UNIVERSITY OF CALIFORNIA
SANTA CRUZ

USING TISSUE BIOMARKERS TO UNDERSTAND THE DEMOGRAPHY AND RECOVERY OF BALEEN WHALES IN A RAPIDLY CHANGING ENVIRONMENT

A dissertation submitted in partial satisfaction
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

ECOLOGY AND EVOLUTIONARY BIOLOGY

By

Logan J. Pallin

December 2022

The Dissertation of Logan J. Pallin is approved:

_____
Professor Daniel P. Costa, Chair

_____
Professor Ari S. Friedlaender, Chair

_____
Professor Rita S. Mehta

_____
Professor C. Scott Baker

_____
Nick M. Kellar, Ph.D.

_____
Peter Biehl
Vice Provost and Dean of Graduate Studies

Table of Contents

List of Tables

List of Figures

Abstract

Using Tissue Biomarkers to Understand the Demography and Recovery of Baleen Whales in a Rapidly Changing Environment

By

Logan J. Pallin

Baleen whale populations in the Southern Ocean are recovering after intense commercial whaling in the 20th century. Along the Western Antarctic Peninsula (WAP), this recovery is occurring in one of the planet's most rapidly changing marine ecosystems. Understanding how climate-driven changes influence the population dynamics of whales in this region is critical for understanding what conservation and management actions must be prioritized to maintain the structure and function of this marine ecosystem. This is even more important as this region has seen extraordinary increases in industrial krill fishery pressure, which overlaps in both time and space with whales foraging in this region, as well as increased human presence in the form of ecotourism. Thus, to begin understanding the dynamics of whale recovery under continued environmental change, we need to study these whales' demography and population dynamics.

My dissertation aimed to examine and describe the demographics and population dynamics of two species of Southern Hemisphere baleen whales (Antarctic minke whales and Southern Hemisphere humpback whales) in the context of a rapidly changing ecosystem. To do this, I used one of the most extensive, non-lethal tissue archives of these two species, collected as part of the National Science Foundations (NSF) Palmer Station long-term ecological research (LTER) project. I found that on average, Antarctic minke whales reproduced each year and estimated that two-thirds of females along the WAP were sexually mature. More importantly, these data represent the first non-lethal

approach to studying this species. Furthermore, I found that broad-scale environmental variation affecting krill abundance and availability along the WAP adversely impacted humpback whale pregnancy rates. This indicates that continued warming along the WAP that results in subsequent changes in the distribution and abundance of prey may adversely affect the recovery of this humpback whale population. Lastly, I found that blubber cortisol levels were not significantly different between male and female humpback whales but were significantly different across different demographic groups of females and across months. Blubber cortisol levels also significantly decreased in 2021, a year when human presence along the WAP was greatly reduced due to the COVID-19 pandemic. These findings provide a critical baseline of cortisol levels for whales in a rapidly changing region and show direct relationships between cortisol levels and human presence.

These are some of the first non-lethal quantitative observations of the demography and population dynamics of recovering whale populations in the Antarctic and provide a critical reference point for future work as the Antarctic climate continues to change and populations continue to recover from whaling.

Acknowledgments

The Research described in this dissertation was made possible by a long-term tissue archive collected under the leadership of Dr. Ari Friedlaender in collaboration with the National Science Foundation's Palmer Station Long-Term Ecological Research program (PAL LTER). Successfully conducting marine research, particularly in polar waters, requires an enormous support team, including students, technicians, and logistical support staff. I would like to thank everyone involved in providing the logistical support needed to complete this thesis, including the PIs, technicians, and grad students of the PAL LTER, the officers and crew aboard the R.V. *Laurence M. Gould* and the R.V. *Nathaniel B. Palmer*, and all the Antarctic Support Staff at Palmer Station and Ukrainian Antarctic Akademik Vernadsky Station. I would also like to extend my gratitude to my committee members and co-authors for their support in completing these chapters.

As scientists, we cannot accomplish our research objectives without the financial support of funders and donors, and I would like to thank them all here. This thesis was supported by the National Science Foundation Office of Polar Programs (grant # 1643877), the Palmer Long-term Ecological Research Program (grant #s 1440435 and 2026045), the National Science Foundation Graduate Research Fellowship Program (DGE1339067), National Geographic Society (NGS-909906R-21), the Marine Mammal Commission, UC Santa Cruz's Graduate Division and Department of Ecology and Evolutionary Biology, the Dr. Earl H. Myers & Ethel M. Myers Oceanographic & Marine Biology Trust, the World Wide Fund for Nature – Australia (WWF-Australia), the International Whaling Commission's Southern Ocean Research Partnership, the US Biological Adaptations to Environmental Change in Antarctica, Scientific Committee of Antarctic Research (SCAR),

American Cetacean Society, OneOcean Expeditions, Hurtigruten Cruises, Cheesemans Ecology Safaris, donation by M.D. Anita North, donation on behalf of Eric Lundeen, the State of the State Institution National Antarctic Scientific Center, and the Ministry of Education and Science of Ukraine's State Institution National Antarctic Scientific Center Special-Purpose Research Program in Antarctica for 2011-2023. Thank you all for your generous support to this research.

First, to my advisors: Ari Friedlaender and Dan Costa. Thank you both for always having my back and providing me the flexibility and independence to grow as a scientist. Ari—it has been a long and exciting road. Thank you sincerely for taking me on as an undergrad in 2012, becoming my master's thesis advisor at Oregon State in 2015, and still taking me on as a Ph.D. student at UCSC in 2017. Thank you for always being there and for answering every phone call, even when they came from a satellite phone at the bottom of the world. Thank you for putting up with my ridiculous Type A tendencies and for always making wherever I am feel like home. It has been a chaotic ten years, and I am immensely grateful to have had the opportunity to have you as my mentor, but most importantly, as someone I genuinely consider a great friend. I look forward to working with you again in the future. Dan—thank you for welcoming me into your lab, even when, in the beginning, I classified myself as "just a cetacean person." You opened my eyes to a world I never knew existed, and you provided me with critical opportunities to grow as a field biologist and gain rare animal handling experience. Thank you for trusting me and allowing me to become a crucial member of the elephant seal team and explore my own scientific curiosities within this system. Don't tell Ari, but between you and I, pinnipeds are pretty awesome; I am hooked!

Next, I would like to thank my global network of mentors, colleagues, and friends who contributed to the intellectual merit of this thesis. Specifically, I would like to extend a special thanks to my committee member Dr. Nick Kellar for his continued support and guidance with all of the hormone work over the last eight years. I would also like to thank my committee member, Dr. Scott Baker, and Debbie Steel at OSU's Marine Mammal Institute, for providing the logistical support to conduct the genetic analyses for this dissertation. To Debbie, Scott, and Anjanette, thank you for always providing a space to call home when during my four-week visits. I also want to thank my committee member, Dr. Rita Mehta, for her continued support throughout the dissertation. Additionally, I want to express my gratitude to the Duke University crew, specifically Drs. Andrew Read, Dave Johnston, and Doug Nowacek. You all invested early on in my future as an emerging scientist and contributed significantly to this work. Thank you to the South Pacific Whale Research Consortium members, especially Drs. Claire Garrigue and Rochelle Constantine, for your insight and support. Finally, thank you to my two very dear friends and colleagues, Dr. Natalia Botero-Acosta and Oksana Savenko for your continued support and contributions to this dissertation. I could not have completely my thesis without each and every one of you.

As a graduate student, one does not simply complete a Ph.D. without the constant support of their lab. I am immensely grateful for every lab member I have had the privilege of getting to know over the years. You are all remarkable people, and each of you helped me grow as a researcher and person. Ross Nichols—even though we never actually worked together in the Antarctic, I have learned so much from you. Thank you for always supporting me and for being a fantastic friend and colleague. Arianna Torello and Lauren

Fritz—as the newest members of the lab, thank you for always knowing how to make me smile. I cannot wait to watch you grow as scientists, and I am very excited to sail with you both in the Antarctic this year. Theresa Keates, Arina Favilla, and Jessie Kendall-Bar—it has been an incredible five years working with you all, thank you. Caitie Kroeger—though many people believe we are two sides of the same coin, I have learned a lot from you and have laughed a lot with you. Thank you for always being willing to troubleshoot my self-diagnosed medical mysteries. Luis Hückstädt—I am grateful for all the knowledge you have shared with me. Thank you for being a fantastic friend and for always being willing to grill some tri-tip, no questions asked. Lastly, there is always that one colleague and friend whose guidance and support played a significant role in shaping the course of your Ph.D. Rachel Holser—thank you for being that person for me. Thank you for always listening to my sassy rants and for teaching me so much over the years. I am a better field biologist and scientist because of you. Thank you for answering every call, even when I call at the most inconvenient of times. Thank you for letting me safely store your field writing utensils in my pants pockets and later helping me find my pants that somehow wandered off. Lastly and most of all, thank you for allowing me to bring you comic relief over the last five years. You are an incredible scientist and friend.

I cannot forget my friends who helped me through hard times, cheered me on, celebrated every accomplishment, and always listened to me ramble on about whales. Your love and support over these five years were not trivial. Rebecca Pelofsky, Mathew Howard, Goose, Nevé Baker, Dom Kone, and Alexa Kownacki—thank you for all you have given me over the years. I must express my gratitude to my dear friends Alexa and Dom. We were the infamous trio, always full of love, laughter, and wicked dance moves. We always talked

about conquering the world together. Alexa—you are missed more than you know. I will embody your love and passion always. To my friends—you are all the best. It is simple but says it all.

Lastly, I would be remiss in not mentioning my family, especially my parents, Brenda and Ken, and my two younger sisters, Kendra and Madison. My family has always been my pillars of support. My family has lovingly nurtured and supported my passion for science ever since I was a young boy romping around the forests and streams of the family land in rural Minnesota. Mom, Dad, Kendra and Madi—your belief in me over the years have kept my spirits and motivation high during this process. You never once questioned my goals and only ever supported me so I could achieve them. Mom and Dad, you have been steadfast in your love and support and I thank you for your never-ending commitment to my education. Thank you for your sacrifices over the years. I would not be who I am today without you. Additionally, I could not have undertaken this journey without the support and love of my grandparents, Maxine (Gaga) and Archie Putnam, and Char and Dave Pallin. From an early age, they inspired me to pursue my interests in science, showering me with their love and encouragement in all my endeavors. To my grandpas, Archie and Dave, you were often in my thoughts on this journey. I miss you both immensely. To my entire family—thank you for everything you have done for me. I cannot fully convey my gratitude for what you all have provided and continue to give me.

Thank you all,

Logan J. Pallin

Statement of Contribution

The text of this dissertation includes reprints of the previously published material:

Chapter 1: Pallin L., Bierlich K. C., Durban J., Fearnbach H., Savenko O., Baker C. S., Bell E., Double M. C., de la Mare W., Goldbogen J., Johnston D., Kellar N., Nichols R., Nowacek D., Read A. J., Steel D. and Friedlaender A. 2022. Demography of an ice-obligate mysticete in a region of rapid environmental change. *R. Soc. open sci.* 9, 220724. http://doi.org/10.1098/rsos.220724

This manuscript was published open access under a CC-BY license; as such, no permission is necessary, according to the *Royal Society Editing Team*. This work was original research by Logan Pallin (LP), with contributions from co-authors as minor revisions of text or analytical advice. Specifically, LP conducted all laboratory analyses. LP, AF, JD, and HF assisted in the project concept and design and data collection and analyses. DJ, DN, AR, OS, EB, MD, and JG assisted in the project concept and design and data collection. CB, NK, and DS assisted in the project concept and design as well as analyses. KB assisted in data collection and analyses. RN assisted in data collection. WM assisted in analyses. All authors participated in drafting the manuscript and gave final approval for publication.

Dedication

To @lexa

Forever my dancing partner. We always intended to conquer the world together while living in our homes in San Diego. You are forever in my heart, on land and while at sea, reminding me to ALWAYS live life to the fullest, to fight for what I believe in, and to be caring and understanding, of whatever I may encounter.

Introduction

Broad Context

Climate change is one of the primary forces inhibiting and deteriorating the growth and health of biological populations worldwide [1]. These changes are likely to have the most dramatic impacts on polar-dependent species [2]. Specifically, we might expect direct impacts on life history and behavioral traits (i.e. fecundity and migration), but these effects may also manifest through ecological processes, with demographic shifts (i.e. skewed age structure, earlier sexual maturity, and skewed sex ratio) occurring as a result of changes to bottom-up forcings [3]. Understanding how and when populations will respond to these ecosystem shifts is critical for effective management and conservation.

The potential response to climate change by cetaceans (i.e. whales and dolphins) has been considered multiple times by expert panels and working groups (i.e. International Whaling Commission(IWC)), however, no clear consensus has been established [4]. Rather, it is thought that cetacean vulnerability to climate change will likely depend on a multitude of factors, such as population size and growth, habitat range and change, and diet specificity and/or plasticity [5]. In some areas of the Southern Ocean, whales are encountering environmental conditions that are very different from those that existed before their exploitation. Specifically, along the Western Antarctic Peninsula (WAP), the recovery of baleen whales is occurring in an environment that is experiencing rapid climatic warming [6]. This region has experienced a rise in winter air temperature of nearly 5°C since the 1950s, resulting in the collapse of ice shelves, the retreat of glaciers, and the exposure of new terrestrial and marine habitats [6-8]. An overall decline in sea ice has been observed along the WAP and has resulted in an annual sea ice extent that is,

on average, 80 days shorter than it was four decades ago [9]. The biological and physical productivity of the WAP marine ecosystem is intimately tied to the amount of sea ice cover in this region [10]. This warming is proceeding rapidly, and we do not fully understand the potential ecosystem effects this change may have. Given the regime shifts that we have already documented among sympatric krill predators along the WAP (like the population shift between ice obligate Adélie and non-ice requiring Gentoo penguins), this is quite alarming [9]. Thus, to discern the possible impacts of climate change on cetaceans we need to describe and monitor their demography over time.

Broadly defined, the study of animal demography is the study of the characteristics of populations (i.e. population size, density, age structure, fecundity, mortality). Studies on large vertebrate populations have used such demographic information to make better-informed decisions and predictions on their population health, growth, and recovery from exploitation [11, 12]. Anthropogenic exploitation of wild animals occurs across all biomes and is often unregulated. This exploitation can alter selective pressures that can strongly influence the life history and demography of individuals within a population and has even been linked to the direct extinction of several populations and species [13-15]. Baleen whales in particular, were heavily exploited during the height of the commercial whaling era and as a result face numerous conservation challenges today. It is estimated that during the 20th century, more than two-million baleen whales were killed in the Southern Hemisphere [16]. The primary challenge now is to maintain and/or restore their current population levels in the face of habitat alteration (i.e. climate change), human disturbance (i.e. hunting, noise pollution, fishing), and environmental disasters

(i.e. oil spills). The generally low reproductive rates, delayed sexual maturity, and longevity in cetaceans exacerbate these conservation challenges.

Some of the earliest efforts to assess the demographics of wild animal populations relied solely on sex and age-structure data collected from animal sightings and/or hunter game checks. In using these sex and age-structured data, scientists are able to estimate vital population sex ratios [17], reproductive output [18], survival [19], population abundance [20], and rates of population change [21]; all metrics necessary to manage and conserve populations. However, assessing these demographics can be quite challenging, especially in marine species which are often logistically difficult to study. Fortunately, the development of non-lethal tissue sampling techniques [22], and the advancement of biochemical techniques to isolate and quantify hormone levels from skin–blubber biopsy samples, now provides the capacity to assess vital health and demographic rates in wild cetacean populations overtime [23-25].

Here, I use one of the largest, non-lethal tissue archives of two Antarctic baleen whales, Antarctic minke whales (*Balaenoptera bonaerensis*) and humpback whales (*Megaptera novaeangliae*), which has been collected over the last 12 years as part of the National Science Foundations (NSF) long-term ecological research (LTER) project based out of Palmer Station, an American research base located on the southern end of Anvers Island. Humpback and Antarctic minke whales are one of a few of the principal sympatric krill predators along the WAP, central to the Palmer LTER. The Palmer LTER began in 1990 on the premise of documenting nearshore biological and physical processes, of which included extensive studies on penguin demography and feeding ecology which began in the 1970's [26, 27]. Having the capacity to establish demographic baselines and link the

biological and physical factors that drive whale demographic variability and population dynamics long term will be crucial to accurately assess population trends and relations in an ecosystem undergoing rapid environmental change.

Study System

Humpback whales are a cosmopolitan species, found in most of the world's oceans. In the Southern Hemisphere, humpback whales annually migrates from their low-latitude breeding grounds to high latitude feeding grounds where they exploit high levels of seasonal productivity in the circumpolar waters around the Antarctic. Seven breeding populations are recognized by the International Whaling Commission (IWC) in the Southern Hemisphere, and these are linked to six defined feeding areas in the Antarctic, including the WAP (IWC management area I) used by Breeding Stock G [28-30]. Little is known about the exact timing of migratory movements and residency times of various demographic classes of humpback whales along the WAP, even though such knowledge could allow us to better understand their recovery from whaling. This lack of knowledge is largely a result of the rapid depletion of humpbacks at the very beginning of Antarctic whaling during the first part of the last century [16]. It is estimated that a total of 200,000 humpback whales were killed across both their foraging and breeding grounds in the Southern Hemisphere, with over 15,000 catches belonging to Breeding Stock G.

During the foraging season along the WAP, humpback whales range broadly over continental shelf waters, utilizing nearshore bays and fjords where they target high densities of krill [31]. Feeding rates are highest early in the feeding season when whale body condition is at its lowest [32]. Understanding these energetic demands takes on even greater importance for pregnant whales. Successfully getting pregnant and

maintaining pregnancy is contingent on having access to sufficient prey resources (*i.e.* Antarctic krill) which are needed to support the high energetic costs of this particular life history stage. For migratory baleen whales, like humpbacks, pregnancy comes at an additional cost (~15%) on top of the needed ~65% increase in body mass these whales must achieve to survive to the next summer feeding season. More specifically, an increase in stored energy reserves is required prior to pregnancy (i.e. feeding season prior to breeding) [33], followed by a continued accumulation of energy stores to support the development and growth of the fetus throughout the pregnancy [34]. This is further supported by evidence from commercial whaling data that showed pregnant and/or lactating humpback females taken along the coasts of Australia yielded roughly twice as much oil as resting females [35].

Breeding Stock G humpback whales breed off the eastern coast of Central and South America, between Peru and Costa Rica [36]. Breeding in humpback whales is strongly seasonal. Females are seasonally polyestrous, with estrus cycles occurring while females are on the breeding grounds from June to October [37]. Termination of these cycles is not well understood but is believed to occur upon a successful copulation or when the migration southward to the feeding grounds begins[38, 39]. These cycles are generally characterized by a single ovulation [40, 41]. The total number of ovulations over a 2-3 year breeding cycle, however, is much less well understood [39]. Females gestate for around 11.5 months, with peak calving occurring in mid-winter. Age at sexual maturity varies greatly among populations and occurs between five years to ten years of age[40]. Interbirth intervals in females are most commonly 2 years, although annual reproductive events have been observed [25, 42].

Unlike humpback whales, Antarctic minke whales (AMWs) an abundant ice-dependent species found year-round in the Antarctic [43, 44]. They have a circumpolar distribution, likely breeding between 7° and 35°S [45]. AMWs have a strong affinity for ice-covered regions or sheltered bays, especially in areas with high densities of krill, their preferred prey [31, 43, 46-50]. An estimated 1,500 AMWs (95% CI: 1,221–1,953; [48]), inhabit the continental shelf waters around the WAP. Age at attainment of sexual maturity is 7–8 years in and pregnancy rates remain at or near 90%, suggesting that this species breeds on an annual cycle [44]. Due to the logistical challenges of studying pagophilic animals, particularly a cryptic marine species like AMWs, little is known about their life history or demography [51, 52].

As both iconic animals and top predators, assessing and monitoring changes in demographic parameters of these two species of baleen whales allows us to better understand how individuals and populations are affected by continuous environmental change, as well as provide insight as to the health, structure, and function of the Antarctic marine ecosystem of which they are a part.

Dissertation Summary

In my dissertation, I examine and describe the demographics of two species of southern hemisphere baleen whales (Antarctic minke whales and Southern Hemisphere humpback whales) in the context of a rapidly changing ecosystem. In my first chapter I utilizes an archive of skin-blubber biopsy samples and UAS-derived measurements of individual AMWs around the WAP to characterize, for the first time, their maturity, sex ratio, and pregnancy rates using non-lethal methods. My second chapter leverages an eight-year time series on pregnancy rates of humpback whales to assess how inter-annual variation

in pregnancy rates responds as a function of environmental variation along the WAP. Finally, in my third chapter, I develop a baseline assessment of temporal changes in stress hormone levels among different demographic groups of humpback whales around the Antarctic Peninsula and investigate whether the reduction in the 2021 Antarctic tourism season due to the COVID-19 pandemic resulted in lower stress levels due to reductions in human activity. This work establishes further baselines on the demography and life history of two Antarctic baleen whales and outlines how the variability of resources may affect their demography and population growth. Further, these data provide the basis for more targeted and adaptive conservation and management practices for two recovering species that feed in a rapidly changing ecosystem with a growing anthropogenic presence.

Animal Ethics

Permission to carry out the research and procedures for ensuring animal welfare during biopsy collection and UAS operations was approved as part of the scientific research permits issued by the National Marine Fisheries Service (NMFS) under the authority of the Marine Mammal Protection Act of 1972 (permit numbers: 14097, 14809, 19091, and 23095). The National Science Foundation (NSF) Antarctic Conservation Act (ACA) permits (2015-001, 2015-011, 2016–024, 2017-029, and 2020-016) were obtained to conduct biopsy sampling and UAS photogrammetry of baleen whales in the Antarctic Peninsula Region. Oregon State University's Institutional Animal Care and Use Committee (IACUC) and UC Santa Cruz's IACUC approved protocols for the collection of biopsy samples (OSU permits 4513 & 4943 and UCSC permits Friea1706 and Friea2004). Additionally, research was conducted under the Ministry of Education and Science of

Ukraine Permit Series AP № 075-19/2. The samples originating from outside the US jurisdiction were imported under the Convention on International Trade in Endangered Species (CITES) import permit numbers 16US50849B/9 through 19US504849/B, and 20US60410D/9.

References

1. Root, T.L., D. Liverman, and C. Newman, *Managing biodiversity in the light of climate change: current biological effects and future impacts.* Key Topics in Conservation Biology, 2006: p. 85-104.

2. Bernstein, L., et al., *IPCC, 2007: climate change 2007: synthesis report*. 2008: IPCC.

3. Leaper, R., et al., *Global climate drives southern right whale (Eubalaena australis) population dynamics.* Biology Letters, 2006. **2**(2): p. 289-292.

4. Clapham, P.J., *MANAGING LEVIATHAN Conservation Challenges for the Great Whales in a Post-Whaling World.* Oceanography, 2016. **29**(3): p. 214-225.

5. Laidre, K.L., et al., *Quantifying the sensitivity of Arctic marine mammals to climate-induced habitat change.* Ecological Applications, 2008. **18**(sp2).

6. Smith, R.C. and S.E. Stammerjohn, *Variations of surface air temperature and sea-ice extent in the western Antarctic Peninsula region.* Annals of Glaciology, 2001. **33**(1): p. 493-500.

7. Vaughan, D.G., et al., *Recent rapid regional climate warming on the Antarctic Peninsula.* Climatic change, 2003. **60**(3): p. 243-274.

8. Meredith, M.P. and J.C. King, *Rapid climate change in the ocean west of the Antarctic Peninsula during the second half of the 20th century.* Geophysical Research Letters, 2005. **32**(19).

9. Ducklow, H.W., et al., *West Antarctic Peninsula: an ice-dependent coastal marine ecosystem in transition.* 2013.

10. Massom, R.A. and S.E. Stammerjohn, *Antarctic sea ice change and variability–physical and ecological implications.* Polar Science, 2010. **4**(2): p. 149-186.

11. Servanty, S., et al., *Influence of harvesting pressure on demographic tactics: implications for wildlife management.* Journal of Applied Ecology, 2011. **48**(4): p. 835-843.

12. Gates, C.C., et al., *American bison: status survey and conservation guidelines 2010*. 2010: IUCN.

13. Allendorf, F.W., et al., *Genetic effects of harvest on wild animal populations.* Trends in Ecology & Evolution, 2008. **23**(6): p. 327-337.

14. Burney, D.A. and T.F. Flannery, *Fifty millennia of catastrophic extinctions after human contact.* Trends in Ecology & Evolution, 2005. **20**(7): p. 395-401.

15. Proaktor, G., T. Coulson, and E. MILNER-GULLAND, *Evolutionary responses to harvesting in ungulates.* Journal of animal ecology, 2007. **76**(4): p. 669-678.

16. Rocha, R., P.J. Clapham, and Y.V. Ivashchenko, *Emptying the oceans: a summary of industrial whaling catches in the 20th century.* Mar. Fish. Rev, 2014. **76**: p. 37-48.

17. Severinghaus, C. and H. Maguire, *Use of age composition data for determining sex ratios among adult deer.* New York Fish and Game Journal, 1955. **2**(2): p. 242-246.

18. Hanson, W.R., *Calculation of productivity, survival, and abundance of selected vertebrates from sex and age ratios.* Wildlife Monographs, 1963(9): p. 3-60.

19. Hayne, D.W. and L.L. Eberhardt, *Notes on the estimation of survival rates from age distributions of deer*. 1960: Michigan Department of Conservation, Game Division.

20. DeLury, D.B., *On the estimation of biological populations.* Biometrics, 1947. **3**(4): p. 145-167.

21. Cole, L.C., *The population consequences of life history phenomena.* The Quarterly Review of Biology, 1954. **29**(2): p. 103-137.

22. Palsbøll, P.J., F. Larsen, and E.S. Hansen, *Sampling of Skin Biopsies from Free-Raging Large Cetaceans in West Greenland: Development of new Biopsy Tips and Bolt Designs.* International Whaling Commission Special Issue Series, 1991(13).

23. Mansour, A.A., et al., *Determination of pregnancy status from blubber samples in minke whales (Balaenoptera acutorostrata).* Marine Mammal Science, 2002. **18**(1): p. 112-120.

24. Kellar, N.M., et al., *Determining pregnancy from blubber in three species of delphinids.* Marine Mammal Science, 2006. **22**(1): p. 1-16.

25. Pallin, L.J., et al., *High pregnancy rates in humpback whales (Megaptera novaeangliae) around the Western Antarctic Peninsula, evidence of a rapidly growing population.* Royal Society Open Science, 2018. **5**(5).

26. Ducklow, H.W., et al., *Marine pelagic ecosystems: the west Antarctic Peninsula.* Philosophical Transactions of the Royal Society of London B: Biological Sciences, 2007. **362**(1477): p. 67-94.

27. Fraser, W.R. and W.Z. Trivelpiece, *Factors Controlling the Distribution of Seabirds: Winter-Summer Heterogeneity in the Distribution of AdéLie Penguin Populations*. 1996: Wiley Online Library.

28. Johnson, J.H. and A.A. Wolman, *The humpback whale, Megaptera novaeangliae.* Marine Fisheries Review, 1984. **46**(4): p. 30-37.

29. Rice, D.W., *Marine mammals of the world: systematics and distribution*. 1998: Society for Marine Mammalogy.

30. Gales, N., et al., *Humpback Whales: Status in the Southern Hemisphere.* Journal of Cetacean Research and Management, 2011(Special Issue 3).

31. Friedlaender, A., et al., *Sympatry and resource partitioning between the largest krill consumers around the Antarctic Peninsula.* Marine Ecology Progress Series, 2021. **669**: p. 1-16.

32. Nichols, R.C., et al., *Intra-seasonal variation in feeding rates and diel foraging behaviour in a seasonally fasting mammal, the humpback whale.* Royal Society Open Science, 2022. **9**(7).

33. Miller, C.A., et al., *Body shape changes associated with reproductive status, nutritive condition and growth in right whales Eubalaena glacialis and E. australis.* Marine Ecology Progress Series, 2012. **459**: p. 135-156.

34. Lockyer, C., *Growth and energy budgets of large baleen whales from the Southern Hemisphere.* Food and Agriculture Organization, 1981. **3**: p. 379-487.

35. Dawbin, W.H., *The seasonal migratory cycle of humpback whales.* Whales, dolphins and porpoises. University of California Press, Berkeley, 1966: p. 145-170.

36. Johnston, S.J., A.N. Zerbini, and D.S. Butterworth, *A Bayesian approach to assess the status of Southern Hemipshere humpback whales (Megaptera novaeangliae) with an application to Breeding Stock G.* J. Cetacean Res. Manage., 2020: p. 309-317.

37. Matthews, L.H., *The humpback whale, Megaptera nodosa*. 1937: publisher not identified.

38. Chittleborough, R., *Studies on the Ovaries of the Humback Whale, Megaptera nodosa (Bonnaterre), on the Western Australian Coast.* Marine and Freshwater Research, 1954. **5**(1): p. 35-63.

39. Chittleborough, R., *The breeding cycle of the female humpback whale, Megaptera nodosa (Bonnaterre).* Marine and Freshwater Research, 1958. **9**(1): p. 1-18.

