Integrative Studies of Southern Ocean Food-Webs and *Pygoscelis* Penguin Demography: Mechanisms of Population Response to Environmental Change

by

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Approval

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Ethics Statement

The author, whose name appears on the title page of this work, has obtained, for the research described in this work, either:

a. human research ethics approval from the Simon Fraser University Office of Research Ethics,

or

b. advance approval of the animal care protocol from the University Animal Care Committee of Simon Fraser University;

or has conducted the research

c. as a co-investigator, collaborator or research assistant in a research project approved in advance,

or

d. as a member of a course approved in advance for minimal risk human research, by the Office of Research Ethics.

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Abstract

Unraveling the proximate physiological, ecological and evolutionary mechanisms that underlie population responses to environmental variability is an increasingly important challenge facing conservation biologists and is the primary motivation of this thesis. I studied *Pygoscelis* penguins that breed west of the Antarctica Peninsula (AP). Here, breeding populations of Adélie (*P. adeliae*), chinstrap (*P. antarctica*) and gentoo (*P. papua*) penguins are undergoing pole-ward range shifts that correlate with long-term warming trends of the region. However, mechanistic relationships that link marine ecosystem variability with demographic parameters that drive population change are rarely quantified. My data support the following conclusions. At Anvers Island, where reductions in sea ice have been notable, *Pygoscelis* penguins became more similar isotopically throughout the breeding season, due to a depletion in $\delta^{13}C$ and $\delta^{15}N$ of blood tissue. Here, crèched chicks of all species occupied similar trophic levels and isotopic mixing models indicated that the proportions of prey provisioned to crèched chicks were generally similar across species within years. Crèched Adélie chicks were isotopically enriched at Avian and Charcot Islands, southern rookeries where sea ice is more prominent, and their trophic level increased with latitude. Penguin food-webs are isotopically wider in regions with greater sea ice coverage. Molecular primers (P2/P8) amplified DNA of all species to discern sex. At Anvers Island, I detected little evidence of trophic foraging influencing body and egg mass of adult penguins and chick body mass. However, regional analyses suggested that a provisioning diet enriched in $\delta^{15}N$, such as that found at southern rookeries, resulted in heavier five-week-old Adélie penguin chicks. Corticosterone hormone did not mediate individual variation in *Pygoscelis* breeding performance. I found no overall genetic structure among Adélie penguins based on microsatellite and mitochondrial DNA markers, however, pairwise comparisons including Charcot Island were significant. Gene flow was asymmetrical from the species' regional core to its northern range. In conclusion, variability in sea ice-associated food-webs of the western AP is an important determinant of *Pygoscelis* penguin reproductive performance, but not at the physiological level. A lack of genetic structure implicates dispersal as an important demographic factor that may structure population responses to future ecosystem change.

Keywords: corticosterone; $\delta^{13}C$ and $\delta^{15}N$ stable isotopes; demography; population genetics; *Pygoscelis* penguins; sea ice

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This thesis is dedicated to my father for his support of my work as a wildlife biologist.

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Introductory Image

Adélie Penguin Rookery, Charcot Island, Antarctica.

Photo by K. B. Gorman

Chapter 1.

Thesis Synthesis

Background, Objectives and Outline

Although factors determining the spatial distribution of species occurrence have long fascinated biologists (e.g., Darwin 1859, MacArthur and Wilson 1967), the topic has become a pivotal subject in modern ecology and evolutionary biology due to the increasing need to forecast species and population responses to anthropogenic influences (Gaston 2009). Pollution, the introduction of invasive species or disease, critical habitat loss, interactions with agriculture or marine fisheries, and climate change are all varied agents of selection that can shape species' distributions and abundances across their range. Basic ecological and evolutionary studies that explore a species ecological niche, identify which environments confer high fitness and how populations are connected through dispersal stand to greatly inform our understanding of how and why geographic ranges expand or contract (Sexton et al. 2009).

For Antarctic penguins, interactions with marine fisheries and ocean-climate variability are two particularly important issues that can impact penguin populations, and especially synergistic effects of both (Ainley and Blight 2009, Forcada and Trathan 2009, Trathan and Agnew 2010, Trivelpiece et al. 2011, Ducklow et al. 2013, Trathan et al. 2014). Throughout the marine ecosystem west of the Antarctic Peninsula (AP) and in regions of the Scotia Sea, *Pygoscelis* penguins are undergoing pole-ward range shifts that correlate with long-term warming trends of the region (Forcada and Trathan 2009, Lynch et al. 2012, Ducklow et al. 2013). Changes in sea ice-associated food-webs and subsequent energetic effects on young chicks that influence their survival and recruitment probability as breeding adults is a hypothesized demographic mechanism underlying penguin population response to regional environmental variability (Fraser et al. 1992,

Trivelpiece et al. 2011) that might be exacerbated by fisheries pressures or competition with other predators such as whales and seals (Trivelpiece et al. 2011, Ducklow et al. 2013). Despite decades of research by several long-term programs in the region directed at studying the breeding ecology and population dynamics of these penguins, I argue that to better predict how Antarctic penguins might respond to changes in Southern Ocean marine ecosystems, integrative and novel studies of linkages between marine food-webs and demographic parameters are needed, and especially studies on how these relationships change across regions with differing marine ecosystem properties to reveal key environmental drivers of demographic variability that contribute to population change, which is the overall objective of this thesis.

The foundation of the work presented here relies on the use of naturally occurring carbon ($^{13}C/^{12}C$, $\delta^{13}C$) and nitrogen ($^{15}N/^{14}N$, $\delta^{15}N$) stable isotopes as integrated proxies of individual penguin trophic foraging and marine food-web structure. Stable isotopes have revolutionized research in animal ecology because these parameters directly reflect an individual's dietary history (Kelly 2000, Inger and Bearhop 2008). The ratio of heavy to light isotopes of carbon and nitrogen in a sample can provide both *source* and *process* information due to isotopic fractionation (Peterson and Fry 1987). For example, the fractionation of carbon during photosynthesis can distinguish sources of primary productivity such as between near-shore and pelagic phytoplankton (Kelly 2000). Thus, $\delta^{13}C$ values provide *source* information due to the origins of the carbon signature and the fact that $\delta^{13}C$ tends to not vary greatly across trophic levels (Peterson and Fry 1987). Nitrogen ($\delta^{15}N$) however captures a distinct signature of trophic position due to the differential excretion of the lighter isotope by consumers (Peterson and Fry 1987). Thus,

as elements such as carbon and nitrogen are produced and consumed throughout a food-web, they fractionate and are reflected in the tissues of predators at varying time scales due to isotopic turnover rates related to metabolic activity, and for these reasons provide fantastic information on the distribution or trophic level of consumers (Deniro and Epstein 1978, 1981). Stable isotope data are really exciting in ecology when they can be related to performance traits of animals such as reproduction or survival. One of the primary objectives for this thesis was to incorporate stable isotopes in the evolutionary ecology of *Pygoscelis* penguins, and therefore directly relate marine food-web variability to the demography of these species in order to provide a novel test of the long standing hypothesis that changes in sea ice-associated marine food-webs have energetic effects on the reproductive survival of young chicks that shape population changes.

To this end, Chapter 2 evaluated regional variation in reproductive isotopic niche among Adélie (*Pygoscelis adeliae*), chinstrap (*P. antarctica*), and gentoo (*P. papua*) penguins breeding west of the AP to test a hypothesis for sea ice-associated food-web correlates of population change. This chapter sets the stage by characterizing the food-webs study populations are linked to throughout the breeding season and provides quantitative estimates of the diet used to provision older penguin chicks that are considered a demographically important group. This chapter genuinely advances our understanding of Southern Ocean penguin food-webs by considering more novel metrics such as trophic level and niche width all within an isotopic framework.

Chapter 3 also used stable isotope methodology, but in the context of ecological sexual dimorphism. Here, molecular primers to discern sex of penguins were validated and used to examine whether environmental variability in the form of winter sea ice is

associated with differences in male and female pre-breeding foraging niche. This paper highlights one of the exceptional features of stable isotope methods, which is the technique's ability to infer diet during phases of the annual cycle outside of breeding based on the isotopic turnover rate of whole blood. Further, this paper was central to validating molecular primers that were subsequently used to discern the sex of chicks in Chapter 4 since young male and female penguins are not dimorphic in plumage or structural size at this stage, in addition to the sex of all Adélie penguins sampled for analyses presented in Chapter 6.

Chapter 4 also used stable isotope methods to examine individual-level indices of trophic foraging, based on $\delta^{13}C$ and $\delta^{15}N$ stable isotopes, and relationships with breeding performance parameters such as body and egg mass variation among all *Pygoscelis* species. This chapter directly integrates marine food-webs in the reproductive performance of penguins and by doing so examines a possible nutritional mechanism the might contribute to population changes. Importantly, this chapter relates some findings in Chapter 2 to synthesize results across studies and also relies on the primer validation developed in Chapter 3 in order to include a parameter for sex in models accounting for body mass variation.

Chapter 5 continues to build off the previous 3 data chapters in terms of food-web drivers of body mass variation and asks whether corticosterone, a physiological parameter associated with energy balance in birds, mediates these issues. Taking an endocrine perspective on this system was another specific objective of this thesis that was designed to be novel and integrative in using an array of techniques that might help

reveal the energetic mechanisms underlying penguin breeding population change in this region of Antarctica.

Lastly, and further along the lines of integrative research, population genetic methods are used in Chapter 6 to provide a better understanding of the spatial genetic structure of Adélie penguin breeding rookeries and gene flow among them to help evaluate the species adaptive capacity in terms of genetic diversity and dispersal, and hence their vulnerability to current and future environmental change. This project contributes to a growing interest in using molecular methods to assess dispersal in Antarctic penguins, a topic that has been difficult to quantify using traditional demographic studies such as banding (Gauthier-Clerc et al. 2004). This study expands the current literature in two key ways. First, the study presented here applied two genetic markers to the same dataset with differing modes of inheritance in order to assess sex-biased patterns of breeding population genetic structure. Secondly, genetic data from this study was obtained from penguins of known reproductive status, or their chicks, at breeding rookeries. Thus, my data speak directly to breeding rookery genetic structure, unlike other studies that have relied on obtaining genetic information from feather samples where the reproductive status of individuals was not always specifically known. Importantly, this study is representative of breeding rookeries not sampled by other researchers, therefore, the combined results with other studies offer a more complete understanding of Adélie penguin population genetic structure of the region.

This project also involved data collection for two other studies not presented in the thesis. First, immunocompetence was measured in blood plasma samples following methods outlined by Matson et al. (2005) as an additional physiological measure of

quality to compliment the corticosterone work. In addition, the molecular primers validated in Chapter 3 can be used to assess offspring sex allocation and whether environmental conditions might select for the differential quality of female versus male penguin chicks, relationships that might be mediated by physiological parameters previously measured (e.g., Love et al. 2005).

This thesis is arranged as a series of five data chapters produced as stand-alone journal articles that each address a particular aspect of the overall topic as summarized above. Chapter 3, which is entitled *Ecological Sexual Dimorphism and Environmental Variability within a Community of Antarctic Penguins (genus Pygoscelis)*, has been previously published by PLoS ONE. The other data chapters are in the process of being submitted to peer-reviewed journals. Although each chapter was prepared separately, they have been formatted for consistency in the thesis in terms of style, arrangement and references. However, because each chapter is its own article, there is some redundancy in the text and figures presented because similar information is relevant to each chapter. Chapters 4 and 5 also include Appendices for the candidate model sets considered in analyses mainly as a reference for the thesis defense. Other candidate model sets considered in analyses have been discussed extensively (Chapter 2) or previously published (Chapter 3), and therefore have not been included.

Major Findings and Implications of Results

This thesis confirms that variability in sea ice-associated food-webs of the western AP was correlated with changes in the number of *Pygoscelis* penguins breeding at regional rookeries over the course of this study (Chapter 2). Isotopic signatures of adults during

egg production by all three species at Anvers Island in the northwestern AP, where reductions in sea ice have been most pronounced, confirm that each species relied on markedly different food-webs in the 30 – 60 days prior to egg laying. Hence, *Pygoscelis* penguin egg production appears to be dependent on differing nutritional resources found in association with each species' preferred late winter/early spring habitat - Adélie penguins are known to be associated with sea ice habitats during winter, while wintering chinstrap and gentoo penguins are associated with open water habitats more characteristic of the sub-Antarctic. This conclusion is further supported by the fact that female Adélie and chinstrap penguins, the two species with enriched $\delta^{15}N$ values of blood at the onset of breeding, produce eggs with pale yellow yolk, yet have very different isotopic responses to over-winter sea ice dynamics. Further, female gentoo penguins produce more orange colored yolk and have relatively depleted $\delta^{15}N$ values at the onset of breeding, unlike Adélie and chinstrap females.

Adult pre-breeding sex-specific foraging niche was associated with overall SSD indices across species - male chinstrap and gentoo penguins were enriched in $\delta^{15}N$ relative to females. Therefore, each sex within a species should respond similarly in pre-breeding trophic foraging to changes in future sea ice or sea ice-free winter habitats as there was no evidence for an interaction between sex and pre-breeding foraging niche among adults (Chapter 3).

Chicks of all three species reared within the northwestern AP became very similar isotopically by the time they were 5 weeks old, suggesting that similar nutritional resources were used to produce chicks across species in this region, which was also confirmed by trophic level and isotopic mixing model analyses. Thus, Adélie penguins at

Anvers Island that have declined by nearly 80% produced chicks that were isotopically similar to their sub-Antarctic congeners, which contrasts with Adélie penguins breeding at more southern rookeries along the western AP where winter sea ice is more prominent and numbers of breeding penguins at these rookeries have increased or have been more stable. Here, Adélie penguins chicks are noticeably enriched in $\delta^{15}N$ as they are provisioned greater proportions of prey typical of more high Antarctic food-webs, which increased their trophic position (Chapter 2).

