Sex Ratios of Migrating Southern Hemisphere Humpback Whales: A new Sentinel Parameter of Ecosystem Health

Authors Alison Druskat ^{1,2}, Ruma Ghosh¹; Juliana Castrillon¹; ^δSusan M. Bengtson Nash¹

Affiliations

¹ Griffith University, The Environmental Futures Research Institute, Southern Ocean Persistent Organic Pollutants Program, 170 Kessels Road, Nathan, QLD 4111, Australia

² University of York, York, YO10 5DD, England, UK

^δCorresponding Author: Susan M. Bengtson Nash

Email: s.bengtsonnash@griffith.edu.au

1.0 Abstract

Southern hemisphere humpback whales have evolved energetically demanding capital breeding and migratory life-history behaviours. It has been hypothesised that not all individuals of a population participate in the seasonal migration each year, or only undertake partial migrations. Given the cost of migration and reproduction, we explored the possibility that specifically, not all mature females participate in the seasonal migration every year, or significantly delay or shorten their migration, in response to poor feeding conditions. That is, females must attain a minimum threshold of accumulated energy reserves to commit to a reproductive event that likely occurs as a product of mating during migration. With a 1:1 male to female birth ratio, yet a male bias observed along the main migratory corridor; this study utilised inter-annual migratory cohort sex ratios to explore their potential to serve as measures of population fecundity, as a function of ecosystem health. The sex ratios of randomly biopsied adult humpback whales, sampled at a defined location and set time-points along the main migratory corridor from 2008 to 2016 were investigated. Northward migration sex ratios in 2009, 2014 and 2016 revealed a lower male bias suggesting good female participation in the migration and therefore apparent optimal provisioning during the two preceding summers. By contrast, the 2011 southward migration, revealed the highest male bias recorded of 5.75:1. Southward migration sex ratios were found to oscillate closely with measures of population adiposity, a sentinel parameter employed for long-term surveillance of the Antarctic sea-ice ecosystems under the Southern Ocean Observing System-endorsed Humpback Whale Sentinel Program. Anomalously poor humpback whale body condition recorded in 2011 was attributed to poor Antarctic feeding conditions during the extreme La Niña event of 2010/11. These findings lend support for the application of migratory cohort sex ratios, standardised by time and location, as a measure of relative inter-annual

population fecundity. This work therefore contributes a new non-lethal tool for the study of population health, as a function of ecosystem productivity, and facilitates the inclusion of fecundity as a sentinel parameter into long-term Antarctic ecosystem surveillance under the Humpback Whale Sentinel Program.

Key Words

Southern Hemisphere Humpback Whales; Antarctic Sea-ice Ecosystem; Sentinel Parameter; Fecundity; Energetic Health; Population Dynamics; Migration; Climate; Trends

2.0 Introduction

Southern hemisphere (SH) humpback whales (*Megaptera novaeangliae*) undertake some of the longest migrations known for any mammal on the planet, moving seasonally between Antarctic summer feeding grounds and equatorial winter breeding grounds (Rasmussen et al., 2007). This extreme migratory life-history allows these populations to exploit the seasonal productivity of the Antarctic sea-ice ecosystem whilst also satisfying the physiological needs of new-born calves, born without a substantial blubber layer, and therefore the ability to adequately thermoregulate (Bengtson Nash, 2018). The preference of mothers and calves, returning from the breeding ground, for warm shallow waters, facilitates effective nursing and energy conversion to body mass (Bengtson Nash and Castrillon, 2017, Bejder et al., 2019) and therefore optimal calf development and survivorship (Corkeron and Connor, 1999, Rasmussen et al., 2007).

Humpback whales are ‘capital breeders’, meaning they fuel the cost of breeding and migration with energy stores accumulated during intensive summer feeding (Eisenmann et al., 2016, Bengtson Nash et al., 2013). Reproductive investment in mammals is especially costly, and encompasses energy allocation to: competitive breeding behavior, gestation, parturition, lactation and parental care (Gittleman and Thompson, 1988, Castrillon and Bengtson Nash, Accepted). Maternal investment towards their young is greatest during lactation (Yasui and Gaskin, 1986, Lockyer, 1987, Lockyer, 2007, Oftedal, 1997, Gittleman and Thompson, 1988). Amongst baleen whale species, humpback whales have the longest lactation period (11-12 months); lasting three months longer than any other baleen whale species and thus coming at greater energy expenditure (Chittleborough, 1958, Lockyer, 1984). Unsurprisingly, females are estimated to lose as much as 25 to 50% of their post-feeding body mass during a reproductive season