40. Chittleborough, R., *Puberty, physical maturity, and relative growth of the female humpback whale, Megaptera nodosa (Bonnaterre), on the Western Australian coast.* Marine and Freshwater Research, 1955. **6**(3): p. 315-327.

41. Chittleborough, R., *Dynamics of two populations of the humpback whale, Megaptera novaeangliae (Borowski).* Marine and Freshwater Research, 1965. **16**(1): p. 33-128.

42. Glockner-Ferrari, D.A. and M.J. Ferrari, *Reproduction in the humpback whale (Megaptera novaeangliae) in Hawaiian waters, 1975–1988: the life history, reproductive rates and behavior of known individuals identified through surface and underwater photography.* Reports of the International Whaling Commission, 1990. **12**: p. 161-169.

43. Risch, D., et al., *Common and Antarctic Minke Whales: Conservation Status and Future Research Directions.* Frontiers in Marine Science, 2019. **6**.

44. Perrin, W.F., S.D. Mallette, and R.L. Brownell Jr, *Minke whales: Balaenoptera acutorostrata and B. bonaerensis*, in *Encyclopedia of marine mammals*. 2018, Elsevier. p. 608-613.

45. Esposito, C., O. Bichet, and M. Petit, *First Sightings of Antarctic Minke Whale (Balaenoptera bonaerensis) Mother-Calf Pairs in French Polynesia.* Aquatic Mammals, 2021. **47**(2): p. 175-180.

46. Friedlaender, A.S., et al., *Whale distribution in relation to prey abundance and oceanographic processes in shelf waters of the Western Antarctic Peninsula.* 2006.

47. Thiele, D., et al., *Seasonal variability in whale encounters in the Western Antarctic Peninsula.* Deep sea research part II: topical studies in oceanography, 2004. **51**(17-19): p. 2311-2325.

48. Murase, H., et al., *Spatial distribution of Antarctic minke whales (Balaenoptera bonaerensis) in relation to spatial distributions of krill in the Ross Sea, Antarctica.* Fisheries Oceanography, 2013. **22**(3): p. 154-173.

49. Lee, J.F., et al., *Behavior of satellite-tracked Antarctic minke whales (Balaenoptera bonaerensis) in relation to environmental factors around the western Antarctic Peninsula.* Animal Biotelemetry, 2017. **5**(1): p. 23.

50. Dominello, T. and A. Širović, *Seasonality of Antarctic minke whale (Balaenoptera bonaerensis) calls off the western Antarctic Peninsula.* Marine Mammal Science, 2016. **32**(3): p. 826-838.

51. Inoue, S., G. Yasunaga, and L.A. Pastene, *Comparison of progesterone concentrations in blubber and plasma among female Antarctic minke whales of known reproductive status.* Fisheries Science, 2019.

52. Mogoe, T., et al., *Results of biological sampling of Antarctic minke whale during the first field survey of NEWREP-A in Area V during the 2015/16 austral summer season.* Paper SC/66b/SP presented to the IWC Scientific Committee. 20pp, 2016.

Chapter 1: Demography of an Ice-Obligate Mysticete in a Region of Rapid Environmental Change

Logan Pallin, KC Bierlich, John Durban, Holly Fearnbach, Oksana Savenko, C. Scott Baker, Elanor Bell, Mike Double, William de la Mare, Jeremy Goldbogen, David Johnston, Nick Kellar, Ross Nichols, Doug Nowacek, Andrew Read, Debbie Steel, Ari Friedlaender

1.1 Abstract

Antarctic minke whales (Balaenoptera bonaerensis, AMW) are an abundant, ice-dependent species susceptible to rapid climatic changes occurring in parts of the Antarctic. Here, we used remote biopsy samples and estimates of length derived from unoccupied aircraft system (UAS) to characterize for the first time the sex ratio, maturity, and pregnancy rates of AMWs around the Western Antarctic Peninsula (WAP). DNA profiling of 82 biopsy samples (2013–2020) identified 29 individual males and 40 individual females. Blubber progesterone levels indicated 59% of all sampled females were pregnant, irrespective of maturity. When corrected for sexual maturity, the median pregnancy rate was 92.3%, indicating that most mature females become pregnant each year. We measured 68 individuals by UAS (mean = 8.04 m) and estimated that 66.5% of females were mature. This study provides the first data on the demography of AMWs along the WAP and represents the first use of non-lethal approaches to studying this species. Furthermore, these results provide baselines against which future changes in population status can be assessed in this rapidly changing marine ecosystem.

1.2 Introduction

The status of biological populations can be inferred by monitoring changes in parameters such as abundance, fecundity, mortality, and age structure [1]. In the absence of direct

estimates of abundance, demographic metrics may serve as indicators of population growth, viability, and, response to environmental changes [2-5]. In long-lived species, changes in demography are more likely to be detected over shorter timescales compared to changes in abundance, particularly for large populations such as baleen whales, where estimating abundance is difficult and often imprecise due to their marine distribution and cryptic behavior.

Among long-lived large vertebrates, the effects of climate change have been well-studied in ungulates primarily focused on how climate variability impacts births, survival, and age structure [2, 6, 7]. For example, in 21 populations of woodland caribou, colder temperatures and increasing snowfall increase juvenile recruitment and population growth [8]. However, as climate anomalies (i.e. warmer temperatures, freeze-thaw events) become more common and larger in magnitude, an overall decrease in habitat availability and forage quality, an increase in adult energy expenditure, a decrease in pregnancy rates, and an increase in predation risk have been observed [8-10]. Similar climate-driven shifts in demography and dynamics have been shown in elk [7], pronghorn antelope [11], moose, owls, and wolves [12].

Understanding the impacts of climate-driven changes on polar species is particularly important given the rapid changes occurring at the poles in both marine and terrestrial ecosystems [13, 14]. Antarctic minke whales (*Balaenoptera bonaerensis,* AMW) are an abundant ice-dependent species found year-round in the Antarctic [15, 16]. They have a circumpolar distribution, likely breeding between 7° and 35°S [17]. AMWs have a strong affinity for ice-covered regions or sheltered bays, especially in areas with high densities of krill, their preferred prey [15, 18-23]. AMWs are well-adapted to feed on krill under

sea ice [24] and use sea-ice habitat to avoid predation by killer whales [25, 26]. Due to the logistical challenges of studying pagophilic animals, particularly a cryptic marine species like AMWs, little is known about their life history or demography [27, 28].

The extent of annual sea ice appears to be constant or expanding around most of the Antarctic, but the Western Antarctic Peninsula (WAP) is experiencing some of the most pronounced loss of sea ice in polar regions [29]. An estimated 1,500 AMWs (95% CI: 1,221–1,953; [20]), inhabit the continental shelf waters around the WAP, which has experienced significant warming [30] and substantial reductions in the extent and duration of sea ice cover over the last 50 years [31]. These changes have resulted in a cascade of effects throughout the WAP ecosystem, and are likely impacting the demography, behavior, and ecology of AMWs [32]. Recent advances in both molecular ecology and unoccupied aircraft systems (UAS) technology allow us to study the demography of these whales using non-lethal techniques. In this study, we analyzed skin and blubber biopsy samples and UAS-derived measurements of individual AMWs around the WAP to characterize, for the first time, their maturity, sex ratio, and pregnancy rates. Our findings help fill key data gaps on the demographic structure and population trajectory of AMWs in this rapidly changing region.

1.3 Methods

1.3.1 Biopsy collection

We collected skin and blubber biopsy samples from AMWs during the 2013-2020 austral summer and fall (January-July) field seasons using standard techniques [33]. Samples were collected opportunistically during dedicated research cruises or from platforms of

opportunity, including ecotour vessels, in the nearshore waters of the WAP (Fig. 1.1). We used a crossbow to project modified bolts and 40mm stainless steel biopsy tips (CetaDart) to obtain samples from a distance of 10-30 meters, targeting the area of the body below the dorsal fin. All age and sex classes of AMWs were sampled, except dependent calves. Samples were stored frozen whole at -20° C until used for analysis. Supplementary data, (including location, group size) were recorded at every biopsy event.

1.3.2 DNA Profiling

We used standard molecular methods to identify the sex of individuals from DNA extracted from biopsies [34, 35]. We used a standard DNA profile, including sex-specific markers, and microsatellite genotypes, to identify individual whales. Genomic DNA was extracted from the skin-blubber interface using a commercially available kit (Dneasy 96 Blood & Tissue Kit, Qiagen, Hilden, Germany). The sex of each sampled whale was determined by amplification of sex-specific markers following the protocols of Gilson et al. [36]. Results were compared to controls for a known male and female using gel electrophoresis. Sex ratios, depicted as the ratio of males to females (M:F), were calculated for the entire dataset and for specific sampling years.

Samples were genotyped using 10 previously published microsatellite loci to resolve the identity of each sampled whale and remove potential replicate s16amples (Table 1.1) [37-40]. Alleles were sized and binned using the software program Genemapper v3.7 (Applied Biosystems). The total number of amplified loci for a given sample was considered as an added quality control threshold. Given the estimated probability of identity for these loci from previous studies [41], we considered samples matching at a

minimum of seven loci to be recaptures of the same individual. Samples with fewer than seven microsatellite loci were repeated or excluded. The expected probability of identity (P_{ID}; the probability that two individuals are drawn at random from a population will have the same genotype by chance) for each locus was calculated in GenAlEx v6.5 [42]. Cervus 3.0.7 [43] was used to compute the number of alleles (K), observed and expected heterozygosity (H_O and H_E), and the probability of identity for all individual matches.

1.3.3 Hormone extraction and quantification

To develop an assay for pregnancy, we extracted progesterone from the blubber portion of the biopsy samples following standard methods [44, 45]. A cross-sectional sub-sample (~0.15g) spanning from the epidermis-blubber interface to the most internal layer of the biopsy was sub-sectioned. These sub-samples were then homogenized multiple times using an automated, multi-tube homogenizer (Omni International). Following the completion of the homogenization process, progesterone was isolated using a series of chemical washes, evaporations, and separations. The final hormone residue was stored at -20° C until analysis. The amount of hormone in each extract was quantified using a commercially available enzyme immunoassay. Before analysis, samples were re-suspended in phosphate-buffered saline and then assayed. The progesterone EIA kit (EIA kit 900-011, ENZO Life Sciences, Farmingdale, NY) used in this study has 100% reactivity with progesterone and an assay detection limit between 15 and 500 pg/ml. Two additional standard dilutions were added to allow for a lower detection limit of the standard curve to 3.81pg/mL. If reliable hormone concentrations were not obtained during the initial assay process, extracts were further diluted and re-run.

As part of our routine quality control, we determined the extraction efficiency by spiking subsamples of blubber from a dead animal with the target hormone [44]. The percentage of hormone recovered after the extraction was calculated, and each sample concentration was adjusted to this efficiency before statistical analyses. An extraction efficiency greater than 60% was acceptable. If the extraction efficiency was less than 60%, the sample extracts were discarded, and the blubber samples re-extracted. Additionally, we conducted a parallelism test to gauge the performance of using the AMW blubber extracts with the progesterone EIA kit. This was done by taking a serially diluted pool of sample extracts and running them along with the standard controls of the assay to determine whether the linear decrease in measured values of the pooled sample was parallel to the standard curve. This would indicate that the assay measures the same antigens in the blubber as in the standards. Extracts from 6 individual females were pooled together, and the pooled sample concentrations were made by diluting five times from the neat preparation to 1/32, decreasing by a factor of two. Each dilution was run two times, and the resulting curve of the concentrations as a function of the mean optical density was then compared to the standard curve.

1.3.4 Pregnancy classification

To assign pregnancy in sampled AMWs, we adapted two methods used by Pallin et al. [46] for humpback whales (*Megaptera novaeangliae*). Similar distributions in the progesterone concentrations were observed for both humpback whales and AMWs and as such, we first assigned the pregnancy status of female AMWs based on the relationship of their progesterone concentration with a reference model developed from known pregnant humpback whales [46]. We then used the range in concentrations of

progesterone from female common minke whales (*Balaenoptera acutorostrata*) of known pregnancy status as described in Mansour et al. [47] to build a second model from our sampled AMWs which fell within those bounds. Specifically, a gap in progesterone concentrations was observed between a maximum of 3.43 ng g^{-1} in not-pregnant females and a minimum of 22.84 ng g^{-1}in pregnant animals, with an almost 60-fold difference observed between the mean blubber progesterone concentrations among these two pregnancy state designations. For the AMW samples which fell between the ranges for not-pregnant and pregnant common minkes (N = 5), we interpreted their pregnancy state based on the relationship of their progesterone concentrations with the reference levels from the second model. In both cases, the models determined the probability of pregnancy (point estimate) and 95% confidence envelope. Using both the point estimate and associated error, we were then able to assess confidence of the pregnancy assay (e.g., >99.9% is pregnant, <0.1% not-pregnant, 0.1% < p < 99.9% undetermined) [46]. Moreover, we were also able to provide an estimate of the proportion of pregnant females (pregnancy rate) in all samples, including those with an assignment probability between 0.1% and 99.9%. This was accomplished by taking the sum of the probabilities for all samples at each individual model bootstrap replicate and dividing by the sample size to obtain the proportion pregnant. The resulting pregnancy rates from each model were compared to each other (Fig. 1.2).

1.3.5 UAS image collection and photogrammetry

To determine the distribution of size classes of AMWs in the population, high-resolution aerial images were collected using unoccupied aircraft systems (UAS, or drones) and analyzed to estimate the total length of AMWs. These images were collected during the

2017-2019 austral summer (January-March) field seasons using three different hexacopters: APH-22 (Aerial Imaging Solutions), Alta 6 (FreeFly), and LemHex-44 (Mikrokopter). The APH-22 was fitted with an Olympus E-PM2 camera with a Micro Four Thirds (17.3 x 13 mm) sensor, 4608 x 3456-pixel resolution, and an Olympus M. Zuiko 25 mm f1.8 focal length lens. Both the LemHex-44 and Alta 6 were equipped with a Sony Alpha a5100 camera with an APS-C (23.5 x 15.6 mm) sensor, 6000 x 4000-pixel resolution, and either 35 or 50 mm f1.8 Sony SEL focal length lens. Each aircraft had an onboard barometer, while the LemHex-44 and Alta 6 were also equipped with a LightWare SF11/C laser altimeter to determine the altitude of each image. Details for flight operations and image collection are described in Kahane-Rapport et al. [48] for the Alta 6 and LemHex-44 and in Durban et al. [49] for the APH-22. Individuals were identified from external marking and pigmentation patterns that were visible in the aerial and/or boat-based photo-identification images.

Images were selected for each individual and ranked for quality in measurability following Christiansen et al. [50], in which a score of 1 (good quality), 2 (medium quality), or 3 (poor quality) was applied to seven attributes: camera focus, straightness of body, body roll, body arch, body pitch, total length measurability, and body width measurability. Images with a score of 3 in any attribute were removed from the analysis, together with any images that received a score of 2 in both roll and arch, roll and pitch, or arch and pitch [50]. Measurements from up to five images were used per individual. We used MorphoMetriX open-source photogrammetry software to measure (in pixels) total length, from the tip of the rostrum to the fluke notch (Fig. 1.4) [51]. MorphoMetriX outputs were collated using CollatriX open-source software [52].

To account for measurement uncertainty associated with each UAS, we used the Bayesian statistical model described in Bierlich et al. [53], in which training data of known-sized objects measured at various altitudes are used to predict length measurements and associated uncertainty of objects of unknown size (e.g. each individual whale). For the Alta 6 and LemHex-44, we employed the dataset used by Bierlich et al. [53] for training the model with measurements from images of known-sized floating objects (n = 110) collected between 10 – 120 m altitude along the WAP (length = 1.33 or 1.40 m), Monterey, California (length = 1.27), and Beaufort, North Carolina (length = 1.48). For the APH-22, we used images of the rail on the RHIB (length = 2.95 m) collected between altitudes of 22-47 m as training data. The training data encompassed the range in altitude that images of AMWs were collected for each aircraft (Alta 6 and LemHex-44 (m): min = 15, max = 83 mean = 42.30, sd = 16.92; APH-22 (m); min = 30, max = 42, mean = 36.67, sd = 2.85). Rather than a single-point estimate, the model generated a posterior predictive distribution for the total length (m) of each individual (Fig. 1.3). We then estimated the total length of each individual as the mean of its posterior predictive distribution and assessed measurement uncertainty by constructing the 95% highest posterior density (HPD) intervals, which is an interval that represents the region with a 95% probability of encompassing the parameter of interest (e.g., total length; Fig. 1.4). Model development and analyses were conducted in R (Version 3.6.1, R Core Team 2019), as described in Bierlich et al. [53].

1.3.6 Estimating proportion of mature females using an undifferentiated sample of animals of known length

If length, sex, and pregnancy data were available from the same individual AMW, it would be possible to estimate the length at maturity directly and use this to calculate the total number of mature animals in the sample. However, this was not possible for AMWs imaged in this study and as such, the pregnancy rate presented here was based solely on hormonal estimates and these data do not distinguish between sexually immature and mature not-pregnant females. Unfortunately, sex and maturity status were not available for our sample of whales whose lengths have been estimated via UAS imaging. However, the lengths of whales can be used to estimate the expected proportion of mature females in our sample if the probabilities that an animal of a given length is both female and mature can be calculated from other sources. This is possible for the Antarctic Peninsula region using data from historical commercial whaling catches (1972-1987) in the region bounded by the longitudes 55°W to 70°W (data provided by IWC archives). It is not necessary that the distributions of lengths in our samples and from the commercial data are comparable, only that the proportions of animals that are female and mature are similar at a given length. The methods for estimating the sex ratios and maturity status at given lengths are described in supplemental text S1.1. These calculations include Bayesian estimates of the sex ratio and maturity at-length distributions, and consequently the distributions of the sex ratio and pregnancy rates based on our length and pregnancy data can be estimated.

1.3.7 Data preparation and statistical analyses

We used a two-tailed Exact Binomial Test [54] to test for deviations from a 1:1 sex ratio (parity) across the entire dataset and within a given year. Additionally, to avoid re-sample bias in our analyses, we removed all within-year replicates. In both cases, the most recent

sample was retained for the analyses. For all statistical tests, we considered a *p*-value of less than 0.05 to be significant. All values are expressed as mean ± s.d. unless otherwise stated.

1.4 Results

1.4.1 Individual identification and sex

We collected 82 skin and blubber biopsy samples along the WAP from 2013-14 and 2016-20 (Fig. 1.1). Samples were collected from January through July, with most (64%) collected in February. An average of 9.4 microsatellite loci were successfully genotyped per individual. Fifteen samples failed the initial genotype quality control and were re-analyzed. Three samples (2013, 1 male, 1 female; 2018, 1 male) never yielded a high-quality genotype and were not included in further analyses. The average P_{ID} for any given combination of seven loci ranged from 5.74×10^{-14} to 1.57×10^{-11}, consistent with similar studies [45]. DNA profiling was sufficient to identify and determine the sex of 69 individual whales from these samples (Table 1.2). In total, we sampled 29 individual males and 40 individual females throughout the study. Details on annual sampling can be found in Table 1.2. We resampled nine individuals within the same year. Five individuals were resampled on the same day (2014, 2 females; 2020 2 males, 1 female), including one female that was sampled 3 times (2014, 1 female). Additionally, one individual was resampled one day apart (2017, 1 male), one individual was sampled three days apart (2013, 1 female), one individual was sampled six days apart (2019, 1 female), and one individual was sampled 24 days apart (2020, 1 male). We did not recapture any individuals across years. Overall, we sampled more females than males (0.73 M:F), but

this deviation from parity was not significant (p = 0.228, Exact Binomial Test, Table 1.2). In addition, the sex ratios did not differ from unity in any sampling year (Table 1.2).

1.4.2 Variation in progesterone concentrations

Based on the concentrations observed from a series of spiked controls, our average extraction efficiency for the progesterone assay was 82.77% ± 14.46 (minimum 65.78%, maximum 100.81%). Additionally, our calculated intra-assay and inter-assay coefficient of variation from a series of replicated samples was 3.10% and 7.21%, respectively. The EIA standards and the pooled serially diluted blubber extracts exhibited statistical parallelism (Fig. 1.3, r^2 = 0.982, slope = 0.997); an indication that the assay is measuring the same antigens in the blubber as in the standards and therefore is suitable for use with AMW blubber tissues extracts.

We measured progesterone concentrations in 39 samples obtained from 34 individual female AMWs (Fig. 1.1). A small number of samples were excluded from the analysis due to within year re-sampling, insufficient blubber for extraction, or a poor-quality genotype. In both probability assignment methods, 13 individual females were estimated to have a probability of being pregnant of < 0.1% (assigned as not-pregnant; p <0.1%; blubber progesterone: mean = 1.98 ± 1.58 ng g^{-1}) and 20 were estimated to have a higher than 99.9% probability of being pregnant (assigned as pregnant, p > 99.9%; blubber progesterone: mean = 144.86 ± 96.53 ng g^{-1}; Table 1.3; Fig. 1.2). Additionally, one individual whose progesterone concentrations fell within the 95% confidence envelope in both models (blubber progesterone: 10.20 ng g^{-1}), received a mean probability of being pregnant of 0.53%, with a lower CI of 0.00% and an upper CI of 99.30%; Table 1.3; Fig. 1.2). This individual received an undetermined pregnancy assignment. The mean

estimated proportion of pregnant females, across both models, across all 34 samples was 58.84% (CI = 58.82—61.74%). The within-year replicate samples provided further validation of the assay by demonstrating that re-sampled females continued to fall within the same pregnancy designation. Specifically, two females were consistently classified as not-pregnant (gBbo19AP006: mean = 3.43 ± 0.68 ng g^{-1}, six days between resampling; gBbo20AP08: mean = 2.48 ± 2.16 ng g^{-1}, sampled same day) and two females as pregnant (gBbo14AP001: mean = 391.66 ± 123.36 ng g^{-1}, sampled same day; gBbo14AP005: mean = 110.60 ± 99.39 ng g^{-1}, sampled same day). Lastly, the distribution in progesterone concentrations across our two designated pregnancy states for female AMWs sampled along the WAP was distributed similarly to common minke whales as outlined in Mansour et al. [47], as well as to samples collected from female humpback whales also sampled along the WAP [45].

1.4.3 Group compositions

The group composition of AMWs sampled throughout this study varied from single animals, pairs, to large aggregations of up to 25 individuals. Of the whales sampled with reliable sighting data in this study, 24 were encountered as singletons (16 M and 8 F), seven were found in pairs, and 36 were found in groups of three or more. Additionally, we fully sampled all individuals present in four groups, including two pairs (FF, MF), one group of three (MFF), and one group of four (MMMM). No calves were observed during this study.

1.4.4 Length frequencies

A total of 68 AMWs were photographed by UAS along the WAP between January and March during the 2017-2019 field seasons (Figs. 1.1, 1.4, 1.5; mean = 8.04 m, SD = 1.06, min = 4.65, max = 9.74). Measurement uncertainty, measured as the width (m) of the 95% HPD interval for each individual (see Fig. 1.4), was similar across each UAS aircraft: mean = 0.45, sd = 0.28, min = 0.15, max = 1.55. No individual was measured more than once during the study period, nor did we observe any behavioral responses of the AMWs toward the UAS.

1.4.5 Adjusted demographic parameters using commercial catch data

Applying the proportions of sex at length from the catch data to our length data provides a median value for the sex ratio of 1.04 M:F (49% female, 95% credible interval 44% - 54%). Figure 1.6A shows the maximum likelihood estimate from the commercial catches of the probabilities at each length that an animal is a mature female. The median estimate of the female length at 50% sexual maturity from the catch data was 8.20 m (95% credible interval 8.10 – 8.28; supplemental text S1.1). Applying the product of this curve with the maximum likelihood estimates of the commercial catch length at maturity to our length data gives the proportion of females that are mature as 66.5%. Thus, the maximum likelihood estimate for the number of mature whales in our sample of 33 females is 22. Twenty of the known sampled females were pregnant and hence the estimated pregnancy rate of adult females is 90.09% under the assumption that the length frequency distribution of the UAS measured animals is the same as the unknown length frequency distribution of the biopsied animals. Uncertainty in the estimates of the proportion of mature females was calculated using a Monte Carlo Markov Chain (MCMC) analysis (see appendix I) for the two functions. This produced the distribution for pregnancy rate

shown in Fig. 1.6B. This distribution had a median pregnancy rate of 92.3% (95% credible interval 83.8% – 102.8 8%). The distribution has a tail above 100%, reflecting the uncertainty in the estimated proportion of mature females as shown in the distribution given in Fig. 1.6C.

1.5 Discussion

Our results provide the first estimates of sex ratio, maturity status and pregnancy rates for Antarctic minke whales (AMWs) inhabiting the waters surrounding the Western Antarctic Peninsula (WAP), a region undergoing rapid environmental change. Our comparisons may have more uncertainty than we can account for as we adjusted our data using data collected during commercial whaling operations that occurred 40 years earlier. This study also represents the first demographic study of this species using non-lethal techniques.

1.5.1 Variation in sex ratios

The sex ratio of the sampled population was biased towards females (0.73 M:F), but this was not statistically different from parity. In addition, the Bayesian estimated sex ratio for this region was 1.04 M:F. Similar sex ratios (mean: 1.18 M:F, range: 0.48-3.26) were observed among 4,383 individual AMWs lethally sampled between 1990-2006 in East Antarctica [55]. Sex ratio biases occur in other parts of the Antarctic that seem to be related to latitude: in a study of minke whales killed under a Special Permit whaling program, females represented 80% of the catch near the ice edge, but males dominated in waters north of 65° S, further from the ice edge [56]. Similarly, skewed sex ratios have been observed as a result of demographic segregation in common minke whales off

Greenland [57]. Population segregation is found in a wide variety of species [58]. In cetaceans, spatial sex segregation is broadly observed [59, 60], with adaptive advantages to social structure, environmental constraints, niche selection, and timed with life-history events. Our samples come from a small region of the WAP relative to the range of the species in this region [61]. AMWs satellite-tagged in our study area maintained a coastal distribution along a large latitudinal gradient that affords them coastal shelter and proximity to available sea ice [21, 23]. If there is spatial segregation related to distance from shore or ice shelf (i.e., sea ice vs. open water), then our sample population could be skewed towards females, which show a strong affinity towards structure in other areas of the Antarctic.

1.5.2 Variation in pregnancy rates

We calculated an unadjusted pregnancy rate of 58.84% for all females sampled during this study and a corrected pregnancy rate (including only sexually mature females) of 92.3%, which is similar to East Antarctica (mean 90%) [62]. It is likely that the high abundance of krill along the WAP [63, 64] in combination with AMWs unique ecological niche [24] supports the high observed reproductive rates within this population. Similar high rates of pregnancy among other baleen whales in this region have also been attributed to the high productivity of the WAP [45] and lack of other baleen whales that have yet to recover from commercial whaling (i.e. blue and fin whales). Unfortunately, it is not possible to reconstruct the demographic trajectory of these whales over the last half-century because there is no good historical baseline for this population. However, continued demographic monitoring will allow us to understand how this population is

responding to climatic change which will likely lead to a decline in the amount of physical habitat and prey available to them over time [23, 64].

1.5.3 Variation in length frequencies and sexual maturity

Our overall mean length of AMWs was 8.04 m and we estimated that 66.5% of females were sexually mature. Both our mean calculated length and the proportion of presumed sexually mature female AMWs from this study were lower than the reported mean length (8.59 m) [65] and reported proportion of sexually mature female AMWs (76%) [27] killed under the Special Permit whaling programs. This is not surprising given that these whaling programs may have been biased towards catching larger individuals near the ice edge [66]. The East Australian humpback whale, a closely related long-lived species, has a high proportion of young and sexually immature individuals, indicative of a rapidly growing population [67]. Continued monitoring of AMWs along the WAP is needed to better understand their age structure in the context of a rapidly changing environment.

1.5.4 Potential effects of spatial and temporal heterogeneity

In the present study, we sampled whales opportunistically, and avoided re-sampling individuals whenever possible. The distribution of AMWs is segregated by sex, age, and reproductive status. Specifically, in the East Antarctic, immature whales are normally solitary and occur in lower latitudes further offshore. Mature males are more abundant in middle latitudes, and mature females occur in greater frequency in higher latitudes near the marginal pack ice zone [56]. For the subset of whales that migrate for reproductive purposes, males tend to arrive in the Antarctic in November, with females on average arriving one month later as a result of weaning their calves in lower latitudes

[68] and then remain in the region. By February, mature females dominated 85% of the catch south of 65 degrees [65]. Mature females' high affinity for the ice zones, may make them susceptible to changes in their environment and as a result they are likely to be the best indicators of changing population dynamics. Although data on the spatial and temporal segregation of AMWs comes from East Antarctica, it is possible that similar spatial and temporal dynamics exist for this species along the WAP, and that our results reflect a similar distribution pattern. Almost two-thirds of our tissue samples and 50% of our UAS images were collected in February and spatially, our sampling was focused within a subset of the known range of AMWs in this region. To better understand these potential biases, a more systematic and comprehensive spatio-temporal sampling effort is required. For example, we suggest future work to pair remote biopsy sampling and UAS imaging of individual whales across the entire continental shelf during a more protracted summer season. Finally, this study has successfully demonstrated the ability to assess the length, sex, maturity and pregnancy status of AMWs sampled non-lethally and these methodologies can now be employed in more comprehensive, long-term studies.