At Anvers Island where sea ice loss has been most prominent, I detected little evidence of an effect of trophic foraging on body and egg mass of adult penguins, as well as body mass of chicks within species and years (Chapter 4). However, there were some species-specific effects of yearly winter sea ice conditions on body mass of chicks. For example, Adélie penguins provisioned a larger proportion of the krill, *Thysanoessa macrura*, during year 2 of the study that followed an extremely low winter sea ice season, were heavier, opposite our main prediction. *T. macrura* holds the highest prey quality in terms of lipid content and energy density among dominant krill species such as Antarctic krill (*Euphausia superba*) and crystal krill (*E. crystallorophias*, Ruck et al. 2014). Thus, the availability of *T. macrura* actually enhanced the quality of chicks in that year, which may be an important mechanism by which Adélie penguins at Anvers Island are able to compensate for changing environmental conditions and still rear chicks of enhanced quality. Conversely, regional analyses of Adélie penguin chick body mass variation suggested that a provisioning diet enriched in $\delta^{15}N$, which is found at Avian Island, a southern rookery characterized by greater over-winter sea ice conditions, resulted in heavier five-week-old chicks. Thus, at the regional scale, variability is sea ice-associated

food-webs does predict chick body condition, which would support a general hypothesis for food-webs and energetics enhancing the reproductive survival of Adélie chicks at southern rookeries (Chapter 4).

Interestingly and opposite my main prediction, I found limited evidence for corticosterone modulation of individual variation in breeding performance across all three species (Chapter 5). Corticosterone levels were an important predictor of body condition among 15-day-old chinstrap penguin chicks only. For all other analyses, there was no hormonal control of body or egg mass variation detected. This result indicates that *Pygoscelis* penguins of the western AP are of adequate energetic quality so as to not require the up-regulation of corticosterone. This is a novel perspective on this system - breeding rookeries with differing trajectories are comprised of individuals that are of sufficient quality at the physiological level. This is a very important result, because it questions the long standing hypothesis that changes in sea ice-associated food-webs have energetic effects on young chicks that influence their survival and recruitment probability as breeding adults. Although I showed in Chapter 4 that at the regional scale, variability is sea ice-associated food-webs does predict chick body condition of Adélie penguins, this is not reflected in the physiology of these chicks.

Lastly, Chapter 6 confirms that Adélie penguins breeding throughout the western AP are a panmictic population that appears to hold adequate genetic diversity and dispersal capacity to be resilient to environmental change. However, sex-biased philopatry/dispersal may be an underappreciated feature of the Adélie penguin system that has been decoupled where environmental conditions are less stable such as the northwestern AP, given results for Charcot Island specifically. The genetic signature of

Adélie penguin breeding rookeries is another very important result, which leads to an overall conclusion that variability is sea ice-associated food-webs of the western AP, which is marked as evidenced by the data presented in this thesis, likely influences dispersal behavior of *Pygoscelis* penguins more so than direct energetic effects on chick reproductive survival. Thus, interactions between changes in marine food-webs and dispersal behavior likely play a disproportionate role in shaping regional population trajectories among these penguins, which contrasts with the general paradigm of how these systems are typically understood that is rooted in the ideas of philopatry and the effects of changing marine food-webs on energetic condition and survival of chicks being the key demographic driver of population change (e.g., Trivelpiece et al. 2011).

Future Directions

The overall conclusion of this thesis suggests that future studies should be focused on links between marine food-web variability and dispersal behavior of both adult and chick penguins. This will require new advances in either how penguins are marked or tracked. Regarding marking, many problems exist for flipper bands that have been typically used in demographic studies such as reduced overall reproductive survival in the propensity to breed, timing of arrival at colonies, chick production and survival (Gauthier-Clerc et al. 2004), presumably due to increased drag asserted by the bands on birds that have energetic effects due to foraging efficiency. In order to circumvent this issue, alternative methods such as implanted electronic tags are of interest and they have been used previously (Gauthier-Clerc et al. 2004), but these approaches require that rookeries be outfitted with automated receivers to detect tags, in addition to physically implanting the

bird in the first place, which may hold its own suite of issues in permitting and ethics. Currently, technology does exist to track penguins such as geolocator or gps tags. Unfortunately, there are many limitations due to battery life and the spatial resolution of these tags. The development of tags that could last a year in between breeding seasons with a high spatial resolution and remote transmission of data would be ideal as they could be deployed on adults in between breeding seasons. In terms of tagging newly fledged chicks, the situation is more difficult since they typically don't return to breed for several seasons. If a tag could be developed with the battery capacity to last several years, and also transmit data remotely, this would greatly improve our understanding of where these young birds recruit and allow for testing ideas highlighted in this thesis about interactions between food-webs and dispersal.

Other research directions that seem interesting and important related to dispersal are the interactions between conditions at the rookery such as sub-colony size and subsequent decisions by parents regarding the location of future breeding attempts. This idea comes from my own observations of adult penguins at breeding rookeries where some sub-colonies are quite small. These small groups of breeding adults are often disproportionately harassed by brown skuas and often lose their eggs or chicks to these avian predators. Once the sub-colony completely fails in one season, i.e., all eggs or chicks have been lost to skuas, the adults typically do not return to that sub-colony to nest in subsequent seasons. However, skuas did not kill these adults, only their chicks. It seems unlikely that parents would completely abandon breeding for the remainder of their lives and that they likely simply dispersed to a different colony where numbers of penguins are more adequate to protect them from skua depredation or even the impacts of

snow. Thus, there could be very important landscape effects of rookeries that determine the dispersal decisions by previously recruited adults, whereas variability in marine food-webs might play a larger role in the initial recruitment decisions of young birds to rookeries as breeding adults. These possible age specific differences in dispersal patterns seem highly relevant to future studies of population change among *Pygocelis* penguins.

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Chapter 2.

Regional Variation in Reproductive Isotopic Niche among *Pygoscelis* Penguins in Relation to Southern Ocean Sea Ice: Evidence for Food-Web Match-Mismatch

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Abstract

Physical oceanographic variability is an important source of natural selection for polar marine predators that can influence populations through match-mismatch dynamics and associated energetic effects on reproductive survival. Here, we evaluated regional variation in reproductive isotopic niche among *matched* and *mismatched* breeding populations of Adélie (*Pygoscelis adeliae*), chinstrap (*P. antarctica*), and gentoo (*P. papua*) penguins west of the Antarctic Peninsula (AP) to test a hypothesis for sea ice-associated food-web correlates of breeding population change. We rely on signatures of naturally occurring carbon ($^{13}C/^{12}C$, $\delta^{13}C$) and nitrogen ($^{15}N/^{14}N$, $\delta^{15}N$) stable isotopes (SI) as integrated proxies of penguin trophic foraging and food-web structure.

At Anvers Island, where reductions in sea ice have been notable, our analyses demonstrated that *Pygoscelis* penguins became increasingly more similar isotopically, due to a depletion in both $\delta^{13}C$ and $\delta^{15}N$ of blood tissue. Crèched chicks at Anvers Island, the most isotopically similar breeding stage, also occupied similar trophic levels. Isotopic mixing models indicated that the proportions of prey provisioned by adults to crèched chicks were generally similar across species within years, consisting primarily of Antarctic krill (*Euphausia superba*). Crèched Adélie chicks were isotopically enriched at Avian and Charcot Islands, southern rookeries where sea ice is more prominent. Trophic level increased with latitude, while the proportions of prey provisioned by Adélie adults to chicks at southern rookeries included species typical of high Antarctic marine food-webs. Several metrics suggested that penguin food-webs are isotopically wider in regions with greater sea ice coverage.

Our results lend insight on key marine food-web drivers of *Pygoscelis* penguin reproduction at the regional scale, and question the long-standing paradigm that Antarctic krill are the only food-web component critical to penguin reproductive survival. Myctophid and notothenioid fishes may be important nutritional drivers of egg production for chinstraps and Adélies, respectively. Adélie penguin reproduction may be enhanced in regions where prey typical of high Antarctic food-webs is provisioned. Future research should focus more broadly on nutritional drivers of *Pygoscelis* demography, especially cross-seasonal effects of marine food-webs on performance. Ideas regarding food-web niche width deserve further consideration given the issue's relevance for altered energy pathways and ecosystem resilience.

Key words: Bayesian methods; $\delta^{13}C$ and $\delta^{15}N$; food-web; foraging niche; information-theoretic methods; match-mismatch; Pygoscelis penguin; reproduction; sea ice; western Antarctic Peninsula

Introduction

The polar oceans are important systems for advancing our understanding of mechanisms underlying upper trophic level biodiversity responses to environmental change (Smetacek and Nicol 2005, Grebmeier et al. 2006, Ducklow et al. 2007). Specific regions of the Arctic and Southern Oceans have experienced significant alternations in physical features, particularly reductions in the seasonal distribution of sea ice (Stammerjohn et al. 2012) that is driven by coupled atmospheric and oceanic circulation (Stammerjohn et al. 2008b, Turner et al. 2009, Turner and Overland 2009) often occurring in association with increases in air temperature (Comiso 2002, Vaughan et al. 2003, Bromwich et al. 2013). From an evolutionary perspective, climate-induced change in sea ice can be an important source of natural selection for high trophic level polar predators (Smetacek and Nicol 2005, Moline et al. 2008). Although reproductive survival of highly sea ice-obligate species may be affected through direct loss of critical habitat, i.e., sea ice used as a platform for breeding, indirect food-web effects operating through trophic match or mismatch dynamics (Cushing 1982, 1990) can have serious implications for energetic strategies that influence individual fitness (Moline et al. 2008). Trophic mismatch occurs when a predator's foraging niche is altered, via physical drivers, resulting in energetically demanding phases of reproduction occurring outside the window of optimal prey availability in either time or space (Durant et al. 2007, Moline et al. 2008). Where match or mismatch dynamics operate, the balance of species occurrence should reflect long-standing ocean-climate selective regimes for or against predator life histories.

Polar marine ecosystem studies have demonstrated broad correlations between climate-driven variation in physical oceanography and associated change in key trophic

levels including predator populations based on time series data (e.g., Grebmeier et al. 2006, Ducklow et al. 2007). Although informative in terms of identifying potentially important ecosystem components involved in predator match-mismatch dynamics, the specific trophic interactions that link predator populations with change in sea ice habitats have not been well resolved. As for example, ecological drivers of Antarctic penguin population change, i.e., *Pygoscelis* and *Aptenodytes* species (see Baker et al. 2006 for Spheniscidae phylogeny) occurring throughout the Scotia Sea and Antarctic Peninsula (AP) regions (Fig. 2.1a, b), have been classic discussions in Antarctic ecology, originally in terms of competitive release due to increases in food availability as a result of the commercial removal of baleen whales (Laws 1977, 1985). The issue remains at the forefront of considerable debate, but now within the context of regional climate warming (Fraser et al. 1992, Croxall et al. 2002, Forcada et al. 2006, Ducklow et al. 2007, Trathan et al. 2007, Forcada and Trathan 2009, Bestelmeyer et al. 2011, Trathan et al. 2011) and synergistic effects with marine resource harvesting (Ainley and Blight 2009, Trathan and Agnew 2010, Trivelpiece et al. 2011). Fraser et al. (1992) hypothesized that climate-driven reductions in winter sea ice mediated divergent breeding population responses by chinstrap (*P. antarctica*) and Adélie (*P. adeliae*) penguins by increasing or decreasing over-winter survival and subsequent recruitment of each species, respectively, given that chinstraps winter in pelagic, sea ice-free regions across the Scotia Sea (Williams 1995, Trivelpiece et al. 2007), while Adélies rely on southerly distributed pack ice for winter habitat (Ainley 2002). Although sea ice was recognized as important in mediating many trophic interactions, the physical extent of this critical winter habitat was considered the key ecological factor that allowed penguins to fully exploit their prey, particularly

Antarctic krill (*Euphausia superba*, see Fraser et al. 1992 p. 529). This paradigm of species' opposing life history affinities for the presence of sea ice has been invoked to explain population change, particularly at range margins, by Antarctic penguins including also gentoo (*P. papua*) and emperor (*A. forsteri*) penguins of the western AP (Ducklow et al. 2007, Forcada and Trathan 2009, Trathan et al. 2011, but see Trivelpiece et al. 2011). However, no studies to date have considered this issue from an integrated food-web perspective as most have exclusively focused on variability in Antarctic krill stocks as the mechanistic link between climate and sea ice change in this region of the Southern Ocean and facets of *Pygoscelis* penguin demography that underlie breeding population change (e.g., Fraser and Hofmann 2003, Hinke et al. 2007, Trivelpiece et al. 2011, Ducklow et al. 2013).