(Lockyer, 1981). As such, it is critical that particularly mature females acquire sufficient energy reserves prior to migration and reproductive investment. Not only are adequate energy reserves necessary for the mother's survival, but the body condition of mothers has been found to carry direct consequences for the size and survivorship of offspring in numerous marine mammal species (Huang et al., 2011, Lunn and Boyd, 1993, Castrillon and Bengtson Nash, Accepted). Indeed, in times of nutritional stress, marine mammals may forego reproduction or restrict maternal investment. This has most frequently been evidenced in pinnipeds (Maniscalco and Parker, 2017). For example, in a study of Antarctic fur seals (*Arctocephalus gazella*), lower pup weights were found to be associated with a decline in maternal body condition, resulting in a significant percentage of pup mortalities (Lunn and Boyd, 1993). In the case of Southern Ocean foraging baleen whales, research concerning southern right whales (*Eubalaena australis*) found a positive correlation between krill densities in the known feeding area of the study population, and the number of calves recorded in a season (Seyboth et al., 2016). Similarly, right whale maternal body condition has been found to influence calf growth (Christiansen et al., 2018). An individual female's foraging success and body condition therefore has direct implications for her reproductive success and population fecundity.

As with most other cetaceans, calving years in humpback whales have been presumed to be interspersed with years of nursing, replenishment of energy reserves, and a new pregnancy (Kraus et al., 2001, Knowlton et al., 1994, Miller et al., 2011), although an annual reproductive cycle has recently been observed in some SH humpback whales (Pallin et al., 2018, Barendse et al., 2013). Barlow and Clapham (1997) produced a demographic model that estimated the mean calving interval of northern hemisphere humpback whales to be 2.4 years, though ranging from

one to more than five years (Baker et al., 1987). If mature female humpback whales are not consistently expected to produce a calf every year, this brings into question the participation of non-reproducing females in the energetically costly seasonal migration. Whilst it is clear that if a female is pregnant, the annual migration is essential for calf survival (Chittleborough, 1958, Seyboth et al., 2016), much uncertainty remains surrounding the timing and frequency of migratory participation of individual females, and more broadly, the proportion of the population as a whole that undertakes the annual migration from year to year. Specifically, it would seem plausible that certain demographic groups, such as juveniles or non-pregnant females that have recently weaned a calf, may forego or substantially delay or shorten the annual migration in favour of conserving and re-/building their energy reserves. Recently, immature individuals have been observed to make up a significant proportion of the rapidly expanding South Pacific humpback whale migratory cohorts (Riekkola et al., 2018, Franklin et al., 2017), suggesting a social role or maternally directed philopatry associated with the migration event (Franklin et al., 2017). Nonetheless, a number of data from multi-disciplinary fields of research have indeed provided clues that some individuals of the population may forego the full annual migration.

In 2013, Van Opzeeland *et al.* (Van Opzeeland et al., 2013) reported acoustic recordings that evidenced the presence of humpback whales in coastal Antarctic waters throughout the austral winter, a time when it is presumed that individuals would seek lower latitudes. Further, whilst the fetal sex ratio of humpback whales has been found to correspond to 1:1 at birth (Chittleborough, 1965), (Brown et al., 1995) found that the sex-ratio of migrating humpback whales along the main migratory corridor of southeast Queensland was highly skewed towards males, revealing an average male to female ratio of 2.4:1 across the entire migration season

(May-October). Similar results were also reported from commercial whaling data which found sex ratios to be occasionally skewed slightly towards females in the feeding grounds, but skewed towards males on migratory paths near the breeding grounds (Omura, 1953, Chittleborough, 1965), leading the authors to suggest that 50% of females do not undertake the annual migration.

It is hypothesized that on account of the considerable energetic demands on female humpback whales for a successful reproductive event, individuals must “decide” in the late summer whether to commit to the migration. Whilst it is clear that pregnant females must migrate to reach optimal calving grounds, the oestrus cycle of humpback whales remains cryptic (Dalle Luche et al., Accepted). Mating may theoretically occur in the Antarctic feeding grounds, although with a gestation period close to a year, and no physiological evidence of delayed implantation in the species, it is favorable for females to fall pregnant during the winter months. Further, the migration journey itself, and the competitive displays by males along the journey, are likely employed by females to select the strongest mate (Tyack and Whitehead, 1983).

Under the partial population migration hypothesis, the seasonal migratory cohort would be made up of pregnant females, non-pregnant females that have accumulated sufficient energy reserves to commit to reproduction, accompanying yearlings/juveniles, and sexually mature males. The most dynamic component of the inter-annual migratory cohort therefore, is the number of migrating females. This component will in turn be influenced by the foraging success and energy reserves acquired by females during the two summers prior to migration (currently pregnant), and of the summer immediately preceding migration (preparation for pregnancy). In this manner, the sex ratio of the migratory cohort may provide information about relative, inter-annual

population fecundity as a function of female foraging success and the productivity of the Antarctic sea-ice ecosystem in their corresponding feeding ground.