1.5.5 Climate change effects on population dynamics of AMWs

The rates of population decline in birds and mammals globally are greater in locations where the temperature has increased at higher rates [69]. The marine environment of the WAP is experiencing some of the most significant warming on Earth, resulting in a rapidly diminishing extent and duration of sea ice. Taken together, these climatic changes represent some of the most dramatic changes in the physical environment on the planet [29]. The distribution and ecology of AMWs are directly tied to sea ice and prey availability and changes that impact both the quantity and quality of their habitat and

food availability may result in significant effects on fitness. We are already witnessing temporal contraction of critical habitat for AMWs in this region [29], as shown by satellite tracking data [61]. The WAP population of humpback whales, which is growing at rates at or near maximum values [45], and AMWs partition prey by feeding in different habitats (sea ice versus open water, and vertically in the water column) [70], but with continued declines in sea ice, the ability to successfully partition foraging habitat may also decline, and competition for prey could increase between the two species. If AMWs are forced to broaden their distribution to sub-optimal areas, they would be at higher predation risk from Type A killer whales in open water [26]. Similar population-level responses among other ice-dependent krill consumers along the WAP have been documented in response to environmental change. Over the last 50 years, Adélie penguin populations have decreased dramatically and ice-intolerant chinstrap and gentoo penguin populations have increased substantially [71]. In light of the better-documented population responses of penguins to changes in physical substrates, such as sea ice, it is not implausible that WAP marine mammal populations (e.g., AMWs and killer whales [72, 73]) that are similarly associated with these variables have responded or will respond in the same way.

1.6 Conclusions

Our study provides the first data on the demographics and population structure of AMWs along the WAP and the first non-lethal study of the demography of this species. Our results provide key information for the population status of AMWs in a rapidly changing system. As the extent of seasonal sea ice and krill continues to decline around the WAP, AMWs may become displaced by lack of preferred habitat and/or increasingly susceptible

to competition and predation, as has been observed in other baleen whales [74] and ice-obligate marine predators [32].

References

1. Lebreton, J.-D., et al., *Modeling survival and testing biological hypotheses using marked animals: a unified approach with case studies.* Ecological monographs, 1992. **62**(1): p. 67-118.

2. Gaillard, J.-M., et al., *Temporal variation in fitness components and population dynamics of large herbivores.* Annual Review of ecology and Systematics, 2000. **31**(1): p. 367-393.

3. Moss, C.J., *The demography of an African elephant (Loxodonta africana) population in Amboseli, Kenya.* Journal of Zoology, 2001. **255**(02): p. 145-156.

4. Trimble, M., S. Ferreira, and R. Van Aarde, *Drivers of megaherbivore demographic fluctuations: inference from elephants.* Journal of Zoology, 2009. **279**(1): p. 18-26.

5. Pacifici, M., et al., *Species' traits influenced their response to recent climate change.* Nature Climate Change, 2017. **7**(3): p. 205-208.

6. DeCesare, N.J., et al., *Estimating ungulate recruitment and growth rates using age ratios.* The Journal of Wildlife Management, 2012. **76**(1): p. 144-153.

7. Cook, J.G., et al., *Effects of summer-autumn nutrition and parturition date on reproduction and survival of elk.* Wildlife monographs, 2004. **155**(1): p. 1-61.

8. DeMars, C., Robert Serrouya, Sophie Gilbert, Allicia Kelly, Nic Larter, Stan Boutin, *The Influence of Climate on the Demography of Woodland Caribou* 2017: BC Oil and Gas Research and Innovation Society.

9. Adams, L.G. and B.W. Dale, *Reproductive performance of female Alaskan caribou.* The Journal of wildlife management, 1998: p. 1184-1195.

10. Parker, K.L., P.S. Barboza, and M.P. Gillingham, *Nutrition integrates environmental responses of ungulates.* Functional ecology, 2009. **23**(1): p. 57-69.

11. Gedir, J.V., et al., *Effects of climate change on long-term population growth of pronghorn in an arid environment.* Ecosphere, 2015. **6**(10): p. 1-20.

12. Hoy, S.R., et al., *Fluctuations in age structure and their variable influence on population growth.* Functional Ecology, 2020. **34**(1): p. 203-216.

13. Chen, D., *Impact of climate change on sensitive marine and extreme terrestrial ecosystems: Recent progresses and future challenges.* Ambio, 2021. **50**(6): p. 1141-1144.

14. Rogers, A., et al., *Antarctic futures: an assessment of climate-driven changes in ecosystem structure, function, and service provisioning in the Southern Ocean.* Annual Review of Marine Science, 2020. **12**: p. 87-120.

15. Risch, D., et al., *Common and Antarctic Minke Whales: Conservation Status and Future Research Directions.* Frontiers in Marine Science, 2019. **6**.

16. Perrin, W.F., S.D. Mallette, and R.L. Brownell Jr, *Minke whales: Balaenoptera acutorostrata and B. bonaerensis*, in *Encyclopedia of marine mammals*. 2018, Elsevier. p. 608-613.

17. Esposito, C., O. Bichet, and M. Petit, *First Sightings of Antarctic Minke Whale (Balaenoptera bonaerensis) Mother-Calf Pairs in French Polynesia.* Aquatic Mammals, 2021. **47**(2): p. 175-180.

18. Friedlaender, A.S., et al., *Whale distribution in relation to prey abundance and oceanographic processes in shelf waters of the Western Antarctic Peninsula.* 2006.

19. Thiele, D., et al., *Seasonal variability in whale encounters in the Western Antarctic Peninsula.* Deep sea research part II: topical studies in oceanography, 2004. **51**(17-19): p. 2311-2325.

20. Murase, H., et al., *Spatial distribution of Antarctic minke whales (Balaenoptera bonaerensis) in relation to spatial distributions of krill in the Ross Sea, Antarctica.* Fisheries Oceanography, 2013. **22**(3): p. 154-173.

21. Lee, J.F., et al., *Behavior of satellite-tracked Antarctic minke whales (Balaenoptera bonaerensis) in relation to environmental factors around the western Antarctic Peninsula.* Animal Biotelemetry, 2017. **5**(1): p. 23.

22. Dominello, T. and A. Širović, *Seasonality of Antarctic minke whale (Balaenoptera bonaerensis) calls off the western Antarctic Peninsula.* Marine Mammal Science, 2016. **32**(3): p. 826-838.

23. Friedlaender, A., et al., *Sympatry and resource partitioning between the largest krill consumers around the Antarctic Peninsula.* Marine Ecology Progress Series, 2021. **669**: p. 1-16.

24. Friedlaender, A.S., et al., *Feeding rates and under-ice foraging strategies of the smallest lunge filter feeder, the Antarctic minke whale (Balaenoptera bonaerensis).* Journal of Experimental Biology, 2014. **217**(16): p. 2851-2854.

25. Herr, H., et al., *Aerial surveys for Antarctic minke whales (Balaenoptera bonaerensis) reveal sea ice dependent distribution patterns.* Ecology and evolution, 2019. **9**(10): p. 5664-5682.

26. Fearnbach, H., et al., *Abundance of Type A killer whales (Orcinus orca) in the coastal waters off the western Antarctic Peninsula.* Polar Biology, 2019. **42**(8): p. 1477-1488.

27. Inoue, S., G. Yasunaga, and L.A. Pastene, *Comparison of progesterone concentrations in blubber and plasma among female Antarctic minke whales of known reproductive status.* Fisheries Science, 2019.

28. Mogoe, T., et al., *Results of biological sampling of Antarctic minke whale during the first field survey of NEWREP-A in Area V during the 2015/16 austral summer season.* Paper SC/66b/SP presented to the IWC Scientific Committee. 20pp, 2016.

29. Stammerjohn, S., et al., *Regions of rapid sea ice change: An inter-hemispheric seasonal comparison.* Geophysical Research Letters, 2012. **39**(6).

30. Vaughan, D.G., et al., *Recent rapid regional climate warming on the Antarctic Peninsula.* Climatic change, 2003. **60**(3): p. 243-274.

31. Stammerjohn, S.E., et al., *Sea ice in the western Antarctic Peninsula region: Spatio-temporal variability from ecological and climate change perspectives.* Deep Sea Research Part II: Topical Studies in Oceanography, 2008. **55**(18): p. 2041-2058.

32. Ducklow, H.W., et al., *West Antarctic Peninsula: an ice-dependent coastal marine ecosystem in transition.* Oceanography, 2013. **26**(3): p. 190-203.

33. Palsbøll, P.J., F. Larsen, and E.S. Hansen, *Sampling of Skin Biopsies from Free-Raging Large Cetaceans in West Greenland: Development of new Biopsy Tips and Bolt Designs.* International Whaling Commission Special Issue Series, 1991(13).

34. Bérubé, M. and P. Palsbøll, *Identification of sex in cetaceans by multiplexing with three ZFX and ZFY specific primers.* Molecular Ecology, 1996. **5**(2): p. 283-287.

35. Palsbøll, P.J., et al., *Determination of gender in cetaceans by the polymerase chain reaction.* Canadian Journal of Zoology, 1992. **70**(11): p. 2166-2170.

36. Gilson, A., et al., *Deer gender determination by polymerase chain reaction.* California Fish and Game, 1998. **84**(4): p. 159-169.

37. Valsecchi, E. and W. Amos, *Microsatellite markers for the study of cetacean populations.* Molecular Ecology, 1996. **5**(1): p. 151-156.

38. Berube, M., et al., *Polymorphic di-nucleotide microsatellite loci isolated from the humpback whale, Megaptera novaeangliae.* Molecular Ecology, 2000. **9**(12): p. 2181-2183.

39. Palsbøll, P., et al., *Primers for the amplification of tri-and tetramer microsatellite loci in baleen whales.* Molecular Ecology, 1997. **6**(9): p. 893-895.

40. Waldick, R., M. Brown, and B. White, *Characterization and isolation of microsatellite loci from the endangered North Atlantic right whale.* Molecular Ecology, 1999. **8**(10): p. 1763-1765.

41. Constantine, R., et al., *Abundance of humpback whales in Oceania using photo-identification and microsatellite genotyping.* Marine Ecology Progress Series, 2012. **453**: p. 249-261.

42. Peakall, R. and P.E. Smouse, *GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research.* Molecular ecology notes, 2006. **6**(1): p. 288-295.

43. Kalinowski, S.T., M.L. Taper, and T.C. Marshall, *Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment.* Molecular ecology, 2007. **16**(5): p. 1099-1106.

44. Kellar, N.M., et al., *Determining pregnancy from blubber in three species of delphinids.* Marine Mammal Science, 2006. **22**(1): p. 1-16.

45. Pallin, L.J., et al., *High pregnancy rates in humpback whales (Megaptera novaeangliae) around the Western Antarctic Peninsula, evidence of a rapidly growing population.* Royal Society Open Science, 2018. **5**(5).

46. Pallin, L., et al., *Validation of a blubber-based endocrine pregnancy test for humpback whales.* Conservation Physiology, 2018. **6**(1): p. coy031-coy031.

47. Mansour, A.A., et al., *Determination of pregnancy status from blubber samples in minke whales (Balaenoptera acutorostrata).* Marine Mammal Science, 2002. **18**(1): p. 112-120.

48. Kahane-Rapport, S., et al., *Lunge filter feeding biomechanics constrain rorqual foraging ecology across scale.* Journal of Experimental Biology, 2020. **223**(20): p. jeb224196.

49. Durban, J.W., et al., *Photogrammetry of killer whales using a small hexacopter launched at sea.* Journal of Unmanned Vehicle Systems, 2015. **3**(3): p. 131-135.

50. Christiansen, F., et al., *Maternal body size and condition determine calf growth rates in southern right whales.* Marine Ecology Progress Series, 2018. **592**: p. 267-281.

51. Torres, W.I. and K. Bierlich, *MorphoMetriX: a photogrammetric measurement GUI for morphometric analysis of megafauna.* Journal of Open Source Software, 2020. **5**(45): p. 1825.

52. Bird, C.N. and K. Bierlich, *CollatriX: A GUI to collate MorphoMetriX outputs.* Journal of Open Source Software, 2020. **5**(51): p. 2328.

53. Bierlich, K., et al., *Bayesian approach for predicting photogrammetric uncertainty in morphometric measurements derived from drones.* Marine Ecology Progress Series, 2021. **673**: p. 193-210.

54. Thompson, K.F., et al., *A novel conservation approach provides insights into the management of rare cetaceans.* Biological conservation, 2013. **157**: p. 331-340.

55. Bando, T., et al., *Biological parameters of Antarctic minke whale based on materials collected by the JARPA survey in 1987/88 to 2004/05.* JARPA review document SC D, 2006. **6**.

56. Horwood, J.W., *Biology and exploitation of the minke whale*. 1989: CRC press.

57. Laidre, K.L., et al., *Sexual segregation of common minke whales (Balaenoptera acutorostrata) in Greenland, and the influence of sea temperature on the sex ratio of catches.* ICES Journal of Marine Science, 2009. **66**(10): p. 2253-2266.

58. Ruckstuhl, K. and P. Neuhaus, *Sexual segregation in vertebrates*. 2006: Cambridge University Press.

59. Laws, R.M., *Reproduction, growth and age of southern fin whales.* Discovery Rep, 1961. **31**: p. 327-486.

60. Whitehead, H., *Sperm whales: social evolution in the ocean*. 2003: University of Chicago press.

61. Lee, J.F., et al., *Behavior of satellite-tracked Antarctic minke whales (Balaenoptera bonaerensis) in relation to environmental factors around the western Antarctic Peninsula.* Animal Biotelemetry, 2017. **5**(1).

62. Murase, H., et al., *Review of the assessment of two stocks of Antarctic minke whales (eastern Indian Ocean and western South Pacific).* J. Cetacean Res. Manage., 2020. **21**(1): p. 95-122.

63. Nowacek, D.P., et al., *Super-aggregations of krill and humpback whales in Wilhelmina Bay, Antarctic Peninsula.* PLoS One, 2011. **6**(4): p. e19173.

64. Atkinson, A., et al., *Krill (Euphausia superba) distribution contracts southward during rapid regional warming.* Nature Climate Change, 2019. **9**(2): p. 142-147.

65. Horwood, J.W., *Biology and exploitation of the minke whale.* 1989.

66. Bando, T., *Laboratory and analytical approaches to estimate biological parameters in the Antarctic minke whale and summary of results.* INSTITUTE OF CETACEAN RESEARCH, 2017: p. 22.

67. Polanowski, A.M., et al., *Epigenetic estimation of age in humpback whales.* Molecular Ecology Resources, 2014. **14**(5): p. 976-987.

68. Kato, H., *Migration strategy of southern minke whales in relation to reproductive cycle estimated from foetal lengths.* Rep. Int. Whal. Commn, 1991. **41**: p. 363-369.

69. Spooner, F.E.B., R.G. Pearson, and R. Freeman, *Rapid warming is associated with population decline among terrestrial birds and mammals globally.* Global Change Biology, 2018. **24**(10): p. 4521-4531.

70. Friedlaender, A.S., et al., *The effects of prey demography on humpback whale (Megaptera novaeangliae) abundance around Anvers Island, Antarctica.* Polar Biology, 2008. **31**(10): p. 1217-1224.

71. Ducklow, H.W., et al., *Marine pelagic ecosystems: the west Antarctic Peninsula.* Philosophical Transactions of the Royal Society of London B: Biological Sciences, 2007. **362**(1477): p. 67-94.

72. Durban, J., et al., *Size and body condition of sympatric killer whale ecotypes around the Antarctic Peninsula.* Marine Ecology Progress Series, 2021. **677**: p. 209-217.

73. Fearnbach, H., et al., *A decade of photo-identification reveals contrasting abundance and trends of Type B killer whales in the coastal waters of the Antarctic Peninsula.* Marine Mammal Science, 2021.

74. Leaper, R., et al., *Global climate drives southern right whale (Eubalaena australis) population dynamics.* Biology Letters, 2006. **2**(2): p. 289-292.

1.8 Tables

Table 1.1. Summary of microsatellite loci used for individual identification of Antarctic minke whales (*Balaenoptera bonaerensis*) along the Western Antarctic Peninsula. The number of alleles, observed (HO), and expected (HE) heterozygosity was calculated using *Cervus 3.0.1*. The expected probability of identity (PID) of each locus was calculated with the program *GenAlEx v6.5.*

Locus	Source	Label	[$mgCl_2$] mM	Size range (bp)	No. of alleles	H_E	H_O	P_{ID}
Ev1	Valsecchi & Amos (1996)	NED	4	114-155	13	0.844	0.841	0.043
Ev37	Valsecchi & Amos (1996)	NED	3.5	184-220	16	0.915	0.380	0.014
Ev104	Valsecchi & Amos (1996)	FAM	2.5	126-160	16	0.894	0.918	0.021
GATA98	Palsbøll *et al.* (1997)	NED	2.5	80-108	8	0.772	0.758	0.082
GT23	Berube *et al.* (2000)	VIC	2.5	88-120	16	0.889	0.922	0.022
GT211	Palsbøll *et al.* (1997)	FAM	2.5	80-114	14	0.887	0.921	0.023
GT509	Berube *et al.* (2000)	HEX	2.5	179-217	31	0.952	0.967	0.004
GT575	Berube *et al.* (2000)	FAM	1.5	129-161	15	0.906	0.872	0.016
rw4-10	Waldick *et al.* (1999)	VIC	2.5	188-219	28	0.947	0.924	0.005
rw48	Waldick *et al.* (1999)	NED	3	108-133	10	0.882	0.500	0.026

Table 1.2. Sample summary statistics for Antarctic minke whales sampled along the Western Antarctic Peninsula (2013-14, 2016-20) with known genetic sex. † Designates that all replicates in the dataset have been removed (i.e. this is the true number of individuals in the dataset). ᵝDoes not include samples that yielded a poor genotype quality score (2013-1 Male, 1 Female; 2018-1 Male).

Temporal Scale	N	# Genotypes	Male		95% CL	Female		95% CL	Sex Ratio	Difference to Parity	Pregnant (females only)		
			N	*%*	Lower-Upper	*N*	*%*	Lower-Upper	*(M:F)*	Exact Binomial Test	$N_{ind.\ analyzed}$	$N_{pregnant}$	%
2013 Total	**19**	**16ᵝ**	**5β**	**31.25**	**11.02-58.66**	**11ᵝ**	**68.75**	**41.34-88.98**	**0.45**	**p = 0.210**	**9**	**6**	**66.67**
2014 Total	**10**	**7**	**1**	**14.29**	**0.36-57.87**	**6**	**85.71**	**42.12-99.64**	**0.17**	**p = 0.125**	**4**	**3**	**75.50**
2016 Total	**5**	**5**	**3**	**60.00**	**14.66-94.73**	**2**	**40.00**	**5.27-85.34**	**1.50**	**p = 1.000**	**2**	**2**	**100.00**
2017 Total	**10**	**9**	**6**	**66.67**	**29.93-92.51**	**3**	**33.33**	**7.49-70.07**	**2.00**	**p = 0.508**	**3**	**3**	**100.00**
2018 Total	**8**	**7ᵝ**	**3β**	**42.86**	**9.90-81.59**	**4**	**57.14**	**18.41-90.10**	**0.75**	**p = 1.000**	**3**	**2**	**66.67**
2019 Total	**14**	**13**	**4**	**30.77**	**9.09-61.43**	**9**	**69.23**	**38.57-90.90**	**0.44**	**p = 0.267**	**9**	**2 (1 UND)**	**22.22**
2020 Total	**16**	**12**	**7**	**58.33**	**27.67-84.83**	**5**	**41.67**	**15.17-72.33**	**1.40**	**p = 0.774**	**4**	**2**	**50.00**
Total	**82**	**69†**	**29**	**42.03**	**30.24-54.52**	**40**	**57.97**	**45-47-69.76**	**0.73**	**p = 0.228**	**34**	**20**	**58.82**

Table 1.3. Progesterone concentrations (ng g $^{-1}$) of presumed pregnant and not-pregnant Antarctic minke whales biopsied along the Western Antarctic Peninsula. $^{\beta}$Does not include samples that yielded a poor genotype quality score (2013-1 Female).

	Mean (ng g^{-1})	*S.d*	*min*	*max*	*N*
not-pregnant	1.98	1.58	0.36	5.92	13
pregnant	144.86	96.53	18.85	307.01	20 $^{\beta}$
undetermined	10.20				1
total					*34$^{\beta}$*

1.8 Figures

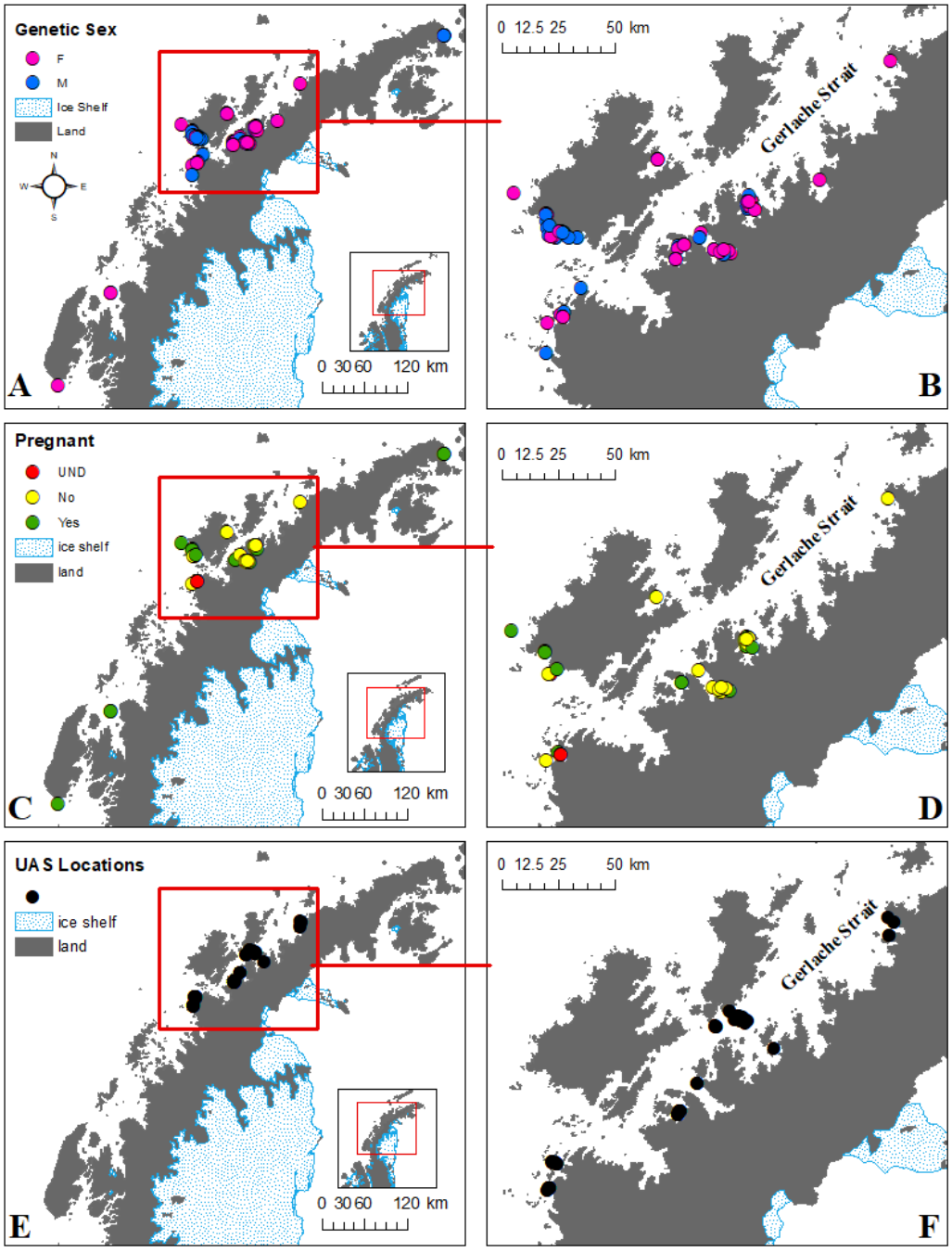

Figure 1.1. Genetic sex of Antarctic minke whales (AMWs) sampled along the Western Antarctic Peninsula (WAP) (A) and in the Gerlache Strait and adjacent bays (B), pregnancy status of female AMWs sampled along the WAP (C) and in the Gerlache Strait and adjacent bays (D), and location of AMWs imaged along the WAP (E) and in the Gerlache Strait and adjacent bays (F).

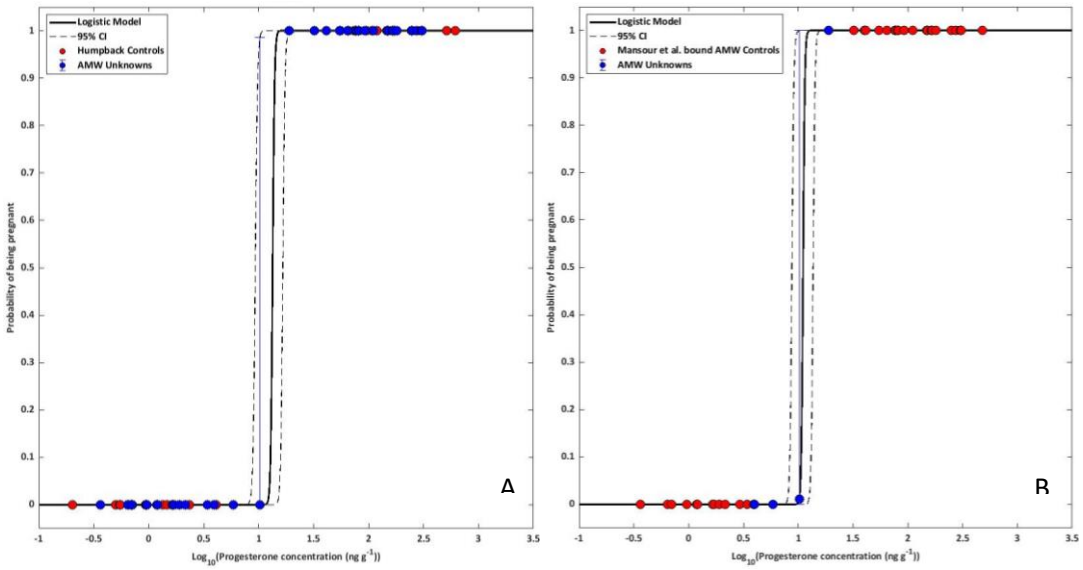

Figure 1.2. Logistic regression models for the probability of pregnancy in Antarctic minke whales (AMWs) relative to blubber progesterone concentration. (A) Model based on the relationship of AMW progesterone concentration with a reference model developed from known pregnant humpback whales adapted from Pallin et al. [46] and (B) Mansour et al. [47] adapted model built by bounding sampled AMWs according to common minke whale progesterone concentrations. Red circles represent mature female humpback whale females (A) and mature female AMWs (B) used to develop the model. Blue circles represent the AMW females of unknown pregnancy status sampled along the Western Antarctic Peninsula with an associated error around their probability of pregnancy. Dashed lines represent the 95% confidence envelopes developed from 10,000 bootstrap iterations. x-axis values are log10 transformed.

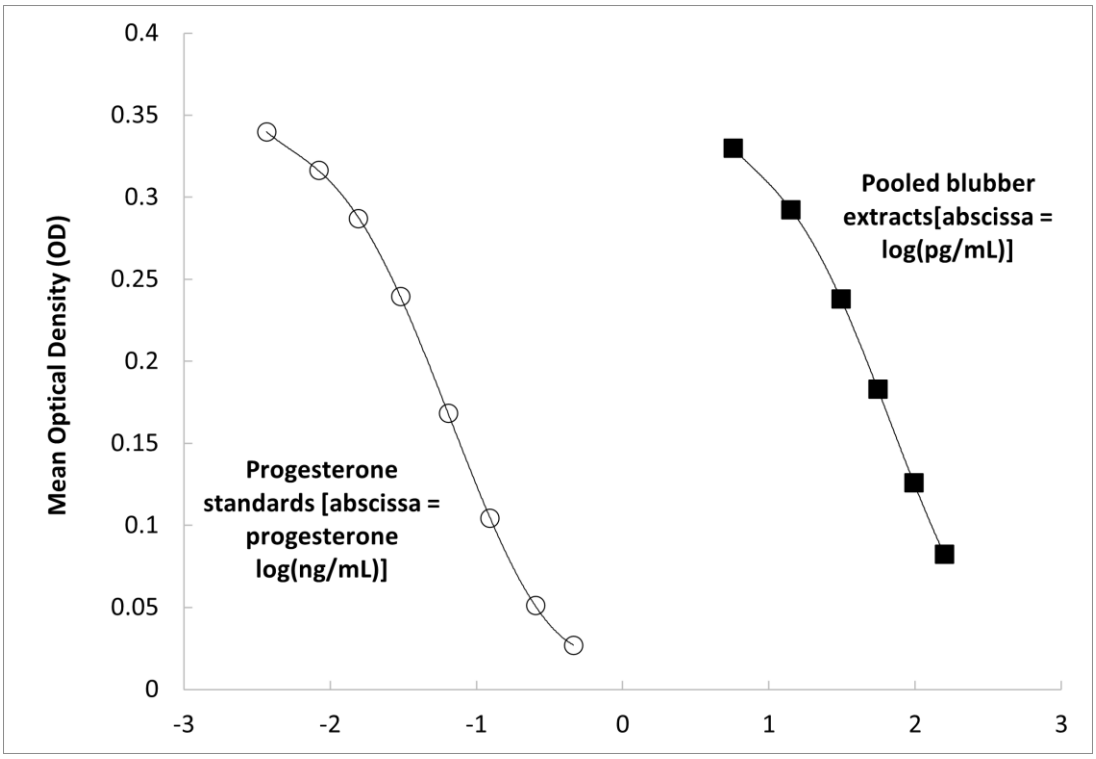

Figure 1.3. Results from linearity assessment of progesterone enzyme immunoassay (EIA) with Antarctic minke whale (AMW) blubber tissue extracts. Serial dilutions of extracts (shaded squares) show parallelism with the standards of the progesterone EIA (open circles) (r^2 = 0.982, slope = 0.997); an indication that the assay is measuring the same antigens in the blubber as in the standards and therefore suitable for use with AMW blubber tissues extracts. Six individual females were represented in the pooled blubber extracts.

