

AN ABSTRACT OF THE THESIS OF

Logan Pallin for the degree of Master of Science in Wildlife Science presented on May 26, 2017.

Title: Temporal Variation in Humpback Whale (*Megaptera novaeangliae*) Demographics Along the Western Antarctic Peninsula

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Abstract approved:

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Humpback whale populations in Antarctica are recovering after intense commercial whaling in the 20th century. Along the Western Antarctic Peninsula (WAP) this recovery is occurring in an environment that is experiencing the fastest warming of any region on the planet. To begin to understand the dynamics of this recovery under such dramatic climate change, we are studying the demography of these whales. To date, we have collected 583 biopsy samples from 239 individual males and 268 individual females during the austral feeding season from 2010, 2013-2016. The overall sex ratio of our sample population is 0.89 M:F, supporting early observations that sexes mix randomly on the feeding grounds. Additionally, we did document a significant seasonal increase in the proportion of females along the WAP into the fall. We believe that this shift represents a tendency for pregnant female humpback whales to depart last from the feeding grounds. Furthermore, we examined progesterone levels of females to assign a

pregnancy status; providing to our knowledge, the first non-lethal estimation of pregnancy rates in Antarctic whales. A series of female humpback whales of known pregnancy status (n=29) from the Northwestern Atlantic, verified from field observations, were used as control samples to develop a logistic regression model, modelling the probability of pregnancy relative to blubber progesterone concentrations. A pregnancy state was then assigned to females biopsied along the WAP by modelling their probability of being pregnant across the control model. Based on our assignment of females as pregnant/not-pregnant, mean progesterone levels for pregnant assigned humpback whales from the WAP was 250 ng progesterone/g blubber (n = 155). The mean value for not-pregnant assigned females was 2.10 ng progesterone/g blubber (n = 89). Pregnancy rates varied significantly across all years, from 36% in 2010 to 86% in 2014. We detected a significant increase in the proportion of pregnant females (58% to 72%) from summer to autumn across all years. Some female whales in this population appear to experience a post-partum ovulation followed by conception (annual pregnancy); on average, more than half (52%) of female whales accompanied by calves were pregnant. These are some of the first quantitative observations of the demography of recovering humpback whale populations in the Antarctic and provides a critical reference point as the Antarctic climate continues to change and populations recover from whaling.

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Temporal Variation in Humpback Whale (*Megaptera novaeangliae*) Demographics
Along the Western Antarctic Peninsula

by
Logan J. Pallin

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APPROVED

Major Professor, representing Wildlife Science

Head of the Department of Fisheries and Wildlife

Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Logan Pallin, Author

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CHAPTER 1: General Introduction

Conservation biology as a scientific discipline is growing rapidly in many countries across the globe as our natural ecosystems and populations continue to experience increased environmental and anthropogenic perturbations. Even so, it remains unclear if the pace of our conservation efforts will precede the rates of these perturbations. To start, we must better understand the population dynamics and demography of species contained in these systems to use as a baseline (Lande 1988). Demographics within animal populations are shaped by external selective pressures. To recognize how these perturbations influence and shift the current health and dynamics of a population requires a current assessment of demographic parameters and an understanding of variability across different temporal scales (Previtali et al. 2009).

Studies on large terrestrial mammals have used demographic information to make better informed decisions and predictions on population health, growth, and recovery from exploitation (Gates et al. 2010, Servanty et al. 2011). Anthropogenic exploitation on animals in the wild occurs in both the marine and terrestrial environments and is often excessive. This exploitation increases the selective pressures that can strongly influence the life history and demography of individuals within a population (i.e. skewed age structure, earlier sexual maturity, skewed sex ratio), and has even been linked to the direct extinction of several populations and species (Burney & Flannery 2005, Proaktor et al. 2007, Allendorf et al. 2008). For example, by the late 1800s we had witnessed an almost complete collapse of the American bison population in North America because of over hunting (Lueck 2002, Gates et al. 2010). It was only by understanding the effects of

this exploitation on their demography were conservationists capable of recovering this species. Thus, understanding demography is important in biological populations.

However, assessing these demographics can be quite challenging, especially in polar dependent, long-lived, mobile species, where it is often logistically difficult to study these animals due to their cryptic nature and the harsh environment in which they are found. Paradoxically, even though the study of polar species is arduous, it is of the utmost importance: species dependent on polar regions are under some of the most intense pressures with respect to climatic changes in environment (Bernstein et al. 2008).

Climate change is now considered one of the primary forcings inhibiting and deteriorating the growth and health of biological populations worldwide (Root et al. 2006). These climatic changes are likely to have dramatic impacts on polar dependent species and the elasticity of their habitats (Bernstein et al. 2008). Once such example is the observed decline in numbers of polar bears along the western Hudson Bay due to climate change (Stirling & Parkinson 2006). These scientists assessed the effects of climate change on this population by developing a comprehensive assessment between the relationship of satellite derived sea ice and basal demographic parameters, such as adult health and associated reproductive rates. This information was then incorporated into essential planning and management actions needed to help conserve this species throughout the Arctic.

The potential response to climate change by cetaceans has been visited multiple times by expert panels and working groups (i.e. International Whaling Commission (IWC)), however, no clear consensus has been established (Clapham 2016). 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 (Laidre et al. 2008). In order to discern the possible impacts of climate change on cetaceans that utilize polar waters in the future, we require a better understanding of their current demography.

Understanding the recovery of a species from intense exploitation places current knowledge on their health and growth into ongoing and future environmental changes. Populations of baleen whales were heavily exploited during the height of the industrial whaling era in the early 20th century (Rocha et al. 2014). As these populations of baleen whales recover, they may re-occupy ecological roles, particularly on their high latitude feeding grounds, from which they may have been historically absent from many decades. Specifically, most humpback whale populations in the Southern Hemisphere are recovering rapidly after intense commercial whaling in the 20th century, whereby more than 215,000 individuals were removed by the early 1970s for commercial purposes (Gales & Commission 2011, Johnston et al. 2011, Rocha et al. 2014).

Like many other mysticetes, humpback whales exhibit an annual migration from low-latitude breeding grounds to high latitude feeding grounds where they exploit high levels of seasonal productivity (Dawbin 1966). Because they migrate close to shore and distribute themselves coastally through large portions of the year, the migratory behaviors of humpback whales are well studied. During the austral summer, humpback whales feed in at least six defined feeding grounds in the Southern Ocean, including the Western Antarctic Peninsula (WAP) (Johnson & Wolman 1984, Rice 1998, Gales et al. 2011). Many humpbacks that feed along the WAP winter along the Pacific coast of Central America and Colombia, and as a result, engage in one of the longest annual migrations of

any mammal (Rasmussen et al. 2007). Two individuals have even been identified in both the WAP and the American Samoa/French Polynesia region, an exceptional migratory record (Robbins et al. 2011, Albertson et al. In Press).

Little is known about the exact timing of migratory movements and residency times of various age and sex 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 (Rocha et al. 2014). During the earliest phase of Antarctic whaling, humpback populations were rapidly depleted because they were relatively slow and thus easy to catch, abundant in protected bays and floated when killed (Hart 2006). Humpbacks comprised the vast majority of the catch at South Georgia from 1904 to 1910, but by the 1913-14 season this species comprised less than 20% of the whales taken (Burnett 2012). The same story of unconstrained over-exploitation unfolded in the South Shetlands and along the WAP (Hart 2006). Humpbacks encompassed 70% of the catch in the 1908 whaling season in the South Shetlands, however by the 1915 season they comprised less than 5% of the total catch in this region (Hart 2006). An estimated total of 9,800 humpback whales were killed in this region (Hart 2006). As a result, there was very little opportunity to study humpback whales in this area before they were reduced to remnant populations. An improved understanding of the demography and seasonal patterns of movements of humpback whales in this area would help place current research on their regional density and population dynamics into a broader ecological context (Nowacek et al. 2011, Johnston et al. 2012).

Insights into the timing of migration for various age and sex classes is available from other populations of humpback whales in the Southern Ocean that were exploited later than those along the WAP, particularly in areas where biologists were able to monitor the age and sex composition of the whaling catch (Chittleborough 1965). Such areas included shore-based whaling stations in Western Australia (i.e. Albany), Eastern Australia (i.e. Byron Bay), and in New Zealand at Cook Strait. In these areas, pregnant female humpback whales were the first to arrive on the feeding grounds, followed by immature animals, resting females and mature males (Dawbin 1966). Lactating females and their young calves are typically the last to arrive in the high polar latitudes. The order is reversed in autumn, with lactating females and their calves leaving first, followed by immature individuals and mature males and, finally, females in late pregnancy (Dawbin 1966). Whilst on the feeding grounds, age and sex classes are believed to mix randomly (Lockyer & Brown 1981). These observations were generated during the height of commercial whaling, and there have been no attempts to assess these relationships, until now, following the cessation of whaling in the Southern Ocean and the recovery of these populations. Thus, based on these early observations, one would expect a sex bias in favor of females both early and late in the feeding season on the feeding grounds, with a ratio closer to parity during the remainder of the feeding season. Assessing deviations from these sex ratio expectations based off historical records would provide further insight into the effects of whaling on these population's recovery.

Awareness of the sex of individuals in a population facilitates the study of population health, behavior, breeding patterns, and social systems (Rosel 2003). Such knowledge allows us to better understand how these animals interact and organize

themselves within their environment, ultimately enhancing our knowledge of their life history characteristics and ecology. Additionally, assessing sex ratios may allow us to make certain inference about that populations adaptations to external perturbations (Clutton-Brock & Iason 1986, West et al. 2002). Fisher (1930) outlined this adaptation response by illustrating that the sex ratio of mammalian populations should be 1:1 on the premise that each sex must supply half the ancestry of all future generations within the population. Additionally, Fisher believed that because of this, the sex ratio will adjust itself under the influence of natural selection and external perturbations (Fisher 1930). However, to truly discern these effects, requires a long-term assessment. This becomes of great importance for populations, like the Southern Hemisphere humpback whale, that experienced intense historical exploitation and is now currently faced with potential ecosystem shifts due to environmental variation from climatic change.

Such demographic adaptations have previously been documented in more recent work done by Komdeur et al. (1997), who showed that birds, specifically warblers, adaptively alter the sex of their offspring as a direct response to breeding territory quality. More specifically, warblers would produce more male chicks on poor quality territory, as males are more likely to disperse from the breeding territory. Conversely, females on sustainable terrain produced a bias in favor of daughters, who would be more likely to remain on the breeding grounds. Thus, assessing variations in sex ratios of humpback whales over time could provide insight on the capacity for these whales to adapt to a changing environment as they recover along the WAP.

In some areas of the Southern Ocean, whales are encountering environmental conditions that are very different from those that existed prior to their exploitation.

Specifically, along the WAP, the recovery of humpbacks is occurring in an environment that is experiencing the fastest climatic warming of any region on the planet (Smith & Stammerjohn 2001). 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 habitat (Vaughan et al. 2003, Meredith & King 2005). The biological and physical productivity of the WAP marine ecosystem is intimately tied to the amount of sea ice cover in this region (Massom & Stammerjohn 2010). Additionally, 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 four decades ago (Ducklow et al. 2013). This warming is proceeding with uncertain impacts and, 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 (Ducklow et al. 2013).

Understanding this complex chain of environmental regime shifts becomes of great importance for species that rely heavily on this Antarctic marine ecosystem as an essential feeding ground, including humpback whales that feed predominantly on Antarctic krill (*Euphausia superba*). The life history of Antarctic krill is closely tied to sea ice cover, as during the juvenile stage they spend majority of their time under the sea ice foraging on phytoplankton and maturing (Ikeda 1985). Thus, the vast climatic changes that we are currently witnessing along the WAP may result in an overall decrease in the abundance of krill in this region over time (Atkinson et al. 2004, Stammerjohn et al. 2008). The potential collapse or depletion of this main food source

around the Antarctic could prove catastrophic for humpback whales, as well as other krill predators.

Feeding is only thought to occur during summer where these whales migrate poleward from their low latitude breeding grounds (Dawbin 1966). Feeding during this time becomes pivotal, if these whales are to survive the next year's migration and fasting while on the breeding grounds. Energetic studies in other baleen whales, particularly blue and fin whales, have shown that more than 65% of their annual lipid reserves are used over the course of the annual migration (Lockyer 1981b). Humpback whales seem to migrate much greater distances and thus likely consume an even larger portion of their energy stores during the migration. This energetic hypothesis seems to be supported by some of the work that Lockyer (1981b) documented, noting that humpbacks appear to double their body mass during the Antarctic feeding season.

Understanding these energetic demands becomes of even greater importance for those whales that are currently gestating or lactating. Lockyer (1981b) suggested that lactation and pregnancy will consume roughly 19% of total energy stores in mature female fin whales. Again, for humpback whales this energetic cost would be even greater as humpbacks likely lactate three months longer than fin whales (Lockyer 1984). This is further supported by evidence from commercial whaling data that showed that pregnant and or lactating humpback females that were taken in the same region, yielded roughly twice as much oil as resting females (Dawbin 1966).

Pregnancy rates are a good estimator for a population's overall health and growth. Obtaining such information will greatly aid in the understanding of the recovery of humpback whales along the WAP. Historically, understanding cetacean reproductive

status was only obtainable from animals taken as part of commercial hunts (Mansour et al. 2002, Kellar et al. 2006). For example, during the commencement of commercial whaling on the Australian coast in the 1940's and 1950's, scientific observers would study the reproductive tracts of animals being flensed, allowing for the determination of pregnancy (Chittleborough 1965). With the moratorium of commercial whaling in 1982 by the IWC, our current understanding of pregnancy in cetaceans has become limited and has now only been obtainable from necropsied dead or captive individuals (Mansour et al. 2002). Given this lack of contemporary knowledge on reproduction parameters in wild cetaceans following the moratorium, techniques have been developed to detect pregnancy using non-lethal methods.