In investigating this hypothesis it is important to consider a number of factors known to influence observed sex-ratios along the migration corridor. Notably, timing and location of observations will carry a significant impact on the sex ratios observed. Staggered migrations of different demographic groups are known to result in measurable temporal variation (Chittleborough, 1965). Immature individuals and non-pregnant females arrive on the breeding ground early in the season, followed by mature males and finally pregnant females (Chittleborough, 1965). The return journey to Antarctic feeding grounds is similarly subject to sex-specific behavioral influences. Predominantly females and immature individuals may divert from the main migratory corridor to utilize resting stop-offs, leading to a female bias in these locations (Franklin et al., 2017). Similarly, feeding stop-offs have been found to present a female bias (Barendse et al., 2010).

Controlling for these influencing factors through standardisation of observations, both in time and at a set location on the main migratory corridor not associated with either feeding nor resting may pave the way for application of the migratory sex ratio as a measure of fecundity, a “sentinel parameter” of ecosystem health. The theory of using marine predators as sentinels of ecosystem health is not a new concept but can only be employed when specific indicators or sentinel parameters are identified as being sufficiently sensitive to detect an observable environmental change (Bengtson Nash et al., 2018), and can be confidently measured. Recently, SH humpback whales were implemented for long term surveillance of the Antarctic sea-ice ecosystem under

the Southern Ocean Persistent Organic Pollutants Program (SOPOPP), using population adiposity and diet as sentinel parameters (Bengtson Nash et al., 2018). Fecundity of SH baleen whales is known to be heavily influenced by krill availability (Seyboth et al., 2016). This study therefore sought to investigate whether population fecundity, as indicated by the migratory cohort sex ratio across 9 years, would reveal a similar trend to the other sentinel parameters employed under the SOPOPP Humpback Whale Sentinel Program (HWSP), thereby lending support for the implementation of this measure into long-term surveillance of the Antarctic sea-ice ecosystem.

3.0 Materials and Methods

3.1 Sampling Method

Skin samples were obtained from free-swimming humpback whales by remote biopsy, as described in detail elsewhere (Waugh et al., 2011). Animals were sampled on their annual migration as they passed North Stradbroke Island, south-east Queensland, Australia (27°26S, 153°34E) (Figure 1). Individuals utilizing this migratory corridor are associated with breeding stock E1, as categorized by the International Whaling Commission (Gales, 2011). Samples were collected from 2008 to 2016 at one of two seasonal time points. These time points were categorized as: (1) Northward migration, i.e. individuals arriving from Antarctica on their way to the breeding ground (sampling took place the last 2 weeks of June/first 2 weeks of July), or (2) Southward migration, i.e. individuals returning from the breeding ground 3 months later in the year (sampled during the last 2 weeks of September/first 2 weeks of October). All sampling was conducted under the approval of The University of Queensland and Griffith University Animal

179 Research Ethics Committee (GU Ref No: NRCET/309/08/SBN, NRCET/273/07/SBN,
180 ENTOX/207/09/SBN, ENV/17/10/AEC, ENV/10/15/AEC).

181
182 **Figure 1** Map depicting the migration route of the E1 breeding stock between Antarctic
183 feeding area V and the east coast of Australia. The insert and outlined area depicts the regional
184 area off North Stradbroke Island where sampling for the study took place

185

186 3.2 Sample Selection

This study utilized sexed samples collected from 2008 to 2016. Only samples that were considered to be collected through random sampling of adult individuals, that is 'first pod encounter' when travelling in the opposite direction to the whales' direction of travel, were used in this work (full list of samples provided in Table S2). On this basis, the southward migrations of 2008 and 2009 were excluded as researchers were targeting males during these field events and did not sample pods with accompanying calves. In order to minimize the possibility of repeat sampling of the same individual, no more than half the number of individuals in a pod were targeted. A total of 264 sexed biopsies from both northward and southward migrations were used for this study.

3.3 DNA Extraction

Genomic DNA (gDNA) was extracted using a GenElute™ Mammalian Genomic DNA Miniprep Kit (Sigma-Aldrich, Sydney, Australia) according to manufacturer's protocols. In brief, prior to gDNA isolation, finely sliced 25 mg of frozen humpback whale skin/blubber tissue was agitated in proteinase K solution (50 mg/mL) and manufacturer's lysis solutions at 65 °C for 24 hours, and an additional 10 minutes at 70°C, as per manufacturers specifications. The resulting lysate containing gDNA was prepared for binding to silica binding columns and washed before eluting purified gDNA with 200 µL of 10mM Tris-HCl, 0.5 mM EDTA, (pH 9.0). The DNA concentration (ng/µL) and purity was quantified by Nanodrop, Spectrophotometer ND-1000.