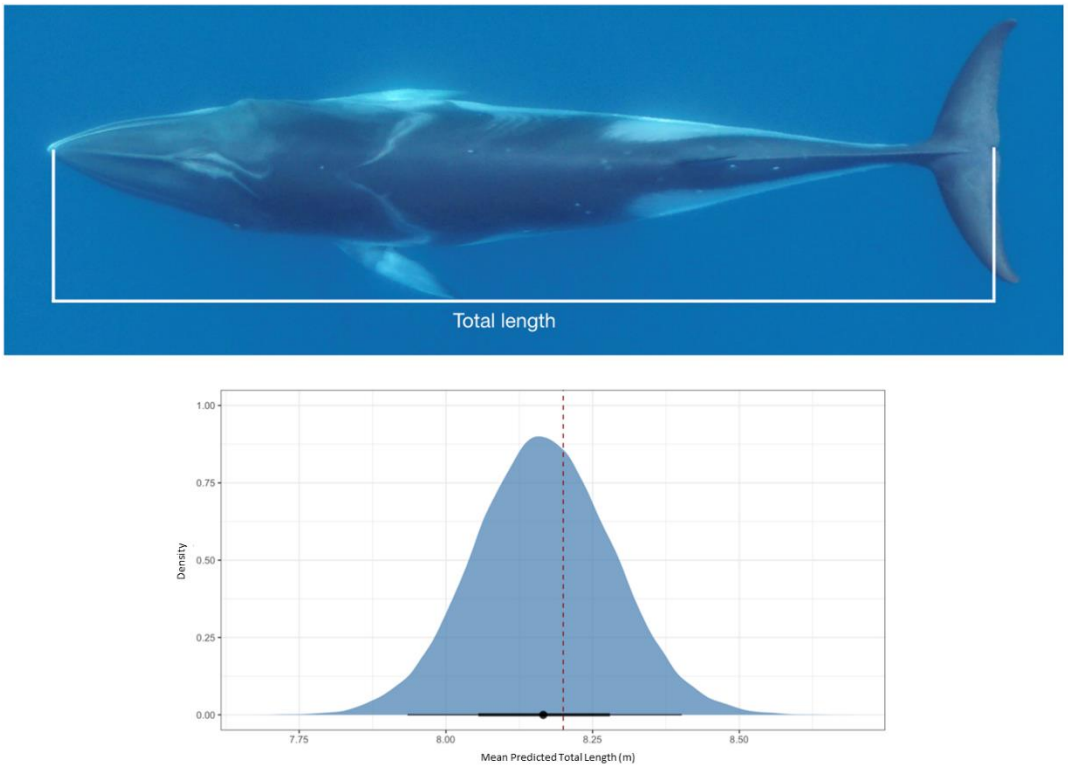

Figure 1.4. An example of a UAS image of an Antarctic minke whale (AMW). Total length is measured from the tip of the rostrum to the fluke notch. The bottom panel shows an example of a posterior predictive total length distribution for a single individual. The longer black bars represent the 95% highest posterior density (HPD) intervals, the thicker shorter black bars represent the 65% HPD interval, and the black dot represents the mean value. The red dashed line represents the median length at sexual maturity for female AMWs generated from commercial catch data (8.20 m).

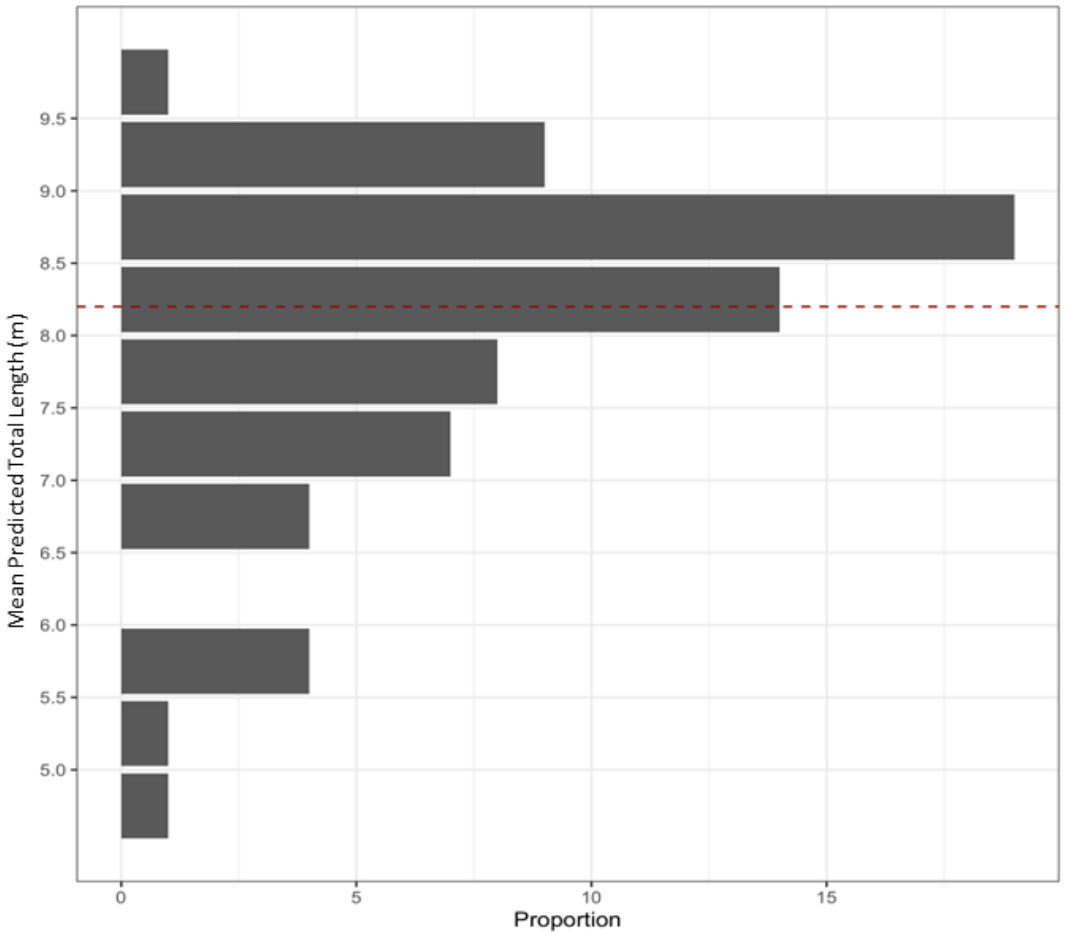

Figure 1.5. Length frequency distribution of Antarctic minke whales (AMWs, N=68) with the 95% highest posterior density (HPD) interval. The total length of each individual is represented by the mean of its predicted posterior total length distribution (see Fig. 1.4). The dashed line represents the median length at sexual maturity for female AMWs generated from commercial catch data (8.20 m).

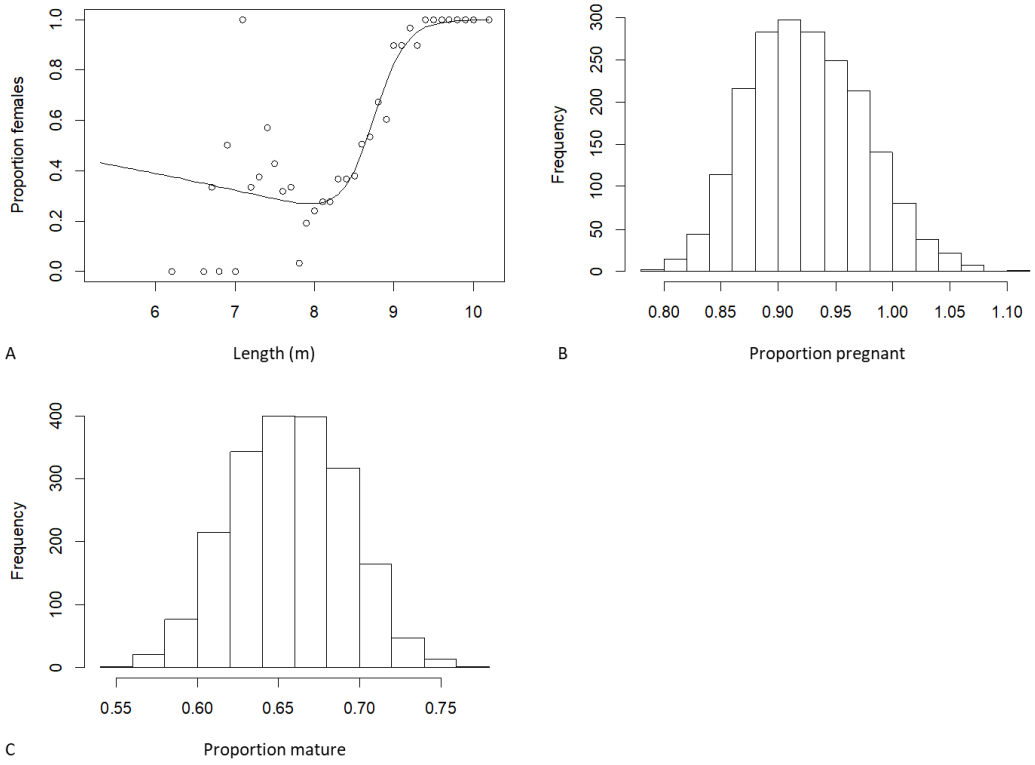

Figure 1.6: (A) Proportion of Antarctic minke whale females at length from commercial catch data in the vicinity of the Antarctic Peninsula (points) and the maximum likelihood estimate of the product of two logistic functions (line) and the posterior distribution of pregnancy rates (B) and proportion of mature females (C) derived from the observed lengths using the MCMC calculations of the proportions of mature females at given lengths derived from commercial catch data (see supplemental Text S1.1).

Chapter 2: A surplus no more? Variation in krill availability impacts reproductive rates of Antarctic baleen whales

Logan Pallin, Nick Kellar, Debbie Steel, Natalia Botero-Acosta, C. Scott Baker, Jack Conroy, Dan Costa, Chris Johnson, David Johnston, Ross Nichols, Doug Nowacek, Andrew Read, Oksana Savenko, Oscar Schofield, Sharon Stammerjohn, Debbie Steinberg, Ari Friedlaender

2.1 Abstract

The krill surplus hypothesis of unlimited prey resources available for Antarctic predators due to commercial whaling in the 20th century has remained unchallenged since the 1970s. Rapid warming of the Western Antarctic Peninsula (WAP) over the past 50 years has resulted in decreased seasonal ice cover and a reduction of krill. The latter is being exacerbated by a commercial krill fishery in the region. Despite this, humpback whale populations have increased but may be at a threshold for growth based on these human-induced changes. Understanding how climate-mediated variation in prey availability influences humpback whale population dynamics is critical for focused management and conservation actions. Using a long-term dataset (2013-2020), we show that inter-annual humpback whale pregnancy rates, as determined from skin-blubber biopsy samples (n =669), are positively correlated with krill availability and fluctuations in ice cover. Annual pregnancy rates showed significant inter-annual variability, between 29% and 86%. Our results indicate that krill availability is in fact limiting and affecting reproductive rates, in contrast to the krill surplus hypothesis. This may suggest that this population of humpback whales has limited resilience to environmental change in this region. As a result, continued warming and increased fishing along the WAP, which continue to reduce krill stocks, will likely impact this humpback whale population and other krill predators

in the region. Humpback whales are sentinel species of ecosystem health, and changes in pregnancy rates can provide quantifiable signals of the impact of environmental change at the population level. Our findings must be considered paramount in developing new and more restrictive conservation and management plans for the Antarctic marine ecosystem and minimizing the negative impacts of human activities in the region.

2.2 Introduction

One of the most significant ecological disturbances to occur over the past 200 years was the removal of more than two million baleen whales during the 20th century in the Southern Ocean [1]. In the 1970s, Laws [2] proposed that this reduction in whales resulted in a "surplus" of Antarctic krill (*Euphausia superba*). This, in turn, could have allowed remaining krill predators (including other whales, seals, and penguins) to eat more, grow, and reproduce faster. Today, most populations of humpback whales in the Southern Hemisphere have recovered and are at or near their carrying capacity. This recovery has been particularly pronounced for humpback whales along the Western Antarctic Peninsula (WAP)[3-5]. However, recent work in the WAP also shows localized krill declines and a southward shift in krill distribution from 1976-2016 [6].

For humpback whales, reproductive success is largely contingent on the accessibility of adequate prey resources on their high-latitude feeding grounds [7]. As capital breeders, humpback whales and other migratory baleen whales exploit high-latitude prey resources during the summer. They then use this stored energy or "capital" to support their annual migration and breeding activities in low-latitude regions during winter [8]. Humpback whale feeding rates are highest early in the feeding season when their body condition is at its lowest[9]. Pregnant whales, in particular, must store sufficient energy

reserves to support gestation and early lactation, when the females are fasting. This contention is supported by data obtained from whales harvested in commercial whaling operations that showed pregnant and/or lactating humpback females taken along the coasts of Australia yielded roughly twice as much oil as non-pregnant females [10].

Several studies demonstrate clear connections between whale pregnancy rates, prey availability/quality, and changing oceanic conditions. For example, in grey whales, reproductive rates increased after seasons in which sea-ice conditions allowed more time to access feeding grounds in the Bering Sea [11]. However, once climatic conditions shifted, and access to these vital feeding regions was truncated, reproductive rates dropped [11]. Similarly, in North Atlantic right whales, significant increases in reproductive rates during the 1990s were closely related to increases in the availability of their prey. This increase in prey was driven by warming oceanic conditions in the Gulf of Maine [12]. These prey resources declined when a distinct climatic shift flipped the oceanic conditions in subsequent years, followed by a decline in North Atlantic right whale fecundity rates [12, 13]. Additional studies have observed a similar phenomenon in Southern Hemisphere baleen whales[14]. These studies revealed mechanisms by which whale reproductive rates can respond to varying prey availability and environmental conditions.

Along the WAP, over half a century of environmental change has been documented as part of the Palmer Long Term Ecological Research (PAL LTER) program. During the austral summer, the breeding stock G (International Whaling Commission (IWC) management group; Eastern South Pacific) population of humpback whales feeds along the WAP [15, 16], making one of the longest annual migrations of any mammal [17]. This

region has experienced a rise in winter air temperature of nearly 5°C since the 1950s, resulting in the collapse of ice shelves, the retreat of glaciers, and the exposure of new terrestrial and marine habitats [18, 19]. The biological and physical productivity of the WAP marine ecosystem is strongly influenced by the amount of sea ice cover in this region [20]. Additionally, an overall decline in sea ice has been observed along the WAP, resulting in an annual sea ice extent that is, on average, 80 days shorter than four decades ago [21]. Thus, the recovery of this humpback whale population is occurring in an environment experiencing some of the fastest climatic warming of any region on the planet [22].

Understanding how climate-driven processes influence the population dynamics of humpback whales is critical for prioritizing internationally-developed conservation actions intended to maintain the structure and function of this marine ecosystem. Whales are ecosystem engineers that enhance local primary production, stabilizing their prey base (e.g., Savoca et al.[23]), so understanding the factors that affect their demography is critical to managing these whale stocks and thus their contributions to the ecosystem. Ideally, we could forecast periods when ecological conditions are favorable or not for whales and use this knowledge to implement targeted and dynamic management strategies to modify human activities, such as krill fishing, that competes for resources with predators like humpback whales[24].

The development of non-lethal tissue sampling techniques and methods to isolate and quantify reproductive markers from skin-blubber biopsy samples allow us to assess environmental variability's impact on female humpback whales' pregnancy rates. Here c To investigate this question, we (i) quantified the variation in pregnancy rates in female

humpback whales across eight consecutive years and (ii) assessed the variation in annual pregnancy rates as a response to two critical environmental variables (prey availability and sea ice cover) using generalized linear models (GLMs). Our findings illustrate how availability and variability of resources affect the reproduction of a capital breeder that also has significant ecosystem function. Further, our data provide support for more direct conservation and management actions to mitigate a growing krill fishery in this rapidly changing ecosystem.

2.3 Methods

2.3.1 Biopsy collection

We collected skin and blubber samples from female humpback whales during the 2013-2020 austral summers (December-March). This was done in the nearshore waters of the Western Antarctic Peninsula (WAP) using standard biopsy techniques (Fig. 1) [25]. We used a crossbow to project modified bolts and 40mm stainless steel biopsy tips (CetaDart) to obtain samples from a distance of 10-30 meters, targeting the area of the body below the dorsal fin. Samples were collected opportunistically when whales were encountered during prey or visual surveys in an area within ~10 nautical miles of Palmer Station, Anvers Island. Dedicated research cruises or platforms of opportunity, including ecotour vessels, were also used. Dependent calves were not sampled during seasons 2013-2019, but all age and sex classes of humpback whales were sampled during 2020. Because of this change in protocol, samples from calves were not included in any analysis. However, the presence of a calf was recorded and identified, as evident by their smaller size (less than half of the presumed mother's length) and close association with an adult, presumed to be the mother. Supplementary data (including location and group size)

were recorded at every biopsy event. Samples were stored frozen whole at -20° C until used for analysis.

2.3.2 DNA profiling

A standard DNA profile, including sex-specific markers and microsatellite genotypes, was used to identify individual whales. DNA was extracted from the skin-blubber interface using a commercially available kit (DNeasy 96 Blood & Tissue Kit, Qiagen, Hilden, Germany). The sex of each sampled whale was determined by amplification of sex-specific markers following the protocols of Gilson et al. [26, 27]. Results were compared to controls for a known male and female using gel electrophoresis.

Samples were genotyped using 10 previously published microsatellite loci to resolve the individual identity of each sampled whale and remove potential duplicates (Table S2.1) [28-32]. Alleles were sized and binned using the software program Genemapper v3.7 (Applied Biosystems). The total number of amplified loci for a given sample was considered as an added quality control threshold, with samples amplifying for less than 7 loci considered poor quality and repeated or removed from final dataset. Given the estimated probability of identity for these loci from previous studies [3, 33], we assumed that samples matching at a minimum of seven loci to be recaptures of the same individual. Recaptures of the same individual were removed from the analysis. The expected probability of identity (P_{ID}; the probability that two individuals drawn at random from a population will have the same genotype by chance) for each locus was calculated in GenAlEx v6.5 [34]. Cervus 3.0.7 [35] was used to compute the number of alleles (K), observed and expected heterozygosity (HO and HE), and the probability of identity for all individual matches.

2.3.3 Hormone extraction and quantification

We extracted steroid hormones from the blubber portion of the biopsy samples following standard methods [3, 36]. Briefly, to quantify hormone biomarkers (i.e., progesterone), we sub-sectioned a cross-sectional sub-sample (~0.15g) spanning from the epidermis-blubber interface to the most internal layer of the biopsy. These sub-samples were then homogenized multiple times using an automated bead mill homogenizer (Bead Ruptor Elite, Omni International). Following the completion of the homogenization process, we isolated progesterone using a series of chemical washes, evaporations, and separations. The final hormone residue was stored at -20° C until analysis.

We quantified the amount of hormone in each extract using a commercially available enzyme immunoassay used extensively in similar studies [3, 37, 38]. Our progesterone EIA kit (EIA kit 900-011, ENZO Life Sciences, Farmingdale, NY) had a 100% reactivity with progesterone and an assay detection limit between 15 and 500 pg/mL. Two additional standard dilutions were added to allow for a lower detection limit of the standard curve to 3.81 pg/mL. We determined extraction efficiency by spiking subsamples of blubber from a dead, stranded animal of known pregnancy status, with 150ng of progesterone and including these with every extraction [36]. We calculated the percentage of progesterone recovered after each extraction and adjusted each sample concentration to this efficiency prior to statistical analyses. An extraction efficiency greater than 60% was adequate and is based on the reported range of efficiencies seen using these methods [36]. If the efficiency of an extraction set was less than 60%, the sample extracts were discarded, and the blubber samples were re-extracted and re-analyzed. Each assay was evaluated for color development using a Biotek plate reader

Epoch (Gen5™ software [Biotek, USA]) with reading and correction wavelengths of 405 nm and 630 nm. Blubber hormone concentrations were then transformed into nanograms of cortisol per gram of blubber (wet weight).

2.3.4 Pregnancy classification

We assigned pregnancy of female humpback whales following previously published methods [38]. Biopsy samples (n = 29) were collected from individuals of a known life-history stage from the Gulf of Maine feeding aggregation by the Center for Coastal Studies in Provincetown, MA. Using these control samples from the Gulf of Maine, the pregnancy state relative to blubber progesterone concentrations was modeled using a standard logistic regression model [39]. Each WAP humpback sample of unknown pregnancy status was entered into the model, and the model returned a probability of being pregnant for each female sampled [39]. If the probability of being pregnant was greater than 99.9%, that female was given an assignment of pregnant. If the probability of being pregnant was less than 0.1%, that female was assigned as not pregnant. If a biopsied female's probability of being pregnant was between those two bounds, that female was set as undetermined pregnancy.

2.3.5 Pregnancy rates

Using this approach, we could estimate the proportion of pregnant females in all samples, including those with an assignment probability between 0.1 and 99.9%. This was accomplished by taking the sum of the probabilities for all samples and dividing by the sample size. Additionally, while calves were not included in the analysis, we cannot

account for females sampled that are not yet sexually mature. Thus, the pregnancy rates presented here represent an estimate for all females age 1+.

2.3.6 WAP environmental data

Biological and environmental variables were used to describe variation in prey and habitat conditions along the WAP during the summer feeding season. The covariates included two environmental factors (i.e., day of spring ice edge retreat (sIER), and krill abundance). The sIER was generated using previously published methods [40-42]. The sIER was created using the GSFC Bootstrap SMMR-SSM/I Version 3.1 sea ice concentration time series (1979–2020) from the EOS Distributed Active Archive Center (DAAC) at the National Snow and Ice Data Center (NSIDC, University of Colorado at Boulder, http://nsidc.org) [43]. We identified the day of the sIER for each satellite grid cell (25 by 25 km pixel) and for each sea ice year. A regional WAP average was generated by taking the mean of all the satellite pixels within our defined area. Our defined area encompassed, from north to south, the South Shetland Islands to Adelaide Island, including Marguerite Bay, and from east to west, the coast of the WAP to 200km offshore (Fig. 2.1). The day of the retreat is defined as the day in which sea-ice concentration decreases below the nominal 'ice edge' threshold (here defined at 15% concentration) and remains below for at least 5 consecutive days. The day of sIER is reported in year-day for the austral spring-summer and typically ranges from year-day ~250 (Sep 7) to ~370 (Jan 5).

Krill (*Euphausia superba*) abundance was assessed following previously published methods [44]. Briefly, krill were collected in net tows (typically 0-120 m) on PAL LTER annual research cruises during austral summer (~ 1 January to 10 February) since 1993.

The PAL LTER study region extends 700 km along the WAP from Anvers Island to Charcot Island and from coastal to slope waters ~200 km offshore [45]. Sampling grid lines are spaced 100 km apart with grid stations every 20 km along each line. To match the spatial distribution of humpback whale sampling, only data from the North sub-region (400-600 sampling lines, Fig. 2.1) were included in the analysis [44]. During the period considered in this analysis (2011-2019), 12-20 net tows were conducted per year in the North sub-region. The abundance at each sampling station was $\log_{10}$-transformed prior to calculating annual mean abundance [46].

Humpback whales utilize the continental shelf and the coastal bays and fjords along the entirety of the WAP for foraging [47]. Additionally, female humpback whales in this region have previously been described to have high pregnancy rates [3]. Thus, we specifically tested the effects of both a one- and two-year lag of each environmental covariate [48]. We did not test the effects of the year of sampling as we could not account for the time the individual female may have spent along the WAP prior to being sampled.

2.3.7 Data analysis

All statistical analyses were performed in R [49]. We removed all within-year replicates from the data set to avoid re-sample bias in our analyses of interannual variation in pregnancy rates. In this case, the first chronologically collected sample was retained for the analyses. We tested for differences in pregnancy rates across all years by using a χ^2 test of independence. We used a Tukey's post hoc stepwise multiple comparison test to determine if there was a significant difference in pregnancy rates between any two individual years. Lastly, for our analysis of pregnancy rates as a function of environmental covariates, we removed all sample replicates from the analysis, including across-year

recaptures. Again, in this instance, the first chronologically collected sample across all eight years was kept for analysis. We considered all statistical tests with a *p*-value of less than 0.05 significant. All values are expressed as mean ± SD, unless otherwise stated.

We used generalized linear models (GLMs) with binomial distribution and logit link functions to assess the effects of environmental covariates on humpback pregnancy rates. For all models, variance inflation factors (VIF; greater than 5) and Pearson's correlation coefficients (absolute correlation value greater than 0.7) were calculated for covariates to ensure that correlated covariates were not included together. GLM models were optimized using backward selection, accepting the model with the lowest AICc (Akaike Information Criterion corrected).

2.4 Results

2.4.1 Individual identification and sex

We collected 669 biopsy samples from age 1+ female humpback whales in the nearshore waters around the Western Antarctic Peninsula (WAP) in eight field seasons from 2013 to 2020 (Fig. 2.1). On average, 9.96 loci were successfully genotyped per individual. The average PID for any given combination of 7 loci ranged from 1.07×10^{-10} to 6.54×10^{-8}, consistent with previous studies[3]. Consequently, we considered samples with matching genotype recaptures of the same individual. DNA profiling was sufficient to identify and determine the sex of 584 individual non-calf females from these samples (Table S2.2). Details on annual sampling can be found in Table S2.2. We resampled 54 individuals within the same year (Table S2.2). Additionally, we recaptured 32 individuals between years (Table S2.2), with one female recaptured in 2013, 2015, and 2018.

2.4.2 Assignment and annual variation in pregnancy

Based on the concentrations observed from the series of spiked controls, our average extraction efficiency was 79.55% ± 14.48 (minimum 61.63%, maximum 129.634). Additionally, our calculated inter-assay and intra-assay coefficients of variation (CV) from a series of replicated samples were 6.27 and 8.86 %, respectively. We measured progesterone concentrations in 616 samples obtained from 546 individual female humpback whales (Tables 2.1-2.2, Fig. 2.1). A small number of samples were excluded from the analysis due to within year re-sampling or insufficient blubber for an extraction. Based on the relationship of their progesterone concentration with the reference levels from known pregnant animals [38], 297 samples were assigned as not-pregnant (P < 0.1% pregnant; blubber progesterone: mean = 1.37 ± 1.35 ng g-1; Table 2.1) and 306 samples were assigned as pregnant (P > 99.9%; blubber progesterone: mean = 217.20 ± 223.12 ng g-1; Table 2.1). Thirteen samples had a probability of pregnancy between 0.1% and 99.9% (blubber progesterone: mean= 12.59 ± 2.94 ng g-1; Table 2.1) and were classified as undetermined.

The mean pregnancy rate for all individual females with a definitive pregnancy designation across all eight years was 51.97% (Table 2.2, Fig. 2.2). Similarly, the estimated proportion pregnant, including females with an undetermined pregnancy state, derived from a series of 10,000 bootstrap samples (see Pallin et al. 2018 [38]), across all 570 individuals of unknown pregnancy status, was 51.87%. Pregnancy rates varied interannually from 29.50% in 2020 to 86.11% in 2017 (Fig. 2.2). We observed significant variation in pregnancy rates across years ($\chi 2 = 77.85$, df = 7, $p < 0.001$; Fig. 2.2). A post hoc multiple comparisons analysis revealed that the pregnancy rate in 2014

was significantly higher than in all years except 2015, 2016 and 2017 (2013 $p = 0.022$, 2018 $p = 0.046$, 2019 $p = 0.002$, 2020 $p < 0.000$), the rate in 2017 was significantly higher than in all years except 2014 (2013 $p < 0.001$, 2015 $p = 0.003$, 2016 $p = 0.013$, 2018 $p < 0.001$, 2019 $p < 0.001$, 2020 $p < 0.001$), and 2018 was significantly higher than 2020 ($p = 0.004$).