Indirect alternatives to detect pregnancy have been developed for some populations of humpback whales where long term sighting histories have been developed (Clapham & Mayo 1990). Specifically, information on these reproductive rates has been obtainable through repeated observations of adults accompanied by calves (Clapham & Mayo 1987, Glockner-Ferrari & Ferrari 1990). Obtaining this high-resolution sighting data from year to year, however, is not always possible, particularly in remote areas like the Antarctic. Pregnancy status in marine mammals has also been determined by the following methods: assessing the concentrations of progesterone in the milk of lactating bottlenose dolphins (West et al. 2000), sex steroids secreted in the urine of killer whales (Walker et al. 1988), salivary steroids in Hawaiian monk seals (Pietraszek & Atkinson 1994), blood plasma, salivary, ocular, and vaginal secretions of false killer whales (Atkinson et al. 1999), progesterone metabolites in fecal samples from North Atlantic right whales (Rolland et al. 2005), and in field ultra sounds of wild, coastal dolphins that

were part of capture-release health assessments (Wells et al. 2014). For cetaceans at sea, and those that are highly migratory like humpbacks, obtaining pregnancy information via any of these methods has been nearly impossible to achieve.

Progesterone, often referred to the hormone of pregnancy, is a lipophilic circulatory steroid hormone produced by corpus luteum, and is the primary regulator of estrous cycling and pregnancy (Pineda 2003). Following ovulation, progesterone signals the uterine lining to prepare for fertilization and implantation, and then throughout gestation it aids in sustaining this environment for successful fetal development (Wuttke et al. 1998). Parturition (birth) occurs following a series of internal physiological events, and the return to basal levels of progesterone is estimated to occur within 10-20 days in some species of terrestrial related ungulates (Bedford et al. 1972, Hillbrand & Elsaesser 1983). The difference in the progesterone concentrations due to either being not-pregnant or pregnant within mammals allows for accurate assignment of pregnancy (Kellar et al. 2006). Progesterone's lipophilic properties make skin-blubber biopsy samples an easily obtainable, non-lethal, analytical medium for assigning pregnancy in wild, free ranging cetaceans. With the development of remote biopsy sampling techniques as well as the methods to assess hormone levels from skin-blubber biopsy samples, we now have the capacity to assess these vital demographic rates in wild cetacean populations while they are still alive (Mansour et al. 2002, Kellar et al. 2006). Developing a current understanding of pregnancy rates within the WAP population of humpbacks will become crucial to make effective conservation decisions on these whale's recovery now and into the future.

Humpback whales are one of a few of the principal sympatric krill predators central to the long-term ecological research (LTER) project based out of Palmer Station, an American research base located on the southern end of Anvers Island. Anvers island is centrally located within the Palmer LTER study region. The WAP region in general is unique among the rest of the Antarctic, due to its distinctive north-south geographic orientation as well as the magnitude of climatic changes central to the northern WAP region (Meredith & King 2005). The Palmer LTER (PAL) began in 1990 on the premise of documenting nearshore biological and physical processes, of which included the extensive studies on penguin demography and feeding ecology which began in the 1970's (Fraser & Trivelpiece 1996, Ducklow et al. 2007). Having the capacity to link the biological and physical factors that drive humpback whale demographic variability long term will be crucial to accurately assess population trends and relations with other sympatric krill predators.

Here, I aimed to assess the current variation in the population demographics of humpback whales along the WAP, and in doing so, develop a current reference point that will be needed to make future comparisons and predictions on humpback population recovery and the potential effects of climate change. I utilized a multipart demographic analysis to assess the inter-annual and seasonal variation in the sex ratios and pregnancy rates of humpback whales biopsied during the austral summer and fall field seasons along the WAP.

Based on previous studies, primarily demographic literature comprised from whaling data in the Southern Hemisphere, I would expect to observe a 1:1 M:F sex ratio on the feeding grounds (Chittleborough 1965, Dawbin 1966). Furthermore, based on

available migration data, I expect a sex bias in favor of females later into the feeding season as they are more likely to feed longer in order to support the energetic demands associated with reproduction (Dawbin 1966, Lockyer 1981b). These assumptions are based on the observations by Dawbin (1966) that sexes appear to mix randomly on the feeding grounds, however migration south and north is segregated according to life history status. Additionally, for pregnancy rates, I would hypothesize a pregnancy rate between 30 and 50%. These predictions are based off the obtainable reproduction parameters from available whaling data and a complex assessment of reproductive rates of humpbacks from Alaska and Gulf of Maine waters that documented average calving rates between 0.37 and 0.41 calves per year (Baker et al. 1987, Clapham & Mayo 1990, Glockner-Ferrari & Ferrari 1990). Assessing population demographics is paramount in understanding the impacts of previous, as well as current, perturbations on biological populations. Humpback whales were hunted extensively 100 years ago and their subsequent recovery is occurring in the face of rapid climate change along the Western Antarctica Peninsula.

To test my predictions, I assessed the aforementioned demographic parameters from a series of humpback biopsy samples collected over the course of five field seasons along the WAP from 2010, 2013-16. Genetic sex and pregnancy status were determined in the lab. Following this, I estimated proportions and tested for differences in the extent of temporal variability within these demographics.

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CHAPTER 2: Temporal Variation in Humpback Whale (*Megaptera novaeangliae*) Demographics Along the Western Antarctic Peninsula

Abstract

Humpback whale populations in Antarctica are recovering after intense commercial whaling in the 20th century. Along the Western Antarctic Peninsula (WAP) this recovery is occurring in an environment that is experiencing the fastest warming of any region on the planet. To begin to understand the dynamics of this recovery under such dramatic climate change, we are studying the demography of these whales. To date, we have collected 583 biopsy samples from 239 individual males and 268 individual females during the austral feeding season from 2010, 2013-2016. The overall sex ratio of our sample population is 0.89 M:F, supporting early observations that sexes mix randomly on the feeding grounds. Additionally, we did document a significant seasonal increase in the proportion of females along the WAP into the fall. We believe that this shift represents a tendency for pregnant female humpback whales to depart last from the feeding grounds. Furthermore, we examined progesterone levels of females to assign a pregnancy status; providing to our knowledge, the first non-lethal estimation of pregnancy rates in Antarctic whales. A series of female humpback whales of known pregnancy status (n=29) from the Northwestern Atlantic, verified from field observations, were used as control samples to develop a logistic regression model, modelling the probability of pregnancy relative to blubber progesterone concentrations. A pregnancy state was then assigned to females biopsied along the WAP by modelling their probability of being pregnant across the control model. Based on our assignment of

females as pregnant/not-pregnant, mean progesterone levels for pregnant assigned humpback whales from the WAP was 250 ng progesterone/g blubber ($n = 155$). The mean value for not-pregnant assigned females was 2.10 ng progesterone/g blubber ($n = 89$). Pregnancy rates varied significantly across all years, from 36% in 2010 to 86% in 2014. We detected a significant increase in the proportion of pregnant females (58% to 72%) from summer to autumn across all years. Some female whales in this population appear to experience a post-partum ovulation followed by conception (annual pregnancy); on average, more than half (52%) of female whales accompanied by calves were pregnant. These are some of the first quantitative observations of the demography of recovering humpback whale populations in the Antarctic and provides a critical reference point as the Antarctic climate continues to change and populations recover from whaling.

Introduction

Conservation biology as a scientific discipline is growing rapidly in many countries across the globe as our natural ecosystems and populations continue to experience increased environmental and anthropogenic perturbations. Even so, it remains unclear if the pace of our conservation efforts will precede the rates of these perturbations. Anthropogenic exploitation on animals in the wild occurs in both the marine and terrestrial environments and is often excessive. This exploitation increases the 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 (Burney & Flannery 2005, Proaktor et al. 2007, Allendorf et al. 2008). For example, by the late 1800s we had witnessed an almost complete

collapse of the American bison population in North America because of over hunting (Gates et al. 2010). It was only by understanding the effects of this exploitation on their demography were conservationists capable of recovering this species. Thus, understanding demography is important in biological populations. However, assessing these demographics can be quite challenging, especially in polar dependent, long-lived, mobile species, where it is often logistically difficult to study these animals due to their cryptic nature and the harsh environment in which they are found. Paradoxically, even though the study of polar species is arduous, it is of the utmost importance: species dependent on polar regions are likely under some of the most intense pressures with respect to climatic changes in environment (Bernstein et al. 2008).

Climate change is now considered one of the primary forcings inhibiting and deteriorating the growth and health of biological populations worldwide (Root et al. 2006). These climatic changes are likely to have dramatic impacts on polar dependent species and the elasticity of their habitats (Bernstein et al. 2008). The potential response to climate change by cetaceans has been visited multiple times by expert panels and working groups (i.e. International Whaling Commission (IWC)), however, no clear consensus has been established (Clapham 2016). 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 (Laidre et al. 2008). In order to discern the possible impacts of climate change on cetaceans that utilize polar waters in the future, we require a better understanding of their current demography.

Understanding the recovery of a species from vast exploitation places current knowledge on their health and growth into ongoing and future environmental changes. Populations of baleen whales were heavily exploited during the height of the industrial whaling era in the early 20th century (Rocha et al. 2014). As these populations of baleen whales recover, they may re-occupy ecological roles, particularly on their high latitude feeding grounds, from which they may have been historically absent from many decades. Specifically, most humpback whale populations in the Southern Hemisphere are recovering rapidly after intense commercial whaling in the 20th century, whereby more than 215,000 individuals were removed by the early 1970s for commercial purposes (Gales & Commission 2011, Johnston et al. 2011, Rocha et al. 2014).

Like many other mysticetes, humpback whales exhibit an annual migration from low-latitude breeding grounds to high latitude feeding grounds where they exploit high levels of seasonal productivity (Dawbin 1966). During the austral summer, humpback whales feed in at least six defined feeding grounds in the Southern Ocean, including the Western Antarctic Peninsula (WAP) (Johnson & Wolman 1984, Rice 1998, Gales et al. 2011). Little is known about the exact timing of migratory movements and residency times of various age and sex 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 (Rocha et al. 2014). An improved understanding of the demography and seasonal patterns of movements of humpback whales in this area would help place current research on their

regional density and population dynamics into a broader ecological context (Nowacek et al. 2011, Johnston et al. 2012).

Awareness of the sex of individuals in a population facilitates the study of population health, behavior, breeding patterns, and social systems (Rosel 2003). Such knowledge allows us to better understand how these animals interact and organize themselves within their environment, ultimately enhancing our knowledge of their life history characteristics and ecology. Insights into the timing of migration for various age and sex classes is available from other populations of humpback whales in the Southern Ocean that were exploited later than those along the WAP (Chittleborough 1965). In these areas, pregnant female humpback whales were the first to arrive on the feeding grounds, followed immature animals, resting females and mature males, and lastly, lactating females and their young calves. These observations were generated during the height of commercial whaling, and there have been no attempts to assess these relationships, until now, following the cessation of whaling in the Southern Ocean and the recovery of these populations. Additionally, assessing sex ratios may allow us to make certain inference about that populations adaptations to external perturbations (Clutton-Brock & Iason 1986, West et al. 2002). This becomes of great importance for populations, like the Southern Hemisphere humpback whale, that experienced intense historical exploitation and is now currently faced with potential ecosystem shifts due to environmental variation from climatic change.

In some areas of the Southern Ocean, whales are encountering environmental conditions that are very different from those that existed prior to their exploitation. Specifically, along the WAP, the recovery of humpbacks is occurring in an environment

that is experiencing the fastest climatic warming of any region on the planet (Smith & Stammerjohn 2001). 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 marine and terrestrial habitat (Vaughan et al. 2003, Meredith & King 2005). The biological and physical productivity of the WAP marine ecosystem is intimately tied to the amount of sea ice cover in this region (Massom & Stammerjohn 2010). Additionally, 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 four decades ago (Ducklow et al. 2013). This warming is proceeding with uncertain impacts, and 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 (Ducklow et al. 2013).

Feeding is only thought to occur during summer where these whales migrate poleward from their low latitude breeding grounds (Dawbin 1966). Feeding during this time becomes pivotal, if these whales are to survive the next year's migration and fasting while on the breeding grounds. Energetic studies in other baleen whales, particularly blue and fin whales, have shown that more than 65% of their annual lipid reserves are used over the course of the annual migration (Lockyer 1981b). Additionally, these energetic demands become of even greater importance for those whales that are currently gestating or lactating. Lockyer (1981b) suggested that lactation and pregnancy will consume roughly 19% of total energy stores in mature female fin whales. For humpback whales, these energetic costs are likely greater as they migrate much greater distances and likely lactate three months longer (Lockyer 1981a). These energetic hypotheses seem to be

supported by the observations that humpbacks appear to double their body mass during the Antarctic feeding season, and that pregnant and lactating humpback females that were commercially taken in the same region yielded roughly twice as much oil as resting females (Dawbin 1966, Lockyer 1981a).

Pregnancy rates are a good estimator for a population's overall health and growth. Obtaining such information will greatly aid in the understanding of the recovery of humpback whales along the WAP. Historically, understanding cetacean reproductive status was only obtainable from animals taken as part of commercial hunts (Mansour et al. 2002, Kellar et al. 2006). For example, during the commencement of commercial whaling on the Australian coast in the 1940's and 1950's, scientific observers would study the reproductive tracts of animals being flensed, allowing for the determination of pregnancy (Chittleborough 1965). With the moratorium of commercial whaling in 1982 by the IWC, our current understanding of pregnancy in cetaceans has become limited and has now only been obtainable from necropsied dead or captive individuals (Mansour et al. 2002). Given this lack of contemporary knowledge on reproduction parameters in wild cetaceans following the moratorium, techniques have been developed to detect pregnancy using non-lethal methods.

Indirect alternatives to detect pregnancy have been developed for some populations of humpback whales where long term sighting histories have been developed (Clapham & Mayo 1990). Specifically, information on these reproductive rates has been obtainable through repeated observations of adults accompanied by calves (Clapham & Mayo 1987, Glockner-Ferrari & Ferrari 1990). Obtaining this high-resolution sighting data from year to year, however, is not always possible, particularly in remote areas like

the Antarctic. Pregnancy status in marine mammals has also been determined by the following methods: assessing the concentrations of progesterone in the milk of lactating bottlenose dolphins (West et al. 2000), sex steroids secreted in the urine of killer whales (Walker et al. 1988), salivary steroids in Hawaiian monk seals (Pietraszek & Atkinson 1994), blood plasma, salivary, ocular, and vaginal secretions of false killer whales (Atkinson et al. 1999), progesterone metabolites in fecal samples from North Atlantic right whales (Rolland et al. 2005), and in field ultra sounds of wild, coastal dolphins that were part of capture-release health assessments (Wells et al. 2014). For cetaceans at sea, and those that are highly migratory like humpbacks, obtaining pregnancy information via any of these methods has been nearly impossible to achieve.