Here, we further advance the sea ice hypothesis (i.e., Fraser et al. 1992) by considering trophic interactions, over a temporal and spatial gradient in sea ice conditions, occurring among breeding populations of all three *Pygoscelis* species throughout major rookeries of the western AP (Fig. 2.1b, c). Study populations represent species at their relative northern, core, or southern distribution that are characterized by long-term divergent population trajectories at contracting or expanding range margins due to general pole-ward breeding range shifts in response to regional ocean-climate warming (Ducklow et al. 2007, Forcada and Trathan 2009, Ducklow et al. 2013). We rely on signatures of naturally occurring carbon ($^{13}C/^{12}C$, $\delta^{13}C$) and nitrogen ($^{15}N/^{14}N$, $\delta^{15}N$) stable isotopes (SI), a time-integrated, biogeochemical parameter that specifically reflects assimilated prey (Deniro and Epstein 1978, 1981, Peterson and Fry 1987), as proxies of penguin trophic foraging and food-web structure. We examined variation in isotopic

niche among breeding individuals of the sea ice-obligate Adélie penguin, and sea ice-intolerant chinstrap and gentoo penguins along the Palmer Archipelago, near Anvers Island (Fig. 2.1b, c). Here, Adélie penguins occur at their northern breeding range margin along the western AP (Fraser et al. 1992, Polito et al. 2011a), which is contracting given extensive population declines of 80% from historical numbers (Ducklow et al. 2007, see Ducklow et al. 2013 Fig. 7) and reflects trends for the species throughout other rookeries of the region (Trivelpiece et al. 2011, Lynch et al. 2012). Adélie penguin breeding population declines have occurred in association with significant, long-term reductions in annual sea ice coverage within the northern seasonal sea ice zone along the western AP (Jacobs and Comiso 1997, Ducklow et al. 2007, Stammerjohn et al. 2008a, Stammerjohn et al. 2012, Ducklow et al. 2013). Regional environmental warming is also associated with breeding population expansions at southern range margins for sympatric nesting chinstrap and gentoo penguins near Anvers Island given long-term moderate to rapid increases in numbers of breeding individuals, respectively, which has included the establishment of new rookeries (Ducklow et al. 2007, see Ducklow et al. 2013 Fig. 7). Regional trends for breeding gentoo penguins, which unlike other *Pygoscelis* species inhabit near shore, open water areas close to breeding colonies during winter (Tanton et al. 2004), generally corroborate those of our study population (Lynch et al. 2012). However, chinstrap penguins nesting within our study area represent one of only several stable or increasing regional rookeries among many that appear to be declining (Trivelpiece et al. 2011, Lynch et al. 2012).

Annual sea ice coverage increases with higher latitude along the western AP (Ducklow et al. 2007, Stammerjohn et al. 2008a). Importantly, breeding ranges of

chinstrap and gentoo penguins do not extend into these sea ice-dominated regions. However, major Adélie penguin rookeries are located at Avian and Charcot Islands (Schofield et al. 2013) within the species core to most southern distribution along the western AP, respectively (Fig. 2.1b). Here, breeding populations of Adélie penguins have increased (Avian Island, Sailley et al. 2013) or appear stable based on currently available census data (Charcot Island, Henderson 1976, W. R. Fraser unpubl. data). Therefore, Adélie penguins breeding within the southwestern AP are considered *matched* with environmental conditions, similar to increasing breeding populations of chinstrap and gentoo penguins near Anvers Island. These populations contrast with declining numbers of Adélie penguins (i.e., *mismatched* population) within our northern study area where reductions in sea ice have been most prominent.

The current report evaluates regional variation in reproductive isotopic niche among *matched* and *mismatched* breeding populations of *Pygoscelis* penguins along the western AP to test a hypothesis for sea ice-associated food-web correlates of breeding population change (hereafter *sea ice trophic interaction hypothesis*). Progress in understanding marine environmental drivers of *Pygoscelis* penguin breeding population change has been hindered by a staunch focus on Antarctic krill as the only critical trophic link between penguins and sea ice variability. In this spirit, we predicted that blood tissue of reproductive adult *Pygoscelis* penguins, which integrates dietary information over approximately the previous 30 – 60 days given allometric turn-over rates for the cellular fraction of blood (Hobson and Clark 1992, Bearhop et al. 2002, Bearhop et al. 2006, Cherel et al. 2007), would be isotopically distinct during primary reproductive effort (i.e., egg laying) given the differing associations each species' late winter foraging areas have

with sea ice habitats (e.g., Fraser et al. 1992, Williams 1995, Ainley 2002, Tanton et al. 2004, Trivelpiece et al. 2007). However, we predicted that blood tissue of *Pygoscelis* chicks reared from these same northwestern AP rookeries would become increasingly similar isotopically over the breeding season given that all three species depend on local forage generally dominated by Antarctic krill (e.g., Volkman et al. 1980, Fraser and Hofmann 2003, Polito et al. 2011b, Trivelpiece et al. 2011). Conversely, we expected that Adélie penguin chicks reared from southwestern AP rookeries at Avian and Charcot Islands, where winter sea ice coverage is more prominent, would be isotopically enriched relative to conspecifics reared at Anvers Island as neritic (i.e., notothenioid) fishes are critical components of energy transfer to higher trophic levels in this region (Donnelly and Torres 2008, W. R. Fraser unpubl. data), and are isotopically enriched relative to Antarctic krill (Polito et al. 2011b). Our predictions for reproductive isotopic niche were similarly extended to analyses of trophic level and the proportions of prey provisioned to older *Pygoscelis* penguin chicks at five weeks of age, individuals considered a demographically important group within the context of population change, in addition to regional penguin food-web isotopic niche structure and width.

Using an information-theoretic approach, we first (1) asked whether species and year parameters, including their interaction, were important predictors of variation in $\delta^{13}C$ and $\delta^{15}N$ SI signatures of *Pygoscelis* penguin blood tissue across four stages of breeding among populations nesting within our northern study area near Anvers Island - adults at the one-egg stage, and their young chicks at five and 15 days of age, as well as older chicks at a population level age of five weeks. We predicted that species differences in SI signatures would become more similar at later stages of breeding. We (2) asked

whether regional rookery location and year parameters, including their interaction, were important predictors of variation in $\delta^{13}C$ and $\delta^{15}N$ SI signatures of older Adélie penguin chicks at five weeks of age, predicting that individual chicks from southern rookeries would be isotopically enriched relative to their more northern conspecifics. Although we discuss yearly variation in penguin SI signatures within the context of qualitative variation in the previous winter's average sea ice coverage, we made no *a priori* predictions regarding these relationships given that isotopic perspectives of this predator-prey system remain essentially unexplored at this level. Similar to the above analyses, we further (3) examined species, location and year differences in trophic level of five-week-old *Pygoscelis* penguin chicks reared at Anvers Island, as well as Adélie penguin chicks reared from regional rookeries. Analyses are based on individual differences in $\delta^{15}N$ signatures between penguin chicks and the base of regional food-webs established using phytoplankton. Similar to previous analyses, we predicted that five-week-old *Pygoscelis* penguin chicks at Anvers Island would occupy similar trophic levels, however, Adélie penguin chicks from regional rookeries would occupy higher trophic levels at southern rookeries. Our predictions concerning trophic level assume no regional variation in $\delta^{15}N$ signatures of phytoplankton given that relationships between the base of marine food-webs and sea ice variability currently remain unexplored for this region. We additionally (4) employed isotopic mixing models within a Bayesian framework to provide yearly quantitative estimates of the proportion of dietary items used to provision older *Pygoscelis* penguin chicks at five weeks of age throughout regional rookeries. We predicted that *Pygoscelis* chicks reared near Anvers Island in the north would be provisioned similar proportions of various krill species and mesopelagic myctophid fish,

i.e., lanternfish (*Electrona antarctica*). However, Adélie penguin chicks reared from southern rookeries would consume higher proportions of notothenioid fish, i.e., Antarctic silverfish (*Pleuragramma antarcticum*), as well as crystal krill (*E. crystallorophias*). Lastly, we (5) considered isotopic niche structure and width of regional penguin food-webs (i.e., phytoplankton, major prey items and penguin blood tissue only) using six Bayesian-derived metrics. We predicted that southwestern AP penguin food-webs should be generally isotopically wider given the importance of higher trophic level prey in these regions to penguin predators.

Materials and Methods

Field methods

Pygoscelis penguins nesting on several islands within the Palmer Archipelago west of the AP near Anvers Island (64°46’S, 64°03’W, Fig. 2.1a-c) were studied during the austral summers of 2007/08, 2008/09, and 2009/10. Specifically, study nests were located on Biscoe Point (64°48’S, 63°46’W), Torgersen (64°46’S, 64°04’W) and Dream (64°43’S, 64°13’W) Islands (Fig. 2.1c). Each study season, Adélie penguin study nests (n = 30) were distributed equally between the three study islands, with 10 nests located on each island. Gentoo penguin study nests (n = 30) were all located on Biscoe Point, while chinstrap penguin study nests (n = 15) were all located on Dream Island (Fig. 2.1c). The reduced sample size for chinstraps was due to the overall smaller number of individuals breeding at rookeries on Dream Island. Final sample sizes of individuals used in analyses differ from the number of original study nests reported here as at times weather

conditions hindered rookery access resulting in some adults reaching clutch completion before sampling, and therefore, these study nests were not sampled and excluded from subsequent monitoring. In addition, some study chicks were never sampled due to brown skua (*Stercorarius lonnbergi*) depredation.

During annual oceanographic research cruises aboard the A.R.S.V. *Laurence M. Gould*, as part of the Palmer Station, Antarctica, Long-Term Ecological Research Program (PAL-LTER), in January of each study year, we further sampled Adélie penguin chicks from rookeries at Avian (n = 25) and Charcot (n = 15) Islands (Fig. 2.1b).

Each season, study nests, where pairs of adults were present, were individually marked and chosen before the onset of egg laying, and monitored consistently. When study nests were found at the one-egg stage, both adults were captured to obtain blood samples used for SI analyses. At the time of capture, each adult penguin was quickly blood sampled (~1 ml) from the brachial vein using a sterile 3 ml syringe and heparinized infusion needle. After handling, individuals at study nests were further monitored to ensure the pair reached clutch completion, i.e., two eggs. Study nests were monitored for hatch of the first egg. At day six and 16 post-hatch for first chicks, i.e., resulting in an approximate average nest age of five and 15 days given hatching asynchrony, offspring from study nests were captured and quickly blood sampled (≤ ~500 μl for day five chicks, and ≤ ~1 ml for day 15 chicks) from the tarsus vein using a sterile needle and heparinized capillary tubes for five-day-old chicks, and a sterile 3 ml syringe and heparinized infusion needle for 15-day-old chicks, again to obtain blood tissue for SI analyses. Study nests were monitored for chick survival to 25 days, prior to crèche. At five weeks into chick-rearing, based on a seasonal population average hatch date for each

species, older crèched chicks of all three species were captured and quickly blood sampled from study rookeries near Anvers Island. Handling of crèched chicks occurred over a one- or two-day period, which varied seasonally and by species depending on nest initiation dates. Adélie penguin chicks at Avian Island were sampled on the same day Anvers Island Adélie penguin chicks were sampled. Adélie penguin chicks at Charcot Island, sampled during one season only on 25 January 2010, were handled three days after Anvers Island and Avian Island Adélie penguin chicks were sampled that year, i.e., 22 January 2010. Blood samples from crèched chicks (~1 ml) were taken from the brachial vein using a sterile 3 ml syringe and heparinized infusion needle following sampling procedures used for adult penguins to obtain blood tissue for SI analyses. All collected blood was stored in 1.5 ml microcentrifuge tubes and kept cool in the field.

Food-web samples, which included particulate organic matter (i.e., phytoplankton, POM) in addition to primary prey items of penguins such as myctophid (i.e., lanternfish) and notothenioid (i.e., Antarctic silverfish) fishes, as well as three species of euphausiids including Antarctic krill, *Thysanoessa macrura*, and crystal krill were primarily collected during a PAL-LTER oceanographic cruise in January 2009. Additional samples were obtained during oceanographic cruises in January of 2010 and 2011 to increase sample sizes for some taxa. Samples of POM were collected only in January 2009 by filtering continuous flow surface seawater onto glass fiber filters (Whatman GF/F, 25 mm diameter) pre-combusted at 450 ºC for four hrs. Zooplankton were sampled using oblique tows of 1 x 1 m^2 frame net (300 μm mesh) towed to a depth of approximately 300 m or a 2 x 2 m^2 frame net (700 μm mesh) towed to a depth of approximately 120 m. Samples were stored in sterile plastic bags or tubes and frozen at -

80 ºC. Food-web samples were collected along PAL-LTER regional study grid lines in association with rookeries at Anvers (600 - 500 lines), Avian (200 - 100 lines) and Charcot (000 - -100 lines) Islands (Figs. 2.1b, 2.2a) to obtain a representative sample of prey items known to be provisioned to chicks based on the general distribution of prey from stomach lavage of reproductive adults (W. R. Fraser unpubl. data, see also penguin diet datasets within the PAL-LTER data system).

Sea ice data were based on satellite estimates of sea ice concentration (SIC) within two regions, 1-the PAL-LTER regional study grid (Fig. 2.2a) and 2-three 200 km^2 penguin foraging grids associated with major penguin rookeries at Anvers, Avian and Charcot Islands (Fig. 2.3a). Daily and monthly time series of SIC were used to calculate two metrics within the PAL-LTER regional grid, 1-average winter sea ice area (km^2) representing the yearly average area of the ocean covered by winter sea ice, and 2-duration (days) representing the yearly total length of the winter sea ice season. For the 200 km^2 penguin foraging grids, the only sea ice metric calculated was 1-average winter sea ice area within each foraging grid. Sea ice metrics were calculated using time series based on Version 2 of the Goddard Space Flight Center (GSFC) Bootstrap algorithm (Comiso 1999) provided by the U. S. National Snow and Ice Data Center (NSIDC; www.nsidc.org), and derived based on 1-monthly averages between the winter months of sea ice advance and retreat, and 2-the day of first and last appearance of sea ice within the same region and averaged for the PAL-LTER grid. These metrics are useful for describing both the winter spatial and temporal variability of Southern Ocean sea ice (Stammerjohn et al. 2008a).

Laboratory methods

Within 12 hrs of field collection, tubes containing whole blood were centrifuged to separate plasma and red blood cell (RBC) fractions, which were stored separately and frozen at -80 ºC. Collected food-web items were also stored frozen at -80 ºC.