2.4.3 Variation in pregnancy rates as a function of regional environmental variation

Of the 546 unique individuals in the entire pregnancy dataset used in this analysis, 537 had a definitive pregnancy state and were used in the model analysis. Pearson's correlation coefficients among the four tested environmental variables were less than 0.7, and the VIF values were less than two. Thus, all four variables were included in the model selection process. The time series of pregnancy anomalies and each environmental variable can be seen in Fig. 2.3. GLM models identified that both one- and two-year lags of both krill abundance (one-year lag $p < 0.001$, two-year lag $p = 0.044$) and the sIER (one-year lag $p < 0.001$, two-year lag $p < 0.001$) influenced female humpback whale pregnancy rates (Table 2.3, Fig. 2.5). The deviance explained by the best model was 8.98% (Table 2.3). Pregnancy rates increased with increasing krill from the previous year (one-year krill lag, z-value 4.536; Figs. 2.4 & 2.5) as well as the sIER with both a one- and two-year lag (IER one-year lag, z-value 4.222, two-year lag, z-value 3.892; Fig. 2.4). Somewhat surprisingly, pregnancy rates decreased with increasing krill from two years prior (two-year krill lag, z-value -2.019; Fig. 2.4).

2.5 Discussion

This is the first investigation of the relationship between environmental variation and pregnancy rates baleen whales that feed around the Antarctic. Such information provides a baseline against which effective conservation and management plans can be developed. This is especially relevant because a large-scale krill fishery is rapidly expanding in the region and is in direct competition for krill with baleen whales and other krill predators.

2.5.1 Variation in pregnancy rates

We observed a pregnancy rate of 51.97% across all eight years of this study; this rate varied from 29.50% in 2020 to 86.11% in 2017. These rates represent an absolute minimum estimate as we cannot differentiate sexually mature and immature females from our samples. Thus, immature females have not been removed from our analyses. Our measured pregnancy rates are higher than those reported from whales killed during the height of commercial whaling (48 %)[50], as well as projected calving rates for other populations of this species [51]. However, calving rates are not directly comparable because they do not consider pregnancies that may have been lost in utero or perinatal mortality. Such distinctions are critical when drawing comparisons between our work and other studies, particularly those that rely solely on calving rates as indicators for population status.

Globally, the recovery of humpback whales from 20th century commercial whaling, especially in the Southern Hemisphere, has been a story of conservation success [4, 5]. The high pregnancy rates we document here are similar to estimates made for other Southern Hemisphere stocks [5, 37], as well as the initial estimate of stock G along the WAP between 2010-2016 [3]. However, we observe significant variability in the eight years of this study. Similar variation was observed among catches taken during a six-year

period in Antarctic whaling areas IV and V in the early 1950s. However, these latter data are biased because lactating females accompanied by calves were protected by international regulation during this period [50]. It is possible that the inter-annual variation we documented in pregnancy rates could have resulted from temporal match-mismatch among reproductive cohorts, leading to years with heavily inflated or deflated reproductive rates. This variation could also result from sampling bias or spatial heterogeneity in which different reproductive classes distribute preferentially along the WAP. We believe these latter sources of uncertainty to be unlikely in our results because we sampled whales opportunistically over a large portion of their known range in this region [52].

2.5.2 Pregnancy rates vary with environmental conditions

We show that the pregnancy rate of humpback whales was positively related with krill abundance from the previous year but, surprisingly, inversely related with krill abundance from two years prior. Previous studies of baleen whales and their reproductive rates show equivalent responses to variation in prey availability and oceanographic and sea ice conditions [12-14, 53]. Specifically, a lagged negative relationship between krill availability and the breeding success of southern right whales was observed at South Georgia [14]. Similarly, low reproductive rates were observed among female humpback whales within the Gulf of St Lawrence. These were also associated with low prey availability, which led to insufficient energy reserves to maintain pregnancy [53]. Here we show that prey availability in the previous year is the most robust predictor of pregnancy when females are gaining/storing energy for the upcoming pregnancy.

Achieving and maintaining pregnancy is contingent on having access to sufficient prey resources to support the high energetic costs of gestation and lactation. More specifically, an increase in stored energy reserves is required prior to pregnancy (i.e., the feeding season prior to breeding) [54], followed by a continued accumulation of energy stores to support the development and growth of the fetus throughout the pregnancy [55]. While migrating and breeding, these whales rely solely on stored energy reserves [55]. Thus, we observed that higher krill abundances the year prior to sampling led to increased pregnancy rates across the population. What is more challenging to interpret is how higher krill availability two years prior results in lower pregnancy rates. We believe this is likely a result of most females breeding once every two years [48], which would be consistent with our observed mean pregnancy rate of around 52%. This two-year cycle may result in a larger non-reproductive cohort occurring in years of high krill availability two years prior, as observed in this study.

We found significant positive relationships between female humpback whale pregnancy rates and the sIER from the two years prior. The sIER is a powerful physical force affecting biological processes at all trophic levels within the WAP marine ecosystem [56]. During a late sIER, many of the dynamic physical oceanographic properties of the WAP ecosystem are stabilized. For example, higher stratification in the water column is created via two mechanisms. First, reduced wind speeds prevent the formation of a deep mixed layer. Second, salinity-driven density gradients are increased because of higher volumes of sea ice meltwater at the surface [56]. Together, these provide favorable, nutrient-rich conditions high in the water column, triggering intense phytoplankton blooms [57], supporting the growth and survival of large krill cohorts [58]. Thus, from an energetic

perspective, a later sIER would likely provide female humpback whales the required energy reserves for their upcoming migration and pregnancy via a larger prey base on which to forage.

2.5.3 Implications for population growth to conservation and management

Responses in the reproductive rates of baleen whales to climate change have been documented previously [14, 53, 59, 60], and long-term studies are vital to detect such responses. It has been proposed that the rapid recovery of humpback whales in this region is due to a lack of competition due to the slower recovery of other large, krill-consuming predators[2, 3]. In contrast, our study suggests that pregnancy rates of humpback whales are significantly affected by broad-scale ecological variables that directly affect prey abundance and availability. Thus, while this humpback whale population currently has high pregnancy rates, the significant inter-annual variation in these rates (in direct relation to krill availability) shows that this population's trajectory is tightly coupled with prey availability, which can be mediated by environmental change. Therefore, during unfavorable foraging conditions, fewer females will become pregnant [61]. Thus, future warming along the WAP that results in subsequent reduction in prey abundance will likely negatively impact this population of humpback whales and other krill predators in this region [62, 63].

Given the present results and those of previous work [24], we suggest that adaptive management actions be implemented as soon as possible to conserve humpback whales and their prey, Antarctic krill. As the range of WAP humpback whales spans national and international jurisdictions, enhanced multilateral cooperation between international organizations, such as the Convention on the Conservation of Antarctic Marine Living

Resources (CCAMLR) and the IWC, are required to implement effective conservation strategies. Specifically, CCAMLR must implement more restrictive protective measures on spatio-temporal effort by the krill fishery to conserve krill predators. These strategies must strengthen ecological connectivity and mitigate growing human impacts across the range of humpback whale migratory routes or foraging areas [64, 65].

Concurrent with large-scale climatic changes, the WAP has seen an extraordinary increase in the industrial krill fishery. This fishery overlaps in time and space with the known foraging areas of humpback (and other baleen) whales in this region [24, 47]. A recent study by Reisinger et al. [24] showed significant overlap in critical foraging ranges of humpback whales with the krill fishery. This overlap increases the likelihood of direct fishery interactions, including recent incidents of whale bycatch by the fishery [66]. CCAMLR is responsible for the conservation and maintenance of ecosystem function, krill stocks, and commercial fishing. CCAMLR must utilize this along with other new knowledge on baleen whale foraging ecology and demography, along with the impact that both environmental variability and krill fishing have on this group pf krill predators, to develop holistic ecosystem-based management plans. This may help push forward CCAMLR's 2011 commitment to implement a Marine Protected Area (MPAs) system in the region (i.e., CCAMLR Conservation Measure 91-04, see https://cm.ccamlr.org/en/measure-91-04-2011). Other key players have a role in developing and implementing science-based conservation strategies for whales that will influence the outlook of this population including the International Whaling Commission, International Union for the Conservation of Nature (IUCN) Specialist Groups, Convention on Biological Diversity (CBD), Convention on the Conservation of Migratory Species of

Wild Animals (CMS), the commercial fishing industry and the United Nations Framework Convention on Climate Change (UNFCCC). Only with enhanced cooperation, can we ensure this population has the greatest success of recovery from commercial depletion in an environment that is changing rapidly.

2.6 Conclusions

We show significant variation in the pregnancy rates of humpback whales feeding along the WAP. Humpback whales are sentinel species of ecosystem health [67]. As such, changes in vital rates (i.e., pregnancy rates) can provide quantifiable signals of the impact of environmental change at the population level. We found a robust relationship between environmental variation and interannual variability in humpback whale pregnancy rates. These relationships align with similar observations among other baleen whales [14, 53] and other Antarctic krill predators such as Antarctic fur seals and gentoo penguins [68]. This information will assist in monitoring, management, and conservation efforts as changes continue to occur along the WAP. Continued support of long-term ecological programs is critical to understanding the population dynamics of long-lived species relative to environmental trends occurring over long time scales. Our data are in marked contrast to the argument that krill stocks are in surplus and overabundant for the needs of krill predators. On the contrary, we found that variation in krill availability in this region are tightly coupled with the reproductive rates of some of the largest krill predators in the region. As a result, continued warming and increased fishing along the WAP which continue to reduce krill stocks, will likely impact this humpback whale population, and other krill predators in the region. Humpback whales are sentinel species of ecosystem health, and changes in pregnancy rates can provide quantifiable signals of

the impact of environmental change at the population level. This study was fundamental in its methodological approach to a wild species that has a global distribution. A number of populations of related species are experiencing similar changes in their environment and this study can act as a template for similar comparison in those systems.

References

1. Rocha, R., P.J. Clapham, and Y.V. Ivashchenko, *Emptying the oceans: a summary of industrial whaling catches in the 20th century.* Mar. Fish. Rev, 2014. **76**: p. 37-48.

2. Laws, R., *Seals and whales of the Southern Ocean.* Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, 1977: p. 81-96.

3. Pallin, L.J., et al., *High pregnancy rates in humpback whales (Megaptera novaeangliae) around the Western Antarctic Peninsula, evidence of a rapidly growing population.* Royal Society Open Science, 2018. **5**(5).

4. Zerbini, A.N., et al., *Assessing the recovery of an Antarctic predator from historical exploitation.* Royal Society Open Science, 2019. **6**(10).

5. Noad, M.J., E. Kniest, and R.A. Dunlop, *Boom to bust? Implications for the continued rapid growth of the eastern Australian humpback whale population despite recovery.* Population Ecology, 2019.

6. Atkinson, A., et al., *Krill (Euphausia superba) distribution contracts southward during rapid regional warming.* Nature Climate Change, 2019. **9**(2): p. 142-147.

7. Lockyer, C., *Review of baleen whales (Mysticeti) reproduction and implications for management.* Reproduction of Whales, Dolphin and Porpoise, 1984.

8. Baker, C.S., et al., *Migratory movement and population structure of humpback whales (Megaptera novaeangliae) in the central and eastern North Pacific.* Marine Ecology Progress Series, 1986: p. 105-119.

9. Nichols, R.C., et al., *Intra-seasonal variation in feeding rates and diel foraging behaviour in a seasonally fasting mammal, the humpback whale.* Royal Society Open Science, 2022. **9**(7).

10. Dawbin, W.H., *The seasonal migratory cycle of humpback whales.* Whales, dolphins and porpoises. University of California Press, Berkeley, 1966: p. 145-170.

11. Moore, S.E. and H.P. Huntington, *ARCTIC MARINE MAMMALS AND CLIMATE CHANGE: IMPACTS AND RESILIENCE.* Ecological Applications, 2008. **18**(sp2): p. S157-S165.

12. Meyer-Gutbrod, E., et al., *Climate-associated changes in prey availability drive reproductive dynamics of the North Atlantic right whale population.* 2015. **535**: p. 243-258.

13. Meyer-Gutbrod, E.L. and C.H. Greene, *Uncertain recovery of the North Atlantic right whale in a changing ocean.* Global Change Biology, 2018. **24**(1): p. 455-464.

14. Leaper, R., et al., *Global climate drives southern right whale (Eubalaena australis) population dynamics.* Biology Letters, 2006. **2**(2): p. 289-292.

15. Rice, D.W., *Marine mammals of the world: systematics and distribution*. 1998: Society for Marine Mammalogy.

16. Gales, N., et al., *Humpback Whales: Status in the Southern Hemisphere.* Journal of Cetacean Research and Management, 2011(Special Issue 3).

17. Rasmussen, K., et al., *Southern Hemisphere humpback whales wintering off Central America: insights from water temperature into the longest mammalian migration.* Biology Letters, 2007. **3**(3): p. 302-305.

18. Vaughan, D.G., et al., *Recent rapid regional climate warming on the Antarctic Peninsula.* Climatic change, 2003. **60**(3): p. 243-274.

19. Meredith, M.P. and J.C. King, *Rapid climate change in the ocean west of the Antarctic Peninsula during the second half of the 20th century.* Geophysical Research Letters, 2005. **32**(19).

20. Massom, R.A. and S.E. Stammerjohn, *Antarctic sea ice change and variability–physical and ecological implications.* Polar Science, 2010. **4**(2): p. 149-186.

21. Ducklow, H.W., et al., *West Antarctic Peninsula: an ice-dependent coastal marine ecosystem in transition.* 2013.

22. Smith, R.C. and S.E. Stammerjohn, *Variations of surface air temperature and sea-ice extent in the western Antarctic Peninsula region.* Annals of Glaciology, 2001. **33**(1): p. 493-500.

23. Savoca, M.S., et al., *Baleen whale prey consumption based on high-resolution foraging measurements.* Nature, 2021. **599**(7883): p. 85-90.

24. Reisinger, R.R., et al., *Spatiotemporal overlap of baleen whales and krill fisheries in the Western Antarctic Peninsula region.* Frontiers in Marine Science, 2022: p. 1201.

25. Palsbøll, P.J., F. Larsen, and E.S. Hansen, *Sampling of Skin Biopsies from Free-Raging Large Cetaceans in West Greenland: Development of new Biopsy Tips and Bolt Designs.* International Whaling Commission Special Issue Series, 1991(13).

26. Gilson, A., et al., *Deer gender determination by polymerase chain reaction.* California Fish and Game, 1998. **84**(4): p. 159-169.

27. Aasen, E. and J.F. Medrano, *Amplification of the ZFY and ZFX genes for sex identification in humans, cattle, sheep and goats.* Bio/technology, 1990. **8**(12): p. 1279-1281.

28. Valsecchi, E. and W. Amos, *Microsatellite markers for the study of cetacean populations.* Molecular Ecology, 1996. **5**(1): p. 151-156.

29. Palsbøll, P., et al., *Primers for the amplification of tri-and tetramer microsatellite loci in baleen whales.* Molecular Ecology, 1997. **6**(9): p. 893-895.

30. Berube, M., et al., *Polymorphic di-nucleotide microsatellite loci isolated from the humpback whale, Megaptera novaeangliae.* Molecular Ecology, 2000. **9**(12): p. 2181-2183.

31. Waldick, R., M. Brown, and B. White, *Characterization and isolation of microsatellite loci from the endangered North Atlantic right whale.* Molecular Ecology, 1999. **8**(10): p. 1763-1765.

32. Baker, C.S., et al., *Strong maternal fidelity and natal philopatry shape genetic structure in North Pacific humpback whales.* 2013.

33. Constantine, R., et al., *Abundance of humpback whales in Oceania using photo-identification and microsatellite genotyping.* Marine Ecology Progress Series, 2012. **453**: p. 249-261.

34. Peakall, R. and P.E. Smouse, *GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research.* Molecular ecology notes, 2006. **6**(1): p. 288-295.

35. Kalinowski, S.T., M.L. Taper, and T.C. Marshall, *Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment.* Molecular ecology, 2007. **16**(5): p. 1099-1106.

36. Kellar, N.M., et al., *Determining pregnancy from blubber in three species of delphinids.* Marine Mammal Science, 2006. **22**(1): p. 1-16.

37. Riekkola, L., et al., *Application of a multi-disciplinary approach to reveal population structure and Southern Ocean feeding grounds of humpback whales.* Ecological Indicators, 2018. **89**: p. 455-465.

38. Pallin, L., et al., *Validation of a blubber-based endocrine pregnancy test for humpback whales.* Conservation Physiology, 2018. **6**(1): p. coy031-coy031.

39. Kellar, N.M., et al., *Low reproductive success rates of common bottlenose dolphins Tursiops truncatus in the northern Gulf of Mexico following the Deepwater Horizon disaster (2010-2015).* Endangered Species Research, 2017. **33**: p. 143-158.

40. Stammerjohn, S.E., et al., *Trends in Antarctic annual sea ice retreat and advance and their relation to El Niño–Southern Oscillation and Southern Annular Mode variability.* Journal of Geophysical Research, 2008. **113**(C3).

41. Stammerjohn, S., et al., *Regions of rapid sea ice change: An inter-hemispheric seasonal comparison.* Geophysical Research Letters, 2012. **39**(6).

42. Stammerjohn, S. and T. Maksym, *Gaining (and losing) Antarctic sea ice: variability, trends and mechanisms.* Sea Ice, 2017: p. 261-289.

43. Comiso, J., *Bootstrap Sea Ice Concentrations from Nimbus-7 SMMR and DMSP SSM/I-SSMIS, Version 3, [daily from 11/1978 to 12/2020]. boulder, Colorado USA. NASA National Snow and Ice Data Center Distributed Active Archive Center*. 2017.

44. Steinberg, D.K., et al., *Long-term (1993–2013) changes in macrozooplankton off the Western Antarctic Peninsula.* Deep Sea Research Part I: Oceanographic Research Papers, 2015. **101**: p. 54-70.

45. Ducklow, H.W., et al., *Marine pelagic ecosystems: the west Antarctic Peninsula.* Philosophical Transactions of the Royal Society of London B: Biological Sciences, 2007. **362**(1477): p. 67-94.

46. Conroy, J.A., et al., *Linking Antarctic krill larval supply and recruitment along the Antarctic Peninsula.* Integrative and Comparative Biology, 2020. **60**(6): p. 1386-1400.

47. Weinstein, B.G., et al., *Identifying overlap between humpback whale foraging grounds and the Antarctic krill fishery.* Biological Conservation, 2017. **210**: p. 184-191.

48. Chittleborough, R., *The breeding cycle of the female humpback whale, Megaptera nodosa (Bonnaterre).* Marine and Freshwater Research, 1958. **9**(1): p. 1-18.

49. R Core Team, *R: A language and environment for statistical computing.* 2021, R Foundation for Statistical Computing: Vienna, Austria.

50. Chittleborough, R., *An analysis of recent catches of humpback whales from the stocks in Groups IV and V.* Norsk Hvalfangst-Tidende, 1958. **47**: p. 109-137.

51. Clapham, P.J. and C.A. Mayo, *Reproduction of humpback whales (Megaptera novaeangliae) observed in the Gulf of Maine.* Rep. Int. Whal. Commn. Special, 1990(12): p. 171-175.

52. Weinstein, B.G. and A.S. Friedlaender, *Dynamic foraging of a top predator in a seasonal polar marine environment.* Oecologia, 2017. **185**(3): p. 427-435.

53. Kershaw, J.L., et al., *Declining reproductive success in the Gulf of St. Lawrence's humpback whales (Megaptera novaeangliae) reflects ecosystem shifts on their feeding grounds.* Global Change Biology, 2021. **27**(5): p. 1027-1041.

54. Miller, C.A., et al., *Body shape changes associated with reproductive status, nutritive condition and growth in right whales Eubalaena glacialis and E. australis.* Marine Ecology Progress Series, 2012. **459**: p. 135-156.

55. Lockyer, C., *Growth and energy budgets of large baleen whales from the Southern Hemisphere.* Food and Agriculture Organization, 1981. **3**: p. 379-487.

56. Saba, G.K., et al., *Winter and spring controls on the summer food web of the coastal West Antarctic Peninsula.* Nature communications, 2014. **5**(1): p. 1-8.

57. Ducklow, H.W., et al., *West Antarctic Peninsula: an ice-dependent coastal marine ecosystem in transition.* Oceanography, 2013. **26**(3): p. 190-203.

58. Ross, R.M., et al., *Palmer LTER: Patterns of distribution of five dominant zooplankton species in the epipelagic zone west of the Antarctic Peninsula, 1993–2004.* Deep Sea Research Part II: Topical Studies in Oceanography, 2008. **55**(18-19): p. 2086-2105.

59. Simard, Y., et al., *North Atlantic right whale shift to the Gulf of St. Lawrence in 2015, revealed by long-term passive acoustics.* Endangered Species Research, 2019. **40**: p. 271-284.

60. Cartwright, R., et al., *Fluctuating reproductive rates in Hawaii's humpback whales, Megaptera novaeangliae , reflect recent climate anomalies in the North Pacific.* Royal Society Open Science, 2019. **6**(3): p. 181463.

61. Fleming, A.H., et al., *Humpback whale diets respond to variance in ocean climate and ecosystem conditions in the California Current.* Global Change Biology, 2016. **22**(3): p. 1214-1224.

62. Klein, E.S., et al., *Impacts of rising sea temperature on krill increase risks for predators in the Scotia Sea.* PLOS ONE, 2018. **13**(1): p. e0191011.

63. Watters, G.M., J.T. Hinke, and C.S. Reiss, *Long-term observations from Antarctica demonstrate that mismatched scales of fisheries management and predator-prey interaction lead to erroneous conclusions about precaution.* Scientific Reports, 2020. **10**(1).

64. Reisinger, R.R., C. Johnson, and A.S. Friedlaender, *Marine Mammal Movement Ecology in a Conservation and Management Context*. 2022, Springer International Publishing. p. 149-192.

65. Johnson, C., et al., *Protecting Blue Corridors – a collaborative international report highlighting the challenges and solutions for migratory whales navigating national and international seas*. 2022: IWC.

66. Welsford, D., et al., *CCAMLR – IWC coordination: Incidents of whale bycatch in the Antarctic krill fishery*, SC/68D/HIM/04, Editor. 2022, IWC.

67. Bengtson Nash, S.M., et al., *Signals from the south; humpback whales carry messages of Antarctic sea-ice ecosystem variability.* Global Change Biology, 2018. **24**(4): p. 1500-1510.

68. Reid, K., et al., *Population dynamics of Antarctic krill Euphausia superba at South Georgia: sampling with predators provides new insights*, in *Top Predators in Marine Ecosystems: Their Role in Monitoring and Management*, C.J. Camphuysen, I.L. Boyd, and S. Wanless, Editors. 2006, Cambridge University Press: Cambridge. p. 249-261.

2.8 Tables

Table 2.1. Progesterone concentrations (ng g^{-1}) of non-calf female humpback whales biopsied along the WAP with a pregnancy assignment.

	Mean (ng g^{-1})	**SD**	**Min**	**Max**	**N**
Not-pregnant	1.37	1.35	0.08	6.86	297
Pregnant	217.20	223.12	19.50	1,940.52	306
Undetermined	12.59	2.94	8.88	18.47	13
Total					**616**

Table 2.2. Summary statistics of pregnancy assignments for non-calf female humpback whales sampled along the WAP during the summer (2013-2020). Numbers inside parenthesis do not include individuals with an undetermined pregnancy state and are excluded from the following columns. *Designates the total number of individual females analyzed for hormones across all eight years (i.e. across year recaptures have been removed).

Year	# Samples	# Individuals	Not-Pregnant		95% CL	Pregnant		95% CL
			N	%	Lower-Upper	N	%	Lower-Upper
2013	35	33	18	54.55	36.35 – 71.89	15	45.45	28.11 – 63.65
2014	41	40 (39)	7	17.95	7354 – 33.54	32	82.05	66.46 – 92.46
2015	48	48	23	47.92	33.29 – 62.81	25	52.08	37.19 – 66.71
2016	44	39	18	46.15	30.09 – 62.82	21	53.84	37.18 – 69.91
2017	79	75 (72)	10	13.89	6.87 – 24.06	62	86.11	75.94 – 93.13
2018	92	86 (83)	38	45.78	34.79 – 57.08	45	54.22	42.92 – 65.21
2019	116	109 (105)	56	53.33	43.34-63.13	49	46.67	36.87 – 56.66
2020	161	140 (139)	98	70.50	62.18 – 77.93	41	29.50	22.07 – 37.82
Total	**616**	**570 (558) 546***	**268**	**48.03**	**43.81 – 52.26**	**290**	**51.97**	**47.74 – 56.19**

Table 2.3. Results of generalized linear models (GLMs) evaluating variation in annual female humpback pregnancy rates as of function of lagged environmental factors (day of spring ice edge retreat (sIER), and krill abundance (Krill)) along the Western Antarctic Peninsula. The model in bold is the best fit model. n = 537 for all models. Only the top six models are displayed here. Weight – relative model support or probability.

	Pregnancy ~	*AICc*	*R^2 adj.*	*Δ AICc*	*% Dev. Expl.*	*Weight*
1.	**$sIER^{-1}$ + $sIER^{-2}$ + $Krill^{-1}$ + $Krill^{-2}$**	**687.2**	**0.090**	**0**	**8.98**	**0.747**
2.	$sIER^{-1}$ + $sIER^{-2}$ + $Krill^{-1}$	689.4	0.084	2.18	8.41	0.251
3.	$sIER^{-1}$ + $Krill^{-1}$	701.0	0.066	13.82	6.56	0.001
4.	$sIER^{-1}$ + $Krill^{-1}$ + $Krill^{-2}$	701.5	0.068	14.24	6.79	0.001
5.	$sIER^{-2}$ + $Krill^{-1}$ + $Krill^{-2}$	703.4	0.065	16.23	6.52	0.000
6.	$sIER^{-1}$ + $sIER^{-2}$ + $Krill^{-2}$	706.9	0.061	19.68	6.06	0.000

2.9 Figures

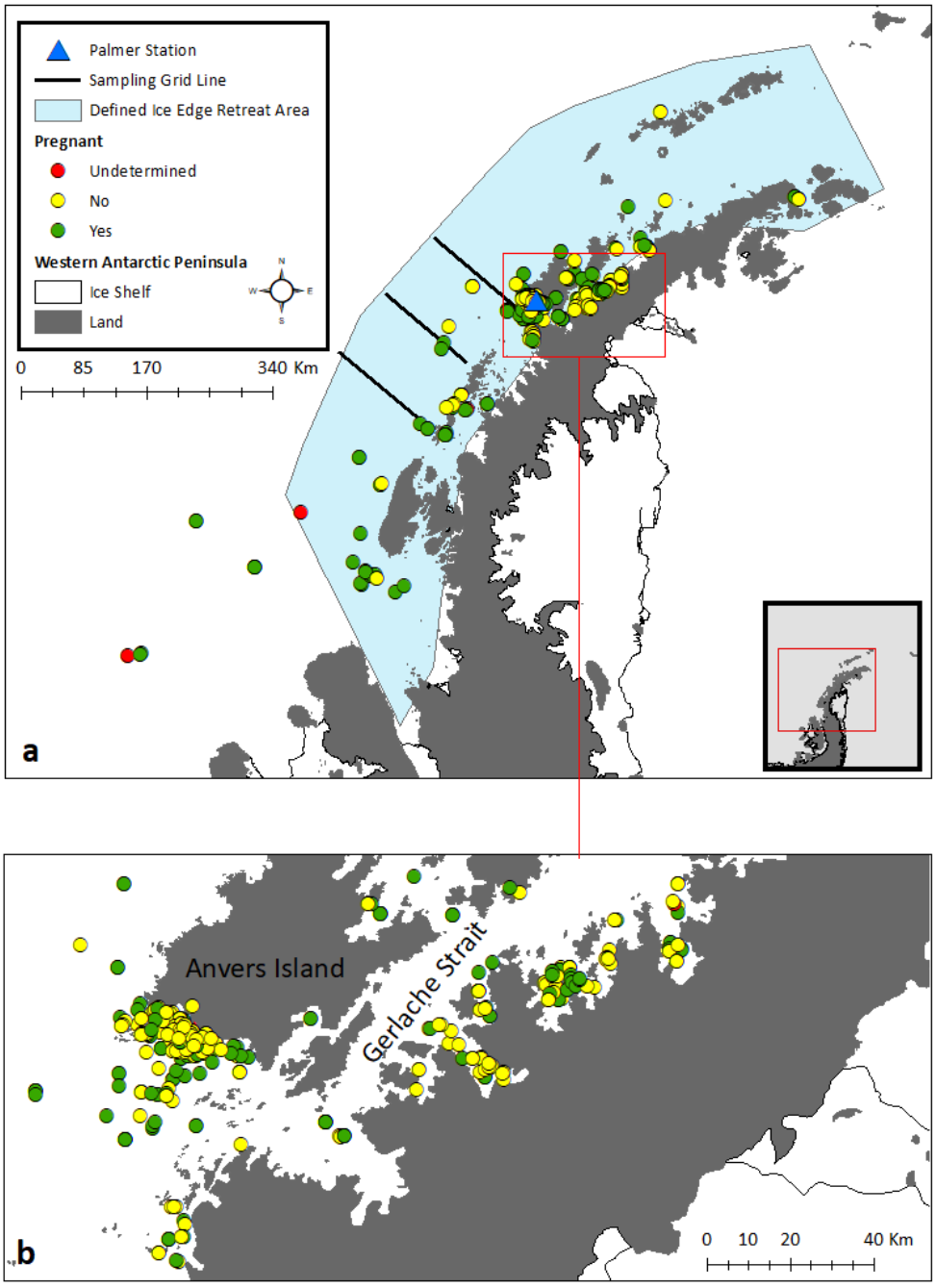

Figure 2.1. Pregnancy status of female humpback whales sampled along the Western Antarctic Peninsula (a) and in the Gerlache Strait and adjacent bays (b) during the 2013-2020 field seasons. Maps were created using ArcMap version 10.8.2 (2022, https://www.esri.com/en-us/home).