Progesterone, often referred to the hormone of pregnancy, is a lipophilic circulatory steroid hormone produced by corpus luteum, and is the primary regulator of estrous cycling and pregnancy (Pineda 2003). Following ovulation, progesterone signals the uterine lining to prepare for fertilization and implantation, and then throughout gestation it aids in sustaining this environment for successful fetal development (Wuttke et al. 1998). Parturition (birth) occurs following a series of internal physiological events, and the return to basal levels of progesterone is estimated to occur within 10-20 days in some species of terrestrial related ungulates (Bedford et al. 1972, Hillbrand & Elsaesser 1983). The difference in the progesterone concentrations due to either being not-pregnant or pregnant within mammals allows for accurate assignment of pregnancy (Kellar et al. 2006). Progesterone's lipophilic properties make skin-blubber biopsy samples an easily obtainable, non-lethal, analytical medium for assigning pregnancy in wild, free ranging cetaceans. With the development of remote biopsy sampling techniques as well as the

methods to assess hormone levels from skin-blubber biopsy samples, we now have the capacity to assess these vital demographic rates in wild cetacean populations while they are still alive (Mansour et al. 2002, Kellar et al. 2006). Developing a current understanding of pregnancy rates within the WAP population of humpbacks will become crucial to make effective conservation decisions on these whale's recovery now and into the future.

Here, I aimed to assess the current variation in the population demographics of humpback whales along the WAP, and in doing so, develop a current reference point that will be needed to make future comparisons and predictions on humpback population recovery and the potential effects of climate change. We utilized a multipart demographic analysis to assess the inter-annual and seasonal variation in the sex ratios and pregnancy rates of humpback whales biopsied during the austral summer and fall field seasons along the WAP.

Based on previous studies, primarily demographic literature comprised from whaling data in the southern hemisphere, I would expect to observe a 1:1 M:F sex ratio on the feeding grounds (Chittleborough 1965, Dawbin 1966). Furthermore, based on available migration data, I expect a sex bias in favor of females later into the feeding season as they are more likely to feed longer in order to support the energetic demands associated with reproduction (Dawbin 1966, Lockyer 1981b). These assumptions are based on the observations by Dawbin (1966) that sexes appear to mix randomly on the feeding grounds, however migration south and north is segregated according to life history status. Additionally, for pregnancy rates, I would hypothesize a pregnancy rate between 30 and 50%. These predictions are based off the obtainable reproduction

parameters from available whaling data and a complex assessment of reproductive rates of humpbacks from the Gulf of Maine and Hawaiian waters (Baker et al. 1987, Clapham & Mayo 1990, Glockner-Ferrari & Ferrari 1990). Assessing population demographics is paramount in understanding the impacts of previous, as well as current, perturbations on biological populations. Humpback whales were hunted extensively 100 years ago and their subsequent recovery is occurring in the face of rapid climate change along the Western Antarctica Peninsula.

To test my predictions, I assessed the aforementioned demographic parameters from a series of humpback biopsy samples collected over the course of five field seasons along the WAP from 2010, 2013-16. Genetic sex and pregnancy status were determined in the lab. Following this, I estimated proportions and tested for differences in the extent of temporal variability within these demographics.

Materials and Methods:

Ethics statement

Procedures for ensuring animal welfare during biopsy collection were approved as part of the scientific research permits issued by the National Marine Fisheries Service (NMFS) issued to Dr. Doug Nowacek (permit 14809-03) under the authority of the Marine Mammal Protection act of 1972. The National Science Foundation Antarctic Conservation Act permit (permit 2016-024 M#1) was obtained by Dr. Ari Friedlaender to conduct tagging and biopsy sampling of baleen whales in the Antarctic Peninsula region. Oregon State University's Institutional Animal Care and Use Committee (IACUC) approved a protocol for the collection of biopsy samples (permit 4513 & 4943). 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. Oregon State University is a registered scientific institution under CITES.

Biopsy collection

Skin and blubber biopsy samples were collected from individual humpback whales during the 2010, 2013-16 field seasons along the Western Antarctic Peninsula (WAP) using standard field methods described below (Figure 2.1). Samples were collected opportunistically whenever whales were encountered during prey or visual surveys in an area within ~10 nautical miles of Palmer Station, Anvers Island. Additionally, samples were collected throughout the Palmer LTER sampling grid during the course of the LTER research cruise in January and February (Figure 2.1). Samples were collected using a crossbow with modified bolts and 40mm tips (CetaDart) from a small vessel from distance of 10-30 meters targeting the area of the body in the vicinity of the dorsal fin (Krützen et al. 2002). Samples were stored frozen whole at -80° C until used for analysis. When possible, fluke photographs were collected to individually identify animals and to be used for inclusion in a catalog of individual whales similar to SPLASH (Barlow et al. 2011).

Sex identification

It is difficult to determine the sex of cetaceans in the field, although adults accompanied by calves are often assumed to be female. However, molecular methods are used routinely to determine the sex of individuals from DNA extracted from skin biopsies (Baker et al. 1991, Palsbøll et al. 1992, Bérubé & Palsbøll 1996, Morin et al. 2005). Thus, to determine the genetic sex of whales that were biopsied, genomic DNA was extracted

from the skin-blubber interface using a proteinase K digestion followed by a standard phenol-chloroform extraction method (Sambrook et al. 1989). The sex of each sampled whale was determined by amplification of sex-specific markers following the protocol of Gilson et al. (1998). This involved a multiplex PCR with primers designed to amplify the male-specific SRY gene and, as positive controls, primers designed to amplify the ZFY/ZFX genes of males and females. We compared results 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, within years, and across seasons.

Microsatellite amplification

To determine the individual identity of each biopsied humpback whale, every sample was genotyped using 10 previously published microsatellite loci (Table 2.1) and PCR'd with labelled primers (Valsecchi & Amos 1996, Palsbøll et al. 1997, Waldick et al. 1999, Baker et al. 2006). Amplifications were carried out in a final volume of 10 μ l at the following concentrations: 1x reaction buffer, 1.5 – 4mM MgCl₂ 0.4 μ M each primer, 0.2mM dNTP's, 0.5U Platinum taq (Invitrogen) and approximately 5ng DNA (Table 1). Two temperature profiles were used for amplification, 'short' and 'long', and cycle number varied depending on demand for the machines across all other laboratories. The 'short' temperature profile consisted of initial denaturation at 94° C for three minutes followed by 35 cycles of denaturation at 94° C for 30 seconds, annealing at 50° C for 30 seconds and extension at 72° C for 30 seconds with a final extension at 72° C for ten minutes. The 'long' profile was as described above with extension at 72° C for 40 seconds each cycle and a final extension at 72° C for 30 minutes. To optimize genotyping, microsatellites were fluorescently labeled (Table 2.1) and mixed post-PCR

into sets of co-loads with non-overlapping loci before being run on an ABI 3730xl sequencer (Applied Biosystems).

Microsatellite quality control and genotype identity

In order to assess the quality of our humpback whale genotypes, alleles were sized and binned using the software program *Genemapper v3.7* (Applied Biosystems). This program also assessed the quality of each allele based on several criteria (e.g. peak height, peak shape, bin fit) and assigned a quality score (QS) for each sample. The peaks for all alleles with a quality score less than 0.80 were visually assessed. Where peaks were deemed to be readable and clean, the alleles were accepted. For some loci, under some conditions a varying ‘plus A’ signal caused problems for the automatic binning component of *Genemapper*, in these instances bins were assigned manually. The expected probability of identity (P_{ID} ; the probability that two individuals drawn at random will have the same genotype) for each locus was calculated in *GenAlEx v6.5* (Peakall & Smouse 2006). *Cervus 3.0.7* (Kalinowski et al. 2007) was used to compute the number of alleles (K), observed and expected heterozygosity (H_O and H_E), deviation from Hardy-Weinberg equilibrium (HWE) with a Bonferroni correction, and the probability of identity for all individual matches (P_{ID}).

Hormone extraction

In order to assess the variation of progesterone concentrations in skin blubber biopsy samples between different female humpback whales, a cross-sectional sub-sample (~0.15g) spanning from the epidermis-blubber interface to the most internal layer of the biopsy was sub-sectioned from already sexed female humpback whales. We modified the extraction methods as were previously reported by Kellar et al. (2006) in order to fit our

lab setup. These sub-samples were then homogenized separately in 1400 μ l of 100% HPLC grade ethanol using an automated, multi-tube homogenizer (Bead Ruptor 12, Omni International). Samples were processed for twelve 60-second periods at a speed of 5m/s in specialized reinforced 2mL homogenization matrix tubes available from Omni International. The homogenization tubes were pre-filled with \sim 0.5mL of 0.7 mm garnet homogenization beads (Omni International). After two 60-second homogenization steps, the samples were placed on ice for ten minutes to prevent the homogenate from overheating. Once cooled, an additional five, two 60-second homogenization steps were conducted.

Following the completion of the homogenization process, the homogenates were transferred using a pipette to a 13x100 mm borosilicate glass test tubes. The remaining beads and connective tissues were transferred from the homogenization tubes to 12 x 100 mm borosilicate test tubes. The beads and connective tissues were washed twice with 500 μ l of 100% ethanol and this solution was transferred to the 13x100 mm test tube containing the original homogenate. The homogenization tubes were washed once with an additional 500 μ l of 100% ethanol and this was transferred to the same 13x100 mm test tube. 2 mL of 4:1 ethanol acetone was combined with the homogenate solution in the 13x100 mm test tube. The samples were then vortexed using a multitube vortexer and centrifuged at 4000 rpm for 15 minutes. The supernatant was transferred to newly labelled 12x100 mm glass tubes and evaporated on an NDK200 sample concentrator (Hangzhou MIU Instruments Co., LTD) using filtered compressed air. In order to isolate the lipids containing the progesterone, 2mL of diethyl ether was added to the tubes, the samples were vortexed and centrifuged again, and the supernatant was collected and

evaporated. Lastly, to extract the hormones, the lipid residue was re-suspended in 1.4mL of acetonitrile, vortexed, and then 1.4mL of hexane was added to the solution. After the solution was vortexed and centrifuged again, the tubes were placed overnight in a -20° C freezer to aid in layer separation. The next day, the acetonitrile layer was extracted and placed in a new tube and the process was repeated with 1.4mL of hexane. The final portion of the acetonitrile layer was collected and evaporated. The remaining hormone residue was stored at -20° C until enough samples were extracted to be run on an assay and quantified.

Quantification of progesterone by enzyme immunoassay (EIA)

The amount of progesterone in each blubber sub-sample was quantified with a progesterone enzyme immunoassay (EIA). Prior to analysis, samples were re-suspended in 1 mL of phosphate buffered saline (pH 7.5) containing 1% bovine serum globulin and vortexed thoroughly using a multitube vortexer for 15 minutes. The progesterone EIA kit utilized in this study has 100% reactivity with progesterone and an assay detection limit between 15 and 500 pg/mL using the standard curve (EIA kit 900-011, ENZO Life Sciences, Farmingdale, NY). Two additional standard dilutions were added to allow for a lower detection limit of the standard curve to 3.81pg/mL. Samples were run blind and in duplicate at both a straight and 1/100 dilution. If the straight dilution maxed out along the assay detection curve while simultaneously providing a concentration for the 1/100 diluted sample, this would verify high progesterone concentrations. If the 1/100 dilution reported a minimum value from the detection curve while simultaneously providing a concentration for the sample run straight, this would verify low progesterone concentrations. If progesterone concentrations were not obtained using either of these

dilutions, they were further diluted in order to be accurately measured. Sampled dilutions consisted of 1:1000, 1:500, 1:100, 1:50, 1:10, 1:5 depending on the original assay measurements such that the final measurement would fall within the range of the detection curve. The reported inter-assay coefficient of variation (COV) and intra-assay COV of the progesterone EIA ranged from 2.7-8.3% and 4.9-7.6% respectively.

In order to assess extraction accuracy, we determined the extraction efficiency using samples from a deceased animal of known pregnancy status by spiking subsamples of blubber with 150ng of progesterone and including these with every extraction (Kellar et al. 2006). The percentage of progesterone that was recovered after the extraction was calculated and each sample concentrations was adjusted to this efficiency prior to statistical analyses. An extraction efficiency greater than 60% was determined to be acceptable. If the extraction efficiency was less than 60%, the sample extracts were discarded and the blubber samples were re-extracted and re-analyzed.

Assay evaluation of parallelism

In order to assess the performance of the progesterone EIA used in our analyses we conducted a parallelism test (Plikaytis et al. 1994). A series of pooled samples from five humpback whale individuals from the WAP were compared against the kit progesterone standard to determine parallelism (Plikaytis et al. 1994, Kellar et al. 2013). Assay parallelism has become a standard calibration technique among the scientific community who utilize EIA in their analyses. Assessing assay parallelism is important as the serially diluted curves between the kit standards and pooled samples must be parallel in order to support the assumptions that the EIA binding antibody (kit progesterone) and bound antigen (blubber progesterone) characteristics are analogous (Plikaytis et al. 1994).

A deviation in parallelism between the two slopes would demonstrate interference within the assay, potentially overestimating or underestimating the true progesterone concentrations in the blubber extracts. The series of serially diluted pooled samples and kit standards were run in duplicate and an analysis of covariance (ANCOVA) was used to determine if the slopes between the two lines were significantly different.

Pregnancy classification

To assign pregnancy within our WAP female humpbacks, a series of biopsy samples ($n = 29$) were collected from individuals of known life history stage from the Gulf of Maine feeding aggregation by the Center for Coastal Studies (CCS) in Provincetown, MA (Dr. Jooke Robbins, personal communication). Pregnant controls included females that were either lactating or considered resting when biopsied, but were observed with a calf the following feeding season. Not-pregnant females were sampled during the summer feeding season and were documented as either lactating or resting during the biopsy collection and verified as not-pregnant by the absence of an accompanied calf the following feeding season. For this scenario, it is important to note that the loss of a calf could have occurred following the collection of the sample, which would show this female as being pregnant.