3.4 DNA Amplification

Approximately 50-80 µg of DNA was used per reaction for amplification with PCR (BioRad C1000™ Thermal Cycler). Each reaction comprised of 0.25 µL GoTaq® Hot Start DNA

Polymerase (1.25 U), 10 μ L 5 $\times$ Go Taq® Flexi buffer (1X), 3 μ L MgCl₂ (2.5 mM) (Promega Corporation, Sydney, Australia), 1 μ L dNTP mix (10mM) (Invitrogen, Sydney, Australia), and 1 μ L of oligonucleotides sequences specific to ZFY/ZFX DNA regions (0.1-1.0 μ M), as previously published (Palsboll et al., 1992) and detailed in Table S1. The final reaction volume of 50 μ L was made up with DEPC treated water (Invitrogen, Sydney, Australia). The following PCR conditions were used: 2 minute initial denaturation step at 94 – 95 °C, 1 minute annealing step at 60 °C, 4 minute extension step at 72 °C (35 cycles) and a final 5 minute extension at 72 °C.

3.5 Sex Determination by Restriction Endo-nuclease Digestion

The amplified product was cut with Taq1 restriction enzyme (ThermoFisher, Australia) at 60°C for 1 hour. Bands specific to ZFY (two bands, 621 and 439 bp long) and ZFX (one band 439 bp long) were compared with phiX174 HaeIII digested lambda DNA marker on 2% Agarose gel (Bio Rad Laboratories, Inc.) stained with 1 μ L RedSafe (Nucleic Acid Staining Solution from Thermo fisher Scientific). (Thermo fisher Scientific) A BioRad PowerPac™ Basic 300V I 400mA I 75 W, was used to run the gel at 100 volts for 45 min. The completed gel was viewed using a BioRad Molecular Imager® Gel Doc™ XR+ with Image Lab™ Software.

3.6 The Inverse Adipocyte Index (AI^{-1})

Sex ratios were compared against population energy reserves or ‘adiposity’ as evaluated by application of the inverse Adipocyte Index (AI^{-1}) (Table S3). The AI is a histological measure of the relative adipocyte area in the outer blubber layer (Castrillon et al., 2017). It is based on the principal that very early on in mammalian development, the number of adipocytes that an

individual develops is set. Any fluctuations in energy stores can therefore be attributed to an increase or decrease in the size of individual adipocytes (Faust et al., 1978). The AI is quantified as the intervacuolar space relative to adipocyte area, in such a way that animals with lower energy reserves receive a higher AI (Castrillon et al., 2017). In order to make the measure more intuitive, that is that a higher index indicates better energetic reserves, the inverse AI (AI^{-1}) was applied. AI measures from the early part of the record (2008-2013), have previously been verified against another Program sentinel parameter of adiposity, namely outer blubber persistent organic pollutant (POP) concentrations (Bengtson Nash et al., 2018). In this study, POP measurements and AI showed a direct correlation to each other and hence only AI measures have been applied in the current work and additional AI measures incorporated for the latter part of the timeline (2014-2016).

3.7 Statistics and Data Analysis

A linear regression was performed for the two parameters of AI and the % of females in the migratory cohort for presentation of data.

4.0 Results

A total of 264 confirmed sex samples were utilised, biopsied from 4 northward and 4 southward migration events spanning 9 years and representing 178 males and 86 females. The northward migration biopsies were obtained in 2008, 2009, 2014 and 2016. Using Brown et al.'s recorded northward migration sex ratio of 2.6:1 as a point of reference, higher female participation in the migration was observed through the northward migrations of 2009 (2.00:1), 2014 (1.53:1) and

2016 (1.35:1) (Table 1; Figure 2; Table S2). By contrast lower female participation was observed during the northward migrations of 2008 (3.50:1).

Southward migration biopsies were collected in 2011, 2013, 2015 and 2016. A slightly elevated female ratio, compared to the northward migration, is expected on the southward migration due to the slower, and more conspicuous travel of pods with accompanying calves. This was previously reported by (Brown et al., 1995) who found a 2:1 male:female ratio in southward migrating cohorts, compared to the 2.6:1 ratio for the northward migration. This was also demonstrated in the current study for 2016 where both northward and southward sampling was undertaken (2.25:1 on southward versus 1.35:1 on the northward). With this in mind, 2015 (1.95:1) appeared a strong year for females. Similarly, 2013 and 2016 approached the previously reported 2.1:1 male:female ratio at 2.44:1 and 2.25:1 respectively. Finally, 2011 appeared as an anomalous dip in female migration participation (5.75:1) (Table 1; Figure 3; Table S2).

The average population AI ranged from 1.26 on the southward migration of 2011 to 3.89 on the northward migration of 2008, with an average AI across all migrations recorded (n=8) of 2.74. Although it was not possible to calculate the significance of the relationship between the sex ratio and AI^{-1} from just four paired data points in the linear regressions performed, a positive relationship trend was suggested in the southward data (Figure 3), primarily driven by the anomalous 2011 year.

Table 1 Migratory cohort male: female ratios, where '*' indicates a sampling event excluded in this study due to conscious male-biased sampling, and '-' indicates a migratory event not captured in the timeline.