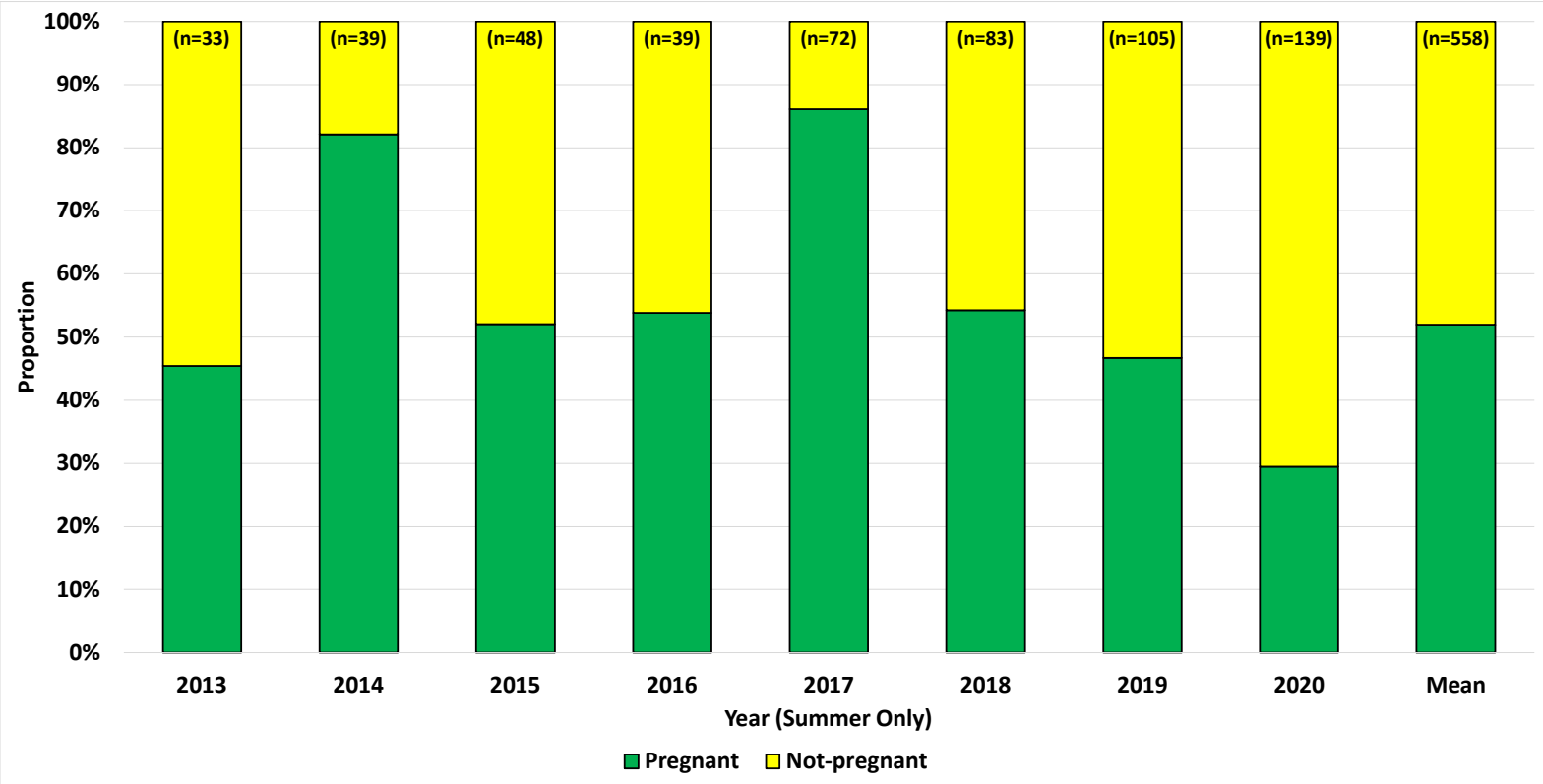

Figure 2.2. Inter-annual variation (summer only) in the proportion of assigned pregnant and not-pregnant (pregnancy rate) non-calf female humpback whales sampled along the WAP based on progesterone concentrations. Within year recaptures have been removed.

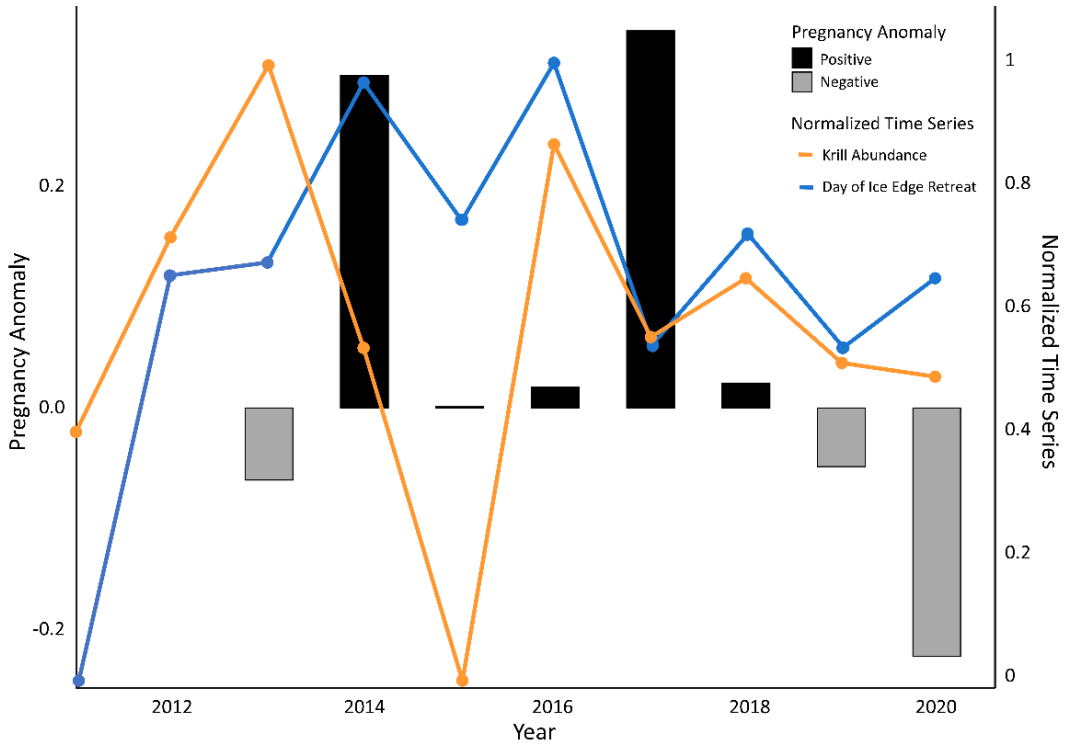

Figure 2.3. Annual time series. Humpback whale pregnancy rate anomalies along the Western Antarctic Peninsula from 2013-2020 and normalized krill abundance and day of ice edge retreat from 2011-2020.

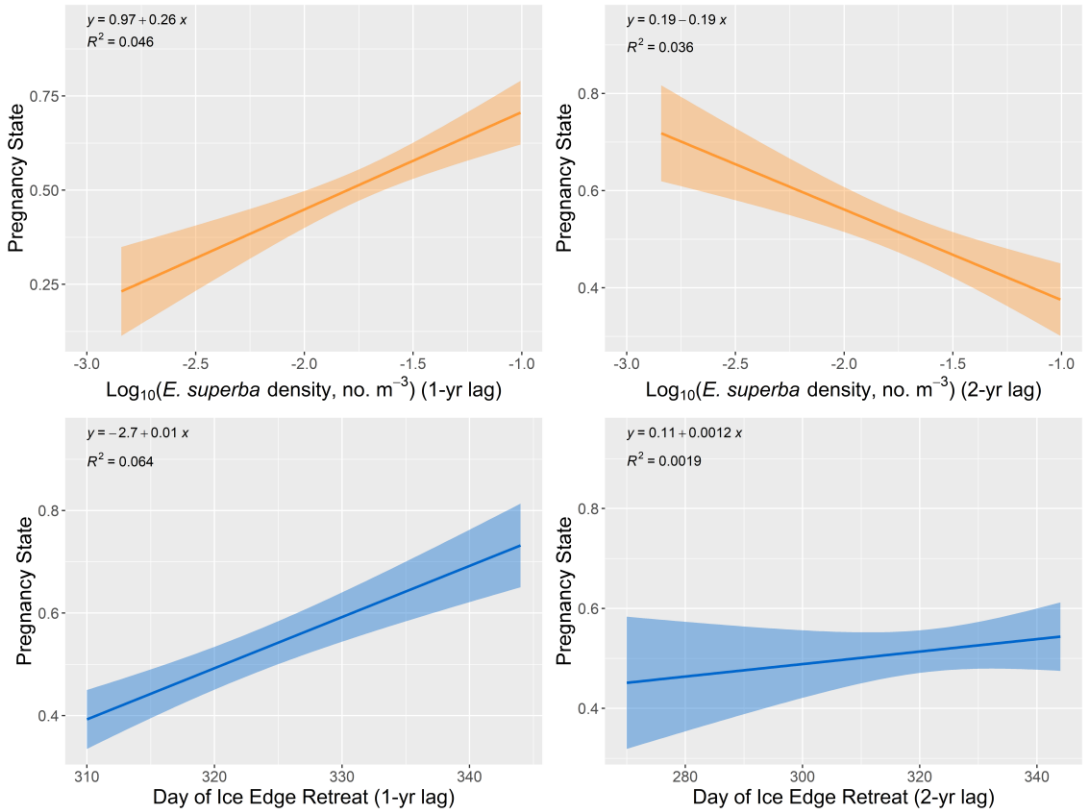

Figure 2.4. Linear relationships between krill abundance (orange lines), spring ice edge retreat (blue lines) and female humpback whale pregnancy rates. Shaded regions represent the confidence intervals.

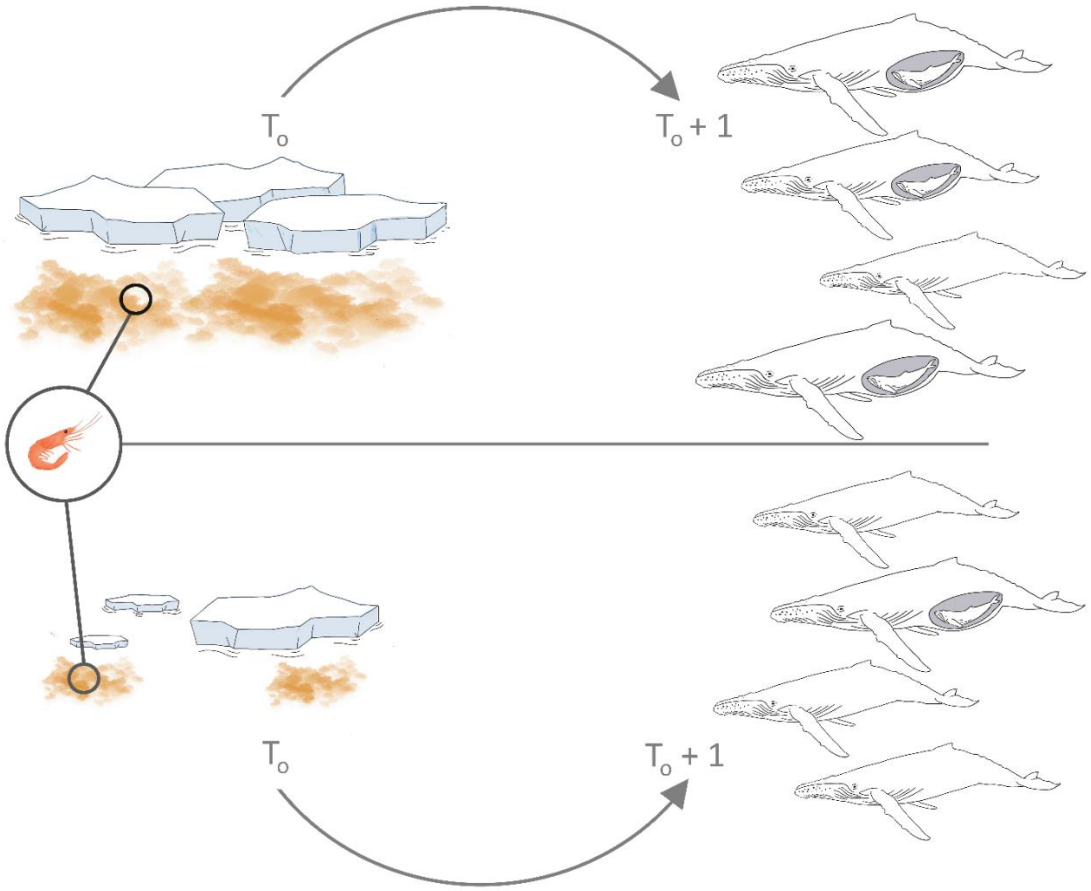

Figure 2.5. Illustration of the results of the generalized linear model showing the effects of krill abundance and the spring ice edge retreat (sIER) on humpback whale pregnancy rates along the WAP. Pregnancy rates were higher in years following high krill and a later sIER (top) and lower in years following low krill and an earlier sIER (bottom). Illustration provided by R. Jones.

Chapter 3: Variation in blubber cortisol levels in a recovering humpback whale population inhabiting a rapidly changing environment

Logan Pallin, Natalia Botero-Acosta, Debbie Steel, C. Scott Baker, Caroline Casey, Dan Costa, Jeremy Goldbogen, David Johnston, Nick Kellar, Michelle Modest, Ross Nichols, Darren Roberts, Megan Roberts, Oksana Savenko, Ari Friedlaender

3.1 Abstract

Glucocorticoids are regularly used as biomarkers of relative condition or health for individuals and populations. Around the Western Antarctic Peninsula (WAP), baleen whales have and continue to experience threats, including direct commercial harvest, prey limitations and habitat change driven by rapid warming, and increased human presence via ecotourism. Here, we measured demographic and monthly variation in blubber cortisol levels of humpback whales (*Megaptera novaeangliae*) over two years around the WAP. Cortisol concentrations were determined from 305 biopsy samples of unique individuals. We found no significant difference in the cortisol concentration between male and female whales. However, we observed significant differences across different demographic groups of females and a significant decrease in the population across months. We also assessed whether COVID-19-related reductions in tourism in 2021 along the WAP correlated with lower cortisol levels across the population. The decline in vessel presence in 2021 was associated with a significant decrease in blubber cortisol concentrations at the population level in humpback whales. Our findings provide critical contextual data on how these hormones vary naturally in a population over time, show direct associations between cortisol levels and human presence, and will enable comparisons among species experiencing different levels of human activity and disturbance.

3.2 Introduction

Wildlife biologists and natural resource managers must identify at-risk individuals and populations and determine why they are threatened. The underlying biological mechanisms associated with these threats are often poorly understood. Increasingly, the physiological response of wildlife to ecological perturbations is used as an indicator of poor population health [1, 2], and the associated costs (e.g., immunosuppression) and mechanisms of these stressors are of particular importance. Physiological indicators provide a directly integrated measure of the response in behavior and physiology and how these manifest to impacts of individual fitness and changes in population dynamics over time [1, 3, 4].

Glucocorticoids (GC) are a group of corticosteroid hormones produced in the adrenal cortex that mainly regulate metabolism and endocrine responses to stressors [5]. For example, GC concentrations primarily suppress glucose uptake, providing energy to the body when an organism's energy demands exceed energy availability (e.g., fight or flight response) [6]. However, it is important to note that GC physiology can be both species- and context-specific, and thus careful consideration needs to be used when interpreting values [6].

In vertebrates, GCs (including cortisol) have been analyzed in several different sample matrices as possible indicators of a stress state. In the last 20 years, captive marine mammal research programs, in which endocrine measures can be easily obtained, have enhanced our understanding of stress responses to acute stimuli [7, 8]. How these short-term responses translate into long-term population impacts, particularly in large, wild, long-lived vertebrates, remains poorly understood [9, 10]. Thus, it is vital to assess the

potential effects that repeated exposure to stressors may have on population dynamics, particularly those experiencing rapid changes in human activities and most susceptible to current and projected climatic changes. If we measure GCs in tissues that reflect long-term exposure to stressors, it is possible to monitor changes in the stress states experienced by populations over prolonged periods[11].

Stressors in natural populations occur in a variety of forms. Studies on marine mammals have documented associations between cortisol levels and human activity, such as vessel traffic and noise[9, 12]. Around the Antarctic Peninsula, baleen whales have and continue to experience a variety of threats ranging from direct depletion from commercial whaling, prey limitations and habitat change driven by rapid warming, and an increase in human activity in the form of ecotourism. Baleen whale populations in the Southern Hemisphere (SH) were severely depleted during the commercial whaling period (1904-1980)[13]. In some regions of the SH, like the nearshore waters along the Western Antarctic Peninsula (WAP), whales are now confronted with some of the most severe impacts of global change. For example, the WAP, an essential feeding ground for an estimated 11,700 humpback whales [14], has experienced a rise in winter temperature of nearly 5°C since the 1950s, resulting in the collapse of ice shelves, the retreat of glaciers, and the exposure of new terrestrial habitat [15]. While the lack of other baleen whales in this region has likely reduced overall competition for resources[16], there is evidence that prey resources are decreasing due to reduced cycling of nutrients[17], and these reductions in prey are manifesting as decreased pregnancy rates in female humpback whales along the WAP [Pallin et al. in prep]. Further, humpback whales are the most abundant baleen whale species in this region and thus are considered sentinel species for ecosystem

processes and climate-related changes. All these ecosystem changes have critical implications for the population health and growth of whales in this region.

Humpback whales along the WAP appear to be recovering quite rapidly [18], even as human activities (i.e., vessel tourism and the commercial krill fishery) in this region are quickly expanding [19]. Specifically, along the WAP, we have seen a rise in tourism from 6,700 visitors among 59 voyages run by 12 vessels in 1990 to over 73,000 visitors among 408 voyages run by 62 vessels during the 2020 season [20]. Previous studies have shown associations between cumulative anthropogenic impacts (i.e., sound pollution, fishing, vessel traffic) and cortisol levels in baleen whales in other parts of the world[12, 21]. Therefore, Antarctic whales may be stressed by human activities related to ecotourism [12, 22-24]. However, to the best of our knowledge, no studies have assessed this relationship in populations that are recovering largely in the absence of direct human activity.

Despite the International Association of Antarctic Tour Operators (IAATO) having self-imposed regulations on approaching whales and, more recently, on vessel speed, there is no way to account for the cumulative numbers of vessels and time these vessels are spending in the presence of whales. Understanding these impacts is crucial for species like humpback whales that exhibit two distinct seasonal modes. Humpback whales along the WAP migrate annually from their low latitude breeding grounds off the Northwest coast of South America to their high latitude feeding grounds along the WAP[25]. These two distinct behaviors make particular times of their annual life history and demography more susceptible to stressors. In whales in the wild, collecting blood for endocrine monitoring is currently not feasible. Likewise, collection of feces and blow sputum,

although non-invasive, is logistically challenging and susceptible to contamination from seawater[26]. Therefore, skin-blubber biopsies, which can be readily collected across both seasonal life-history strategies and archived, provide a viable opportunity to test whether prolonged exposure to potential stressors is related with increased blubber cortisol concentrations.

The COVID-19 pandemic created a unique opportunity by removing humans (e.g., science and tourism) from waters around the WAP in 2021, resulting in an "anthropause." Quantifying these impacts in the absence of anthropogenic disturbance is often challenging. The goals of this study were to (i) describe the demographic and monthly trends in blubber cortisol levels of humpback whales around the Antarctic Peninsula over two years (2019-2020) when human presence was consistent and (ii) test whether reductions in vessel presence along the WAP in 2021 as a result of the COVID-19 pandemic were associated with a decrease in blubber cortisol levels. Our findings provide critical contextual data on how these hormones vary naturally in a population. Further, our data provide the basis for more focused comparisons with other populations of whales in locations with differing levels of human activity.

3.3 Methods

3.3.1 Biopsy collection

We collected skin and blubber biopsy samples from humpback whales during the 2019-2021 austral summer and fall (January-July) field seasons using standard biopsy techniques[27]. Samples were collected opportunistically during dedicated research cruises or from platforms of opportunity, including tour vessels, in the nearshore waters

along the western side of the Antarctic Peninsula (Fig. 1). Further, a majority of our samples were collected in the bays and fjords adjacent to the Gerlache Strait and near Palmer Station at the Southern end of Anvers Island. Both of these regions are highly used by humpback whales[28] and tourism vessels [19].

We used a crossbow with modified bolts and 40mm stainless steel cutting tips (CetaDart) to obtain samples from a distance of 10-30 meters targeting the area of the body below the dorsal fin when the whale surfaced to breathe [29]. Humpback whales were sampled opportunistically from all age and sex classes, including calves. Samples were stored frozen whole at -20^{o} C until used for analysis. Supplementary data (i.e., location, group size, group composition) were also recorded with every biopsy event.

3.3.2 DNA profiling

A standard DNA profile, including sex-specific markers, and microsatellite genotypes, was used to identify individual whales. DNA was extracted from the skin-blubber interface using a commercially available kit (DNeasy 96 Blood & Tissue Kit, Qiagen, Hilden, Germany). The sex of each sampled whale was determined by amplification of sex-specific markers following the protocols of Gilson et al. [30, 31]. Results were compared to controls for a known male and female using gel electrophoresis.

Samples were genotyped using 10 previously published microsatellite loci to resolve the individual identity of each sampled whale and remove potential duplicates (Table 3.1) [32-36]. Alleles were sized and binned using the software program Genemapper v3.7 (Applied Biosystems). The total number of amplified loci for a given sample was considered as an added quality control threshold. Given the estimated probability of

identity for these loci from previous studies [18, 37], we considered samples matching at a minimum of seven loci to be recaptures of the same individual. Samples with fewer than seven microsatellite loci were repeated or excluded. Recaptures of the same individual were removed from analysis. The expected probability of identity (P_{ID}; the probability that two individuals are drawn at random from a population will have the same genotype by chance) for each locus was calculated in GenAlEx v6.5 [38]. Cervus 3.0.7 [39] was used to compute the number of alleles (K), observed and expected heterozygosity (HO and HE), and the probability of identity for all individual matches.

3.3.3 Hormone extraction and quantification

We extracted steroid hormones from the blubber portion of the biopsy samples following standard methods [18, 40]. Briefly, to quantify hormone biomarkers (i.e., progesterone and cortisol), a cross-sectional sub-sample (~0.15g) spanning from the epidermis-blubber interface to the most internal layer of the biopsy was sub-sectioned. These sub-samples were then homogenized multiple times using an automated, multi-tube homogenizer (Omni International). Following the completion of the homogenization process, target hormones were isolated using a series of chemical washes, evaporations, and separations. The final hormone residue was stored at -20^{o} C until analysis. The amount of hormone in each extract was quantified using a commercially available enzyme immunoassay. The progesterone EIA kit (EIA kit 900-011, ENZO Life Sciences, Farmingdale, NY) that was utilized in this study has 100% reactivity with progesterone and an assay detection limit between 15 and 500 pg/mL. Two additional standard dilutions were added to allow for a lower detection limit of the standard curve to 3.81 pg/mL. The cortisol EIA kit (EIA kit K003-H1W, Arbor Assay, Ann Arbor, MI) that was

used in this study has 100% reactivity with cortisol and an assay detection limit between 50 and 3200 pg/mL. Two additional standard dilutions were added to allow for a greater detection limit of the standard curve from 25pg/mL to 6400 pg/mL. All samples were run blind and in duplicate for both hormones. For both assays, extracts were further diluted and re-run if reliable hormone concentrations were not obtained during the initial assay process. For cortisol, to avoid censoring the data, samples that fell below the detectable range of the assay were assigned a value half of the lowest standard curve (i.e. 12.5 pg/mL). Each assay was evaluated for color development using a Biotek plate reader Epoch (Gen5™ software [Biotek, USA]) with read and correction wavelengths of 405 nm and 630 nm for progesterone and 450 nm and 630 nm for cortisol. Blubber hormone concentrations were then transformed into nanograms of cortisol per gram of blubber (wet weight).

As part of our routine quality control, we determined the extraction efficiency by spiking subsamples of blubber from a stranded, dead humpback whale with the target hormone [40]. The percentage of hormone that was recovered after the extraction was calculated and each sample concentration was adjusted to this efficiency before statistical analyses. An extraction efficiency greater than 60% was acceptable. If the extraction efficiency was less than 60%, the sample extracts were discarded, and the blubber samples were re-extracted. Additionally, we conducted a parallelism test to gauge the performance of humpback blubber extracts with the cortisol EIA kit. This was done by taking a serially diluted pool of sample extracts and running them, along with the standard controls of the assay, to determine whether the linear decrease in measured values of the pooled sample was parallel to the standard curve. This would indicate that the assay measures the same

antigens in the blubber as in the standards. Five extracts from four individual whales were pooled together (4-female; 2-biopsied, 2-stranded), and the pooled sample concentrations were made by diluting five times from the neat preparation to 1/32, decreasing by a factor of two. Each dilution was run two times, and the resulting curve of the concentrations as a function of the mean optical density was compared to the standard curve.

3.3.4 Pregnancy classification

Pregnancy of female humpback whales was assigned following previously published methods [41]. Briefly, a series of biopsy samples (n = 29) were collected from individuals of a known life-history stage from the Gulf of Maine feeding aggregation by the Center for Coastal Studies in Provincetown, MA. Using these control samples from the Gulf of Maine, the pregnancy state relative to blubber progesterone concentrations was modeled using a standard logistic regression model [42]. Each WAP humpback sample, of unknown pregnancy status, was entered into the model and the model returned a probability of being pregnant for each female sampled [42]. If the probability of being pregnant was greater than 99%, that female was given a pregnant assignment. If the probability of being pregnant was less than 1%, that female was given a not-pregnant assignment. If a biopsied female's probability of being pregnant was between those two bounds, that female received an undetermined pregnancy assignment.

3.3.5 Classification of life history groups

We classified individual females into four life history categories based on field observations during biopsy events and blubber progesterone concentrations. These

included lactating females, pregnant females, lactating and pregnant females, and lastly resting females. Lactating females were those females that had a calf travelling near the female and whose behavior was in sync with the presumed mother (so although we do not know for certain these females were lactating, their behavior was consistent with females that are known to be lactating). Pregnant females were determined using progesterone concentrations as described above. Lactating and pregnant females were females that were pregnant based on progesterone concentrations and in proximity with a calf. Lastly, resting females included females that were not pregnant and or lactating, but could also have included females that recently lost a pregnancy.

3.3.6 Vessel Activity Data

Vessel activity data for the Antarctic Peninsula region was obtained from the International Association of Antarctic Tour Operators (IAATO) statistics repository (https://iaato.org/information-resources/data-statistics/). IAATO has been carefully monitoring, analyzing, and reporting Antarctic tourism trends since 1991 as part of its commitment to the effective self-management of guest activities in the region. As part of these reports, IAATO generates seasonal statistics among the roughly 100 member vessels, including the number of operators, vessels, and voyages in each season, as well as the number of passengers visited. As part of the IAATO guidelines, member vessels are grouped into four categories: category 1 (12-200 passengers), category 2 (201-500 passengers), cruise only (501+ passengers), and yacht (12 passengers max). We used the total number of voyages across all categories as our explanatory metric to assess the relationship between humpback whale cortisol levels and human activity. This analysis

does not account for the activity of vessels along the peninsula that are not IAATO members.

3.3.7 Data preparation and statistical analyses

All statistical analyses were performed in R [43]. Cortisol concentrations were log-transformed to improve normality. We tested for differences in cortisol concentrations between sexes, pregnancy states, and across years of high and low vessel activity using a *Welch's two sample t-test.* We used an ANOVA to test for differences in cortisol concentrations across months and life history groups. We used a post-hoc Tukey's Honest Significant Difference (HSD) test to check for differences among individual explanatory variables (e.g., life history class). We used a general linear model to examine the relationship between ordinal date and vessel activity on cortisol concentrations. All cortisol values are reported as mean ± standard error (ng/g wet weight) unless otherwise specified. For all statistical tests, we considered a *p*-value of less than 0.05 to be significant. Sampling periods for this study were bounded to the months of January - March of 2019-2021 when sampling was most uniform across the three years.

3.4 Results

We analyzed biopsy samples collected from 305 individual humpback whales in the nearshore waters around the WAP over the course of three field seasons from 2019-2021 (2019 n = 134, 2020 n = 145, 2021 n =26; Fig. 3.1). Sampling logistics were greatly reduced during 2021 due to the COVID-19 pandemic. Annual sampling details can be found in Table 3.2. The mean cortisol concentration for the 305 individual whales sampled was 0.4 ± 0.28 ng/g.