Using these control samples from the Gulf of Maine, we modelled the pregnancy state relative to blubber progesterone concentrations using a standard logistic regression model (Kellar et al. 2017). All the blubber progesterone measurements were log transformed preceding the analysis. Each WAP sample, of unknown pregnancy status, was input into the model and the model returned a probability of being pregnant for each female sampled (Kellar et al. 2017). 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, she received an undetermined pregnancy assignment. Additionally, these control values were plotted on a log scale and overlaid with the progesterone concentrations from WAP humpbacks to visually assess our modelled pregnancy assignments.

Data preparation and analysis

To test for a deviation from an expected 1:1 sex ratio within our entire sample dataset, a two tailed Exact Binomial Test was used (Thompson et al. 2013). To assess within year and season deviations from a 1:1 sex ratio, an Exact Binomial Test was again used. The austral seasons, summer and fall, were split according to the fall equinox on March 19. Additionally, to avoid re-sample bias in our analyses, all within-year replicates were removed from the inter-annual comparisons and only within season replicates from the same year were removed from the seasonal analyses. In both cases, the most chronologically recent sample was kept for the analyses.

To evaluate the overall variation in the sex ratios, pregnancy rates, and reproductive states across seasons and years, we tested for significance using a two-tailed χ^2 test of independence. We used a Tukey's post hoc stepwise multiple comparison test to determine significance between individual years. For all statistical tests a P-value of less than 0.05 was considered significant. Values are expressed as mean \pm SD, unless otherwise stated.

Results:

Sampling overview

We collected a total of 583 humpback whale biopsy samples from the nearshore waters around the Western Antarctic Peninsula (WAP) over the course of five field seasons from 2010 to 2016 (Figure 2.1). A genetic sex was successfully assigned to 577 samples from 507 individual whales (Table 2.2). A total of 239 individual males and 268 individual females were sampled over the course of the 5 years of this study (Table 2.2). Across all samples, females were slightly more abundant (0.89 M:F), however this deviation from an expected 1:1 sex ratio was not significant ($P = 0.213$, Exact Binomial Test; Table 2.2). Twenty-seven samples were collected in 2010 (27 Fall), 86 samples were collected in 2013 (86 Summer), 136 samples were collected in 2014 (109 Summer, 27 Fall), 128 samples were collected in 2015 (99 Summer, 29 Fall), and 200 samples were collected in 2016 (104 Summer, 96 Fall; Table 2.2).

Variation in microsatellites and genotype quality

Overall, microsatellite amplification and the developed genotype quality of the humpbacks analyzed was high. Missing allelic data averaged 0.1% across all the loci with a maximum of 0.47% for the locus GT211. The number of alleles per locus ranged from 5 to 21 (mean = 12.9 ± 4.7 ; Table 2.3). The largest number of alleles (21) was found at locus GATA417, while the smallest was recorded at locus rw48 (Table 2.3). The P_{ID} for each locus ranged from 0.015 to 0.366 and the combined P_{ID} for all ten loci was 4.146×10^{-13} (Table 2.3). All loci were in HWE, as no significant differences were found between the observed and expected heterozygosity for any alleles (Table 2.3).

Genotype ID

In total, we recaptured 54 individuals within the same year; two individuals were resampled in 2010, seven individuals in 2013, 19 individuals in 2014, four individuals in 2015, and 22 individuals in 2016 (Tables 2.2, 2.4). Three individuals were sampled three times (two in 2014, one in 2016) and one individual was sampled four times in 2016 over the course of a year. Additionally, we documented 11 between year genetic re-captures; one between 2010 and 2013, one between 2013 and 2014, three between 2013 and 2015, one between 2013 and 2016, one between 2014 and 2015, two between 2014 and 2016, and lastly, two between 2015 and 2016 (Tables 2.2, 2.4).

Within year variation in sex ratios

The sex ratio in 2010 was 0.47 M:F (8 males, 17 females), which was not significantly different from parity (Exact Binomial Test, $P=0.108$; Figure 2.2, Table 2.2). The sex ratio in 2013 was 1.32 M:F (45 males, 34 females), which was not significantly different from parity (Exact Binomial Test, $P=0.260$; Figure 2.2, Table 2.2). The sex ratio in 2014 was 1.05 M:F (59 males, 56 females), which was not significantly different from parity (Exact Binomial Test, $P=0.852$; Figure 2.2, Table 2.2). The sex ratio in 2015 was 0.75 M:F (53 males, 71 females), which was not significantly different from parity (Exact Binomial Test, $P=0.127$; Figure 2.2, Table 2.2). The sex ratio in 2016 was 0.84 M:F (80 males, 97 females), which was not significantly different from parity (Exact Binomial Test, $P=0.290$; Figure 2.2, Table 2.2). There was no overall significant difference in the sex ratio observed across all years ($\chi^2 = 7.256$, $df = 4$, $P=0.123$; Figure 2.2, Table 2.9).

Within season variation in sex ratios

The sex ratio in the summer was 1.01 M:F (182 males, 180 females), which was not significantly different from parity (Exact Binomial Test, $P=0.960$; Figure 2.3, Table 2.2). The sex ratio in the fall was 0.68 M:F (65 males, 95 females), which was significantly different from parity (Exact Binomial Test, $P=0.020$; Figure 2.3, Table 2.2). Additionally, we observed a significant bias in favor of females into the fall ($\chi^2 = 4.146$, $df = 1$, $P=0.042$; Figure 2.3, Table 2.9).

Validation of humpback progesterone assays

Based on the concentrations observed from the series of spiked controls, our average extraction efficiency was $73.78\% \pm 0.09$ (minimum 61.1%, maximum 95.6%). Additionally, our calculated inter-assay and intra-assay coefficient of variation (COV) from a series of replicated samples was 6.37 and 7.75 respectively. The results from our assay parallelism test between the kit progesterone standard and humpback whale progesterone showed strong parallelism, indicating the use of the progesterone assay to detect humpback whale progesterone is viable ($P=0.848$; Figure 2.4).

Pregnancy assignment from logistic model

In both cases, all 29 female control progesterone pregnancy determinations corroborated with the field life history descriptions recorded by the research group at the Center for Coastal Studies (CCS). Additionally, no loss of a calf was documented. The pregnant female humpback whale blubber progesterone concentrations from the Gulf of Maine (mean = 223.15 ± 185.62 ng progesterone/g blubber; Figure 2.5, Table 2.5) were much greater on average than not-pregnant females (mean = 1.19 ± 1 ng progesterone/g blubber; Figure 2.5, Table 2.5). Additionally, there was no overlap in progesterone

concentration values between 5 and 50 ng progesterone/g blubber observed among the Gulf of Maine controls, providing great specificity for assigning pregnancy within our WAP dataset for the logistic model (Figure 2.5).

Two hundred and sixty-four female humpbacks were measured for progesterone concentrations, representing 244 individual females (Tables 2.6, 2.7). It is important to note here that not all samples were included in the analysis due to within year re-samples and not all samples were analyzed for progesterone due to there not being enough blubber for an extraction to take place. All 244 individuals of an unknown pregnancy state were predicted across the logistic regression model (Figure 2.6). Of these 244 individual females, 89 received a probability of being pregnant of less than 1% (not-pregnant assignment; blubber progesterone: mean = 2.10 ± 1.13 ng progesterone/g blubber; Table 2.6), seven in 2010, 18 in 2013, seven in 2014, 26 in 2015, and 31 in 2016 (Table 2.7). One hundred and fifty-five of the 244 females received a probability of being pregnant of greater than 99% (pregnant assignment; blubber progesterone: mean = 249.96 ± 281.79 ng progesterone/g blubber; Table 2.6), 4 in 2010, 15 in 2013, 44 in 2014, 38 in 2015, and 54 in 2016 (Table 2.7). Additionally, one female received an undetermined pregnancy assignment and was not included in any further analyses (probability of pregnant 1.6%, blubber progesterone: 11.81 ng progesterone/g blubber; Table 2.6). The distribution in progesterone concentrations across the two assigned pregnancy states for females sampled along the WAP were similarly distributed to the control samples from the Gulf of Maine (Figure 2.7). Additionally, we also documented the same bimodal distribution of raw progesterone values between pregnant and not-pregnant assigned individuals that has been documented among several other cetacean species (Figure 2.7, Tables 2.6, 2.8).

It is also important to mention that except for one replicate sample, 19 of the 20 re-sampled female's progesterone values and pregnancy assignments agreed. Upon further investigation, the one individual whose values were inconsistent was biopsied in the fluke. This sample was not included in any further analyses.

Variation in pregnancy rates of WAP humpbacks

The average pregnancy rate of female humpbacks biopsied across all five sampling years was $58.20\% \pm 19.08$. The pregnancy rates for 2010, and 2013-2016 were 36.36%, 45.45%, 86.27%, 59.38%, and 63.53% respectively (Figure 2.8, Table 2.7). We observed an overall significant difference in pregnancy rates across years ($\chi^2 = 20.02$, $df = 4$, $P=0.001$; Figure 2.8, Table 2.10). A post hoc multiple comparisons analysis revealed that the pregnancy rate in the year 2014 was significantly higher than in all other years (Table 2.9). The pregnancy rate of female humpbacks during the summer and fall seasons across all years was 58.75% and 72.09% respectively (Figure 2.9, Table 2.7). We observed a significant increase in the proportion of pregnant females into the fall ($\chi^2 = 4.53$, $df = 1$, $P=0.001$; Table 2.9; Figure 2.9).

Evidence of annual pregnancy

Over the course of this study, we sampled a total of 44 mother calf pairs; one in 2010, four in 2013, 10 in 2014, 17 in 2015, and 12 in 2016 (Table 2.11). Based on our progesterone pregnancy assignments, we found that 24 of the 44 mothers were pregnant at the time they were biopsied; one in 2013, nine in 2014, seven in 2015, and seven in 2016 (Table 2.11). This indicates an annual pregnancy rate observed in females, excluding the year 2010 due to low sample size, of 25% in 2013, 90% in 2014, 41.2% in 2015 and 50% in 2016 and an overall average of 52.3% (Table 2.11). We also sampled

one female between 2015 and 2016, which was pregnant in both years. Additionally, when separated by season, the proportion of females with a calf and that were pregnant was significantly greater in the fall ($\chi^2 = 5.92$, $df = 1$, $P=0.015$; Table 2.12).

Historical comparison

When the proportion of females biopsied along the WAP in the summer that were pregnant and or lactating was compared to the observed annual pregnancy rate of female humpbacks killed in Antarctic whaling areas IV and V between 1950 and 1958, we observed no significant difference ($\chi^2 = 0$, $df = 1$, $P=0.982$; Table 2.12).

Discussion:

In the present study, we assessed the current demography of humpback whales on the feeding grounds in the waters surrounding the WAP at two different temporal scales: within seasons and across years. We found that sex ratios of biopsied humpback whales did not vary significantly between years, but did between seasons within all years. In addition, we found that pregnancy rates of female humpback whales did vary significantly across all years and that the year 2014 was significantly greater than all other years. Pregnancy rates also varied significantly between seasons. Lastly, our pregnancy dataset suggests a high proportion of females experience an annual, post-partum pregnancy. The results presented here provide a current reference point and multi-year understanding of the present demographics of humpback whales along the WAP, building off previous studies where data was mostly derived from catch statistics. The value of this information is in providing a current reference point and baseline to assess how the dynamics of this population of humpback whales will change overtime in the

face of a rapid climate change, extreme inter-annual variation in sea ice, and the growing krill fishery in the WAP region.

Variation in sex ratios

The sex ratio of the entire sampled population was close to unity (0.89 M:F) and we observed no inter-annual differences among sex ratios across all years. Our data supports the early observations from catch data in the Southern Ocean that the sexes mix randomly on the feeding grounds (Matthews 1937, Chittleborough 1965). For example, Chittleborough (1965) showed that males comprised 52.8% of the 18,136 total whales killed in the Antarctic Area IV population (south of Australia and New Zealand) between 1949 and 1962. Additionally, Matthews (1937) reported that of the 1,057 whales killed at South Georgia, 45.4% of them were male. Similar sex ratios have also been found in non-exploited, Northern Hemisphere populations of humpback whales, like in the Gulf of Maine (Clapham et al. 1995).

We also observed a significant bias towards females into the austral fall along the WAP. This result corroborates with what is known about the temporal segregation of humpback migration in the Southern Hemisphere. Specifically, Dawbin (1966) showed that humpbacks were segregated by demographic status during the northbound and southbound migrations. Lactating females were the first to be observed during the northward migration (towards their breeding grounds) near Cook Strait, NZ, starting about one month past the first northbound catches, followed by immature whales 12 days later, mature males 20 days later, then followed by resting females 23 days later, and lastly, pregnant females 31 days later (Dawbin 1966). Pregnant females were observed accruing an additional 10-15% of fat when compared to resting females, resulting from a

greater portion of time spent on the feeding grounds, of which a maximum of 6.5 months has been historically observed (Lockyer 1984).

Variation in pregnancy rates

Pregnancy rates varied significantly among the five years of this study, ranging from 36-86%, with an average rate of 58.2%. Our overall mean rate is comparable to what was observed in humpback whales off the California coast (mean = 48.4%) and what was reported from female humpbacks taken from Antarctic whaling areas IV and V (mean = 48%) from 1950-56 (Chittleborough 1958a, Clark et al. 2016). Even so, our overall pregnancy rate is greater than what might be expected based on the generally assumed calving rate of 0.37-0.41 (Baker et al. 1987, Clapham & Mayo 1990). Both the WAP and North Pacific populations of humpbacks are recovering from past exploitation (Rice 1978, Calambokidis et al. 2008, Jackson et al. 2015), and thus we would expect current pregnancy rates to be higher than prior to exploitation (Lockyer 1984). A recent IWC report projected the abundance of humpback whales from breeding stock G (humpbacks that feed along the WAP) to be 9,687 individuals as of 2015, growing at a rate of 3.4% (Jackson et al. 2015).

The observed difference between our pregnancy rates and expected calving rates raises the issue of comparing different reproductive parameters in cetaceans. Calving rates are representative of the number of calves observed per a mature female in a given year. Intuitively, this is the most direct measure of recruitment within a population, but obtaining this high resolution of sighting data is not always possible, particularly in a remote environment like the Antarctic. The difference between these two reproductive parameters is likely because calving rates incorporate fetal misbirth and re-absorption

that may have occurred after the biopsy sample of a given female was collected. It is also important to mention that the difference between these two methods may be because with our methods, we do not have the capacity to void immature females from our data analyses and thus the pregnancy rates we have presented here are an absolute minimum estimate.