Migration		Ratio	
Year			
	Sample Size (n)	Northward Migration (NM)	Southward Migration (SM)
		(Male: Female)	
2008	27	3.50: 1	*
2009	12	2.00: 1	*
2010		-	-
2011	27	-	5.75: 1
2012		-	-
2013	31	-	2.44: 1
2014	38	1.53: 1	-
2015	62	-	1.95: 1
2016	54 (NM); 13 (SM)	1.35: 1	2.25: 1

Figure 2 Sex distribution of northward migrating cohorts, presented along with the average population inverse adiposity index (AI⁻¹) of the migrating cohort. The AI⁻¹ is a histological measure of relative adipocyte size where a higher index indicates an animal in better body condition. The horizontal red lines indicate a) solid line - the historical 2.6:1 Male:Female migratory ratio reported by (Brown et al., 1995), in the same location for northward migrating cohorts and, b) dashed line – parity.

Figure 3 Sex distribution of southward migrating cohorts, presented along with the average population inverse adiposity index (AI⁻¹) of the migrating cohort. The AI⁻¹ is a histological measure of relative adipocyte size where a higher index indicates an animal in better body condition. The horizontal red lines indicate a) solid line - the historical 2.1:1 Male:Female migratory ratio reported by (Brown et al., 1995), in the same location for southward migrating cohorts and b) dashed line – parity.

5.0 Discussion

Given the substantial energetic cost to female humpback whales for a successful reproductive event, it was hypothesised that relative sex ratios on the migration path, at a set location and time-points, may serve as an indicator of ecosystem productivity in the associated feeding ground. Under this hypothesis, the relative inter-annual sex ratio would reflect the number of mature females within the population that had, over the past two austral summers, accumulated the threshold energetic reserves required to commit to a reproductive event, and indirectly therefore, would provide insight into inter-annual population fecundity.

In order to evaluate this, the migratory sex ratios were established across 9 years and eight separate migration events. These in turn were evaluated against the established humpback whale sentinel parameter of adiposity. The inter-annual variability, and the long-term trend, of migratory sex-ratios for this location and these time points are unknown. The 1992 study by Brown et al. (Brown et al., 1995), is however useful as a historical comparison as measurements of sex ratio were made across the entire migration season in the corresponding geographical location to this study. Reference to these historical measures are therefor made in discussion of current findings.

Despite a slightly higher anticipated female ratio on the southward migration, due to the slower and more conspicuous nature of travel of pods with accompanying calves, the most dramatic results throughout this study were observed on the southward migration of 2011. This migration event captured a strongly male biased sex ratio of 5.75:1, compared to the historical 2.1:1 ratio reported in the same location during the southward migration by Brown et al. in 1992 (Brown et

al., 1995), and consistently lower ratios measured within our own timeline. The unusually low proportion of migrating females corroborates the findings of (Bengtson Nash et al., 2018), who found 2011 to be an anomalously poor year in terms of whale adiposity and concluded that sub-optimal feeding conditions throughout the extreme La Niña event of 2010/11 were reflected in this sentinel parameter. Notably, a recent study by Pallin et al (2018) (Pallin et al., 2018) into the fecundity of SH humpback whales foraging in the western Antarctic Peninsula region, found the lowest proportion of pregnant females occurred in 2010 and 2011 (36.36 and 45.45% respectively), across a 7-year timeline from 2010 to 2016.

By contrast then, years that approach a 1:1 male:female ratio should be viewed as years of optimal summer provisioning in the 2 preceding austral summers, as more females attained sufficient energy reserves to fund migration and reproduction. In our captured timeline, the male to female sex ratios of the northward migrations of 2014 and 2016 approached parity at 1.53:1 and 1.35:1 respectively. The associated adipocyte measures tended towards the mean for all migrations at 2.62 and 2.95 respectively, reflecting good energy reserves for this stage of migration. Notably, the highest proportion of pregnant females in the western Antarctic Peninsula feeding ground were also recorded for 2014 and 2016 (86.27 and 63.53% respectively) (Pallin et al., 2018).

Findings of the current work therefore corroborate both long-term surveillance of the Antarctic ecosystem using humpback whales as a sentinel species (Bengtson Nash et al., 2018), as well a recent work into the pregnancy rates of SH humpback whales foraging in the western Antarctic Peninsula region (Pallin et al., 2018). These supporting findings affirm the close connection

between regional climatic conditions and ecosystem productivity, humpback whale foraging success, adiposity and ultimately female fecundity, underscoring the potential of the latter to serve as a sensitive sentinel parameter of ecosystem health.