Samples of POM, krill and fish were first thawed and gently rinsed with double-distilled water (ddH_2O). Using an acid solution of 0.1 N HCl, POM samples were acid treated to remove carbonates (Levin and Currin 2012, K.A. Hobson pers. comm.) and rinsed a second time with ddH_2O prior to drying. Samples of acid treated POM, whole krill and fish, along with tubes containing RBCs, were allowed to dry to a consistent mass in a drying oven at 60 ºC. Using a mortar and pestle, lined with clean weighing paper, dried krill, fish, and RBCs were homogenized into a powder. Each mortar and pestle was washed and dried in between sample homogenization. Lipids of dried and ground krill and fish samples were removed prior to SI analyses using a 2:1 chloroform:methanol by volume solution (Bligh and Dyer 1959) first for 24 hrs, and 12 hrs thereafter until the solvent was clear. Lipid-free krill and fish samples were allowed to dry under the hood and subsequently in a drying oven for 24 hrs. Lipid-free krill were further treated with 0.1 N HCl for 24 hrs to remove carbonates in exoskeletons (Soreide et al. 2006), and again dried. RBC samples were not removed of lipids since the plasma portion, which tends to be more lipid-rich than RBC fractions (Bearhop et al. 2000 and references therein), was previously separated and others have shown no need to remove lipids from avian blood tissue (Bearhop et al. 2000, Cherel et al. 2005). Aliquots of powdered krill, fish and RBC samples, as well as whole GF/F samples of POM were transferred to 8 x 5 mm pressed tin capsules (Elemental Microanalysis) and weighed (~2

mg for powdered samples and ~30 mg for GF/F samples) using an analytical microbalance. Samples were analyzed for $\delta^{13}C$ and $\delta^{15}N$ SI signatures using an elemental analyzer interfaced with an isotope ratio mass spectrometer at the Stable Isotope Facility, University of California - Davis. Data expressed as $\delta^{13}C$ or $\delta^{15}N$ were calculated using the following equation: $\delta^{13}C$ or $\delta^{15}N = ([R_{sample}/R_{standard}]-1) \times 1000$, where R_{sample} is the ratio of the heavy to light isotope for either $^{13}C/^{12}C$ or $^{15}N/^{14}N$, and $R_{standard}$ is the heavy to light isotope ratios for international standards - Vienna PeeDee Belemnite for carbon, and atmospheric N_2 (Air) for nitrogen.

Statistical methods

Reproductive isotopic niche (RIN)

Statistical analyses were delineated by reproductive stage and location and performed in the R language environment (R Core Team 2012): 1-*Pygoscelis* adults at the one-egg stage (Anvers Island, *n* = 344), 2-*Pygoscelis* chicks at average nest age of five days (Anvers Island, *n* = 242), 3-*Pygoscelis* chicks at average nest age of 15 days (Anvers Island, *n* = 226), 4-*Pygoscelis* crèched chicks at the population average of five weeks old (Anvers Island, *n* = 136), and 5-Adélie penguin crèched chicks at the population average of five weeks old from regional rookeries (Anvers, Avian, and Charcot Islands, *n* = 135). Sample sizes represent all individuals sampled over the three years of study. Final sample sizes used in analyses differ from those reported here as some individuals were excluded. For example, occasionally adult pairs were never observed having reached clutch completion, i.e., two eggs, likely due to brown skua depredation, and therefore, we could not be certain those individuals represented adults exactly at the one egg stage.

Furthermore, at times weather conditions hindered rookery access resulting in some chicks being sampled at the incorrect age.

Pygoscelis adult and crèched chick analyses at Anvers Island (*RIN* analyses 1 and 4) employed least-squares general linear models (hereafter LM) to examine continuous variation in $\delta^{13}C$ and $\delta^{15}N$ SI signatures in relation to two parameters treated as main effects including 1-species and 2-year, both treated as categorical variables. An a priori set of five candidate models consisted of an equal-means (null) model, each predictor variable as a main effect (two models), a multiple predictor model that included both main effect parameters (one model), and an interaction model including both main effect parameters and their interaction (one model). This same candidate model set was evaluated for each isotope separately using unique datasets for each reproductive stage (*Pygoscelis* adults: $n = 296$ for both $\delta^{13}C$ and $\delta^{15}N$ analyses, *Pygoscelis* crèched chicks: $n = 130$ for both $\delta^{13}C$ and $\delta^{15}N$ analyses). For each candidate model, Akaike's Information Criterion (AICc), corrected for small sample size, in addition to ΔAICc and Akaike weight (w) values were calculated and used to compare models (Burnham and Anderson 2002). An r^2 value was also calculated, defined as the fraction of the total variance explained by the model and given by the Multiple R-squared calculation in R (Crawley 2007, p. 399), to provide a measure of fit for each model. Inference was based on the relative support for parameters across all models and weighted parameter estimates (i.e., model averaging). Parameter estimation included calculation of model-averaged parameter estimates based on w values for all candidate models. Standard errors for parameter estimates were based on unconditional variances calculated across the same

models. Parameter likelihood values were evaluated by summing *w* values across all models that included each parameter under consideration (Burnham and Anderson 2002).

Adélie crèched chick analyses at Anvers, Avian and Charcot Islands (*RIN* analysis 5) also used LM to examine continuous variation in $\delta^{13}C$ and $\delta^{15}N$ SI signatures in relation to two parameters treated as main effects including 1-location and 2-year, both treated as categorical variables. An a priori set of five candidate models considered location and year as main effects following the same model structure described above for *RIN* analyses 1 and 4. This same candidate model set was evaluated for each isotope separately (n = 132 for $\delta^{13}C$ analyses, n = 133 for $\delta^{15}N$ analyses). Again, for each candidate model, AICc, ΔAICc and *w* values were calculated and used to compare models (Burnham and Anderson 2002), as well as calculation of an r^2 value. Inference was based on model averaging with parameter estimation following that described previously.

Pygoscelis chick analyses at five and 15 days of age (*RIN* analyses 2 and 3) employed linear mixed effects models (hereafter LME, nlme package R Core Team 2012) to examine continuous variation in $\delta^{13}C$ and $\delta^{15}N$ SI signatures in relation to two parameters treated as fixed main effects including 1-species and 2-year, both treated as categorical variables. An a priori set of five candidate models consisted of an equal-means (null) model, each predictor variable as a fixed main effect (two models), a multiple predictor model that included both fixed main effect parameters (one model), and an interaction model including both fixed main effect parameters and their interaction (one model). This same candidate model set was evaluated for each isotope separately (Day five: n = 223 for both $\delta^{13}C$ and $\delta^{15}N$ analyses, Day 15: n = 202 for both $\delta^{13}C$ and

$\delta^{15}N$ analyses). A random effect of nest on intercept (random = ~1|nest) was included in all candidate LME models to control for non-independence of data given that sibling chicks were sampled and presumably more similar in isotope signatures. Again, for each candidate model, AICc, ΔAICc and *w* values were calculated and used to compare models (Burnham and Anderson 2002). Inference was based on model averaging with parameter estimation following that described previously. The importance of including a random effect of nest on intercept was tested in preliminary analyses by comparing the fit (i.e., AICc value) of two models for each isotope: the most parameterized fixed effect model, both with and without, the random effect. A linear generalized least squares (GLS) model was fit without a random effect where the restricted log-likelihood was maximized (method = REML). A LME model was also fit with a random effect of nest on intercept, again maximizing the restricted log-likelihood. Both models included the same fixed main effect parameters and their interaction.

Crèched chick trophic level (CCTL)

Trophic level of *Pygoscelis* crèched chicks at a population average of five weeks old was calculated at the individual level for all species, locations, and years following Post (2002): trophic level = $\lambda + (\delta^{15}N_{consumer} - \delta^{15}N_{base})/\Delta_n$, where $\lambda = 1$ as phytoplankton, i.e., primary producers, were used to estimate $\delta^{15}N_{base}$, $\delta^{15}N_{consumer}$ = signatures of $\delta^{15}N$ for individual RBCs of *Pygoscelis* crèched chicks at Anvers, Avian and Charcot Islands, $\delta^{15}N_{base}$ = mean $\delta^{15}N$ signatures of phytoplankton collected along PAL-LTER regional study grid lines in association with rookeries at Anvers, Avian and Charcot Islands (Table 2.1), and Δ_n = 2.54 ‰ as a $\delta^{15}N$ trophic enrichment factor (Vanderklift and Ponsard 2003,

Layman et al. 2005). Importantly, when calculating trophic level for crèched chicks from each of the three regional study areas, the corresponding regional $\delta^{15}N_{base}$ value was used, i.e., trophic level of Anvers Island chicks was calculated based on $\delta^{15}N$ signatures of phytoplankton collected in association with Anvers Island rookeries along the 600 - 500 lines of the PAL-LTER regional study grid. The $\delta^{15}N$ trophic enrichment factor we employed is a lower value than has been suggested previously (i.e., 3.4‰ after Minagawa and Wada 1984, Post 2002) and used by other Antarctic researchers (Stowasser et al. 2012). Therefore, our analyses represent upper estimates of predator trophic position following meta-analysis results of Vanderklift and Ponsard (2003). Furthermore, using a $\delta^{15}N$ trophic enrichment factor of 2.54‰ is more similar to the isotopic discrimination factor for $\delta^{15}N$ (2.07‰) we used in our mixing model analyses, described below, following Cherel et al. (2005).

Trophic level of five-week-old *Pygoscelis* crèched chicks reared at Anvers Island (*CCTL* analysis 1), and five-week-old Adélie penguin crèched chicks reared at regional rookeries (*CCTL* analysis 2) was examined exactly following *RIN* analyses 4 and 5 described above. Least-squares general linear models were used to examine continuous variation in trophic level, calculated as described previously, in relation to two parameters treated as main effects, either 1-species and year, or 2-location and year, where all main effects were treated as categorical variables. The same a priori set of five candidate models described in *RIN* analyses 4 and 5 were also considered here (n = 130 for Anvers Island *Pygoscelis* crèched chick analyses, n = 133 for analyses of Adélie penguin crèched chicks along the western AP). For each candidate model, AICc, ΔAICc and w values were calculated and used to compare models (Burnham and Anderson

2002), as well as calculation of an r^2 value. Inference was again based on model averaging with parameter estimation following that described previously.

Crèched chick isotopic mixing models (CCIMM)

The SIAR package, implemented in R (Parnell et al. 2010, R Core Team 2012), was used to generate quantitative estimates of the proportion of diet items provisioned by adult penguins to five-week-old crèched chicks of all three species at Anvers Island (*CCIMM* analysis 1, n = 130 for all species and year), and Adélie penguin chicks at Avian (*CCIMM* analysis 2, n = 70 for all years) and Charcot (*CCIMM* analysis 3, n = 15 for 2010 only) Islands, for each year of the study, using dual $\delta^{13}C$ or $\delta^{15}N$ signatures of various prey items and chick RBCs. Within a Bayesian framework, SIAR functions modeled probability distributions by incorporating sources of variability such as that within isotopic dietary endpoints and consumers, isotopic discrimination, and elemental concentration. We used the same isotopic dietary endpoints collected in association with each regional study rookery to describe yearly variation in SI signatures of five-week-old crèched chicks reared at those same rookeries. The various dietary endpoints considered for each major study rookery reflected the general distribution of dominant prey from stomach lavage of reproductive adults nesting at Anvers, Avian and Charcot Islands (W. R. Fraser unpubl. data, see also penguin diet datasets within the PAL-LTER data system). Mean values for $\delta^{13}C$ and $\delta^{15}N$ SI signatures of dietary endpoints and associated mean elemental concentrations are reported in Table 2.1. We calculated isotopic discrimination factors and associated standard deviations (SD) using data based on king penguin (*A. patagonicus*) whole blood and a diet of Atlantic herring (*Clupea harengus*) and Icelandic capelin (*Mallotus villosus*) (δ13C: -0.806 ± 0.428 SD and δ15N: 2.067 ± 0.165 SD,

Cherel et al. 2005). The same discrimination factors were applied to all prey types. SIAR models were run for one million iterations with a burn in of the first 40,000 iterations, and thinned by 10, resulting in 96,000 posterior draws.

Food-web isotopic niche structure and width (FWINSW)

SIBER functions within the SIAR package (Jackson et al. 2011) also implemented in R (Parnell et al. 2010, R Core Team 2012) were used to generate six Bayesian-derived metrics to describe regional food-web community structure and width from the perspective of penguin predators. Therefore, analyses were restricted to consider only $\delta^{13}C$ and $\delta^{15}N$ SI signatures of phytoplankton, krill and fish prey, and *Pygoscelis* penguin RBCs from each regional rookery (see Table 2.1 for sample sizes used in *FWINSW* analyses 1 – 3). Bayesian-derived community structure and width metrics are based on those described by Layman et al. (2007) including 1-$\delta^{13}C$ range (dCr): Distance between the most depleted and enriched $\delta^{13}C$ values as an estimate of variation in basal resources, 2-$\delta^{15}N$ range (dNr): Distance between the most depleted and enriched $\delta^{15}N$ values as an estimate of community trophic length, 3-Mean distance to centroid (CD): Average distance of community members to the centroid, which is the mean $\delta^{13}C$ and $\delta^{15}N$ value for all community members, as an additional estimate of niche width, 4-Mean nearest neighbor distance (MNND): Average distances to each community member's nearest neighbor as an estimate of species clustering, 5- Standard deviation of nearest neighbor distance (SDNND): An estimate of the spatial evenness of community member density, 6-total area (TA): isotopic area (‰^{2}) based on the convex hull of the data as an estimate of niche width. Within a Bayesian framework, SIBER functions modeled probability

distributions by incorporating sources of variability such as that within the originally derived means of community members, resulting in one million posterior draws.