Pregnancy rates varied significantly across seasons as well (58.75 % in summer and 72.09% in fall), with a bias in favor of pregnant females in the austral fall. We believe this relative increase in the proportion of pregnant females during the austral autumn can be attributed to the fact that these females are maximizing the time spent on the feeding grounds prior to migrating to their low latitude breeding and calving areas. This is supported by the observed significant decline in the proportion of mothers accompanied by a calf into the fall, regardless of whether or not they are pregnant. Additionally, this result continues to support the bias in favor of females that we observed into the fall, as well as the demographic segregation of migration observed from northbound whales that were commercially hunted (Chittleborough 1965, Dawbin 1966). Specifically, there was an observed increased abundance of pregnant females caught in late July and August of Cook Strait, NZ. Following the energetic demands that Lockyer (1981b) suggested, whereby baleen whales must increase their body mass up to 65% to maintain energetic costs for the next year, it makes sense that these pregnant females stay longer (migrate later) on the feeding grounds to exploit the vast abundance of krill biomass in the Antarctic in an effort to increase lipid reserves and support fetal growth.

By assessing the mean mature overall length (39.5 ft.), ear plugs, and the ovaries of 1,603 humpback females, Chittleborough showed that female humpback whales reach

sexual maturity at 4 to 5 years of age (Chittleborough 1954, 1955, 1960, 1965). As we continue our sampling and survey efforts along the WAP, it will become important for us to assess the portion of immature females within our sample data set. With the onset of UAS (unmanned aerial systems) technology, and the capacity to gain accurate estimates of individual cetacean body dimensions, we hope to determine the sexual maturity of biopsied animals based on body length (Christiansen et al. 2016, Durban et al. 2016).

It is important to discuss the significant deviation in pregnancy rates (86%) observed in 2014. Similarly high rates of 72% and 82% were observed in catch data from Antarctic whaling areas IV and V from 1950-1956 and the Bellingshausen Sea, just west of the WAP, in February of 1957 (Chittleborough 1958a, Symons et al. 1958). Additionally, over the course of six years of whaling in Antarctic areas IV and V, pregnancy rates varied from as low as 36% to as high as 72%, again a variation similar to what we have observed in the five years of our study (Chittleborough 1958a). Given the rate of climatic changes occurring in the Antarctic, we might expect to observe some inter-annual variability in pregnancy rates. This variability may coincide with fluctuations in environmental conditions such as varying sea ice as well as krill recruitment. For example, because of the high energy costs associated with reproduction and the annual decrease in winter sea ice, it is possible that some females may stay longer and in fact overwinter on the feeding grounds, allowing them to increase lipid reserves and support an earlier pregnancy (Craig & Herman 1997, Smith & Stammerjohn 2001). Additionally, krill recruitment has been correlated with varying sea ice conditions (Nicol 2006, Steinberg et al. 2015). Rapid demographic responses in Southern Hemisphere cetaceans as a result of varying environmental conditions have already been documented

(Leaper et al. 2006). Therefore, for example, we might expect our 2014 peak in pregnancy to be a delayed response to large krill recruitment years (years preceded by high sea ice), ultimately resulting in a disproportionate increase in the number of both successful pregnancies and females choosing to get pregnant. As we increase our dataset of pregnancy rates across years within this feeding population, we will need to consider these environmental relations to gain a better understanding of these reproductive trends overtime.

Evidence for annual pregnancy

From our pregnancy analyses, we found evidence of a one-year reproductive cycle in which postpartum ovulation and conception occurred among some females that feed along the WAP. We sampled a total of 44 mother-calf pairs over the course of our five field seasons, and 52.3% of these lactating females appear to be pregnant as well. According to Chittleborough (1954) it was assumed that female humpback whales are seasonally polyestrous, with estrus cycles occurring while females are on the breeding grounds in sub-tropical/temperate waters from June to October (Matthews 1937). 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.

These cycles are generally characterized by a single ovulation (Chittleborough 1955, 1965). The total number of ovulations over a 2-3 year breeding cycle, however, is much less well understood (Chittleborough 1958b). Chittleborough (1959) believed that it was possible for annual ovulations to occur, contrary to what others observed in humpback whales around Tonga in the South Pacific, where no evidence of post-partum ovulations within that breeding population was found (Chittleborough 1965).

Additionally, Chittleborough (1958b) documented three types of breeding cycles found in carcasses of sexually mature female humpback whales, of which two supported the fact that annual pregnancy (post-partum ovulations) did occur (Figure 2.10). Specifically, of 19 lactating females observed, eight (42.1%) were both pregnant and lactating (Chittleborough 1958b). This rate is almost identical to the rate we calculated in the summer along the WAP, almost 60 years later.

The lack of this data in historical catch records is likely a result of the International Whaling Commission (IWC) ruling in 1946 whereby mothers accompanied by calves were not allowed to be killed as well as the associated challenges of detecting a fetus in early term in southbound females. Still considered largely a rare phenomenon, a few more recent studies have documented annual reproduction in female humpback whales (Baker et al. 1987, Glockner-Ferrari & Ferrari 1990, Straley et al. 1994). A summary of humpback whale reproductive rates, in which an annual or non-annual pregnancy rate was observed can be found in Table 2.13 (Chittleborough 1958b, Symons et al. 1958, Baker et al. 1987, Clapham & Mayo 1987, 1990, Glockner-Ferrari & Ferrari 1990, Straley et al. 1994, Steiger & Calambokidis 2000). It is important to mention here that most knowledge we have on the reproductive parameters of living humpback whales is derived from populations in the Northern Hemisphere. Specifically, of the calving intervals reported for 34 mothers by Glockner-Ferrari and Ferrari (1990) in the Hawaiian Islands, four females experienced a one-year reproductive cycle in which postpartum ovulation occurred with conception, one female was observed with four different calves four years in a row.

The frequent occurrence of an annual reproductive cycle in female humpbacks could be indicative of an increasing population (Glockner-Ferrari & Ferrari 1990). Indeed, the WAP population of humpbacks (and nearly all in the Southern Hemisphere) is believed to be increasing (Jackson et al. 2015). An increase in pregnancy rates of blue, fin, and sei whales was observed in the Southern Ocean from 1930 to 1970, thought to likely be the result of more food per capita available from reduced intra and inter-specific competition due to commercial whaling (Lockyer 1984). For a similarly exploited Southern Ocean species, like humpback whales, this too likely resulted in an increased proportion of annual pregnancy, as detected by Chittleborough (1958b) in the 1950s, as they were hit hardest early on (Hart 2006, Rocha et al. 2014).

The observed increase of annual pregnancies within this population of humpbacks could be indicative of lasting demographic pressures from post-industrial whaling. Specifically, high rates of exploitation of adults from a population has been linked to an observed decrease in the mean age at sexually maturity and an increase in favor of early reproduction (Lockyer 1984, Proaktor et al. 2007). The growth of a population depends not only on its reproductive rate, but on the survival and recruitment of these calves into the population (Allen 1974). With this in mind, the occurrence of an annual pregnancy cycle in females within this population might be the result of an early loss of a calf or fetus from the previous cycle (Allen 1974, Lockyer 1984). This phenomenon is likely more common in first time, early breeders, as Straley et al. (1994) believed that older females are more likely to achieve a successful annual pregnancy. Even with the higher occurrence in our dataset, it is important to outline the associated biases associated with this data, such as the lack of understanding of neonatal and fetal deaths and the

physiological conditions of the mother. Thus, in order to clearly say whether some female humpback whales that feed along the WAP are engaging in a successful annual pregnancy, we need to better understand calving intervals and survival.

Implications and significance

Understanding and assessing basal demographic rates in a previously exploited population is a critical first step in predicting a population's recovery. The future of humpbacks feeding along the WAP is unclear as we are still unaware of what the WAP ecosystem will look like in the next 50 to 100 years in the face of climate change. To clearly assess population growth and change requires vigilance and continuity of data collection. To this end, we now have the opportunity and methods to conduct this work. We can now begin to predict population level responses to climate change by linking these demographics to key environmental variables as well as assess individual variation through mark recapture studies, allowing us to document reproductive states over consecutive years. Additionally, this work builds on the demographic and life history data of Southern Hemisphere humpbacks that was derived from whales killed roughly 100 years ago and published in papers 50 years ago. This work bridges the existing knowledge gap by placing the current demography and recovery of this population in the scope of current and future climatic trends. With the continued decrease of winter sea ice, we may begin to document both a temporal shift and spatial expansion of the feeding grounds of these whales in the short-term as prey availability will likely increase (Moore & Laidre 2006, MacLeod 2009, Ramp et al. 2015). Long-term however, proves more problematic, due to the tight coupling of sea ice and the recruitment of krill within the WAP ecosystem, and the reliability of krill as the main food source for most of the large

trophic marine vertebrates in the Southern Ocean (Flores et al. 2012, Steinberg et al. 2015).

In this study, we assessed the inter-annual and seasonal variation in two key demographic parameters, sex-ratios and pregnancy rates, of the WAP feeding population of humpback whales. To our knowledge this is the first attempt to document pregnancy in Antarctic whales using non-lethal methods and is among the largest study conducted on blubber progesterone concentrations among free ranging mysticetes. Additionally, the results presented here provide further evidence supporting the use of blubber biopsy samples as an analytical medium for pregnancy assignment in cetaceans, and particularly humpback whales. In agreement with previous studies, our results documented an overall sex ratio close to parity and an average pregnancy rate similar to others documented within other populations of humpback whales. Additionally, we documented a disproportionate number of female humpback in the fall, which we believe is the tendency for pregnant females to stay later on the feeding grounds. We observed some variability within pregnancy rates across years, which may be attributed to environmental variability. Also, we found newly, undocumented evidence, suggesting some females along the WAP are experiencing annual cycles of pregnancy. To conclude, this information provides a baseline and current reference point for further demographic studies of these recovering krill predators in a changing polar ecosystem.

Chapter 2: References

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Figures

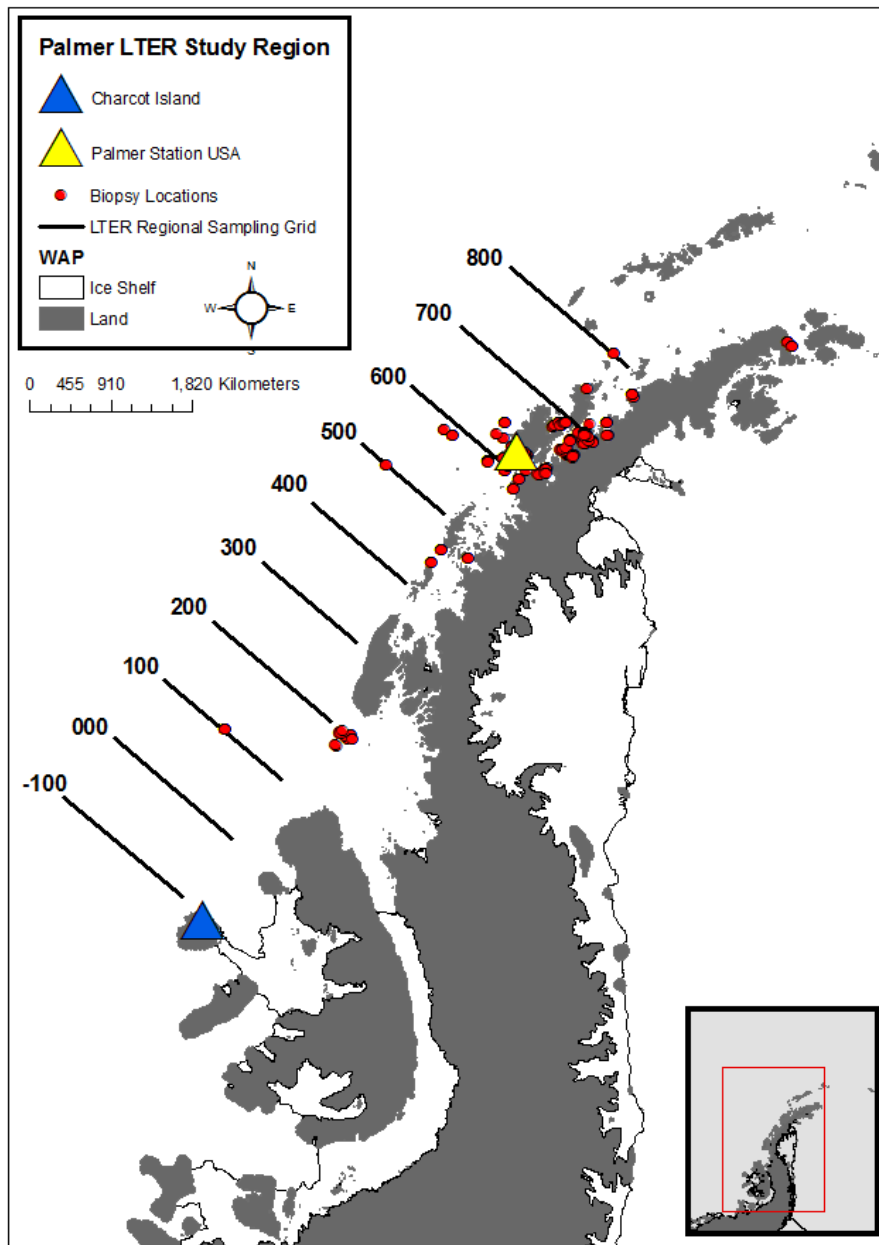


Figure 2.1: Map of humpback whale biopsy samples collected during the 2010, 2013-16 field seasons throughout the Palmer LTER study region along the Western Antarctic Peninsula. Map shows Palmer Station, USA (yellow triangle), Charcot Island (blue triangle), LTER oceanographic sampling grid (black lines), and location of humpback biopsies (red dots).

