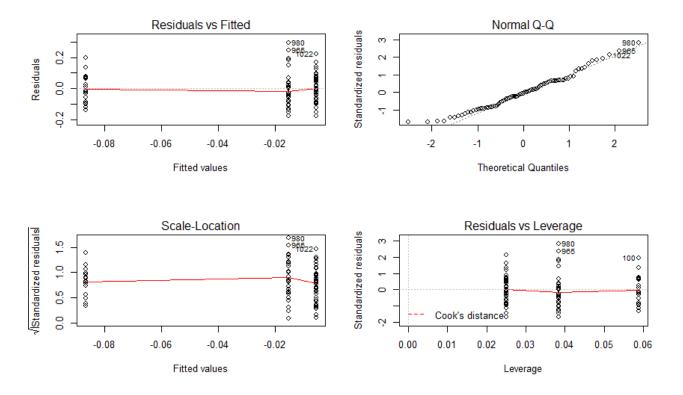


Figure A1.2. Model validation plots of the interpopulation comparison of BCI of lactating females between South Africa (n=17), Australia (n=40) and Argentina (n=26) in 2019, including a scatter plot of model residuals against fitted model values (to evaluate homogenous residuals), frequency histograms of model residuals (to examine normality of residuals) and calculating leverage scores and Cook's distance (to identify influential points and outliers, respectively).

APPENDIX 2

This appendix accompanies Chapter 3 of this dissertation:

Blubber glucocorticoid concentrations in relation to body condition in southern right whales

Appendix 2

 Table A2.1. Blubber GC concentrations for each obtained southern right whale blubber sample, including details on date, reproductive class and grading scores. Body condition index (BCI) and relative calf length is given for those individuals selected for further analyses, based on good quality photogrammetry data.

Sample number	Date	Reproductive class	Focus	Straightness	Rolling	Arching	Pitch	Length measura bility	Width measura bility	Glucocorticoid concentration (ng/g blubber)	BCI	Relative calf length (proportion to maternal length)
1	9/7/2019	Juvenile	1	2	1	1	1	1	1	0.3		
2	9/7/2019	Lactating female	1	1	1	1	1	1	1	0.28	-0.18083	0.4356
3	9/7/2019	Juvenile	1	1	1	1	2	1	1	0.71		
4	9/7/2019	Lactating female	1	1	1	1	1	1	2	0.75	-0.11822	0.4323
5	9/7/2019	Juvenile	1	1	2	1	1	1	2	1.01		
6	9/9/2019	Adult	1	3	1	1	1	1	3	1.17		
7	9/9/2019	Lactating female	1	1	2	1	1	2	3	1.16		
8	9/9/2019	Lactating female	1	1	1	1	1	1	3	1.46		
9	9/9/2019	Lactating female	1	1	1	1	1	2	2	0.76	-0.00677	0.4073

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10	9/10/2019	Lactating female	1	3	1	1	1	2	2	0.6	-0.06278	0.4379
11	9/10/2019	Lactating female	1	1	1	2	1	1	2	1.32	0.06131	0.4311
12	9/11/2019	Lactating female	1	1	1	1	1	2	3	0.72		
13	9/11/2019	Lactating female	1	1	1	1	2	3	3	1		
14	9/11/2019	Lactating female	1	1	1	1	2	2	1	0.78	0.0615	0.4856
15	9/15/2019	Lactating female	1	1	1	1	1	1	2	0.56	-0.11083	0.4442