3.4.1 Individual identification and sex

On average, 9.97 loci were successfully genotyped per individual. The average P_{ID} for any given combination of 7 loci ranged from $8.02x10^{-11}$ – $5.74x10^{-8}$, consistent with previous studies. We analyzed 183 individual females and 122 individual males across all three years (Table 3.2).

3.4.2 Validation of humpback cortisol assays

Based on the concentrations observed from a series of spiked controls, our average extraction efficiency for the cortisol assay was 82.40% ± 11.75 (minimum 60.54%, maximum 100.14%). The EIA standards and the pooled serially diluted blubber extracts exhibited statistical parallelism and high accuracy (Fig. 3.2, R^2 = 0.999, slope = 1.01); an indication that the assay is measuring the same antigens in the blubber as in the standards and is, therefore, suitable for use with humpback whale blubber tissues extracts. Additionally, our calculated intra-assay and inter-assay COV from a series of replicated samples were 6.14 and 14.93%, respectively. These results are consistent with previous studies on humpback whales[10]. Thirty-five samples had values that were undetectable by the assay used in this study.

3.4.3 Demographic and monthly variation in cortisol concentrations across years

The mean cortisol concentration for all female and male humpback whales sampled in 2019 and 2020 was 0.40 ± 0.22 and 0.44 ± 0.37 ng/g, respectively (Table 3.2). These were not significantly different (t = -0.131, df = 196.8, *p* = 0.896, Fig. 3.3a). Of the 172 individual female humpback whales, 71 were classified as pregnant (41.28%, mean = 0.46 ± 0.22 ng/g) and 101 individuals were classified as not pregnant (mean = 0.36 ± 0.21 ng/g).

Pregnant females had significantly higher cortisol levels than not pregnant females (t = -3.250, df = 161.28, p = 0.001, Fig. 3.3b). Of the 71 pregnant females, 57 females were classified as pregnant but presumably not lactating (mean = 0.44 ± 0.22 ng/g) while 14 were assigned as lactating and pregnant (mean = 0.51 ± 0.25 ng/g). Additionally, of the 101 females classified as not pregnant, 79 females were assigned as resting (mean = 0.36 ± 0.2 ng/g), and 22 were assigned as lactating (mean = 0.35 ± 0.23 ng/g). We observed a significant difference in the cortisol concentrations among these different life history groups (r^2 = 0.044, $F_{3,168}$ = 3.603, p = 0.015, Fig. 3.3c). However, a *post hoc* analysis revealed no two life history groups were different from each other.

During the 2019 and 2020 season, we sampled 109 humpbacks in January (mean = 0.43 ± 0.27 ng/g), 85 in February (mean = 0.44 ± 0.23 ng/g), and 85 in March (mean = 0.36 ± 0.35 ng/g), and found a significant negative relationship between blubber cortisol levels and month (r^2 = 0.030, $F_{2,276}$ = 5.253, p = 0.006, Fig. 3.3d). A *post hoc* multiple comparisons analysis revealed that the cortisol concentrations among humpback whales sampled in March were significantly lower than in both January (p = 0.028) and February (p = 0.008). When comparing the relationship between cortisol concentrations and day of year, we found no significant relationship (r^2 = 0.008, $F_{1,303}$ = 2.55, p = 0.111).

3.4.4 Association of population-level stress state and human activity

The number of IAATO-registered vessels along the WAP was greatly reduced in the 2021 ecotourism season. Specifically, IAATO reported 360 voyages among 56 vessels in 2019, 408 voyages among 62 vessels in 2020, and only two voyages among two registered yachts in 2021.The mean cortisol concentrations for 2019, 2020, and 2021 were 0.45 ± 0.34 ng/g, 0.38 ± 0.22 ng/g, and 0.28 ± 0.14 ng/g respectively. When combined based on

similarity in levels of human activity, the mean cortisol concentration during the 2019/2020 seasons (mean = 0.41 ± 0.29 ng/g) was significantly higher than the mean cortisol concentrations during the 2021 season (mean = 0.28 ± 0.14 ng/g; t = 2.913, df = 33.1, p = 0.006, Fig. 3.4).

3.5 Discussion

Our study provides critical contextual data on how blubber cortisol levels vary naturally in a population of humpback whales inhabiting Antarctic waters and shows that a post-COVID-19 decrease in vessel activity along the WAP was related to a decrease in blubber cortisol levels. Cortisol concentrations in humpback whales along the WAP were generally low but comparable to levels measured in the blubber of other baleen whale species [10, 44, 45]. This information is essential for monitoring population dynamics in the face of continued anthropogenic and environmental changes. Furthermore, these results will facilitate inter-population comparisons of cortisol levels among areas with differing levels of human activity and disturbance.

3.5.1 Demographic variation in blubber cortisol

We observed no significant difference in cortisol concentrations between male and female humpback whales. However, we did observe significant variation between different demographic groups of females. Several studies have found that baseline and stress-induced GC levels vary due to intrinsic biological factors, such as sex, reproductive state, and age in other species[3, 46-48]. For example, male North Atlantic right whales show significantly higher levels of cortisol related to the mobilization of energy reserves needed for breeding activity [44, 46]. Given that male humpback whales sampled in the

current study were sampled roughly four months post peak breeding, we would not anticipate that they would have elevated cortisol levels as a result of previous breeding activities. In our study, pregnant and lactating while pregnant females had the highest observed cortisol levels among female-specific demographic groups, similar to other studies[46, 49]. Pregnancy involves a series of metabolic and physiological adjustments to help maintain the increased energy expenditure needed for fetal development[50, 51]. For pregnant whales, endocrine responses reported to date include high progesterone concentrations to help establish and maintain pregnancy[18, 45, 52] and elevated cortisol[46]. Increased cortisol concentrations in pregnant whales is not unexpected, as the adrenal gland responds to endocrine changes throughout gestation[50]. Conversely, resting and lactating but not pregnant females had the lowest cortisol levels, consistent with other studies[53].

Many studies of terrestrial mammals have also shown that age drives changes in baseline GC levels[3, 48]. In our study, the age of sampled whales is unknown; thus, we cannot account for its potential effect on GC levels. It is also important to note that while we found significant differences in cortisol among different life history groups in humpback whales, similar studies on both humpback[10] and blue whales[45] found no effect of life history stage. This could be a result of low sample sizes and/or population-specific differences. Future work should incorporate detailed sighting histories in combination with cortisol monitoring to better understand how susceptible different age and life history groups are to the continued changes occurring along the WAP.

3.5.2 Temporal variation in blubber cortisol

We observed higher cortisol in humpback whales sampled during the early part of the feeding season in January and February than later in March. The WAP is a very dynamic marine ecosystem, and the rapid climatic shifts in this region exacerbate the annual and seasonal variability in the ecosystem [15, 54, 55]. It is thought that cortisol regulation evolved as a mechanism to balance energetic demands between behavioral modes associated with different seasonal states[3]. In Steller sea lions, cortisol levels significantly increased during periods of nutritional stress and were negatively correlated with percent body fat [48]. Thus, it is likely that temporal variability in abiotic (i.e., available habitat) and biotic (i.e., prey availability) stressors may manifest in changes in population cortisol levels in humpback whales along the WAP.

Steroid hormone integration and clearance in blubber, particularly in large whales, is still poorly understood. It is possible that the higher levels early in the feeding season could be a residual carryover from the most recent breeding season[7, 56]. Several species, including North Atlantic right and humpback whales, show increases in baseline cortisol levels in relation to breeding activities[3, 10, 46]. Conversely, these high early season cortisol levels, followed by a decrease later in the season as thermal stores are likely replenished, could also be the result of homeothermic responses (i.e., increase in baseline metabolic rate) associated with polar waters[51, 57, 58]. Similar occurrences have been observed in both dolphins and pinnipeds[57, 58]. However, thermal models suggest that the lower critical temperature for large whales is much lower than the minimal seawater temperature they encounter[59]. Therefore, the monthly effects documented in this study are more likely nutritionally derived, as in the case of the Steller sea lions[48]. This is also supported by recent observations that humpback whale feeding rates are highest

early in the feeding season (Nichols et al.[60]) when whale body condition is at its lowest[61], and they are nutritionally stressed. While cortisol likely plays a role in the physiological response to nutritional stress and changes in energy stores, other hormones work in tandem to maintain homeostasis. Thus, continued annual and seasonal sampling of this species across breeding and feeding grounds and incorporating other nutritional biomarkers (e.g., triiodothyronine) is needed to better understand how short and long-term environmental variability affects population-level variation in cortisol levels.

3.5.3 Biological relevance of low blubber cortisol levels

While we observed significant differences in cortisol concentrations across months and among some demographic groups, these differences might not be biologically significant. Other studies have documented much wider ranges in blubber cortisol concentrations in cetaceans. However, these were done in regions that, on average, contain a greater human presence compared to the WAP. For example, Graham et al.[44] reported a six-fold increase in the concentrations between entangled and healthy North Atlantic right whales. Kellar et al.[62] found that stranded common dolphins had, on average, 6.1 times higher blubber cortisol values than bycaught dolphins in the eastern North Pacific. These studies clearly documented that high adrenal activation due to stressful events can be captured in blubber tissue. We sampled one juvenile humpback whale (female) during the 2022 (January) field season that was actively entangled in fishing gear around its caudal peduncle and fluke (unpublished data). This individual had a mean blubber cortisol concentration of 0.70 ng/g. Though only 1.75 times greater than our overall mean concentration (0.40 ng/g),and a much smaller difference in magnitude than reported

above, this whale was under stress as the entanglement likely occurred outside of the Antarctic, implying the animal had been carrying the gear during its migration and for the entire period it had been on the feeding grounds. If human activity affects cortisol levels in humpback whales along the WAP, the magnitude of this effect appears smaller compared to other studied populations of baleen whales[12]. Furthermore, an individual's behavioral and endocrine response to a stressor are complex and highly variable [6]. As a result, careful consideration must be taken when directly connecting stress physiology to individual and population level effects on fitness and health. Thus, it is important that humpback whale blubber cortisol levels continue to be monitored. This will be necessary to understand how cortisol variability translates into meaningful biological differences at the population and individual level.

3.5.4 Conservation implications and future work

We observed a significant decrease in cortisol concentrations in 2021, a year when human activity along the WAP was greatly reduced due to the COVID-19 pandemic. However, our sample size for the 2021 season is relatively small. Even so, we do not believe these findings result from a temporal artifact in our sampling because the median ordinal dates for which samples were collected across the three seasons (2019-2021) are 36, 46, and 35, respectively. Ecotourism, especially whale watching, is a growing industry worldwide, particularly along the WAP. Vessel disturbance, both from the physical presence of boats and the associated vessel noise, has both short-term and long-term physiological and behavioral impacts on marine mammals, including humpback whales [12, 23, 63-66]. Sound, like that generated from cruise ships, is a persistent source of low-frequency ocean noise and travels effectively in sea water, moving approximately five

times faster than it does in air [67]. These properties allow sound to dramatically alter the soundscape of marine environments, masking essential sounds produced and heard by marine animals, including whales, as well as potentially changing animal behavior and physiology [67]. For example, post-9/11 decreases in background underwater noise from reduced ship traffic corresponded to a decrease in fecal GC levels in North Atlantic right whales [12]. Conversely, in Alaskan humpback whales, cortisol levels in groups of whales in high vessel-use areas were not significantly higher than in other, low vessel regions [9]. A conclusion drawn from this study was that humpback whales in Alaska were likely habituated to vessel presence. Though this study was conducted on a separate population of humpback whales, it is plausible that WAP humpback whales also have some internal habituation to vessel presence. Several studies have documented decreases in cortisol levels as a result of habituation[68]. However, even if habituated, this does not necessarily mean that whales will not become more susceptible to collisions, propeller strikes, and entanglement in fishing gear [69], especially as the ecotourism and krill fishing industry grow along the WAP. Continued monitoring of this population is required to better understand the interplay between demographic, temporal, and anthropogenic stressors and how these manifest in both short and long-term impacts on population health and dynamics.

3.6 Conclusions

While our sample size in 2021 was relatively small, we still detected a significant decrease in cortisol levels when human presence (i.e. number of vessels) along WAP was greatly reduced. Without a planned cessation of ecotourism, future work is needed to further characterize and compare the relationship between human disturbance and blubber

cortisol levels in humpback whales given the natural variability in GC levels. However, this study demonstrates the continued efficacy of using blubber biopsies to measure hormone concentrations from whales in the wild and provide critical contextual information on how these hormones vary naturally in a population of recovering baleen whales. While we found an association between cortisol levels and human activity, this population is relatively naïve in its exposure to human activity as there are minimal human activities in these waters outside of tourism (as well as scientific ships and a commercial krill fishery). Throughout their broad distributions in coastal areas in both hemispheres, humpback whales occur in both some of the most heavily trafficked regions and some of the least. This dichotomy makes humpback whales an ideal species to use for comparing how human disturbance impacts cortisol levels among wildlife. Specifically, as the environment continues to change along the WAP and human activities continue to grow in this region, we may observe changes in the stress states of these whales over the long-term. By combining these measurements with new technologies for assessing biomarkers from additional sample matrices (i.e., blow sputum), more specific markers of nutritional state (i.e., thyroid hormones)[48], as well as photogrammetry to evaluate body condition, we can better understand the relationships between human disturbance and animal stress.

References

1. Wikelski, M. and S.J. Cooke, *Conservation physiology.* Trends in Ecology & Evolution, 2006. **21**(1): p. 38-46.

2. Cattet, M., et al., *Understanding grizzly bear health in the context of changing landscapes.* Foothills Model Forest Grizzly Bear Research Program Annual Report, 2005: p. 80-86.

3. Reeder, D.M. and K.M. Kramer, *STRESS IN FREE-RANGING MAMMALS: INTEGRATING PHYSIOLOGY, ECOLOGY, AND NATURAL HISTORY.* Journal of Mammalogy, 2005. **86**(2): p. 225-235.

4. Dunlop, R.A., et al., *Assessing population-level effects of anthropogenic disturbance on a marine mammal population.* Frontiers in Marine Science, 2021. **8**: p. 230.

5. Atkinson, S., et al., *Stress physiology in marine mammals: how well do they fit the terrestrial model?* Journal of Comparative Physiology B, 2015. **185**(5): p. 463-486.

6. Romero, L.M. and U.K. Beattie, *Common myths of glucocorticoid function in ecology and conservation.* Journal of Experimental Zoology Part A: Ecological and Integrative Physiology, 2022. **337**(1): p. 7-14.

7. Champagne, C.D., et al., *Blubber cortisol qualitatively reflects circulating cortisol concentrations in bottlenose dolphins.* Marine Mammal Science, 2017. **33**(1): p. 134-153.

8. Champagne, C.D., et al., *Comprehensive endocrine response to acute stress in the bottlenose dolphin from serum, blubber, and feces.* General and comparative endocrinology, 2018.

9. Teerlink, S., L. Horstmann, and B. Witteveen, *Humpback Whale (Megaptera novaeangliae) Blubber Steroid Hormone Concentration to Evaluate Chronic Stress Response from Whale-Watching Vessels.* Aquatic Mammals, 2018. **44**(4).

10. Mingramm, F.M., et al., *Blubber cortisol levels in humpback whales (Megaptera novaeangliae): A measure of physiological stress without effects from sampling.* General and Comparative Endocrinology, 2020. **291**: p. 113436.

11. Saco, Y., et al., *Evaluation of serum cortisol, metabolic parameters, acute phase proteins and faecal corticosterone as indicators of stress in cows.* The Veterinary Journal, 2008. **177**(3): p. 439-441.

12. Rolland, R.M., et al., *Evidence that ship noise increases stress in right whales.* Proceedings of the Royal Society B: Biological Sciences, 2012. **279**(1737): p. 2363-2368.

13. Rocha, R., P.J. Clapham, and Y.V. Ivashchenko, *Emptying the oceans: a summary of industrial whaling catches in the 20th century.* Mar. Fish. Rev, 2014. **76**: p. 37-48.

14. Comission, I.W., *Report of the scientific committee.* Journal of Cetacean Research and Management, 2021. **SC/68C**.

15. Ducklow, H.W., et al., *West Antarctic Peninsula: an ice-dependent coastal marine ecosystem in transition.* Oceanography, 2013. **26**(3): p. 190-203.

16. Laws, R., *Seals and whales of the Southern Ocean.* Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, 1977: p. 81-96.

17. Savoca, M.S., et al., *Baleen whale prey consumption based on high-resolution foraging measurements.* Nature, 2021. **599**(7883): p. 85-90.

18. Pallin, L.J., et al., *High pregnancy rates in humpback whales (Megaptera novaeangliae) around the Western Antarctic Peninsula, evidence of a rapidly growing population.* Royal Society Open Science, 2018. **5**(5).

19. Bender, N.A., K. Crosbie, and H.J. Lynch, *Patterns of tourism in the Antarctic Peninsula region: a 20-year analysis.* Antarctic Science, 2016. **28**(3): p. 194-203.

20. Operators, I.A.o.A.T., *International Association of Antarctica Tour Operators*. 2019.

21. Trumble, S.J., et al., *Baleen whale cortisol levels reveal a physiological response to 20th century whaling.* Nature Communications, 2018. **9**(1).

22. Cates, K.A., et al., *Corticosterone in Central North Pacific Male Humpback Whales (Megaptera novaeangliae): Pairing Sighting Histories with Endocrine Markers to Assess Stress.* General and Comparative Endocrinology, 2020: p. 113540.

23. Sprogis, K.R., S. Videsen, and P.T. Madsen, *Vessel noise levels drive behavioural responses of humpback whales with implications for whale-watching.* eLife, 2020. **9**.

24. Rolland, R., et al., *Fecal glucocorticoids and anthropogenic injury and mortality in North Atlantic right whales Eubalaena glacialis.* Endangered Species Research, 2017. **34**: p. 417-429.

25. Modest, M., et al., *First description of migratory behavior of humpback whales from an Antarctic feeding ground to a tropical calving ground.* Animal Biotelemetry, 2021. **9**(1): p. 1-16.

26. Amaral, R.S., *Use of alternative matrices to monitor steroid hormones in aquatic mammals: a review.* Aquatic Mammals, 2010. **36**(2).

27. Palsbøll, P.J., F. Larsen, and E.S. Hansen, *Sampling of Skin Biopsies from Free-Raging Large Cetaceans in West Greenland: Development of new Biopsy Tips and Bolt Designs.* International Whaling Commission Special Issue Series, 1991(13).

28. Weinstein, B.G., et al., *Identifying overlap between humpback whale foraging grounds and the Antarctic krill fishery.* Biological Conservation, 2017. **210**: p. 184-191.

29. Lambertsen, R.H., *A Biopsy System for Large Whales and Its Use for Cytogenetics.* Journal of Mammalogy, 1987. **68**(2): p. 443-445.

30. Gilson, A., et al., *Deer gender determination by polymerase chain reaction.* California Fish and Game, 1998. **84**(4): p. 159-169.

31. Aasen, E. and J.F. Medrano, *Amplification of the ZFY and ZFX genes for sex identification in humans, cattle, sheep and goats.* Bio/technology, 1990. **8**(12): p. 1279-1281.

32. Valsecchi, E. and W. Amos, *Microsatellite markers for the study of cetacean populations.* Molecular Ecology, 1996. **5**(1): p. 151-156.

33. Palsbøll, P., et al., *Primers for the amplification of tri-and tetramer microsatellite loci in baleen whales.* Molecular Ecology, 1997. **6**(9): p. 893-895.

34. Berube, M., et al., *Polymorphic di-nucleotide microsatellite loci isolated from the humpback whale, Megaptera novaeangliae.* Molecular Ecology, 2000. **9**(12): p. 2181-2183.

35. Waldick, R., M. Brown, and B. White, *Characterization and isolation of microsatellite loci from the endangered North Atlantic right whale.* Molecular Ecology, 1999. **8**(10): p. 1763-1765.

36. Baker, C.S., et al., *Strong maternal fidelity and natal philopatry shape genetic structure in North Pacific humpback whales.* 2013.

37. Constantine, R., et al., *Abundance of humpback whales in Oceania using photo-identification and microsatellite genotyping.* Marine Ecology Progress Series, 2012. **453**: p. 249-261.

38. Peakall, R. and P.E. Smouse, *GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research.* Molecular ecology notes, 2006. **6**(1): p. 288-295.

39. Kalinowski, S.T., M.L. Taper, and T.C. Marshall, *Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment.* Molecular ecology, 2007. **16**(5): p. 1099-1106.

40. Kellar, N.M., et al., *Determining pregnancy from blubber in three species of delphinids.* Marine Mammal Science, 2006. **22**(1): p. 1-16.

41. Pallin, L., et al., *Validation of a blubber-based endocrine pregnancy test for humpback whales.* Conservation Physiology, 2018. **6**(1): p. coy031-coy031.

42. Kellar, N.M., et al., *Low reproductive success rates of common bottlenose dolphins Tursiops truncatus in the northern Gulf of Mexico following the Deepwater Horizon disaster (2010-2015).* Endangered Species Research, 2017. **33**: p. 143-158.

43. R Core Team, *R: A language and environment for statistical computing.* 2021, R Foundation for Statistical Computing: Vienna, Austria.

44. Graham, K.M., E.A. Burgess, and R.M. Rolland, *Stress and reproductive events detected in North Atlantic right whale blubber using a simplified hormone extraction protocol.* Conservation Physiology, 2021. **9**(1).

45. Atkinson, S., et al., *Pregnancy rate and biomarker validations from the blubber of eastern North Pacific blue whales.* Marine Mammal Science, 2019.

46. Hunt, K.E., et al., *Analysis of fecal glucocorticoids in the North Atlantic right whale (Eubalaena glacialis).* General and Comparative Endocrinology, 2006. **148**(2): p. 260-272.

47. Mashburn, K.L. and S. Atkinson, *Evaluation of adrenal function in serum and feces of Steller sea lions (Eumetopias jubatus): influences of molt, gender, sample storage, and age on glucocorticoid metabolism.* General and Comparative Endocrinology, 2004. **136**(3): p. 371-381.

48. Jeanniard Du Dot, T., et al., *Changes in glucocorticoids, IGF-I and thyroid hormones as indicators of nutritional stress and subsequent refeeding in Steller sea lions (Eumetopias jubatus).* Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 2009. **152**(4): p. 524-534.

49. Foley, C.A.H., S. Papageorge, and S.K. Wasser, *Noninvasive Stress and Reproductive Measures of Social and Ecological Pressures in Free-Ranging African Elephants.* Conservation Biology, 2001. **15**(4): p. 1134-1142.

50. Challis, J.R., et al., *Endocrine and paracrine regulation of birth at term and preterm.* Endocrine reviews, 2000. **21**(5): p. 514-550.

51. Robeck, T.R., K.J. Steinman, and J.K. O'Brien, *Characterization and longitudinal monitoring of serum androgens and glucocorticoids during normal pregnancy in the killer whale (Orcinus orca).* General and Comparative Endocrinology, 2017. **247**: p. 116-129.

52. Rolland, R.M., et al., *Assessing reproductive status of right whales (Eubalaena glacialis) using fecal hormone metabolites.* General and comparative endocrinology, 2005. **142**(3): p. 308-317.

53. Burgess, E.A., et al., *Adrenal responses of large whales: Integrating fecal aldosterone as a complementary biomarker to glucocorticoids.* General and Comparative Endocrinology, 2017. **252**: p. 103-110.

54. Ducklow, H.W., et al., *Marine pelagic ecosystems: the west Antarctic Peninsula.* Philosophical Transactions of the Royal Society of London B: Biological Sciences, 2007. **362**(1477): p. 67-94.

55. Rogers, A., et al., *Antarctic futures: an assessment of climate-driven changes in ecosystem structure, function, and service provisioning in the Southern Ocean.* Annual Review of Marine Science, 2020. **12**: p. 87-120.

56. Hillbrand, F. and F. Elsaesser, *Concentrations of progesterone in the backfat of pigs during the oestrous cycle and after ovariectomy.* Journal of reproduction and fertility, 1983. **69**(1): p. 73-80.

57. Funasaka, N., et al., *Seasonal difference of diurnal variations in serum melatonin, cortisol, testosterone, and rectal temperature in Indo-Pacific bottlenose dolphins (Tursiops aduncus).* Aquatic Mammals, 2011. **37**(4).

58. Oki, C. and S. Atkinson, *Diurnal patterns of cortisol and thyroid hormones in the Harbor seal (Phoca vitulina) during summer and winter seasons.* General and Comparative Endocrinology, 2004. **136**(2): p. 289-297.

59. Lavigne, D., et al., *Lower critical temperatures of blue whales, Balaenoptera musculus.* Journal of Theoretical Biology, 1990. **144**(2): p. 249-257.

60. Nichols, R.C., et al., *Intra-seasonal variation in feeding rates and diel foraging behaviour in a seasonally fasting mammal, the humpback whale.* Royal Society Open Science, 2022. **9**(7).

61. Bierlich, K.C., *Incorporating Photogrammetric Uncertainty in UAS-based Morphometric Measurements of Baleen Whales*. 2021.

62. Kellar, N.M., et al., *Blubber cortisol: a potential tool for assessing stress response in free-ranging dolphins without effects due to sampling.* PLoS One, 2015. **10**(2): p. e0115257.

63. Bejder, L. and A. Samuels, *Evaluating the effects of nature-based tourism on cetaceans.* Marine mammals: Fisheries, tourism and management issues, 2003. **1**: p. 229-256.

64. New, L.F., et al., *The modelling and assessment of whale-watching impacts.* Ocean & Coastal Management, 2015. **115**: p. 10-16.

65. Avila, I.C., L.M. Correa, and E. Parsons, *Whale-watching activity in Bahía Málaga, on the Pacific coast of Colombia, and its effect on humpback whale (Megaptera novaeangliae) behavior.* Tourism in Marine Environments, 2015. **11**(1): p. 19-32.

66. Amrein, A.M., et al., *Impacts of whale watching on the behavior of humpback whales (Megaptera novaeangliae) in the Coast of Panama.* Frontiers in Marine Science, 2020: p. 1105.

67. Heenehan, H., et al., *Caribbean Sea soundscapes: monitoring humpback whales, biological sounds, geological events, and anthropogenic impacts of vessel noise.* Frontiers in Marine Science, 2019: p. 347.

68. Keay, J.M., et al., *Fecal glucocorticoids and their metabolites as indicators of stress in various mammalian species: a literature review.* Journal of Zoo and Wildlife Medicine, 2006. **37**(3): p. 234-244.

69. Harris, K., et al., *Spatial Pattern Analysis of Cruise Ship–Humpback Whale Interactions in and Near Glacier Bay National Park, Alaska.* Environmental Management, 2012. **49**(1): p. 44-54.

3.8 Tables

Table 3.1. Summary of microsatellite loci used for individual identification of humpback whales (*Megaptera* novaeangliae) along the Western Antarctic Peninsula. The number of alleles observed (HO) and expected (HE) heterozygosity) was calculated using *Cervus 3.0.1*. The expected probability of identity (PID) of each locus was calculated with the program *GenAlEx v6.5.*

Locus	Source	Label	[$mgCl_2$] mM	Size range (bp)	No. of alleles	H_E	H_O	P_{ID}
Ev14	Valsecchi & Amos (1996)	VIC	2.5	125-143	9	0.789	0.800	0.072
Ev37	Valsecchi & Amos (1996)	NED	3.5	192-226	16	0.905	0.915	0.017
Ev96	Valsecchi & Amos (1996)	FAM	1.5	143-173	13	0.870	0.875	0.030
GATA28	Palsbøll *et al.* (1997)	NED	2.5	143-191	11	0.325	0.328	0.016
GATA417	Palsbøll *et al.* (1997)	FAM	2.5	187-282	20	0.909	0.911	0.465
GT211	Palsbøll *et al.* (1997)	FAM	2.5	100-120	10	0.818	0.82	0.056
GT23	Berube *et al.* (2000)	VIC	2.5	101-123	9	0.728	0.728	0.113
GT575	Berube *et al.* (2000)	FAM	1.5	137-177	14	0.814	0.836	0.056
rw4-10	Waldick *et al.* (1999)	VIC	2.5	190-216	14	0.845	0.846	0.044
rw48	Waldick *et al.* (1999)	NED	3	112-120	5	0.740	0.708	0.111

Table 3.2. Blubber cortisol levels (mean ± SD ng/g wet weight) in biopsied humpback whales across years and different sex and demographic groups.