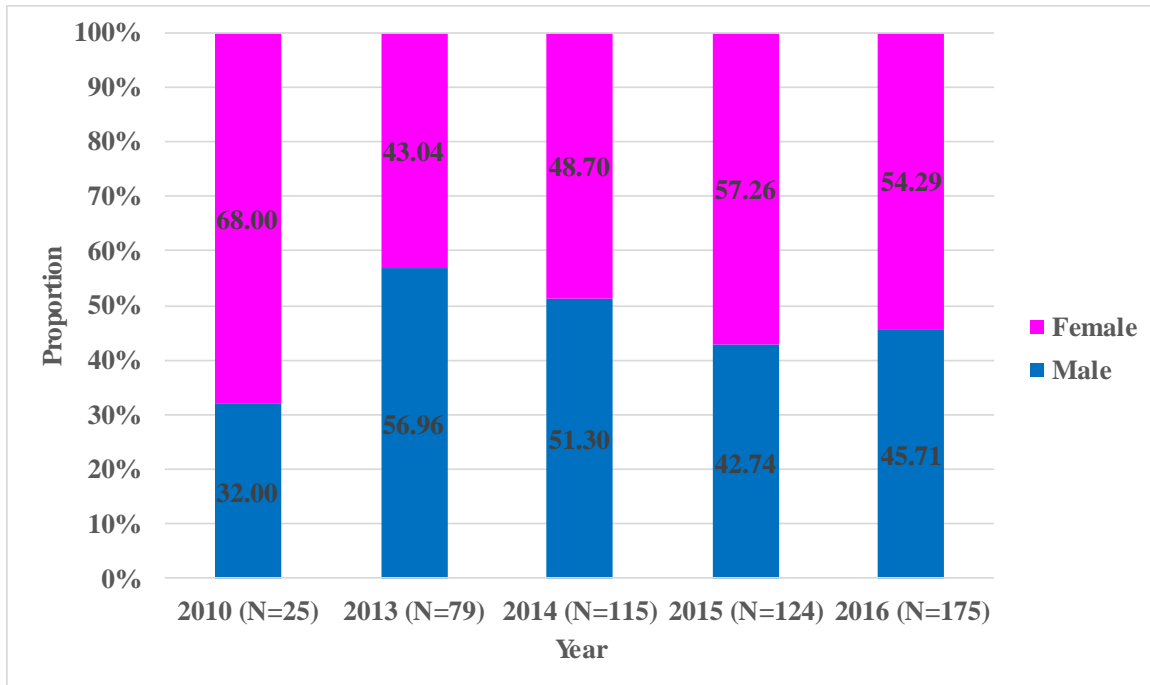


Figure 2.2: Inter-annual changes in the proportion of male to female (sex ratio) humpback whales sampled along the Western Antarctic Peninsula.

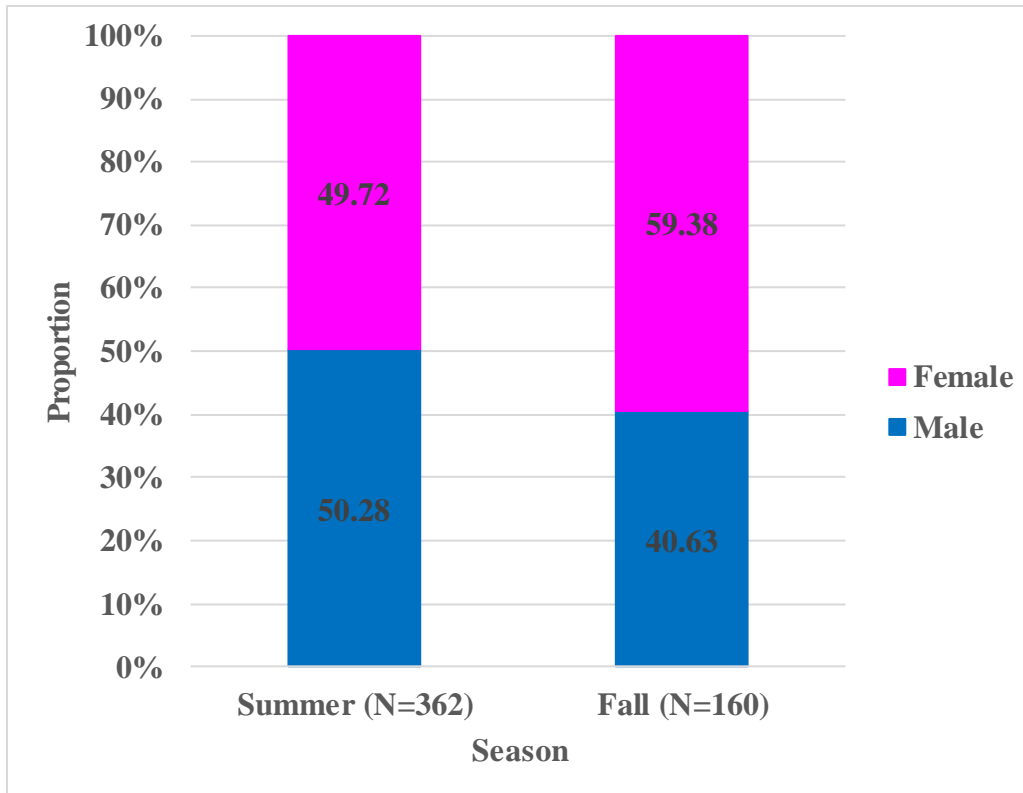


Figure 2.3: Inter-seasonal changes in the proportion of male to female (sex ratio) humpback whales sampled along the Western Antarctic Peninsula.

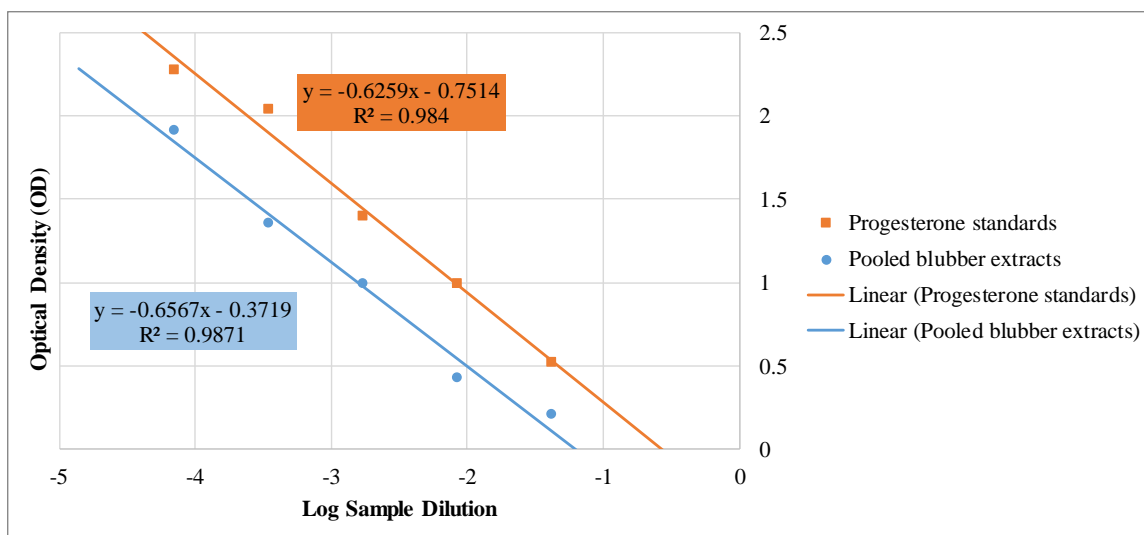


Figure 2.4: Parallelism assessment of the progesterone enzyme immunoassays (EIA) with humpback whale blubber biopsy samples.

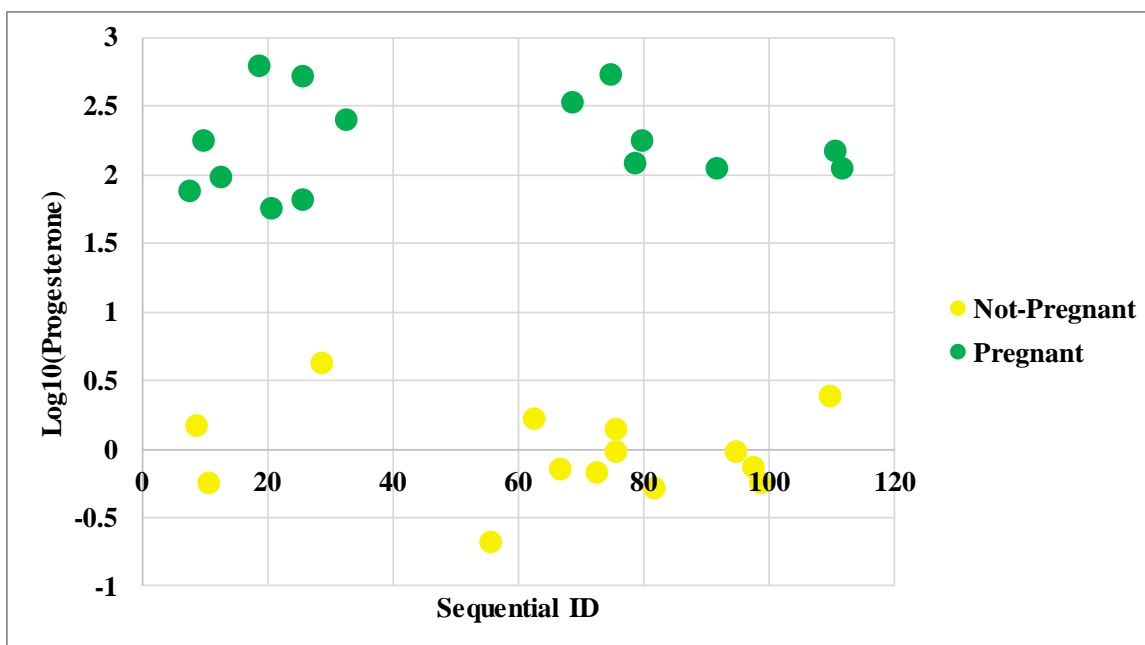


Figure 2.5: Variation in progesterone concentrations (ng P4/g blubber) of humpback control samples from the Gulf of Maine according to pregnancy state.

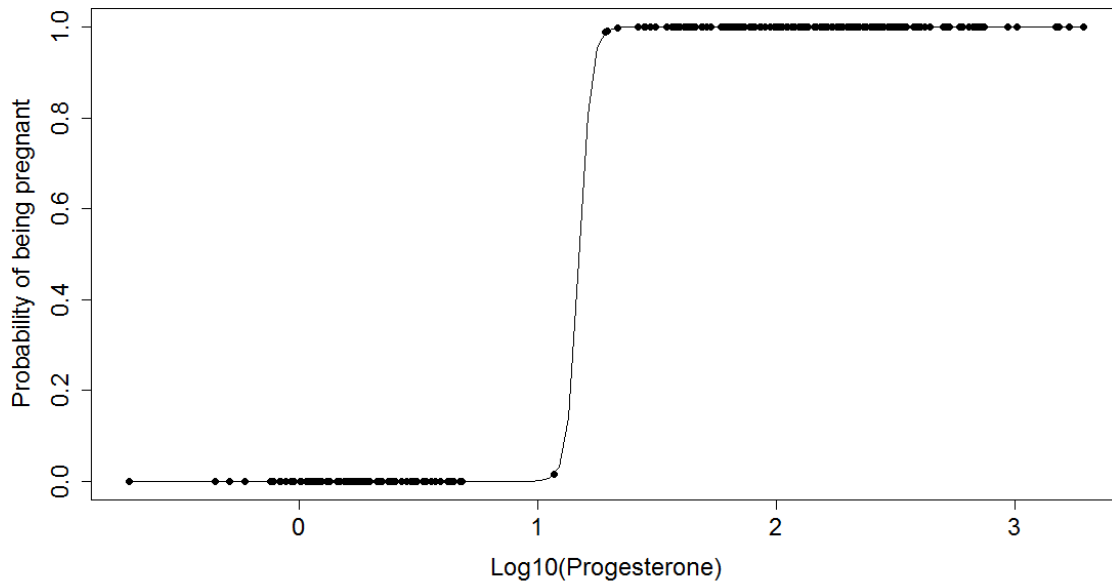


Figure 2.6: Logistic model used to assign the probability of pregnancy in humpback whales sampled along the WAP. Black circles represented measured progesterone concentrations from WAP females of unknown pregnancy status.

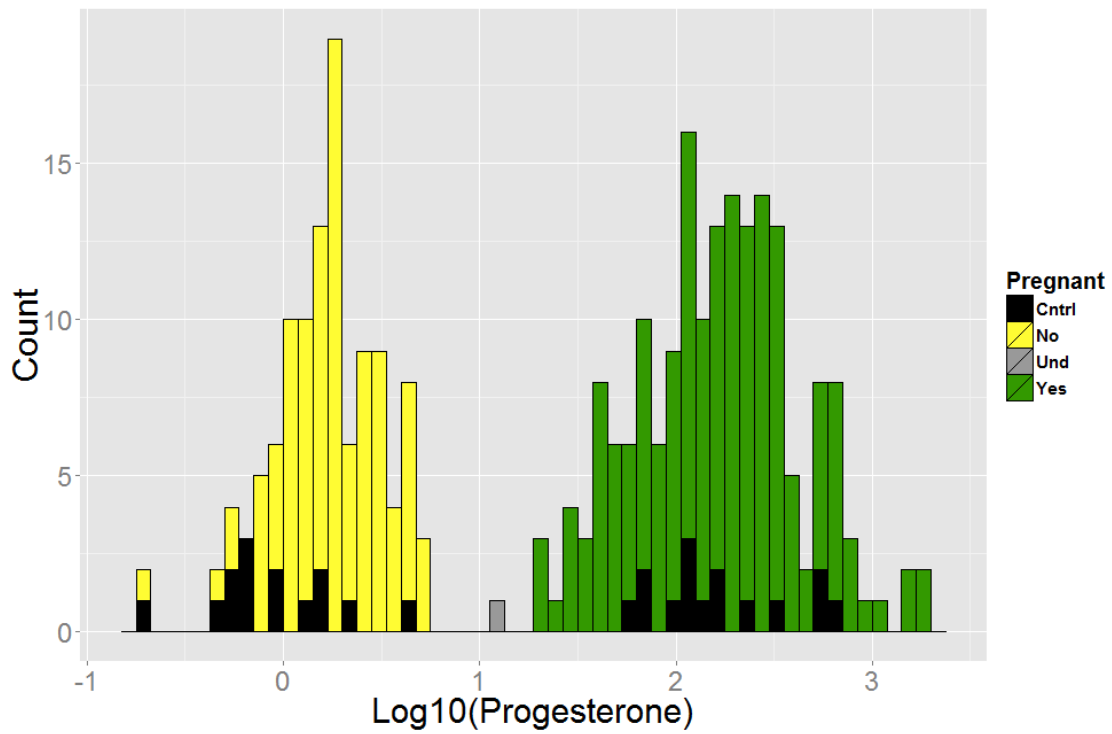


Figure 2.7: Variation in the log10 progesterone concentrations (ng P4/g blubber) of WAP humpbacks and control samples from the Gulf of Maine by pregnancy assignment. Pregnancy among WAP females was assigned via a logistic model developed from animals of known pregnancy status from the Gulf of Maine.