Data management

Data reported here are publicly available within the PAL-LTER data system (datasets #223, 224, 225, 226, and 227): http://oceaninformatics.ucsd.edu/datazoo/data/pallter/datasets. These data are additionally archived within the US LTER Network's Information System Data Portal: https://portal.lternet.edu/. Individuals interested in using these data are therefore expected to follow the US LTER Network's Data Access Policy, Requirements and Use Agreement: http://www.lternet.edu/policies/data-access.

Results

Sea ice metrics

Sea ice metrics indicated that the austral winters preceding each study year were generally characterized by lower than average sea ice coverage, with winter 2008 being the lowest sea ice season in the Palmer LTER record since 1979 (Stammerjohn et al. 2008a, see also sea ice datasets within http://pal.lternet.edu/data/). Relative yearly variability in sea ice area and duration (Fig. 2.2b) over the course of the study suggested that 2007 was an intermediate sea ice season relative to 2008 (low) and 2009 (high). Results for inter-annual variability in isotopic niche of adult *Pygoscelis* penguins at the

one-egg stage are discussed within the context of these qualitative differences in each year's winter sea ice season over the entire PAL-LTER regional study grid (Fig. 2.2a).

Variation in average winter sea ice area across the 200 km^2 penguin foraging grids (Fig. 2.3a) confirmed that in general sea ice area increased to the south during the austral winters preceding each study year (Fig. 2.3b). However, this trend was not as strong during the winter of 2008 (Fig. 2.3b) when sea ice area within the Avian Island penguin foraging grid (27,990 km^2) was slightly greater than that for Charcot Island (26,608 km^2). Yearly variation in average winter sea ice area within penguin foraging grids at Anvers and Charcot Islands confirmed our previous finding based on sea ice metrics calculated over the entire PAL-LTER regional study grid (Fig. 2.2a, b) that 2007 was an intermediate winter sea ice season relative to 2008 (low) and 2009 (high). This trend was not apparent within the Avian Island penguin foraging grid (Fig. 2.3b) where 2009 was an intermediate winter sea ice season relative to 2007 (low) and 2008 (high). Results for inter-annual variability in isotopic niche of *Pygoscelis* penguin chicks at all stages and locations are discussed within the context of these qualitative differences in each foraging grid's winter sea ice season (Fig. 2.3a).

Reproductive isotopic niche

Analyses of variation in $\delta^{13}C$ and $\delta^{15}N$ SI signatures of *Pygoscelis* adults at Anvers Island (*RIN* analysis 1, Fig. 2.1b, c) resulted in only one model receiving a ΔAICc value ≤ 2 for each isotope. The most parsimonious model for each isotope was the global model that included terms for species, year, and an interaction for species*year. Both models received very high *w* values (1.00 for each isotope) and r^2 values ($\delta^{13}C$: 0.91, $\delta^{15}N$: 0.71,

Table 2.2). All other models in the candidate set received no support. Parameter likelihoods indicated strong support for all terms in both analyses (Table 2.3). On average, chinstrap penguin adults were most enriched in $\delta^{13}C$ and $\delta^{15}N$, Adélie penguin adults were intermediate, while gentoo penguin adults were the most isotopically depleted of the three species at the one-egg stage (Fig. 2.4a). Adult Adélie penguins were relatively depleted in $\delta^{13}C$ and especially $\delta^{15}N$ following the 2008 winter of very low sea ice within the PAL-LTER regional study grid (Fig. 2.2a, b) in comparison with the other study seasons when sea ice coverage was greater (Fig. 2.4a). Conversely, adult gentoo penguins were relatively enriched in $\delta^{15}N$, but not $\delta^{13}C$, following the 2008 winter of very low sea ice within the PAL-LTER regional study grid (Figs. 2.2a, b, 2.4a). However, $\delta^{15}N$ SI signatures of adult gentoo penguins were only slightly less depleted following the winter of 2009 characterized by the highest sea ice coverage (Fig. 2.2a, b). Yearly variation in chinstrap penguin signatures showed no apparent relationship with over-winter sea ice conditions as each year of the study adults were increasingly enriched in both $\delta^{13}C$ and $\delta^{15}N$ (Fig. 2.4a).

Analyses of variation in $\delta^{13}C$ signatures of *Pygoscelis* chicks at five days of age provisioned by adults at Anvers Island (*RIN* analysis 2, Fig. 2.1b, c) resulted in only one model receiving a ΔAICc value ≤ 2. Again, the most parsimonious model was the global model that included terms for species, year, and an interaction for species*year. This model received a very high *w* value (0.97, Table 2.2). Parameter likelihoods indicated strong support for all these terms (Table 2.3). For $\delta^{15}N$ analyses of *Pygoscelis* chicks at five days of age, three models received ΔAICc value ≤ 2. The best-fit model included a

term for species only and received more than two-times the support (w = 0.43) of the next best model (w = 0.17, Table 2.2) that included terms for species, year, and an interaction for species*year. The third best-supported model included terms for species and year only, and received the same level of support as the second best model (w = 0.17, Table 2.2). Parameter likelihoods indicated moderately strong support for the species term, and weaker support for the year term (Table 2.3). The interaction of species*year received essentially no support (Table 2.3). On average, five-day-old gentoo penguin chicks were most enriched in $\delta^{13}C$, while Adélie and chinstrap penguin chicks had similar $\delta^{13}C$ SI signatures, particularly in the second and third year of the study (Fig. 2.4b). All species were depleted in $\delta^{13}C$ during the second year of the study (Fig. 2.4b) that was characterized by the lowest over-winter sea ice coverage within the 200 km^2 foraging grid at Anvers Island (Fig. 2.3a, b). Gentoo penguin chicks were on average more enriched in $\delta^{15}N$ than the other species (Fig. 2.4b). However, $\delta^{15}N$ SI signatures were most similar across species following the 2008 winter of the lowest sea ice coverage within the Anvers Island foraging grid (Fig. 2.4b).

Similar to five-day-old chicks, analyses of variation in $\delta^{13}C$ signatures of *Pygoscelis* chicks at 15 days of age provisioned by adults at Anvers Island (*RIN* analysis 3, Fig. 2.1b, c) resulted in only one model receiving a ΔAICc value ≤ 2. The best-fit model was the global model that included terms for species, year, and an interaction for species*year, and received a very high w value (1.0, Table 2.2). Again, parameter likelihoods indicated strong support for all these terms (Table 2.3). Analyses of $\delta^{15}N$ SI signatures of 15-day-old *Pygoscelis* chicks resulted in only one model received ΔAICc value ≤ 2. The best-fit model included terms for species and year only, and received a

very high *w* value (0.77, Table 2.2). Parameter likelihoods indicated strong support for both species and year terms (Table 2.3). Again, all species were depleted in $\delta^{13}C$ during the second year of the study (Fig. 2.4c) that was characterized by the lowest over-winter sea ice coverage within the 200 km^2 foraging grid at Anvers Island (Fig. 2.3a, b). All species had similar $\delta^{15}N$ SI signatures across all years (Fig. 2.4c).

Analyses of variation in $\delta^{13}C$ and $\delta^{15}N$ SI signatures of five-week-old *Pygoscelis* crèched chicks provisioned by adults at Anvers Island (*RIN* analysis 4, Fig. 2.1b, c) resulted in only one model receiving a ΔAICc value ≤ 2 for each isotope. The most parsimonious model for each isotope was the global model that included terms for species, year, and an interaction for species*year. Both models received very high *w* values (1.00 for each isotope) and r^2 values ($\delta^{13}C$: 0.94, $\delta^{15}N$: 0.63, Table 2.2). Parameter likelihoods indicated strong support for all terms in both analyses (Table 2.3). All species were depleted in $\delta^{13}C$ during the second year of the study (Fig. 2.4d) that was characterized by the lowest over-winter sea ice coverage within the 200 km^2 foraging grid at Anvers Island, particularly in comparison with the third year of the study following the winter of highest sea ice coverage (Fig. 2.3a, b). All species had similar $\delta^{15}N$ SI signatures during the second and third years of the study (Fig. 2.4d). However, during the first year of the study, five-week-old gentoo chicks were enriched in $\delta^{15}N$ relative to the other species (Fig. 2.4d).

Analyses of regional variation in $\delta^{13}C$ and $\delta^{15}N$ SI signatures of five-week-old crèched Adélie chicks at Anvers, Avian and Charcot Island rookeries (*RIN* analysis 5, Fig. 2.1b) resulted in only one model receiving a ΔAICc value ≤ 2 for each isotope. The

most parsimonious model for each isotope was again the global model that included terms for location, year, and an interaction for location*year. Both models received very high *w* values (1.00 for each isotope) and r^2 values ($\delta^{13}C$: 0.97, $\delta^{15}N$: 0.86, Table 2.2). Parameter likelihoods indicated strong support for all terms in both analyses (Table 2.3). Across regional rookeries, Adélie penguin crèched chicks were relatively enriched in $\delta^{13}C$ and $\delta^{15}N$ at more southern rookeries (Fig. 2.5) that are subject to higher over-winter sea ice conditions (Fig. 2.3a, b). Chicks provisioned at Avian Island had more similar SI signatures across years than their northern conspecifics reared at Anvers Island (Fig. 2.5). Yearly variation in $\delta^{13}C$ and $\delta^{15}N$ SI signatures of Adélie penguin crèched chicks reared at Anvers and Avian Islands did not clearly associate with the previous winter's sea ice coverage within each rookery's 200 km^2 foraging grid. Anvers Island crèched chicks were most enriched during the second and third years of the study when sea ice coverage was the lowest and highest, respectively (Fig. 2.3a, b). Also, Avian Island crèched chicks had similar $\delta^{13}C$ and $\delta^{15}N$ SI signatures across years following winters that varied by approximately 7000 km^2 on average in winter sea ice coverage (Fig. 2.3a, b).

In summary, across all reproductive stages at Anvers Island (Fig. 2.1b, c), the species term was consistently selected in all best-fit models for describing variation in community level $\delta^{13}C$ and $\delta^{15}N$ SI signatures of *Pygoscelis* penguins (Table 2.2). Importantly, species were most different in $\delta^{13}C$ and $\delta^{15}N$ SI signatures among adults at the one-egg stage. As parents provisioned chicks from local prey found within the foraging range of Anvers Island, *Pygoscelis* chicks at five weeks of age were much more similar isotopically than their parents in becoming notably depleted in both $\delta^{13}C$ and $\delta^{15}N$

(Fig. 2.6). Chicks at five and 15 days of age tracked the progressive depletion of community $\delta^{13}C$ and $\delta^{15}N$ SI signatures where chicks at five days of age were relatively enriched in comparison with chicks at 15 days of age (Fig. 2.6). Regional analyses of five-week-old crèched Adélie penguin chicks reared at Anvers, Avian and Charcot Islands (Fig. 2.1b) also demonstrated a gradient in isotopic enrichment where Adélie penguin chicks reared from more southern rookeries, which are associated with relatively more over-winter sea ice coverage (Fig. 2.3a, b), were more enriched in $\delta^{13}C$ and $\delta^{15}N$ than their northern conspecifics (Fig. 2.6).

Crèched chick trophic level

Analyses of variation in trophic level of five-week-old *Pygoscelis* crèched chicks at Anvers Island (*CCTL* analysis 1, Fig. 2.1b, c) resulted in only one model receiving a ΔAICc value ≤ 2. The most parsimonious model was the global model that included terms for species, year, and an interaction for species*year, which received a very high *w* value (1.00) and moderately high r^2 value (0.63, Table 2.4). Parameter likelihoods indicated strong support for all terms (Table 2.5). In the first year of the study, five-week-old gentoo penguin chicks occupied a higher trophic level (4.03 ± 0.07 CI) than Adélie and chinstrap penguin chicks, which held similar trophic levels of 3.8 ± 0.03 CI and 3.7 ± 0.07 CI, respectively (Fig. 2.7a). In subsequent study years, five-week-old chicks of all species occupied very similar trophic levels (2008: Adélie = 4.04 ± 0.07 CI, chinstrap = 4.09 ± 0.04 CI, and gentoo = 4.00 ± 0.04 CI, 2009: Adélie = 3.96 ± 0.04 CI, chinstrap = 4.01 ± 0.03 CI, and gentoo = 4.02 ± 0.04 CI, Fig. 2.7a). Analyses of variation in trophic level of five-week-old crèched Adélie penguin chicks across regional rookeries (*CCTL*

analysis 2, Fig. 2.1b) again resulted in only one model receiving a ΔAICc value ≤ 2. The most parsimonious model was the global model that included terms for location, year, and an interaction for location*year, which received a very high *w* value (1.00) and moderately high r^2 value (0.68, Table 2.4). Parameter likelihoods indicated strong support for all terms (Table 2.5). In year one, five-week-old Adélie penguin chicks reared at Avian Island occupied a higher trophic level (4.17 ± 0.04 CI) than Adélie penguin chicks at Anvers Island (3.75 ± 0.03 CI, Fig. 2.7b). However, in year two of the study, five-week-old Adélie penguin chicks provisioned from these same regional rookeries occupied a more similar trophic level (Anvers Island = 4.04 ± 0.07 CI, Avian Island = 4.07 ± 0.03 CI, Fig. 2.7b). Finally, in the last year of the study, and the only year or which isotope data were obtained from five-week-old Adélie penguin chicks at Charcot Island, chicks reared from more southern rookeries occupied higher trophic levels (Charcot Island: 4.15 ± 0.04 CI, Avian Island: 4.06 ± 0.03 CI) than Adélie penguin chicks at Anvers Island (3.96 ± 0.04 CI, Fig. 2.7b).