The findings of the current study further provide important new information regarding the population migratory patterns of SH humpback whales. It has been postulated that not all individuals of a population migrate every year, or undertake only partial migrations, however only few data have been presented in support of this theory (Van Opzeeland et al., 2013, Brown et al., 1995). Here we present inter-annual data, spanning 9 years, regarding the sex ratio of randomly biopsied adult whales, standardised by location and time point to control for the known staggered migration of reproductive cohorts and age groups of these populations. These control measures do not exclude the possibility that lower measured female participation in the migration through this sampling design is not reflective of delayed or abbreviated migration by females rather than a missed migration. Indeed, (Chittleborough, 1965) report observed parturition as far south as Albany. Nonetheless, the driver behind such a delay or higher latitude migration terminus is similarly theorized to be driven by acquired energy reserves prior to migration.

Male to female ratios ranged from 1.35:1 to 3.75 on the northward migration and 2.25:1-5.75:1 on the southward migration. High relative levels of female participation in the migration, and therefore inferred population fecundity, observed through 2014, 2015 and 2016 is supported by both high pregnancy rates (Pallin et al., 2018) and the high rate of post-whaling recovery of

several SH humpback whale reported in recent years (Bejder et al., Noad et al., 2008, Riekkola et al., 2018).

6.0 Conclusions

The results from this study support the application of relative migratory sex ratios, standardised by location and time-point, as an indirect measure of relative, inter-annual SH humpback whale fecundity. The work therefore contributes a new non-lethal tool for the study of population health, as a function of ecosystem productivity, and facilitates the inclusion of fecundity as a sentinel parameter into long-term Antarctic ecosystem surveillance under the HWSP.

The work further provides valuable new information to a poorly understood area of SH humpback whale population ecology, namely inter-annual migratory participation. The findings of the current study suggest that not all mature females participate in the full annual migration, or delay migration, in response to foraging success. Further, the proportion of females that implement such strategies is highly variable from year to year. Application of AI measures alongside steroid hormone analysis would provide further indications of implications of female body condition for female fecundity, whilst new drone and acoustic applications in the Southern Ocean over winter months may serve to further qualify current theory on non-participation in winter migrations.

7.0 Acknowledgements

387 This work was funded, in part, by an Ocean Foundation Pacific Life grant. SOPOPP
388 acknowledge the support of Moreton Bay Research Station staff and the contributions of field
389 volunteers during each field campaign.

390

8.0 References

- BAKER, C. S., PERRY, A. & HERMAN, L. M. 1987. Reproductive histories of female humpback whales *Megaptera novaeangliae* in the North Pacific. *Marine Ecology Progress Series*, 103-114.
- BARENDSE, J., BEST, P. B., CARVALHO, I. & POMILLA, C. 2013. Mother Knows Best: Occurrence and Associations of Resighted Humpback Whales Suggest Maternally Derived Fidelity to a Southern Hemisphere Coastal Feeding Ground. *PLOS ONE*, 8, e81238.
- BARENDSE, J., BEST, P. B., THORNTON, M., POMILLA, C., CAVALHO, I. & ROSENBAUM, H. C. 2010. Migration redefined? Seasonality, movements and group composition of humpback whales *Megaptera novaeangliae* off the west coast of South Africa. *African Journal of Marine Science*, 32, 1-22.
- BARLOW, J. & CLAPHAM, P. J. 1997. A new birth-interval approach to estimating parameters of humpback whales. *Ecology*, 78, 535-546.
- BEJDER, L., VIDESEN, S., HERMANNSEN, L., SIMON, M., HANF, D. & MADSEN, P. T. 2019. Low energy expenditure and resting behaviour of humpback whale mother-calf pairs highlights conservation importance of sheltered breeding areas. *Scientific Reports*, 9, 771.
- BEJDER, M., JOHNSTON, D. W., SMITH, J., FRIEDLAENDER, A. & BEJDER, L. Embracing conservation success of recovering humpback whale populations: Evaluating the case for downlisting their conservation status in Australia. *Marine Policy*.
- BENGTON NASH, S. 2018. Toxicological Risks and Considerations Associated with Lipophilic Contaminant Burdens of Southern Ocean Mysticetes In: FOSSI, C. & PANTI, C. (eds.) *Marine Mammal Ecotoxicology: impacts of multiple stressors on population health* 1st Edition ed.: Elsevier.
- BENGTON NASH, S. & CASTRILLON, J. 2017. Evaluating the risk of ship strikes in Moreton Bay. Griffith University and Port of Brisbane Pty. Ltd.
- BENGTON NASH, S., CASTRILLON, J., EISENMANN, P., FRY, B., SHUKER, J., CROPP, R., DAWSON, A., BIGNERT, A., BOHLIN-NIZZETTO, P., WAUGH, C. A., POLKINGHORNE, B., DALLE LUCHE, G. & MCLAGAN, D. 2018. Signals from the South; Humpback Whales Carry Messages of Antarctic Sea-ice Ecosystem Variability. *Global Change Biology*, 24, 1500-1510.
- BENGTON NASH, S. M., WAUGH, C. A. & SCHLABACH, M. 2013. Metabolic Concentration of Lipid Soluble Organochlorine Burdens in Humpback Whales Through Migration and Fasting. *Environmental Science and Technology*, 47, 9404-9413.
- BROWN, M. R., CORKERON, P. J., HALE, P. T., SCHULTZ, K. W. & BRYDEN, M. M. 1995. Evidence for a Sex-Segregated Migration in the Humpback Whale (*Megaptera novaeangliae*). *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 259, 229-234.
- CASTRILLON, J. & BENGTON NASH, S. Accepted. Evaluating Cetacean Body Condition; a Review of Traditional Approaches and New Developments. *Ecology and Evolution*.
- CASTRILLON, J., HUSTON, W. & BENGTON NASH, S. 2017. The blubber adipocyte index: A nondestructive biomarker of adiposity in humpback whales (*Megaptera novaeangliae*). *Ecology and Evolution*, 7, 5131-5139.
- CHITTLEBOROUGH, R. G. 1958. The breeding cycle of the female humpback whale, *Megaptera nodosa* (Bonnaterre). *Marine and Freshwater Research*, 9, 1-18.