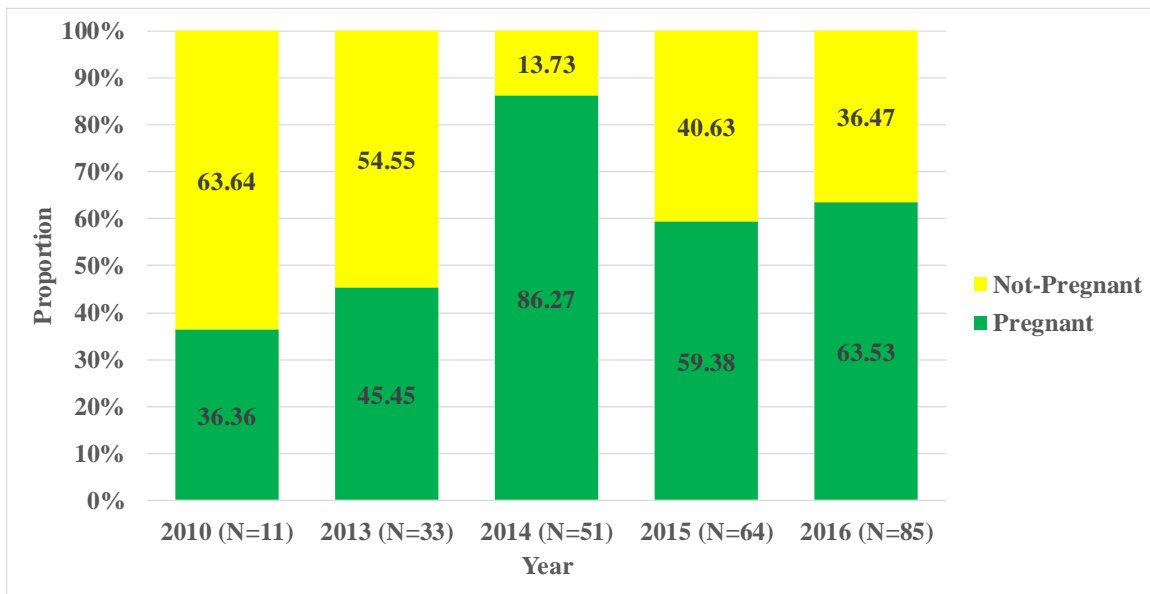


Figure 2.8: Inter-annual variation in the proportion of assigned pregnant and not-pregnant (pregnancy rate) female humpback whales sampled along the WAP based on progesterone concentrations.

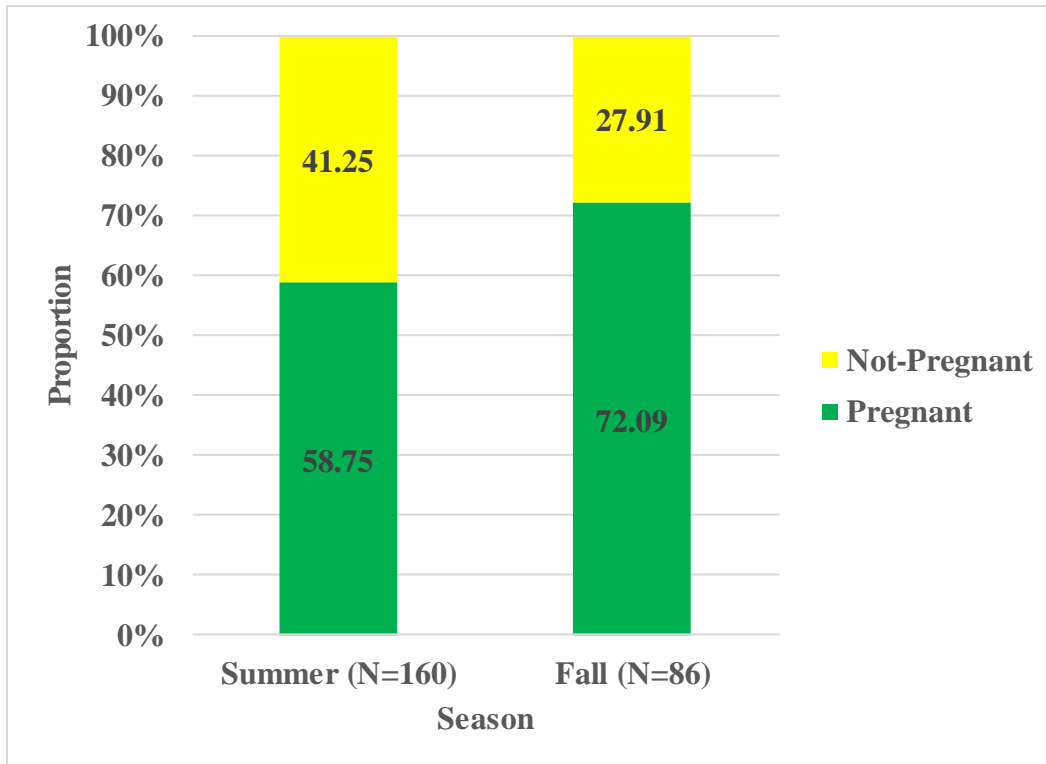


Figure 2.9: Seasonal variation in the assigned proportion of pregnant and not-pregnant (pregnancy rate) female humpback whales sampled along the WAP based on progesterone concentrations.

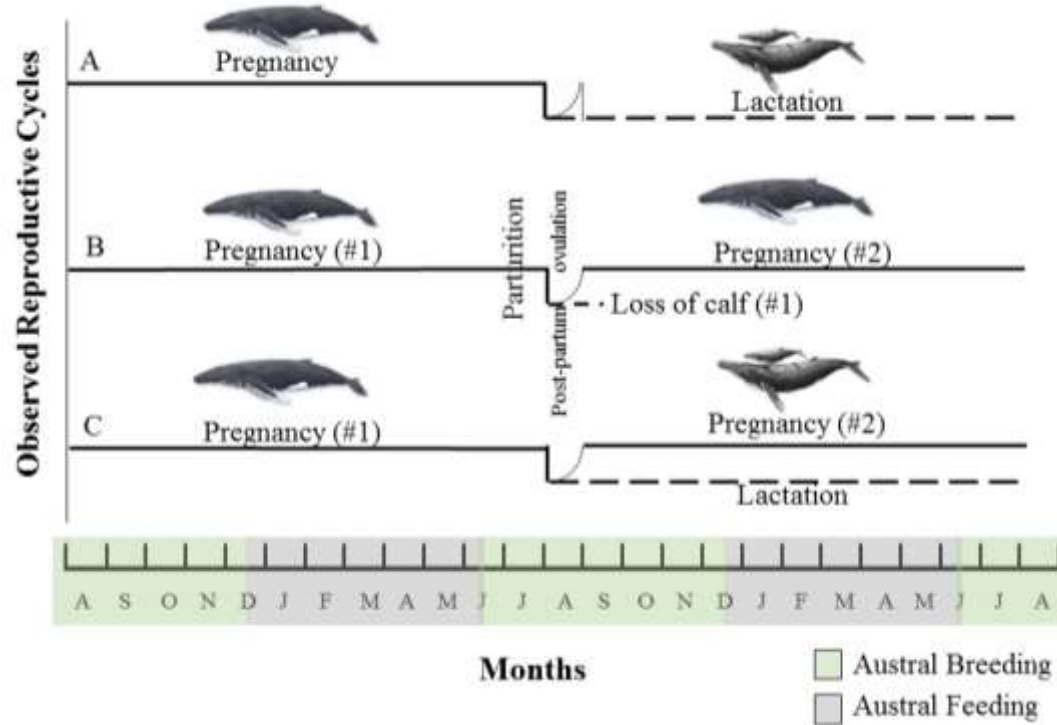


Figure 2.10: Observed breeding cycles in mature female humpback whales killed along the west coast of Australia. Modified from Chittleborough (1958b).

Tables

Table 2.1: Summary of microsatellite loci used for individual identification of humpback whales along the WAP. The short and long profiles refer to PCR extension times as described in the aforementioned text.

Locus	Source	Label	profile	[mgCl₂] mM
Ev14	Valsecchi and Amos 1996	VIC	Long	2.5
Ev37	Valsecchi and Amos 1996	NED	Short	3.5
Ev96	Valsecchi and Amos 1996	FAM	Long	1.5
GATA417	Palsboll et al. 1997	FAM	Long	2.5
GATA28	Palsboll et al. 1997	NED	Long	2.5
GT211	Palsboll et al. 1997	FAM	Short	2.5
GT23	Palsboll et al. 1997	VIC	Long	2.5
GT575	Palsboll et al. 1997	FAM	Short	1.5
rw4-10	Waldick et al. 1999	VIC	Short	2.5
rw48	Waldick et al. 1999	NED	short	3

Table 2.2: Sample summary statistics for humpback whales sampled along the WAP (2010, 2013-16) with a known genetic sex. Note: *denotes where an individual(s) was recaptured across a season, numbers inside () designated when all replicates have been removed from the sample data set.

Temporal Scale	# Samples	# Genotypes	Male		95% CL Lower-Upper	Female		95% CL Lower-Upper	Sex Ratio (M:F)
			N	%		N	%		
2010 Fall	27	25	8	32.00	14.9-53.5	17	68.00	46.5-85.05	0.47
2010 Total	27	25	8	32.00	14.9-53.5	17	68.00	46.5-85.05	0.47
2013 Summer	86	79	45	56.96	45.33-68.06	34	43.04	31.94-54.67	1.32
2013 Total	86	79	45	56.96	45.33-68.06	34	43.04	31.94-54.67	1.32
2014 Summer	109	92	48	52.17	41.5-62.7	44	47.83	37.3-58.5	1.09
2014 Fall	27	24	12	50.00	29.12-70.88	12	50.00	29.12-70.88	1
2014 Total	136	116 (115)	60 (59)*	51.30	41.81-60.73	56	48.70	39.27-58.19	1.05
2015 Summer	99	96	43	44.79	34.63-55.29	53	55.21	44.71-65.37	0.81
2015 Fall	29	28	10	35.71	18.64-55.93	18	64.29	44.07-81.36	0.56
2015 Total	128	124	53	42.74	33.9-51.94	71	57.26	48.06-66.10	0.75
2016 Summer	104	95	46	48.42	38.04-58.9	49	51.58	41.1-61.96	0.94
2016 Fall	96	83	35	42.17	31.4-53.51	48	57.83	46.49-68.6	0.73
2016 Total	200	178 (175)	81 (80)*	45.71	38.18-53.40	97 (95)*	54.29	46.6-91.82	0.84
Total	577	518 (507)	245 (239)	47.14	42.72-51.59	273 (268)	52.86	48.41-57.28	0.89

Table 2.3: Summary of microsatellite variation for humpback whales biopsied along the Western Antarctic Peninsula. The number of alleles (K), observed (H_o) and expected (H_e) heterozygosity, and deviation from Hardy Weinberg Equilibrium (HWE) was calculated using *cervus 3.0.1*. Significant deviations from HWE are represented by s and non-significant deviations ns. The expected probability of identity (P_{ID}) of each locus was calculated with the program *GenAlEx v6.5*.

Locus	Range	K	H_e	H_o	HWE	P_{ID}
Ev14	125-143	9	0.787	0.748	ns	0.074
Ev37	192-228	18	0.898	0.891	ns	0.019
Ev96	141-173	15	0.869	0.862	ns	0.030
GATA417	143-199	21	0.912	0.891	ns	0.37
GATA28	187-282	14	0.404	0.402	ns	0.015
GT211	100-120	10	0.82	0.82	ns	0.056
GT23	101-123	9	0.749	0.712	ns	0.1
GT575	137-177	14	0.804	0.787	ns	0.061
rw4-10	190-216	14	0.845	0.824	ns	0.043
rw48	112-120	5	0.724	0.742	ns	0.12

Table 2.4: Within and between year genotype recaptures of humpback whales biopsied along the WAP. Recaptures within the same year are presented as Male/Female. Blue shaded cells indicated male recaptures and pink shaded cells indicate female recaptures.

Year	n	2010	2013	2014	2015	2016
2010	27	2/0	1			
2013	86		3/4	1	1	
2014	136			16/5		1
2015	128		2	1	4/0	1
2016	200		1	1	1	7/18

Table 2.5: Progesterone concentrations (ng P/g blubber) of control samples from the Gulf of Maine.

Reproductive State	Mean P4 (ng P/g blubber)	SD	Min	Max	N
Not-pregnant	1.19	1.00	0.20	4.08	14
Pregnant	223.15	185.62	54.97	616.36	15
Total					29

Table 2.6: Progesterone concentrations (ng P4/g blubber) of humpback whales biopsied along the WAP with a pregnancy assignment. Note: values in () denotes the exclusion of within year replicates, *total does not include undetermined individual.

	Mean P4 (ng P/g blubber)	SD	Min	Max	N
Not-pregnant	2.06 (2.10)	1.12 (1.13)	0.20	4.86	98 (89)
Pregnant	254.65 (249.96)	293.94 (281.79)	19.28	1,940.52	166 (155)
Undetermined	11.81				1
Total					264 (244)*

Table 2.7: Summary statistics of pregnancy assignments for female humpback whales sampled along the WAP (2010, 2013-16). Note: *denotes where an individual(s) was recaptured across seasons, numbers inside () designate when all replicates have been removed from the sample data set.