Crèched chick isotopic mixing models

Bayesian mixing model analyses estimating the proportion of diet items provisioned by adult penguins to five-week-old crèched *Pygoscelis* chicks from Anvers Island (*CCIMM* analysis 1, Fig. 2.1b, c) considered three main prey types (Table 2.1, Fig. 2.8) including two species of krill (Antarctic krill and *T. macrura*) and the myctophid, lanternfish. Within all three study years, the proportion of these diet items provisioned by adults to chicks were generally similar across *Pygoscelis* species at Anvers Island, which included anywhere from 42% - 91% of the diet each season consisting of Antarctic krill based on

95% credibility intervals for all species and years (Fig. 2.9a). During the first study year gentoo penguin crèched chicks consumed a lower proportion of Antarctic krill based on 95% credibility intervals (48% – 66%) than Adélie (66% - 85%) and chinstrap (68% - 91%) penguin chicks (Fig. 2.9a). Only small proportions of each species diet consisted of lanternfish based on 95% credibility intervals for all years (0% - 27%).

Five main prey types were considered within mixing model analyses estimating the proportion of diet items provisioned by adult penguins to five-week-old Adélie penguin chicks reared at Avian Island (*CCIMM* analysis 2, Fig. 2.1b, Table 2.1, Fig. 2.8). These prey types included all species considered by Anvers Island analyses, as well as crystal krill and the notothenioid, Antarctic silverfish. Across study years at Avian Island that were characterized by variability in winter sea ice coverage of approximately 7000 km^2 (Fig. 2.3a, b), the proportion of various diet items consumed by crèched Adélie penguin chicks was remarkably similar, with Antarctic krill consisting of 48 - 71% of the diet based on the spread of 95% credibility intervals across years (Fig. 2.9b). Smaller proportions of the diet consisted of crystal krill (1.7% - 26%) and *T. macrura* (1.4% - 34%), while fish (lanternfish and Antarctic silverfish) again comprised a small proportion (0% - 17%, Fig. 2.9b) based on the spread of 95% credibility intervals across year.

Three main prey types were considered by mixing model analyses estimating dietary proportions consumed by five-week-old Adélie penguin chicks reared at Charcot Island during the breeding season of 2010 only (*CCIMM* analysis 3, Fig. 2.1b, Table 2.1, Fig. 2.8). These prey types included dominant species found within high latitude, continental-shelf regions of the Southern Ocean including Antarctic and crystal krill, as well as Antarctic silverfish. Charcot Island is subject to the highest winter sea ice

coverage within the PAL-LTER regional study grid (Fig. 2.3a, b). Interestingly, crystal krill comprised 37% - 67% of the diet provisioned to Adélie penguin chicks at Charcot Island, while Antarctic krill represented 33% - 55% (Fig. 2.9c) based on 95% credibility intervals. Antarctic silverfish comprised again only a very small proportion of the diet (0% – 9.1%, Fig. 2.9c).

Food-web isotopic niche structure and width

Bayesian-derived metrics of western AP marine food-web structure and width (FWINSW analyses 1-3) provided some evidence that penguin food-webs increased in isotopic width in association with increases in over-winter sea ice coverage. Firstly, distance to centroid was greater in food-webs subject to increased winter sea ice area based on 95% credibility intervals (Anvers Island: 2.43‰ – 2.87‰, Avian Island: 3.32‰ – 3.88‰, Charcot Island: 4.18‰ – 4.62‰). Range in $\delta^{13}C$ was also greater in southwestern AP food-webs although 95% credibility intervals overlapped for Avian and Charcot Islands (Anvers Island: 2.87‰ – 6.63‰, Avian Island: 7.17‰ – 8.84‰, Charcot Island: 8.43‰ – 9.60‰). Range in $\delta^{15}N$ did not increase with latitude as 95% credibility intervals overlapped among all regions (Anvers Island: 8.17‰ – 12.00‰, Avian Island: 11.40‰ – 12.30‰, Charcot Island: 9.99‰ – 11.50‰). However, mean nearest neighbor distance was greater in food-webs subject to increased winter sea ice area (Anvers Island: 1.62‰ – 2.18‰, Avian Island: 2.44‰ – 2.91‰, Charcot Island: 3.01‰ – 3.52‰). Standard deviation of nearest neighbor distance did not increase with latitude as 95% credibility intervals overlapped among all regions (Anvers Island: 1.82‰ – 2.42‰, Avian Island: 1.44‰ – 2.01‰, Charcot Island: 2.31‰ – 2.97‰). Lastly, total isotopic area was not

different across regions as 95% credibility intervals overlapped (Anvers Island: 10.40‰^{2} – 18.60‰^{2}, Avian Island: 17.00‰^{2} – 31.80‰^{2}, Charcot Island: 18.3‰^{2} – 29.00‰^{2}). In summary, three of six metrics (distance to centroid, $\delta^{13}C$ range, and mean nearest neighbor distance) provided some evidence that southwestern AP regional penguin food-webs, which are subject to greater over-winter sea ice coverage, are isotopically wider (Figs. 2.3a, b and 2.10a).

Discussion

Elucidating relationships between sea ice change and associated food-web effects on penguin demography has been highlighted as a key component to understanding population responses to climate variability in the Southern Ocean (Forcada and Trathan 2009). To this end, our study is the first to consider regional breeding population trends among western AP *Pygoscelis* penguins from an integrated food-web perspective based on variation in $\delta^{13}C$ and $\delta^{15}N$ signatures of penguin blood tissue measured at the individual level, as well as SI signatures of dominant prey and phytoplankton. Many of our specific predictions were supported by the data. First, our analyses at Anvers Island demonstrated that over the course of the breeding season, individuals became increasingly more similar isotopically, as predicted, due to a general depletion in both $\delta^{13}C$ and $\delta^{15}N$ of blood tissue (2.6a-d). Older chicks at five weeks of age near Anvers Island, the most isotopically similar breeding stage (Figs. 2.4a-d, 2.6a-d), also occupied generally similar trophic levels as would be expected, particularly in the second and third year of study (Fig. 2.7a). Furthermore, isotopic mixing models indicated that the

proportion of diet items provisioned by adults to older chicks reared near Anvers Island were generally similar across *Pygoscelis* species within years (Fig. 2.9a). Secondly, as we predicted, Adélie penguin chicks reared at regional rookeries were isotopically enriched at more southern rookeries (Fig. 2.5). Within this context, occupied trophic level increased with latitude (Fig. 2.7b), while the proportions of dietary items provisioned by Adélie penguin adults to chicks at regional rookeries were notably different, particularly at Charcot Island (Fig. 2.9a-c).

Pygoscelis adults at Anvers Island

Adults at the one egg stage were the most isotopically distinct breeding stage, which suggests that species are foraging at different trophic levels across the western AP or Scotia Sea during the 30 – 60 days prior to nest initiation in association with their differing preferred late winter/early spring habitat. Chinstrap penguins were most enriched at this stage, followed by Adélie penguins, whereas gentoo penguins were the most depleted in $\delta^{13}C$ and $\delta^{15}N$ among species (Fig. 2.6a). These data, coupled with simple field observations, knowledge of other seabird systems, in addition to previous research on our study populations demonstrating that male and female *Pygoscelis* penguins are not greatly segregated in their pre-breeding trophic foraging niche (Gorman et al. 2014), suggest the possibility that adult male and female chinstrap and Adélie penguins may be relying on a fish based diet prior to egg production in association with off-shore winter habitats in sea ice-free or sea ice-associated regions, respectively. However, gentoo penguins may rely primarily on krill while wintering near-shore close to breeding colonies. Over the course of our study, brown skua depredation of *Pygoscelis*

penguin eggs at breeding colonies near Anvers Island revealed species-specific variability in the color of penguin egg yolks where chinstrap and Adélie females appear to produce pale yellow colored yolk, while gentoo females produce bright orange to red yolk (K. B. Gorman pers. obs.). Research on alcids of the north Pacific has shown yolk coloration to be related to carotenoid deposition by females, including both the types and concentration of carotenoids, as a function of trophic foraging (Hipfner et al. 2010a, Hipfner et al. 2010b). These studies confirmed marked variability in carotenoids among crustacean versus fish prey; lower trophic level crustaceans contained high concentrations of astaxanthin, while higher trophic level fish prey contained only low levels of the carotenoids astaxanthin, lutein, and zeaxanthin (Hipfner et al. 2010b). Similarly, yolk of these seabirds with a more red hue contained only astaxanthin, while yolk with a more yellow hue contained all three carotenoid types (Hipfner et al. 2010b). Inter- and intra-specific analyses showed a negative relationship between carotenoid concentration and $\delta^{15}N$ signatures of the yolk tissue itself, indicating that red colored yolks are derived from nutrients obtained at lower trophic levels with high levels of carotenoids, while yellow colored yolks are derived from higher trophic level diets with lower levels of carotenoids (Hipfner et al. 2010a, Hipfner et al. 2010b). Similar trophic relationships may govern patterns of carotenoid allocation among *Pygoscelis* penguins of the western AP, which would account for the fact that females of the two species with enriched $\delta^{15}N$ values of blood at the onset of breeding also produce eggs with pale yellow yolk. In accordance, our $\delta^{15}N$ data show forage fishes to be enriched relative to krill (Table 2.1, Fig. 2.8) similar to results by Polito et al. (2011b) regarding isotopic variability of penguin prey within this same general region of the Antarctic. However, unlike Polito et al. (2011a)

that concluded female Adélie and gentoo penguins both rely on higher trophic level prey prior to breeding due to their similarity in eggshell $\delta^{15}N$ enrichment, our results indicate that adults of these two species may rely on very different trophic level prey during the 30 – 60 days prior to nest initiation. Our data suggest that *Pygoscelis* penguins are fundamentally linked to different food-webs during late winter/early spring, presumably as a function of each species preferred winter habitat. Our results have important implications for understanding community variation in nutritional resources critical to the individuals that return to breed, and more specifically egg production.

Our study revealed important relationships between each species trophic foraging during late winter/early spring and the most recent winter's average sea ice coverage (Figs. 2.2b, 2.4a). Notably, foraging by Adélie penguins appeared most closely associated with sea ice variability as blood $\delta^{15}N$ values of adults were similarly enriched in years of relatively higher sea ice conditions, and depleted in winter 2008 with the lowest sea ice conditions. Conversely, blood $\delta^{15}N$ values of gentoo penguins were relatively enriched following the 2008 winter of extremely low winter sea ice coverage. Although gentoo penguins were relatively depleted following the winter with the highest average sea ice conditions (year three), individuals were not as greatly depleted as in year one. Variation in blood $\delta^{15}N$ values of adult chinstrap penguins appeared independent of winter sea ice conditions as individuals became increasingly enriched across years. Community level foraging responses during late winter/early spring by *Pygoscelis* penguins to over-winter sea ice variability have not been studied previously (but see Gorman et al. 2014). Our data suggest that species do in fact hold differing foraging affinities with sea ice, where Adélie penguins are most tightly coupled and chinstrap penguins least tightly coupled,

with winter sea ice coverage. In this sense, it is possible that the higher trophic level prey, likely fish, these two species appear to rely upon during late winter/early spring include different communities given each species differing foraging response to sea ice variability and generally disparate wintering locations.

Previous work on pelagic fish communities of the western AP has shown that mesopelagic and neritic assemblages are typically segregated by sea ice coverage, as well as hydrographic properties (Kellerman 1996, Donnelly and Torres 2008). Mesopelagic fishes, including the genera *Electrona*, *Gymnoscopelus*, *Protomyctophum*, *Bathylagus*, *Cyclothone*, and *Notolepis*, typically occur in association with circumpolar deep water (CDW), a relatively warm and more saline water mass derived from the Antarctic Circumpolar Current found both at the continental shelf break, and in association with cross-shelf submarine canyons that act as a conduit delivering CDW to more coastal regions (Kellerman 1996, Donnelly and Torres 2008). Conversely, notothenioids, in particular *Pleuragramma*, are found in colder and less saline coastal waters subject to sea ice formation along the western AP (Kellerman 1996, Donnelly and Torres 2008). The notothenioids, which includes approximately 80 species, are critical components of energy transfer within high Antarctic continental shelf regions such as the Ross Sea, with Antarctic silverfish comprising a majority of the mid-water fish biomass (La Mesa et al. 2004). Importantly, Antarctic silverfish hold a strong affinity for the presence of sea ice (Kellerman 1996), particularly during early life stages (Vacchi et al. 2004, Bottaro et al. 2009). Therefore, we interpret our data to suggest that adult chinstrap penguins may be relying on mesopelagic fish assemblages associated with open water habitats, while adult Adélie penguins may depend on sea ice-associated neritic assemblages during the 30 – 60

days prior to egg production given each species produces pale yellow colored yolk, forages at relatively high trophic levels, but have very different foraging associations with over winter sea ice variability. Emslie et al. (2003) corroborate these ideas in studying prey remains at active chinstrap and Adélie penguin breeding colonies at King George Island where chinstraps preferred the myctophid, lanternfish, while Adélie prey remains primarily included Antarctic silverfish.