- CHITTLEBOROUGH, R. G. 1965. Dynamics of two populations of the humpback whale, *Megaptera novaeangliae* *Australian Journal of Marine Freshwater Research*, 16, 33-128.
- CHRISTIANSEN, F., VIVIER, F., CHARLTON, C., WARD, R., AMERSON, A., BURNELL, S. & BEJDER, L. 2018. Maternal body size and condition determine calf growth rates in southern right whales. *Marine Ecology Progress Series*, 592, 267-281.
- CORKERON, P. J. & CONNOR, R. C. 1999. Why do baleen whales migrate? *Marine Mammal Science*, 15, 1228-1245.
- DALLE LUCHE, G., BENGTSON NASH, S., KUCKLICK, J., MINGRAMM, F. & BOGGS, A. Accepted. Quantification of steroid hormone profiles in stranded humpback whales by Liquid chromatography tandem mass spectrometry *Conservation Physiology*.
- EISENMANN, P., FRY, B., HOLYOAKE, C., COUGHRAN, D., NICOL, S. & NASH, S. B. 2016. Isotopic Evidence of a Wide Spectrum of Feeding Strategies in Southern Hemisphere Humpback Whale Baleen Records. *Plos One*, 11.
- FAUST, I. M., JOHNSON, P. R., STERN, J. S. & HIRSCH, J. 1978. Diet-induced adipocyte number increase in adult rats: a new model of obesity. *American Journal of Physiology; Endocrinology and Metabolism* 235, E279.
- FRANKLIN, T., FRANKLIN, W., BROOKS, L. & HARRISON, P. 2017. Site-specific female-biased sex ratio of humpback whales (*Megaptera novaeangliae*) during a stopover early in the southern migration. *Canadian Journal of Zoology*, 96, 533-544.
- GALES, N. 2011. *Humpback whales: status in the southern hemisphere*, International Whaling Commission.
- GITTLEMAN, J. L. & THOMPSON, S. D. 1988. Energy allocation in mammalian reproduction. *American zoologist*, 28, 863-875.
- HUANG, S. L., CHOU, L. S., SHIH, N. T. & NI, I. H. 2011. Implication of life history strategies for prenatal investment in cetaceans. *Marine Mammal Science*, 27, 182-194.
- KNOWLTON, A. R., KRAUS, S. D. & KENNEY, R. D. 1994. Reproduction in North Atlantic right whales (*Eubalaena glacialis*). *Canadian Journal of Zoology*, 72, 1297-1305.
- KRAUS, S. D., HAMILTON, P. K., KENNEY, R. D., KNOWLTON, A. R. & SLAY, C. K. 2001. Reproductive parameters of the North Atlantic right whale. *J. Cetacean Res. Manage*, 2, 213-236.
- LOCKYER, C. 1981. Growth and energy budgets of large baleen whales from the Southern Hemisphere. *Food and Agriculture Organization*, 3, 379-487.
- LOCKYER, C. 1984. Review of baleen whale (Mysticeti) reproduction and implications for management. *Reports of the International Whaling Commission, Special*, 27-50.
- LOCKYER, C. The relationship between body fat, food resource and reproductive energy costs in north Atlantic fin whales (*Balaenoptera physalus*). Symposia of the Zoological Society of London, 1987. Published for the Zoological Society by Academic Press., 343-361.
- LOCKYER, C. 2007. All creatures great and smaller: a study in cetacean life history energetics. *Journal of the Marine Biological Association of the United Kingdom*, 87, 1035-1045.
- LUNN, N. & BOYD, I. 1993. Effects of maternal age and condition on parturition and the perinatal period of Antarctic fur seals. *Journal of Zoology*, 229, 55-67.
- MANISCALCO, J. M. & PARKER, P. 2017. Maternal and offspring effects on the timing of parturition in western Steller sea lions (*Eumetopias jubatus*). *Canadian Journal of Zoology*.