Temporal Scale	# Females	# Individuals	Not-Pregnant		95% CL Lower-Upper	Pregnant		95% CL Lower-Upper
			N	%		N	%	
2010 Fall	11	11	7	63.64	30.79-89.07	4	36.36	10.93-69.21
2010 Total	11	11	7	63.64	30.79-89.07	4	36.36	10.93-69.21
2013 Summer	35	33	18	54.55	36.35-71.89	15	45.45	28.11-63.65
2013 Total	35	33	18	54.55	36.35-71.89	15	45.45	28.11-63.65
2014 Summer	41	40	7	17.50	7.34-32.78	33	82.50	67.22-92.66
2014 Fall	11	11	0	0.00	0-28.49	11	100.00	71.51-100
2014 Total	52	51	7	13.73	5.7-26.26	44	86.27	73.74-94.3
2015 Summer	48	48	23	47.92	33.29-62.81	25	52.08	37.19-66.71
2015 Fall	16	16	3	18.75	4.05-45.65	13	81.25	54.35-95.95
2015 Total	64	64	26	40.63	28.51-53.63	38	59.38	46.37-71.49
2016 Summer	44	39	18	46.15	30.09-62.82	21	53.85	37.18-69.91
2016 Fall	58	48	14	29.17	16.95-44.06	34	70.83	55.94-83.05
2016 Total	102	87 (85)	32 (31)*	36.47	26.29-47.62	55 (54)*	63.53	52.38-73.71
Total	264	246 (244)	90 (89)	36.48	30.43-42.86	156 (155)	63.52	57.14-69.57

Table 2.8: Blubber progesterone concentrations from 12 cetacean species including those reported here for the WAP. The progesterone concentrations are reported as ng P4/g blubber. ^a(Kellar et al. 2006), ^b(Mansour et al. 2002), ^c(Clark et al. 2016), ^d(Pérez et al. 2011), ^e(Kellar et al. 2013), ^f(Trego et al. 2013).

Status	<i>T. truncatus</i> ^d	<i>G. melas</i> ^d	<i>D. delphis</i> ^a	<i>L. borealis</i> ^a	<i>L. obliquidens</i> ^a	<i>B. acutorostata</i> ^b	<i>D. capensis</i> ^f
Pregnant							
Average	54.82 ± 22.86	45.28 ± 28.40	45.28 ± 28.40	312 ± 44	161	132 ± 22	152.75
Min	31.96	16.89	132	196		22.8	
Max	77.7	73.68	415	402		454	
N	2	2	8	5	1	22	2
Not-pregnant							
Average	6.16 ± 3.62	Not available	13.7	15 ± 7.5	12.1 ± 8.4	Not available	3.67
Min	1.65		6.75	2.11	3.75		
Max	12.7		33.3	34.7	20.5		
N	9		19	18	2		8

Status	<i>S. attenuata</i> ^f	<i>S. longirostris</i> ^f	<i>P. dalli</i> ^f	<i>B. mysticetus</i> ^e	<i>M. novaeangliae</i> (Cal) ^c	<i>M. novaeangliae</i> (WAP)
Pregnant						
Average	435.08	596.19	1250.22	615.7 ± 1938	122.28 ± 13.95	249.96 ± 281.79
Min					46.05	26.40
Max					286.53	1940.52
N	2	2	1	6	20	154
Not-pregnant						
Average	1.05	0.58	8.29	7.12 ± 3.4	0.24 ± 0.06	2.10 ± 1.13
Min					0.13	0.20
Max					0.32	4.86
N	1	1	4	3	7	89

Table 2.9: Post hoc multiple comparisons table of pregnancy rates across years for WAP.

Year 1	Year 2	Mean Difference (Year 2- Year1)	P-value (adjusted)
2010	2013	0.09	0.981
	2014	0.5	0.013
	2015	0.23	0.555
	2016	0.27	0.365
2013	2014	0.41	0.001
	2015	0.14	0.632
	2016	0.18	0.325
2014	2015	-0.27	0.02
	2016	-0.23	0.049
2015	2016	0.04	0.983

Table 2.10: Results of chi-square test of independence for the inter-annual and seasonal variation in sex ratios and pregnancy rates of humpbacks sampled along the WAP.

Statistical Comparison	χ^2	df	P-value
Sex-Year	7.256	4	0.123
Sex-Season	4.146	1	0.042
Pregnancy-Year	20.02	4	0.001
Pregnancy-Season	4.53	1	0.033

Table 2.11: Percentage of females accompanied by a calf along the WAP and assigned a pregnancy state from progesterone concentrations measured from biopsy samples. Replicates in the same year have been removed. Note: due to the small sample size, 2010 was not included in the mean. This is our best estimate of annual pregnancy within our dataset. Additionally, values are fit with a binomial confidence limit.

Year	# Mother Calf Pairs	# Pregnant	% Annual Pregnancy	95% CL Lower-Upper
2010	1	1	100%	NA
2013	4	1	25%	.6-80.6
2014	10	9	90%	55.5-99.8
2015	17	7	41.2%	18.4-67.1
2016	12	6	50.0%	47.6-92.7
Total	44	24	52.3%	38.9-69.6

Table 2.12: Proportion of varying reproductive states of female humpback whales biopsied along the WAP across seasons. Includes reproductive assessments from 94 humpback whales hunted in Antarctic whaling areas IV and V from 1950-1955 (Chittleborough 1958b). Percent annual pregnancy is derived from females accompanied with calves only. Across season replicates have not been removed.

Reproductive State	WAP Summer		WAP Fall		Total	Whaling IV and V	
	N	%	N	%	N	N	%
Not Pregnant, No Calf	47	29.4	22	25.6	69	8	8.5
Pregnant, No Calf	80	50.0	52	60.5	132	67	71.3
Not Pregnant, Calf	19	11.9	2	2.3	21	11	11.7
Pregnant, Calf	14	8.7	10	11.6	24	8	8.5
Total	160	100	86	100	246	94	100
% Annual Pregnancy	42.42		83.33		53.33	42.11	

Table 2.13: Literature summary table of humpback whale reproductive rates in which an annual or non-annual reproduction was observed. Evidence is either presented as an annual pregnancy rate from carcasses or the observation from consecutive calving intervals from photo-ID recaptures.

Methods	Years	Annual Pregnancy	Breeding/Feeding Ground	Region	Notes	Paper
Biopsy	2010-16	Yes	Feeding	WAP	44 moms with calves observed, 24 pregnant	Thesis
Photo ID	1975-88	Yes	Breeding	Hawaii	584 individuals, 7 annual calving intervals	Glockner-Ferrari & Ferrari 1990
Photo ID	1978-84	Yes	Feeding	Alaska	138 sighted females, 1 annual calving interval	Baker 1987
Photo ID	1978-84	Yes	Breeding	Hawaii	40 sighted females, 1 annual calving interval	Baker 1987
Photo ID	1997-92	Yes	Feeding	Alaska	Paper specific to 5 annual calving events	Straley, 1994
Photo ID	1986-96	Yes	Feeding	West USA	6,494 sighted whales, 2 annual calving intervals	Steiger, 2000
Photo ID	1985-14	No	Feeding	Alaska	662 individuals, no annual calving	Gabriele et al 2017
Photo ID	1979-85	Yes	Feeding	Massachusetts Bay	44 individual females, 1 annual calving interval	Clapham and Mayo 1987
Photo ID	1979-87	Yes	Feeding	Gulf of Maine	65 individual females, 2 annual calving intervals	Clapham and Mayo 1990
Whaling	1957	Yes	Feeding	Antarctic Area I	4 mothers with calves observed, 1 pregnant	Symons, 1958
Whaling	1950-58	Yes	Feeding	Western Australia	27 mothers with calves observed, 8 pregnant	Chittleborough 1958

CHAPTER 3: General Conclusions and Future Directions

Demographic studies on humpback whales in the Southern Hemisphere have historically been comprised of data from whaling statistics of animals killed during the 20th century. The goals of my thesis were to describe the current demography of humpback whales that feed within the nearshore waters of the Western Antarctic Peninsula (WAP). Combining a temporal assessment across years and within seasons of both the variation in sex ratios and pregnancy rates over the five years of this study, I developed a current demographic reference point for this population of humpback whales. In turn, this will help facilitate future comparisons and predictions on humpback whale population level responses to climate change (and its myriad impacts to the marine ecosystem) in this region. My analyses resulted in a quantitative description of both the temporal composition and variation in sex ratios and pregnancy rates within humpback whales along the WAP.

Primary findings: sex ratios

Humpback whales were heavily exploited during the height of the industrial whaling era in the Southern Hemisphere with more than 200,000 killed (Rocha et al. 2014). Due to the rapid depletion of humpbacks at the beginning of Antarctic whaling (1900-1940) along the WAP, little was revealed about the exact timing of migratory movements and residency times of various sex classes of humpback whales feeding in this region (Hart 2006, Rocha et al. 2014, Cressey 2015). The genetic sex was determined for 577 of 583 humpbacks biopsied over the course of the five years of this study, representing 239 and 268 individual males and females respectively. Through my

analyses on the temporal variation in sex ratios, I found consistent evidence documenting a sex ratio close to parity across all sampling years and in the summer season. However, I did demonstrate a seasonal shift in the sex ratio of humpback whales feeding along the WAP, such that females were disproportionally represented in the fall. I propose that this shift represents a tendency for pregnant female whales to depart last from the feeding grounds, prior to undertaking a long migration to low latitude breeding grounds off the coasts of central and South America. Even after several decades of intense exploitation, my results support historical records from other populations of humpback whales in the Southern Ocean, documenting that male and female humpback whales still appear to mix randomly while on the feeding grounds (Chittleborough 1965, Dawbin 1966). This consistent sex ratio supports the ideas that humpback whales in this region are recovering at a healthy state and that their life history has not changed (Jackson et al. 2015).

Primary findings: pregnancy rates

Historically, obtaining knowledge on cetacean reproductive status has only been quantifiable by examining the reproductive tracts of hunted, necropsied, or captive individuals. To my knowledge this work is the first attempt to detect pregnancy in Antarctic baleen whales using non-lethal methods. Furthermore, I believe this is among the most recent assessment of vital reproductive rates of humpbacks in this region, as well as the largest study conducted on blubber progesterone concentrations among free ranging mysticetes in the Southern Hemisphere. I successfully assigned pregnancy in 244 individually identified female humpbacks sampled during this study and documented both across year and seasonal variation in pregnancy rates. I found evidence suggesting that pregnancy rates varied significantly across years, with 2014 being the most variable

year. Furthermore, I observed a bias in favor of pregnant females during fall, supporting my hypothesis that females choose to stay on the feeding grounds to support the energetic demands associated with pregnancy. After assessing the change in proportion of mothers with calves that were both pregnant and not pregnant across seasons, we found that staying later in the season to likely provision a calf, significantly decreased for those mothers that were not pregnant. Overall, our pregnancy rates were comparable to historical measures and those currently being observed in other previously exploited and recovering humpback whale populations in the Southern Hemisphere, but inconsistent with observed calving and pregnancy rates in the Northern Hemisphere (Symons et al. 1958, Clark et al. 2016). The utilization of these methods now allows us to better assess both individual and population level variation in the health and growth of humpbacks over time.

This research presents multiple novelties regarding humpback whale demography along the WAP, the most striking of which was my observation of a high rate of annual pregnancies; being both pregnant and nursing a calf simultaneously. Annual pregnancies were rarely documented in whaling data, likely due to the take of females migrating south when the fetus would be challenging to detect as well as the suspension by the International Whaling Commission (IWC) on the killing of mothers accompanied by calves in 1946. Additionally, today, such highly fecund events in humpback whales are only supported by the few observed occurrences of mothers accompanied by different calves at least two years in a row. However, this information is only currently available for Northern Hemisphere humpbacks. With continued biopsy sampling efforts in the WAP region, combined with photo identification, and field observations of mothers

accompanied by calves, we will likely be able to better quantify annual reproduction in this feeding aggregation overtime.

Future directions

While I am confident that my classifications of pregnancy were correctly assigned, we are still unaware of the maturity of all females sampled, as well as the rates of fetal and neonatal/calf mortality, both factors that likely drive an adult female's opportunity to reproduce annually. I documented strong evidence that a high proportion of the females we biopsied demonstrated annual pregnancy. The use of unmanned aerial systems (UAs) in the biological sciences has grown rapidly in the last few years. The combination of this technology with continued biopsy sampling and photo identification of humpbacks along the WAP can be used to generate estimates of body condition, age, and growth rates over time (Christiansen et al. 2016). Additionally, with continued sampling in this region we will be able to begin to assess other reproduction parameters like calving rates and can relate these to our estimates of pregnancy. Ultimately, this will provide a more robust understanding on the health of this population in the face of a rapidly changing marine ecosystem.

Robust knowledge of the short and long-term adaptability to climate change of any marine mammal species remains poorly understood. To bridge this knowledge gap, we need to begin by assessing vital demographic rates, as has been presented in this thesis, in order to have the capacity to assess their variability over time. Shifts in baleen whale demography as a response to climate change has previously been shown in southern right whales (Leaper et al. 2006, MacLeod 2009). As we would expect, a top predator's demography and behavior will be affected by prey, changes in the prey

distribution and or abundance, and environmental variability (Simmonds & Isaac 2007).

Due to the rapid warming and depletion of sea ice and ice shelves at the poles, it is predicted that polar dependent cetacean species will continue to shift their distributions poleward, resulting in a diminished global range, but concurrently expanding into new and previously unoccupied habitat (Simmonds & Isaac 2007, MacLeod 2009).

Additionally, with the continued deployment of long-term satellite tags on humpback whales along the WAP, we can begin to link the behavior of individuals with their demography to further understand how different age, sex, and reproductive classes behave in this region. Thus, I recommend that as sampling continues in this region we begin to assess the interactions between observed demographic shifts and changes in environmental variables, such as sea ice cover and regional krill biomass.

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 ecological change through the collection of long term datasets. The data presented in this thesis 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 of humpback whales in this region and in turn, provide continuous insight on the ecological and biological responses of humpback whales to climate change.

In summary, there now exists a baseline demographic description of humpback whales that occupy the near-shore waters of the WAP during the austral feeding season

that can act as a reference point for future comparisons and upon which a basis for conservation and management can be established.

Chapter 3: References

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