Pygoscelis chicks at Anvers Island

Chicks at day five, 15, and five weeks of age at Anvers Island became increasingly isotopically similar, specifically due to the depletion of blood $\delta^{13}C$ and $\delta^{15}N$ values (Figs. 2.4b-d and 2.6b-d). We interpret this trend in isotopic variability over the chick stage to reflect the turnover of nutrients from those originally allocated to egg tissue during late winter/early spring, to those nutrients provisioned by parents to chicks from local foraging areas during summer. Interestingly, gentoo chicks appeared to have a slower rate of isotopic depletion as compared to Adélie or chinstrap chicks (Fig. 2.6b-d). This may reflect, in part, the fact that gentoo chicks have the slowest growth rate of the three *Pygoscelis* species, broadly corresponding to their more northerly distribution (Volkman and Trivelpiece 1980, Williams 1995). Given the extreme similarity in five-week-old chick isotope signatures across species, our data suggest that parents of all three species rely on very similar sources of nutrients to raise chicks near Anvers Island. Within this context, our trophic level analyses confirmed that older chicks do occupy generally similar trophic positions across species (Fig. 2.7a), which appears to be due to the provisioning of similar proportions of prey items as identified by isotopic mixing models

(Fig. 2.9a). Only in year one did gentoo chicks appear to hold a slightly higher trophic level than the other species, which was driven by a larger proportion - although not significantly larger based on confidence intervals - of *T. macrura* being provisioned by adult gentoo penguins (Fig. 2.9a) that are isotopically enriched in $\delta^{15}N$ relative to Antarctic krill (Table 2.1, Fig. 2.8). Importantly, Antarctic krill was the dominant prey species provisioned to chicks by all species during each study year (Fig. 2.9a).

Unlike the adult stage, there were no strong inter-specific associations between chick isotope signatures at all stages and over-winter sea ice variability within the Anvers Island foraging grid (Figs. 2.3a, b and 2.4a-d). Gentoo penguin chicks clearly showed no isotopic association with the previous winter's average sea ice coverage. Isotopic signatures of five-day-old Adélie and chinstrap chicks were both enriched in $\delta^{15}N$ during the second year of study following the winter of very low sea ice coverage (Fig. 2.4b). However, this relationship was not apparent during later chick stages (Fig. 2.4c, d). Despite the apparent lack of species-specific isotopic associations with winter sea ice variability during chick rearing, isotopic mixing models did indicate that parents of all species provisioned their chicks greater proportions of *T. macrura* following the winter of very low sea ice coverage (Figs. 2.3a, b and 2.9a), which accounted for all species occupying a higher trophic level in year two of the study (Fig. 2.7a). During the third year of the study, parent penguins appeared to provision their chicks slightly higher proportions, again not significantly higher based on confidence intervals, of the myctophid, *E. antarctica*. Provisioning of *E. antarctica* during year three appeared to have no relationship with sea ice variability as this type of foraging was not observed during the first year of study characterized by similar winter sea ice conditions, and was

likely related to increased CDW intrusion into the Palmer Deep canyon, where penguins typically forage near Anvers Island (Kahl et al. 2010, Schofield et al. 2013), due to wind forcing (Martinson et al. 2008).

Adélie chicks at regional islands

Five-week-old Adélie penguin chicks at regional rookeries became increasingly enriched in both $\delta^{13}C$ and $\delta^{15}N$ at higher latitude rookeries (Fig. 2.5) associated with greater over-winter sea ice coverage within foraging grids (Fig. 2.3a, b). This trend in isotopic variability was strong as it persisted across years at both Anvers and Avian Island rookeries, and was additionally confirmed by the one year of data collected at Charcot Island. Within this context, older Adélie chicks occupied higher trophic levels at regional rookeries in association with greater sea ice coverage, even after accounting for variation in the base of marine food-webs due to the apparent enrichment in $\delta^{15}N$ of phytoplankton at higher latitudes (Table 2.1, Fig. 2.8). Our mixing model analyses suggested that regional enrichment of Adélie chicks, particularly at Charcot Island, was primarily due to greater proportions of crystal krill being provisioned to chicks at higher latitude rookeries, as opposed to greater proportions of Antarctic silverfish as we originally predicted. Like Antarctic silverfish, crystal krill are key components of high Antarctic continental shelf food-webs (La Mesa et al. 2004). Therefore, it is not surprising that crystal krill are provisioned by adult Adélie penguins to chicks in greater proportions at our highest latitude study site associated with the greatest over-winter sea ice coverage (Fig. 2.3a, b). However, the lack of a substantial fish component at Charcot Island during the final year of our study was surprising and may be related to a lack of recruitment

events of Antarctic silverfish given the relatively low sea ice conditions of the previous winters since 2006 (see Ducklow et al. 2013 Fig. 2). These conditions may have interacted with cyclonic gyres of the western AP that typically retain larval fish in the system, determining their regional distribution at older life stages (Kellerman 1996), resulting in the absence of juvenile fish at specific age classes that are appropriate sizes for capture by provisioning adult penguins.

There was no strong relationship between regional Adélie penguin chick isotope signatures and over-winter sea ice variability within each region's corresponding foraging grid at both Anvers and Avian Islands (Fig. 2.5). However, there was less variability among years in chick isotope signatures at Avian Island than Anvers Island. Within this context, mixing model results indicated that across years, prey provisioned to chicks at Avian Island was more similar than that modeled for Anvers Island, and importantly suggest the idea that marine foraging areas near Avian Island may be more predictable in regions with greater over-winter sea ice coverage. Environmental predictability is critical to the evolved life history strategies of penguins (Forcada and Trathan 2009), particularly during the breeding season given that adult penguins must maximize their foraging effort within a restricted range in order to provision and protect chicks at terrestrial breeding rookeries. Within the northern sector of our regional study area, where changes in sea ice have been most prominent, ocean-climate variability may be impacting penguin populations by altering the predictability of prey critical for successful reproduction.

Food-web structure and width

To our knowledge, the Bayesian metrics we calculated of regional penguin food-web isotopic structure and width along the western AP have not been applied previously to Antarctic marine food-webs. Variation in the base of regional marine food-webs was evident in our data, due to the apparent enrichment in $\delta^{15}N$ of phytoplankton at higher latitudes (Table 2.1, Fig. 2.8). We interpret this result to reflect the fact that phytoplankton communities that dominate the northern region of the western AP, where winter sea ice loss has been most prominent, differ from communities that dominate southern regions with greater sea ice coverage (Montes-Hugo et al. 2009). Even after accounting for this variation, our results provide some evidence that penguin food-webs in regions with greater over-winter sea ice coverage along the western AP are isotopically wider based on distance to centroid, $\delta^{13}C$ range, and mean nearest neighbor distance calculations. The concept of differences in penguin food-web niche width between high and low Antarctic marine systems is intuitive given the importance of higher trophic level prey, particularly Antarctic silverfish, in the diets of Adélie and emperor penguins in high Antarctic systems (Cherel and Kooyman 1998, Ainley 2002), in comparison with regions where Antarctic krill dominate penguin diets (Fraser and Hofmann 2003, Trivelpiece et al. 2011). Our data provide some of the first evidence that food-web niche width may be correlated with breeding population trends for Adélie penguin along the western AP, which may indicate that niche width collapse for Adélie penguins at more northern rookeries leaves these populations less resilient to environmental variability (see also Chapman et al. 2011).

Isotopic baselines

An important issue in the interpretation of the data presented here is whether or not the temporal and spatial isotopic variability detected among penguins reflects actual differences in their diet (as has been interpreted here) versus simply reflecting variability in isotopic baselines of the marine ecosystem (i.e., signal to noise ratio). Isotopic baselines are an important aspect of ecological studies that incorporate stable isotope data (Casey and Post 2011), however, they can be extremely difficult to quantify for predators. For example, the isotopic signatures of the predators studied here reflects isotopic baselines from various regions along the western AP during late winter and throughout the summer breeding season. It is logistically difficult to obtain baseline information during winter in remote regions such as the Southern Ocean when researchers typically work with penguins during the summer breeding season only.

This study quantified phytoplankton baseline information across regions along the western AP in one year only. These data suggest a shifting isotopic baseline for phytoplankton in association with a latitudinal gradient in sea ice coverage (Table 2.1 and Figure 2.8). However, Antarctic krill do not show this same trend (Table 2.1 and Figure 2.8). Thus, I argue that isotopic baselines in primary productivity are much smaller than the observed differences in penguin isotope signatures, as well as their prey, which would indicate that diet is in fact driving the differences in predator isotope variability as discussed previously. Nonetheless, baseline variability undoubtedly contributes to the variability in predator isotope signatures reported here and this study would have been stronger had we been able to quantify baseline variability at least across the years of study during the summer months.

Importantly, the analyses regarding food-web structure and width accounted for regional differences in baseline isotopic variability of phytoplankton by incorporating these data in the analyses directly. Interestingly, these analyses detected differences in some metrics of interest, which indicates again that observed differences in upper trophic level isotope signatures are likely driven by diet and not just simple differences in baseline variability.

Sea ice trophic interaction hypothesis

The data we present in the current report lends considerable insight on key marine food-web drivers of *Pygoscelis* penguin reproduction at the regional scale along the western AP. Within the context of divergent breeding population responses among all three species occurring near Anvers Island, our most northern study site, our results call into question the long standing paradigm that Antarctic krill are the key food-web component critical to the reproductive survival of these species in this region of the Antarctic (e.g., Fraser et al. 1992, Fraser and Hofmann 2003, Hinke et al. 2007, Trivelpiece et al. 2011). Recently, Trivelpiece et al. (2011) argued that populations of both chinstrap and Adélie and penguins breeding at the South Shetland Islands (Fig. 2.1b) are regulated by the availability of Antarctic krill, which would explain why both species appear to be declining in this region in association with reductions in krill stocks due to sea ice loss, increased pressures from krill fishery expansions, as well as recovering populations of other krill predators such as seals and whales. Demographically, the decline of these populations is considered driven by reduced survival and recruitment of young birds as breeding adults due to a reduction in krill availability that has reduced fledgling

condition, which impacts energetic buffers following the departure from natal colonies when young birds are learning to self-feed. While our data confirm that Antarctic krill are in fact a dominant prey item in the provisioning diet of both chinstrap and Adélie penguins at Anvers Island (Figs. 2.6d and 2.9a), and therefore, undoubtedly play a major role in determining variation in fledging condition, our data also confirm that adults of these species that return to breed rely on higher trophic level prey that we argue is two differing communities of fish as discussed previously. Within this context, juveniles of these species likely also rely on these same fish communities after the breeding season and throughout the winter months in order to survive. Therefore, an alternative hypothesis to that offered by Trivelpiece et al. (2011) is that variation in the availability of critical fishes may be an important, and highly underappreciated, driver of juvenile survival and recruitment as breeding adults for these species. If in fact chinstrap and Adélie penguins breeding near Anvers Island are linked to mesopelagic (myctophid) and neritic (notothenioid) fish fauna during the non-breeding period, respectively, this may explain differing population trends near Anvers Island in association with regional reductions in sea ice as notothenioids such as Antarctic silverfish hold life histories tightly associated with the presence of sea ice (Kellerman 1996, La Mesa et al. 2004, Vacchi et al. 2004, Bottaro et al. 2009), while mesopelagic fishes are typically found in sea ice free regions in association with ACC-derived warmer water masses (Kellerman 1996, Donnelly and Torres 2008) - an oceanic habitat that has been enhanced by ongoing regional climate changes. Of all three species, gentoo penguins showed less variability across breeding stages in isotope signatures (Fig. 2.6a-d), reflecting a more consistently available nutrient source, likely krill, important to this population's breeding effort. This

foraging niche consistency may partly explain why numbers of gentoo penguins have increased dramatically near Anvers Island since the mid-1990's. However, the rapid rate of increase suggests that immigration of individuals into the population is likely an important factor as well. These issues underscore the importance of future research that will address not only the spatial distribution and foraging ecology of these species outside the breeding season (e.g., Bost et al. 2009), but also carry-over or cross-seasonal effects on performance (e.g., Harrison et al. 2011), as well the role of dispersal as a contributing factor in population change (e.g., Dugger et al. 2010) including perspectives based on historical population genetic structure.

Our results further develop an understanding of marine food-web drivers of Adélie penguin reproduction during late chick-rearing at the regional scale, demonstrating higher latitude breeding populations, which have increased or are stable, to be linked to sea ice-associated food-webs more characteristic of high Antarctic regions due to the greater provisioning of crystal krill to chicks (Fig. 2.9a-c). The paleoecological record during the late Holocene suggests that Antarctic silverfish, a key species in high Antarctic food-webs along with crystal krill, dominated diets of Adélie penguins during periods of colder climate and greater sea ice coverage (Emslie and McDaniel 2002, Lorenzini et al. 2010), which generally corroborates our understanding of regional variation in the dietary niches occupied by Adélie penguins. Again, our results call into question the general paradigm that *Pygoscelis* penguins of the western AP are regulated by the availability of Antarctic krill (sensu Trivelpiece et al. 2011). Our data indicate that crystal krill are the dominant prey item provisioned at our highest latitude study site at Charcot Island, comprising over 50% of the chick diet. We interpret these data to indicate

that declining breeding populations of Adélie penguins within the northwestern AP near Anvers Island are mismatched with the local summer food-web where the chick provisioning diet consists of nearly 75% Antarctic krill and in some extremely low sea ice years a significant proportion of *T. macrura*. However, breeding populations at Charcot Island are matched where more than 50% of the chick provisioning diet includes crystal krill. Therefore, the onset of the historical decline of breeding Adélie penguins near Anvers Island that started in the late 1980's and early 1990's may have resulted from shifts in a high Antarctic food-web dominated by sea ice, crystal krill and Antarctic silverfish to one more typical of sea ice-free, lower latitude regions where Antarctic krill and myctophid fish assemblages dominate. Although the Adélie penguin breeding population at Avian Island has increased in recent years (Sailley et al. 2013), the provisioning diet during the relatively low sea ice years over the course of this study showed only a small amount of crystal krill in the diet (11-16%). These small proportions of crystal krill reflect the fact that the marine food-web in the foraging vicinity of Avian Island is intermediate between the high Antarctic penguin food-web found at Charcot Island, and the low Antarctic penguin food-web at Anvers Island. Should the warming trend continue along the western AP, studies of the marine food-web near Avian and Charcot Islands may prove particularly informative for understanding how and why these systems shift from being dominated by sea ice, crystal krill, Antarctic silverfish, and Adélie penguins to being dominated by open water, Antarctic krill, myctophid fish fauna, as well as chinstrap and gentoo penguins.