- MILLER, C. A., REEB, D., BEST, P. B., KNOWLTON, A. R., BROWN, M. W. & MOORE, M. 2011. Blubber thickness in right whales *Eubalaena glacialis* and *Eubalaena australis* related with reproduction, life history status and prey abundance. *Marine Ecology Progress Series*, 438, 267-283.
- NOAD, M., DUNLOP, R., PATON, D. & CATO, D. 2008. An update of the east Australian humpback whale population (E1) rate of increase. International Whaling Commission.
- OFTEDAL, O. T. 1997. Lactation in whales and dolphins: evidence of divergence between baleen-and toothed-species. *Journal of mammary gland biology and neoplasia*, 2, 205-230.
- OMURA, H. 1953. Biological study on humpback whales in the Antarctic whaling areas IV and V. *Scientific Report of Whales Research Institute*, 8, 81-102.
- PALLIN, L. J., BAKER, C. S., STEEL, D., KELLAR, N. M., ROBBINS, J., JOHNSTON, D. W., NOWACEK, D. P., READ, A. J. & FRIEDLAENDER, A. S. 2018. High pregnancy rates in humpback whales (*Megaptera novaeangliae*) around the Western Antarctic Peninsula, evidence of a rapidly growing population. *Royal Society Open Science*, 5.
- PALSBOLL, P. J., VADER, A., BAKKE, I. & ELGEWELY, M. R. 1992. Determination of Gender in Cetaceans by the polymerase chain-reaction. *Canadian Journal of Zoology-Revue Canadienne De Zoologie*, 70, 2166-2170.
- RASMUSSEN, K., PALACIOS, D. M., CALAMBOKIDIS, J., SABORIO, M. T., DALLA ROSA, L., SECCHI, E. R., STEIGNER, G. H., ALLEN, J. M. & STONE, G. S. 2007. Southern hemisphere humpback whales wintering off Central America: Insights from water temperature into the longest mammalian migration. *Biology Letters*, 3, 302-305.
- RIEKKOLA, L., ZERBINI, A. N., ANDREWS, O., ANDREWS-GOFF, V., BAKER, C. S., CHANDLER, D., CHILDHOUSE, S., CLAPHAM, P., DODÉMONT, R., DONNELLY, D., FRIEDLAENDER, A., GALLEG0, R., GARRIGUE, C., IVASHCHENKO, Y., JARMAN, S., LINDSAY, R., PALLIN, L., ROBBINS, J., STEEL, D., TREMLETT, J., VINDENES, S. & CONSTANTINE, R. 2018. Application of a multi-disciplinary approach to reveal population structure and Southern Ocean feeding grounds of humpback whales. *Ecological Indicators*, 89, 455-465.
- SEYBOTH, E., GROCH, K. R., DALLA ROSA, L., REID, K., FLORES, P. A. C. & SECCHI, E. R. 2016. Southern Right Whale (*Eubalaena australis*) Reproductive Success is Influenced by Krill (*Euphausia superba*) Density and Climate. *Scientific Reports*, 6.
- TYACK, P. & WHITEHEAD, H. 1983. Male competition in large groups of wintering humpback whales. *Behaviour*, 83, 132-154.
- VAN OPZEELAND, I., VAN PARIJS, S., KINDERMANN, L., BURKHARDT, E. & BOEBEL, O. 2013. Calling in the cold: Pervasive acoustic presence of humpback whales (*Megaptera novaeangliae*) in Antarctic coastal waters. *PLoS ONE*, 8, e73007.
- WAUGH, C. A., HUSTON, W., NOAD, M. & BENGTON NASH, S. M. 2011. Cytochrome P450 isoenzyme protein verified in the skin of southern hemisphere humpback whales (*Megaptera novaeangliae*); Implications for biochemical biomarker assessment. *Marine Pollution Bulletin*, 62, 758-761.
- YASUI, W. & GASKIN, D. 1986. Energy budget of a small cetacean, the harbour porpoise, *Phocoena phocoena* (L.). *Ophelia*, 25, 183-197.

Accepted Manuscript

Sex ratios of migrating southern hemisphere humpback whales: A new sentinel parameter of ecosystem health

Alison Druskat, Ruma Ghosh, Juliana Castrillon, Susan M. Bengtson Nash

PII: S0141-1136(18)30653-6

DOI: <https://doi.org/10.1016/j.marenvres.2019.104749>

Article Number: 104749

Reference: MERE 104749

To appear in: *Marine Environmental Research*

Received Date: 18 September 2018

Revised Date: 17 June 2019

Accepted Date: 18 June 2019

Please cite this article as: Druskat, A., Ghosh, R., Castrillon, J., Bengtson Nash, S.M., Sex ratios of migrating southern hemisphere humpback whales: A new sentinel parameter of ecosystem health, *Marine Environmental Research* (2019), doi: <https://doi.org/10.1016/j.marenvres.2019.104749>.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.