Year	Sex	Demographic Class	Blubber Cortisol (ng/g wet weight)
2019 (n = 134)			0.45 ± 0.34
	M (n = 50)		0.53 ± 0.46
	F (n = 84)		0.4 ± 0.24
		Lactating (n= 15)	0.38 ± 0.27
		Lactating and Pregnant (n= 8)	0.56 ± 0.3
		Pregnant (n = 31)	0.42 ± 0.22
		Resting (n = 30)	0.34 ± 0.22
2020 (n = 145)			0.38 ± 0.22
	M (n = 57)		0.36 ± 0.25
	F (n = 88)		0.39 ± 0.2
		Lactating (n = 7)	0.28 ± 0.12
		Lactating and Pregnant (n = 6)	0.46 ± 0.18
		Pregnant (n = 22)	0.47 ± 0.22
		Resting (n = 53)	0.37 ± 0.19
2021 (n = 26)			0.28 ± 0.14
	M (n = 15)		0.31 ± 0.16
	F (n = 11)		0.25 ± 0.1
		Lactating (n = 3)	0.24 ± 0.09
		Lactating and Pregnant (n = 1)	0.24
		Pregnant (n = 2)	0.23 ± 0.08
		Resting (n = 5)	0.26 ± 0.14

3.9 Figures

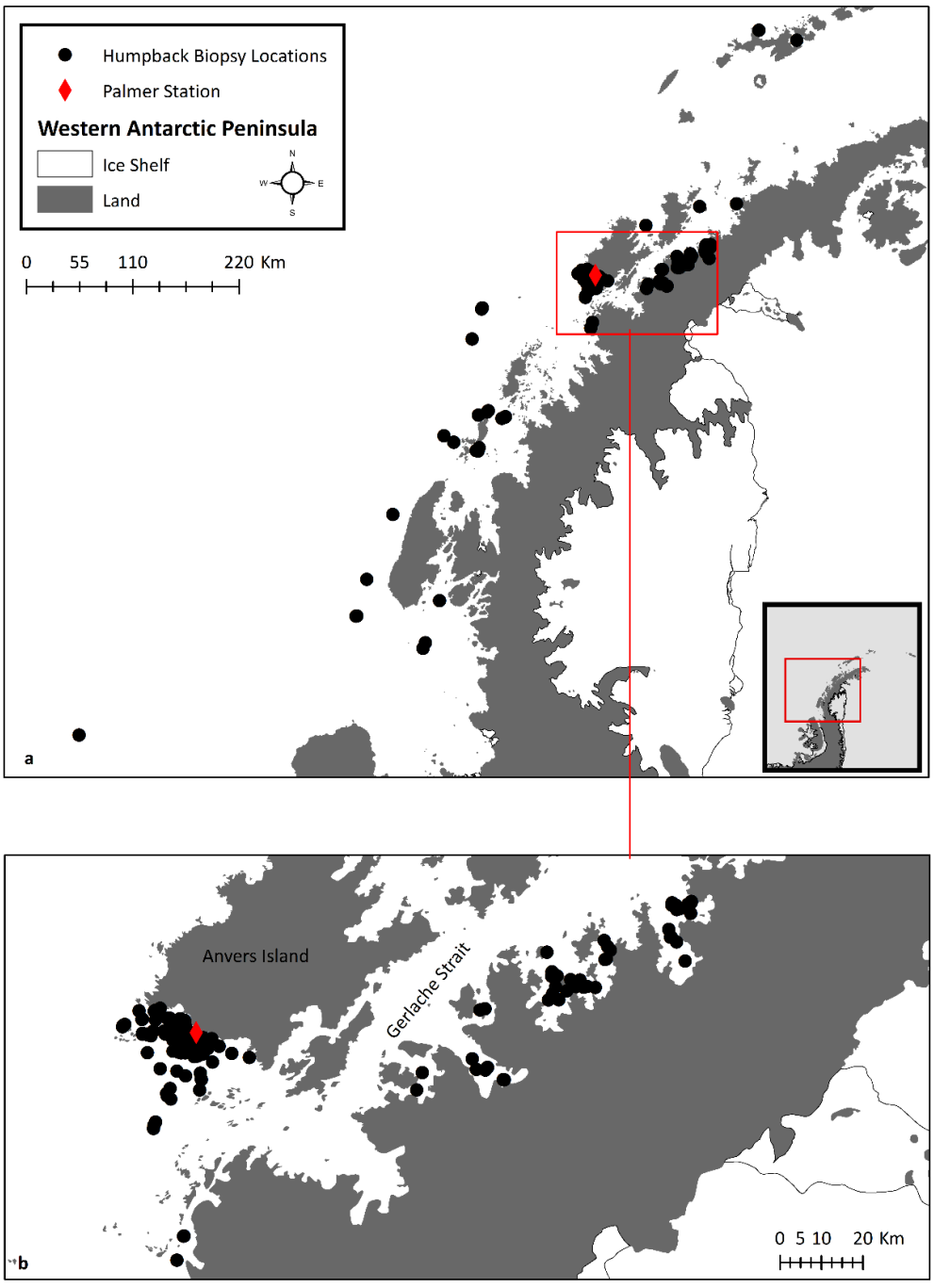

Figure 3.1. Sampling locations of humpback whales along the Western Antarctic Peninsula (**a**) and in the (**b**) Gerlache Strait and adjacent bays during the 2019-2021 field seasons. Maps were created using ArcMap version 10.8.2 (2022, https://www.esri.com/en-us/home).

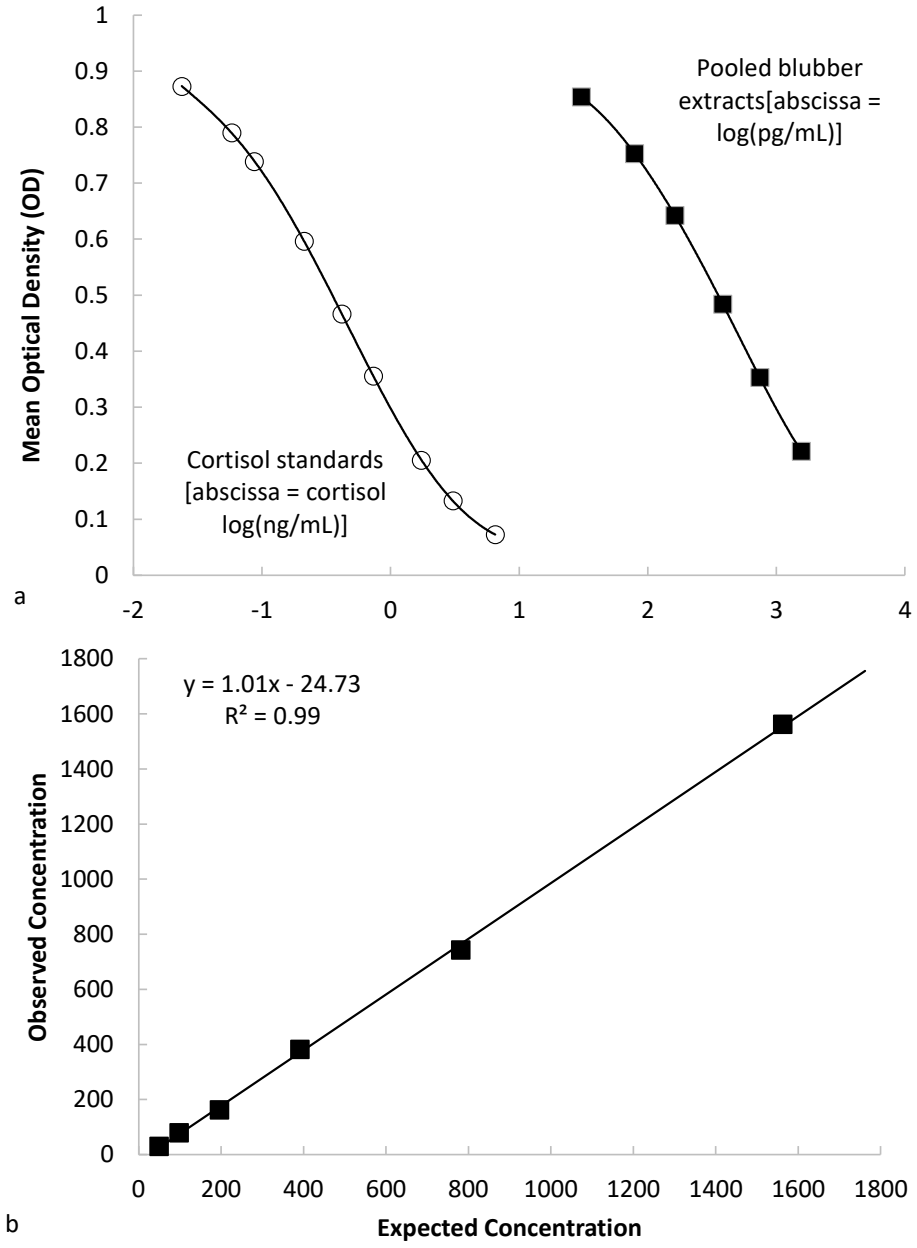

Figure 3.2. Enzyme immunoassay (EIA) validations for cortisol extracted from blubber biopsy samples in humpback whales. (**a**) Serial dilutions of extracts (shaded squares) showed strong parallelism with the standards of the progesterone EIA (open circles) and good accuracy (**b**) demonstrated by the positive linear relationship of known cortisol concentrations against apparent concentrations in spiked samples (R^2 = 0.999, slope = 1.01); Both tests indicate that the assay is measuring the same antigens in the blubber as in the standards and therefore suitable for use with humpback whale blubber tissues extracts. Four individual females were represented in the pooled blubber extracts.

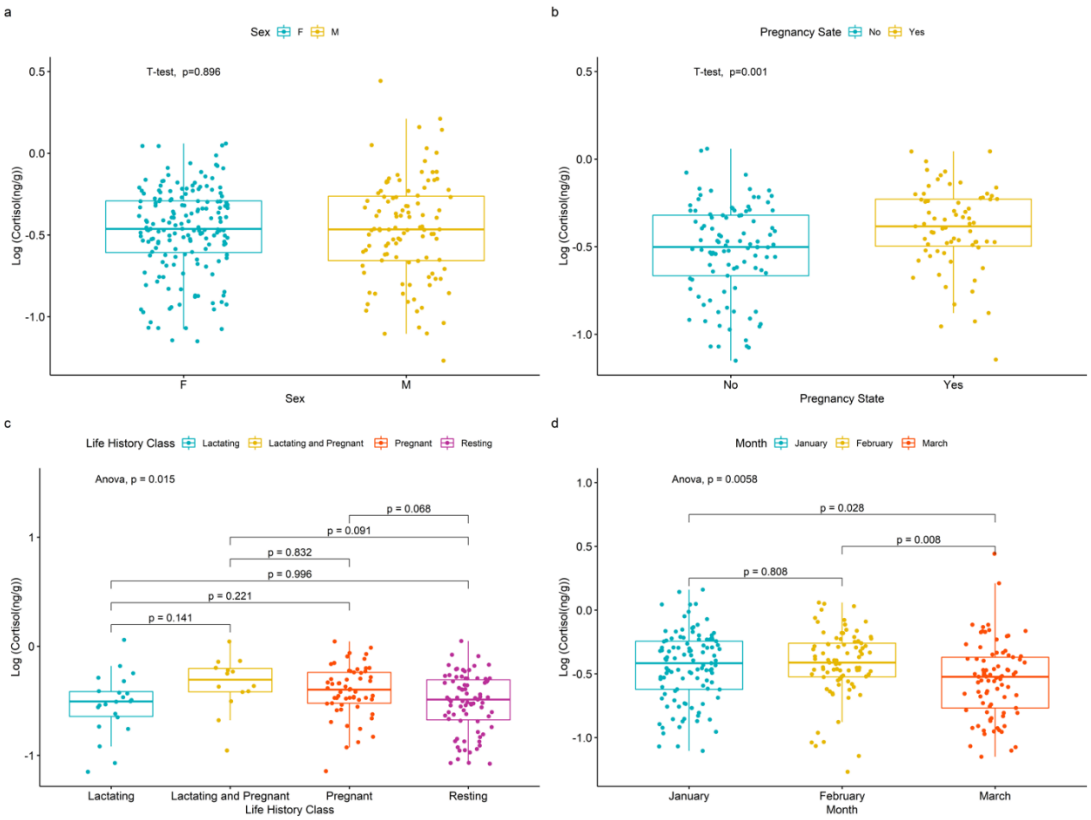

Figure 3.3. Blubber cortisol concentrations (ng/g wet weight) of humpback whales sampled along the Western Antarctic Peninsula during 2019-20. Blubber cortisol concentrations by sex (a), pregnancy state (b), life history class (c), and across months (d).

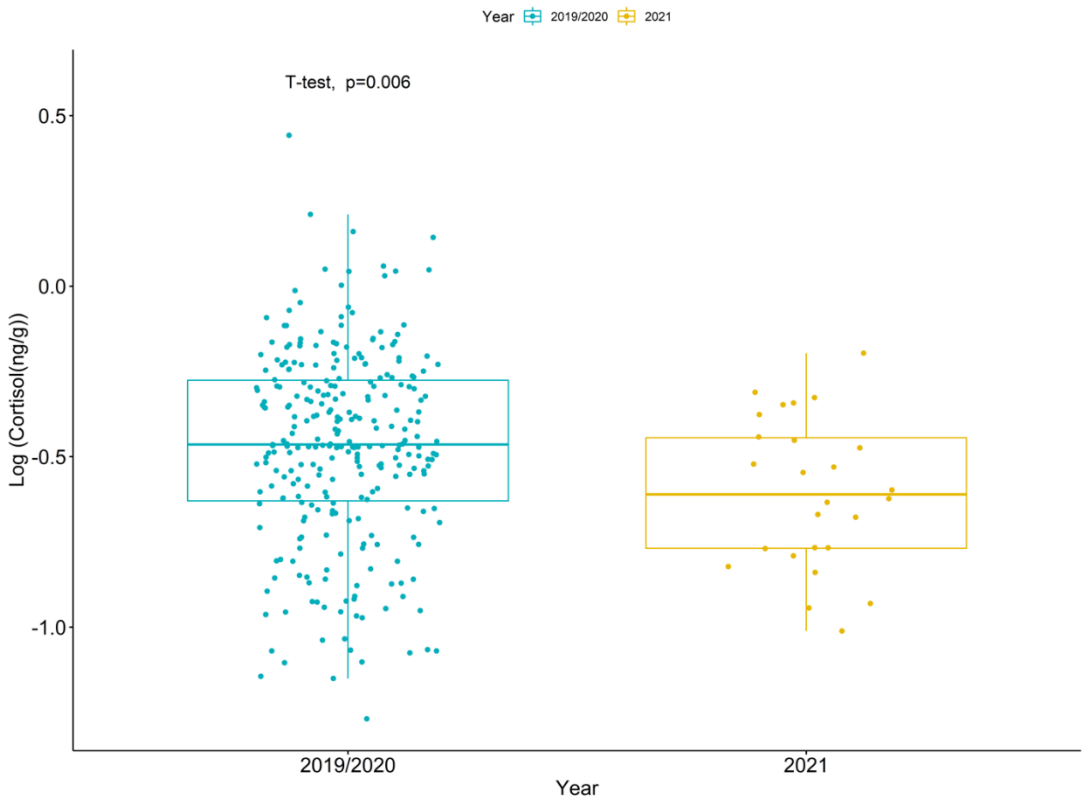

Figure 3.4. Variation in blubber cortisol concentrations (ng/g wet weight) of humpback whales sampled along the Western Antarctic Peninsula during periods of high (2019/2020) and low (2021) vessel presence. Cortisol concentrations are log-transformed.

Synthesis

Climate change is one of the primary forces inhibiting and deteriorating the growth and health of biological populations worldwide [1]. These changes will likely have the most dramatic impacts on polar-dependent species [2]. In the Southern Ocean, baleen whales were heavily exploited during the height of the commercial whaling era and, as a result, face numerous conservation challenges today [3]. As these populations recover, they are re-occupying ecological roles, particularly on their Antarctic feeding grounds from which they have been functionally absent for half a century. This thesis established one of the first non-lethal demographic descriptions of Antarctic minke whales in the context of a rapidly changing marine ecosystem (Chapter 1), examined the direct relationships between environmental variation and humpback pregnancy rates (Chapter 2), and then assessed baseline demographic and temporal variation in blubber cortisol levels among humpback whales in years with varying human activity (Chapter 3). The findings in Chapters 1 – 3 outline: (i) the continued importance of long-term ecological research programs and how they can directly inform adaptive management and conservation efforts, and (ii) the continued efficacy of using blubber biopsies to measure critical health biomarkers from whales in the wild.

Given the continued efficacy of using adipose biomarkers to study the demography of whales and recent advances in unoccupied aircraft systems (UAS) technology, I characterized for the first time the maturity, sex ratio, and pregnancy rates of Antarctica minke whales along the WAP (Chapter 1). While the pregnancy rates of sampled females were high, indicating these whales likely breed annually, the estimated proportion of sexually mature females was lower than expected. The marine environment of the WAP

is experiencing some of the most significant warming on Earth, resulting in a rapidly diminishing extent and duration of sea ice. [4]. The distribution and ecology of AMWs are directly tied to sea ice and prey availability. Changes that impact both the quantity and quality of their habitat and food availability may significantly change their demography and population dynamics. Significant temporal reductions in the critical habitat for AMWs along the WAP are already occurring [4]. Thus, continued demographic monitoring of this population is needed to understand their response to continuous ecological change.

Assessing trends in reproductive rates of recovering populations is vital to informing management strategies and decisions to promote their growth. Determining and monitoring pregnancy rates can indicate population health and provide the opportunity to track the recovery of exploited or threatened populations over time. Perhaps one of the most significant ecological disturbances over the past 200 years was the removal of more than two million large krill-consuming baleen whales during the 20th century in the Southern Ocean [3]. For migratory whales, such as the humpback whale, fitness and reproductive success is entirely contingent on the accessibility of available foraging habitat and adequate prey resources on their high-latitude feeding grounds [5]. The eight-year time series of humpback pregnancy rates analyzed here (Chapter 2) showed significant direct relationships between environmental variation and interannual variability in humpback whale pregnancy rates. These relationships supported similar trends observed among other baleen whales [6, 7] and other Antarctic krill predators [8]. This understanding will assist in monitoring, management, and conservation efforts as the environment continues to change along the Western Antarctic Peninsula (WAP), and human activities continue to grow in this region.

Global ecological change has resulted in the decline of many species (e.g., Wittmer et al. [9]). However, the underlying biological mechanisms responsible are often poorly understood. Glucocorticoids (GC) are one of two corticosteroid hormones produced in the adrenal cortex with the primary function of regulating metabolism and endocrine responses to stressors [10]. In vertebrates, GCs (including cortisol), have been analyzed in several different sample matrices as possible indicators of physiological stress. Humpback whales along the WAP appear to be recovering quite rapidly [11], even as human activities (i.e., vessel tourism and commercial krill fishery) in this region are rapidly expanding [12]. Several studies have linked cumulative anthropogenic impacts to increased physiological stress levels (i.e., increased GCs) in baleen whales. The variation in blubber cortisol levels in humpback whales (Chapter 3) was comparable to similar studies and showed a weak, albeit significant direct relationship between vessel numbers and cortisol levels. These findings provide a critical baseline of cortisol levels for whales in a rapidly changing region, show direct relationships between cortisol levels and human presence, and will enable comparisons among species experiencing different levels of human activity and environmental change.

The foundation of the National Science Foundation's (NSF) Long Term Ecological Research Network (LTER) is to provide a context to evaluate the nature and impact of environmental change through the collection of long-term datasets. The data presented in this dissertation builds off the specific objectives of the Palmer LTER study site, aiding in the further understanding of biological and ecological change within the WAP region as a response to climate change. With the continued support of the Palmer LTER, we can further develop a long-term dataset on the demography and recovery of baleen whales in

this region and, in turn, provide continuous insight into whales' ecological and biological responses to climatic and anthropogenic change. Lastly, by integrating current demographic knowledge with new technologies for assessing biomarkers from additional sample matrices (i.e., blow sputum), more specific markers of nutritional state (i.e., thyroid hormones), as well as photogrammetry to evaluate body condition, we can better understand the relationships between climate change, human disturbance, and population dynamics.

In summary, there now exists a baseline demographic description of two species of baleen whales occupying the nearshore waters along the WAP, a region undergoing rapid environmental change. These data can act as a reference point for future comparisons and upon which a basis for conservation and adaptive management can be established.

References

1. Root, T.L., D. Liverman, and C. Newman, *Managing biodiversity in the light of climate change: current biological effects and future impacts.* Key Topics in Conservation Biology, 2006: p. 85-104.

2. Bernstein, L., et al., *IPCC, 2007: climate change 2007: synthesis report*. 2008: IPCC.

3. Rocha, R., P.J. Clapham, and Y.V. Ivashchenko, *Emptying the oceans: a summary of industrial whaling catches in the 20th century.* Mar. Fish. Rev, 2014. **76**: p. 37-48.

4. Stammerjohn, S., et al., *Regions of rapid sea ice change: An inter-hemispheric seasonal comparison.* Geophysical Research Letters, 2012. **39**(6).

5. Lockyer, C., *Review of baleen whales (Mysticeti) reproduction and implications for management.* Reproduction of Whales, Dolphin and Porpoise, 1984.

6. Leaper, R., et al., *Global climate drives southern right whale (Eubalaena australis) population dynamics.* Biology Letters, 2006. **2**(2): p. 289-292.

7. Kershaw, J.L., et al., *Declining reproductive success in the Gulf of St. Lawrence's humpback whales (Megaptera novaeangliae) reflects ecosystem shifts on their feeding grounds.* Global Change Biology, 2021. **27**(5): p. 1027-1041.

8. Reid, K., et al., *Population dynamics of Antarctic krill Euphausia superba at South Georgia: sampling with predators provides new insights*, in *Top Predators in Marine Ecosystems: Their Role in Monitoring and Management*, C.J. Camphuysen, I.L. Boyd, and S. Wanless, Editors. 2006, Cambridge University Press: Cambridge. p. 249-261.

9. Wittmer, H.U., et al., *Changes in landscape composition influence the decline of a threatened woodland caribou population.* Journal of animal ecology, 2007. **76**(3): p. 568-579.

10. Atkinson, S., et al., *Stress physiology in marine mammals: how well do they fit the terrestrial model?* Journal of Comparative Physiology B, 2015. **185**(5): p. 463-486.

11. Pallin, L.J., et al., *High pregnancy rates in humpback whales (Megaptera novaeangliae) around the Western Antarctic Peninsula, evidence of a rapidly growing population.* Royal Society Open Science, 2018. **5**(5).

12. Bender, N.A., K. Crosbie, and H.J. Lynch, *Patterns of tourism in the Antarctic Peninsula region: a 20-year analysis.* Antarctic Science, 2016. **28**(3): p. 194-203.

Supplemental Materials

Text S1.1. Estimating proportion of mature females using undifferentiated sample of animals of known length.

Estimating the pregnancy rate requires knowledge of the proportion of females that are mature and the proportion pregnant. However, the hormonal dataset does not provide us with that information because it does not distinguish between immature females and mature non-pregnant females. The proportion of mature females can be calculated from our undifferentiated by sex length data provided we can estimate the probability that an animal at a given length is both female and mature. Commercial whaling data on sex, length and pregnancy of Antarctic minke whales (AMW) are available from the International Whaling Commission (IWC) data base, and these data were extracted for all the catches in the region 55°W to 70°W longitude from 1972-1987, which encompasses the tip of the Antarctic Peninsula. Records of 513 AMW females and AMW 509 males were available from this region. 393 females were pregnant, but the maturity state of the non-pregnant females is not recorded in the IWC database. Standard lengths of these animals was recorded to the nearest 0.1 m.

The following logistic function was fitted to the female catch data to estimate the proportion mature in each 0.1m length interval (l):

$$p_f(l) = \alpha\left(1+\mathrm{e}^{\beta\left(l-l_{m,50}\right)}\right)^{-1} \qquad (0.1)$$

Where:

α is the asymptotic proportion of females pregnant at higher lengths,

β is a rate parameter determining the length span over which maturation occurs, and

$l_{m,50}$ is the length at which 50% are pregnant.

To calculate the maturity ogive we assume that the logistic function with α set to unity then describes the proportion of females mature at each length. We estimated the parameters using a Monte Carlo Markov Chain (MCMC) assuming that the proportions pregnant in each length interval have binomial distributions.

The catch data (Fig. 1.6A) shows that the sex-ratio of animals of greater than 9m in length approaches 100% females, but males dominate the sex-ratio at around 8m. Below 8m the sex ratio at length is highly variable because of the small sample sizes. To allow for the high variability in the sex ratio of smaller animals we estimate the probability by fitting the product of two logistic functions so that the female sex ratio for large animals can approach unity, with a dip around 8m, where the smaller males dominate, while being free to accommodate a range of shapes at smaller lengths. However, because the probability of maturity of females under 8m is low, uncertainty in the sex ratio at smaller sizes will not have much effect on the estimates of the proportion mature. The fitted function for the proportion of males in each length interval, l is:

$$p_m(l) = \omega\left(\left(1+\mathrm{e}^{\gamma(l-\nu)}\right)^{-1} \times \left(1+\mathrm{e}^{\eta(l-\phi)}\right)^{-1} \Big/ \sup\left(\left(1+\mathrm{e}^{\gamma(l-\nu)}\right)^{-1} \times \left(1+\mathrm{e}^{\eta(l-\phi)}\right)^{-1}\right)\right) \qquad (0.2)$$

where:

ω is the estimated maximum proportion of males

γ, ν, η, ϕ are parameters to be estimated

The proportion of females in length interval l is thus $1 - p_m(l)$.

The expected number of females, both mature and otherwise, in our length sample is:

$$N_f = \sum_{i=1}^{n} \left(1 - p_m(l_i)\right) \qquad (0.3)$$

The expected number of mature females in our length sample is:

$$N_M = \sum_{i=1}^{n} \left(\left(1 - p_m(l_i)\right) p_f(l_i)\right) \qquad (0.4)$$

Hence, the proportion of mature females in the length samples is N_M / N_f . This proportion is then applied to the number of females identified from the biopsies, so that the calculated pregnancy rate (P) is:

$$P = \frac{n_p}{n_f N_M / N_f} \qquad (0.5)$$

Where:

n_p is the number of pregnant females from our hormone analyses and

n_f is the total number of females identified.

A MCMC of length 6 million was used to generate a set of 1999 parameters for the two functions that describe the proportion of females at length and the proportion pregnant at length using binomial likelihood functions. A distribution of pregnancy rate was

calculated using all instances from the set of posterior parameters. The prior distributions for each parameter and the basic statistics of their posterior distributions are given in the Table S1.1.

Table S1.1. The prior distributions for the parameter used to calculate the sex ratio and maturity functions from the commercial catch data and the basic statistics of their posterior distributions.

Function	Parameter	Prior		Posterior	
		Distribution		Median	95% credible interval
Maturity function	Pregnant asymptote α	Uniform	0.85 – 0.999	0.957	0.909 – 0.995
	Rate of maturation β	Uniform	2.0 – 9.0	4.768	3.494 – 7.040
	Length at 50% mature $l_{m,50}$	Normal	$\mu = 8.21$ $\sigma = 0.5$	8.198	8.111 – 8.280
Proportion male	Max. male proportion ω	Uniform	0.60 – 0.85	0.742	0.686 – 0.825
	γ	Uniform	-7.0 – -2.0	-4.75	-6.57 – -3.27
	ν	Uniform	0.0 – 7.0	3.07	0.14 – 6.06
	η	Uniform	3.0 – 7.0	4.89	3.63 – 6.58
	ϕ	Uniform	8.4 – 9.0	8.72	8.53 – 8.84

Table S2.1. Summary of microsatellite loci used for individual identification of female humpback whales (*Megaptera* novaeangliae) along the Western Antarctic Peninsula. The number of alleles, observed (HO), and expected (HE) heterozygosity was calculated using *Cervus 3.0.1*. The expected probability of identity (PID) of each locus was calculated with the program *GenAlEx v6.5.*

Locus	**Source**	**Label**	**[$mgCl_2$] mM**	**Size range (bp)**	**No. of alleles**	**H_E**	**H_O**	**P_{ID}**
Ev14	Valsecchi and Amos (1996)	VIC	2.5	125-143	9	0.776	0.764	0.079
Ev37	Valsecchi and Amos (1996)	NED	3.5	192-228	17	0.901	0.897	0.018
Ev96	Valsecchi and Amos (1996)	FAM	1.5	143-173	14	0.864	0.848	0.032
GATA28	Palsbøll *et al.* (1997)	NED	2.5	143-199	13	0.365	0.357	0.413
GATA417	Palsbøll *et al.* (1997)	FAM	2.5	187-282	22	0.912	0.901	0.015
GT211	Palsbøll *et al.* (1997)	FAM	2.5	100-120	10	0.816	0.930	0.057
GT23	Berube *et al.* (2000)	VIC	2.5	101-123	9	0.738	0.721	0.107
GT575	Berube *et al.* (2000)	FAM	1.5	137-177	14	0.792	0.800	0.066
rw4-10	Waldick *et al.* (1999)	VIC	2.5	190-216	13	0.847	0.855	0.042
rw48	Waldick *et al.* (1999)	NED	3	112-120	5	0.731	0.731	0.117

Table S2.2. Within- and between-year genotype recaptures of non-calf female humpback whales sampled during the austral summer (Dec – Mar) along the Western Antarctic Peninsula with confirmed genetic sex. Recaptures within the same year are presented along the diagonal. Shaded cells indicate recaptures across years. n: the total number of samples in a year with known genetic sex. Ind: total number of individual females within that sampling year. *Total individual females in the entire sample data set exclude all recaptures. There is one, three-year recaptures of a female, and thus 1 individual has been added to the individual total.

Year	**n**	**Ind.**	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**	**2019**	**2020**
2013	38	34	4	1	1			1		
2014	51	47		4		1		1	3	1
2015	53	53			0		1	1		1
2016	53	48				5	1		2	2
2017	80	76					4	2		4
2018	98	91						7	1	2
2019	124	116							8	6
2020	172	150								22
Total	**669**	**615 (584*)**								