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Table 2.1. Mean $\delta^{13}C$ and $\delta^{15}N$ signatures and elemental concentrations of western Antarctic Peninsula marine community members used in food-web analyses of trophic position, Bayesian isotope mixing models and food-web structure and width.

Study rookery	Community member	Sample size	Mean $\delta^{13}C$ ± SD / SE	Mean $\delta^{15}N$ ± SD / SE	Elemental concentration (%C ± SD)	Elemental concentration (%N ± SD)
Anvers	*Phytoplankton*	*n* = 16	-29.331 ± 1.161 / 0.290	0.145 ± 0.906 / 0.227	NA	NA
Island	*E. superba* (krill)	*n* = 28	-26.102 ± 0.966 / 0.183	4.368 ± 0.651 / 0.123	38.098 ± 5.979	11.891 ± 1.859
	T. macrura (krill)	*n* = 30	-26.521 ± 1.189 / 0.217	7.484 ± 0.588 / 0.107	36.536 ± 3.314	11.268 ± 0.969
	E. antarctica (fish)	*n* = 2	-24.610 ± 0.535 / 0.378	10.222 ± 0.313 / 0.221	39.926 ± 4.287	12.423 ± 1.303
	P. adeliae (penguin)	*n* = 47	-27.401 ± 0.624 / 0.091	7.559 ± 0.397 / 0.058	NA	NA
	P. antarctica (penguin)	*n* = 41	-27.321 ± 0.527 / 0.082	7.681 ± 0.448 / 0.070	NA	NA
	P. papua (penguin)	*n* = 42	-27.067 ± 0.699 / 0.108	7.784 ± 0.266 / 0.041	NA	NA
Avian	*Phytoplankton*	*n* = 20	-29.454 ± 0.412 / 0.092	0.489 ± 0.652 / 0.146	NA	NA
Island	*E. crystallorophias* (krill)	*n* = 7	-21.967 ± 1.976 / 0.747	8.264 ± 1.216 / 0.460	39.583 ± 3.164	12.218 ± 1.078
	E. superba (krill)	*n* = 24	-25.860 ± 1.683 / 0.344	4.862 ± 0.699 / 0.143	40.083 ± 3.298	12.416 ± 1.059
	T. macrura (krill)	*n* = 23	-26.018 ± 2.248 / 0.469	7.890 ± 0.636 / 0.133	37.997 ± 3.224	11.710 ± 0.958
	E. antarctica (fish)	*n* = 13	-24.172 ± 0.421 / 0.117	10.202 ± 0.895 / 0.248	42.393 ± 6.673	13.179 ± 2.051
	P. antarcticum (fish)	*n* = 12	-21.773 ± 0.607 / 0.175	12.341 ± 0.465 / 0.134	41.893 ± 5.133	13.161 ± 1.547
	P. adeliae (penguin)	*n* = 70	-25.774 ± 0.334 / 0.040	8.358 ± 0.255 / 0.030	NA	NA
Charcot	*Phytoplankton*	*n* = 16	-31.576 ± 0.330 / 0.083	1.087 ± 0.467 / 0.117	NA	NA
Island	*E. crystallorophias* (krill)	*n* = 18	-22.575 ± 1.113 / 0.262	8.502 ± 0.697 / 0.164	36.700 ± 3.549	11.237 ± 1.146
	E. superba (krill)	*n* = 14	-25.974 ± 1.874 / 0.501	5.046 ± 0.704 / 0.188	35.990 ± 10.056	10.982 ± 3.070
	P. antarcticum (fish)	*n* = 7	-24.062 ± 0.588 / 0.222	11.861 ± 0.721 / 0.273	38.912 ± 5.534	12.256 ± 1.743
	P. adeliae (penguin)	*n* = 15	-23.110 ± 0.193 / 0.050	9.080 ± 0.190/0.049	NA	NA

Abbreviations: SD = standard deviation, SE = standard error, NA = elemental concentration not applied within food-web analyses.

Table 2.2. Candidate models describing variation in reproductive isotopic niche of *Pygoscelis* penguins throughout the western Antarctic Peninsula (*RIN* analyses 1-5). Models presented are those determined to be most parsimonious, as well as all models receiving ΔAICc values ≤ 2.

Study rookery	Reproductive stage	Response variable	Model number	Explanatory variable	Number of parameters	ΔAICc	*w*	r^2
Anvers	Adults	$\delta^{13}C$	5	Species + Year + Species*Year	10	0.000	1.000	0.91
Island		$\delta^{15}N$	5	Species + Year + Species*Year	10	0.000	1.000	0.71
	Day 5 chicks	$\delta^{13}C$	5	Species + Year + Species*Year	11	0.000	0.974	NA
		$\delta^{15}N$	2	Species	5	0.000	0.430	NA
			5	Species + Year + Species*Year	11	1.828	0.172	NA
			4	Species + Year	7	1.876	0.168	NA
	Day 15 chicks	$\delta^{13}C$	5	Species + Year + Species*Year	11	0.000	0.998	NA
		$\delta^{15}N$	4	Species + Year	7	0.000	0.773	NA
	Week 5 chicks	$\delta^{13}C$	5	Species + Year + Species*Year	10	0.000	0.999	0.94
		$\delta^{15}N$	5	Species + Year + Species*Year	10	0.000	1.000	0.63
Regional	Week 5 Adélie	$\delta^{13}C$	5	Location + Year + Location*Year	8	0.000	1.000	0.97
Islands	chicks	$\delta^{15}N$	5	Location + Year + Location*Year	8	0.000	1.000	0.86

Abbreviations: NA = r^2 value not available for linear mixed effects models.

Table 2.3. Parameter estimates and likelihoods from candidate models describing variation in reproductive isotopic niche of *Pygoscelis* penguins throughout the western Antarctic Peninsula (*RIN* analyses 1-5). Parameter estimates (±1 SE) are weighted averages, and standard errors are based on unconditional variances. Parameter likelihoods are Akaike weight (*w*) values summed across all models that include the variable.

Study rookery	Reproductive stage	Response variable	Explanatory variable	Parameter likelihood		Parameter estimate ± 1SE	
				$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
Anvers	Adults	$\delta^{13}C/\delta^{15}N$	Intercept	1.000	1.000	-25.056 ± 0.041	9.018 ± 0.051
Island			Species CHPE	1.000	1.000	0.468 ± 0.070	-0.002 ± 0.087
			Species GEPE	1.000	1.000	-0.382 ± 0.060	-1.055 ± 0.074
			Year 2008/09	1.000	1.000	-1.167 ± 0.054	-0.461 ± 0.067
			Year 2009/10	1.000	1.000	-0.942 ± 0.055	0.012 ± 0.068
			Species CHPE*Year 2008/09	1.000	1.000	1.137 ± 0.101	0.835 ± 0.126
			Species CHPE*Year 2009/10	1.000	1.000	1.067 ± 0.094	0.546 ± 0.118
			Species GEPE*Year 2008/09	1.000	1.000	-0.150 ± 0.078	0.915 ± 0.098
			Species GEPE*Year 2009/10	1.000	1.000	0.225 ± 0.079	0.302 ± 0.098
	Day 5 chicks	$\delta^{13}C/\delta^{15}N$	Intercept	1.000	1.000	-25.723 ± 0.127	8.399 ± 0.124
			Species CHPE	0.992	0.771	-0.714 ± 0.303	0.055 ± 0.265
			Species GEPE	0.992	0.771	-0.204 ± 0.185	0.195 ± 0.177
			Year2008/09	1.000	0.414	-1.693 ± 0.175	0.065 ± 0.122
			Year 2009/10	1.000	0.414	-0.617 ± 0.177	-0.011 ± 0.096
			Species CHPE*Year 2008/09	0.974	0.172	1.118 ± 0.381	0.165 ± 0.293
			Species CHPE*Year 2009/10	0.974	0.172	1.047 ± 0.370	0.142 ± 0.257
			Species GEPE*Year 2008/09	0.974	0.172	0.580 ± 0.249	0.005 ± 0.055
			Species GEPE*Year 2009/10	0.974	0.172	0.707 ± 0.259	0.080 ± 0.150
	Day 15	$\delta^{13}C/\delta^{15}N$	Intercept	1.000	1.000	-26.906 ± 0.073	7.352 ± 0.103
	chicks		Species CHPE	1.000	0.999	-0.745 ± 0.167	-0.068 ± 0.196

			Species GEPE	1.000	0.999	-0.105 ± 0.147	0.523 ± 0.138
			Year 2008/09	1.000	0.998	-0.948 ± 0.105	0.487 ± 0.132
			Year 2009/10	01.000	0.998	0.323 ± 0.108	0.186 ± 0.139
			Species CHPE*Year 2008/09	0.998	0.225	0.710 ± 0.224	0.121 ± 0.216
			Species CHPE*Year 2009/10	0.998	0.225	0.539 ± 0.220	0.065 ± 0.142
			Species GEPE*Year 2008/09	0.998	0.225	0.681 ± 0.180	-0.050 ± 0.112
			Species GEPE*Year 2009/10	0.998	0.225	0.636 ± 0.189	0.024 ± 0.087
	Week 5	$\delta^{13}C/\delta^{15}N$	Intercept	1.000	1.000	-27.671 ± 0.042	7.117 ± 0.063
	chicks		Species CHPE	1.000	1.000	0.162 ± 0.066	-0.104 ± 0.099
			Species GEPE	1.000	1.000	0.290 ± 0.059	0.722 ± 0.089
			Year 2008/09	1.000	1.000	-0.292 ± 0.058	0.745 ± 0.087
			Year 2009/10	1.000	1.000	1.084 ± 0.058	0.552 ± 0.087
			Species CHPE*Year 2008/09	0.999	1.000	-0.017 ± 0.088	0.238 ± 0.131
			Species CHPE*Year 2009/10	0.999	1.000	-0.241 ± 0.088	0.224 ± 0.132
			Species GEPE*Year 2008/09	0.999	1.000	-0.024 ± 0.085	-0.905 ± 0.127
			Species GEPE*Year 2009/10	0.999	1.000	0.149 ± 0.084	-0.569 ± 0.126
Regional	Week 5	$\delta^{13}C/\delta^{15}N$	Intercept	1.000	1.000	-25.981 ± 0.048	8.547 ± 0.045
Islands	Adélie		Location Anvers	1.000	1.000	-1.690 ± 0.079	-1.429 ± 0.073
	chicks		Location Charcot	1.000	1.000	2.647 ± 0.080	0.825 ± 0.074
			Year 2008/09	1.000	1.000	0.427 ± 0.071	-0.279 ± 0.065
			Year 2009/10	1.000	1.000	0.223 ± 0.070	-0.293 ± 0.065
			Location Anvers*Year 2008/09	1.000	1.000	-0.719 ± 0.112	1.024 ± 0.103
			Location Anvers*Year 2009/10	1.000	1.000	0.861 ± 0.111	0.845 ± 0.103

Abbreviations: SE = standard error, CHPE = chinstrap penguin, GEPE = gentoo penguin.

Table 2.4. Candidate models describing variation in trophic level of five-week-old *Pygoscelis* penguin chicks throughout the western Antarctic Peninsula (*CCTL* analyses 1-2). Models presented are those determined to be most parsimonious, as well as all models receiving ΔAICc values ≤ 2.

Study rookery	Reproductive stage	Response variable	Model number	Explanatory variable	Number of parameters	ΔAICc	*w*	r^2
Anvers	Week 5 chicks	Trophic	5	Species + Year + Species*Year	10	0.000	1.000	0.63
Island		level						
Regional	Week 5 Adélie	Trophic	5	Location + Year + Location*Year	8	0.000	1.000	0.68
Islands	chicks	level						

Table 2.5. Parameter estimates and likelihoods from candidate models describing variation in trophic level of five-week-old *Pygoscelis* penguin chicks throughout the western Antarctic Peninsula (*CCTL* analyses 1-2). Parameter estimates (±1 SE) are weighted averages, and standard errors are based on unconditional variances. Parameter likelihoods are Akaike weight (*w*) values summed across all models that include the variable.

Study rookery	Reproductive stage	Response variable	Explanatory variable	Parameter likelihood	Parameter estimate ± 1SE
Anvers	Week 5 chicks	Trophic level	Intercept	1.000	3.746 ± 0.025
Island			Species CHPE	1.000	-0.043 ± 0.039
			Species GEPE	1.000	0.283 ± 0.035
			Year 2008/09	1.000	0.292 ± 0.034
			Year 2009/10	1.000	0.217 ± 0.034
			Species CHPE*Year 2008/09	1.000	0.096 ± 0.052
			Species CHPE*Year 2009/10	1.000	0.089 ± 0.052
			Species GEPE*Year 2008/09	1.000	-0.354 ± 0.050
			Species GEPE*Year 2009/10	1.000	-0.224 ± 0.050
Regional	Week 5 Adélie	Trophic level	Intercept	1.000	4.172 ± 0.018
Islands	chicks		Location Anvers	1.000	-0.427 ± 0.029
			Location Charcot	1.000	0.090 ± 0.029
			Year 2008/09	1.000	-0.110 ± 0.025
			Year 2009/10	1.000	-0.115 ± 0.025
			Location Anvers*Year 2008/09	1.000	0.403 ± 0.041
			Location Anvers*Year 2009/10	1.000	0.333 ± 0.041

Abbreviations: SE = standard error, CHPE = chinstrap penguin, GEPE = gentoo penguin.

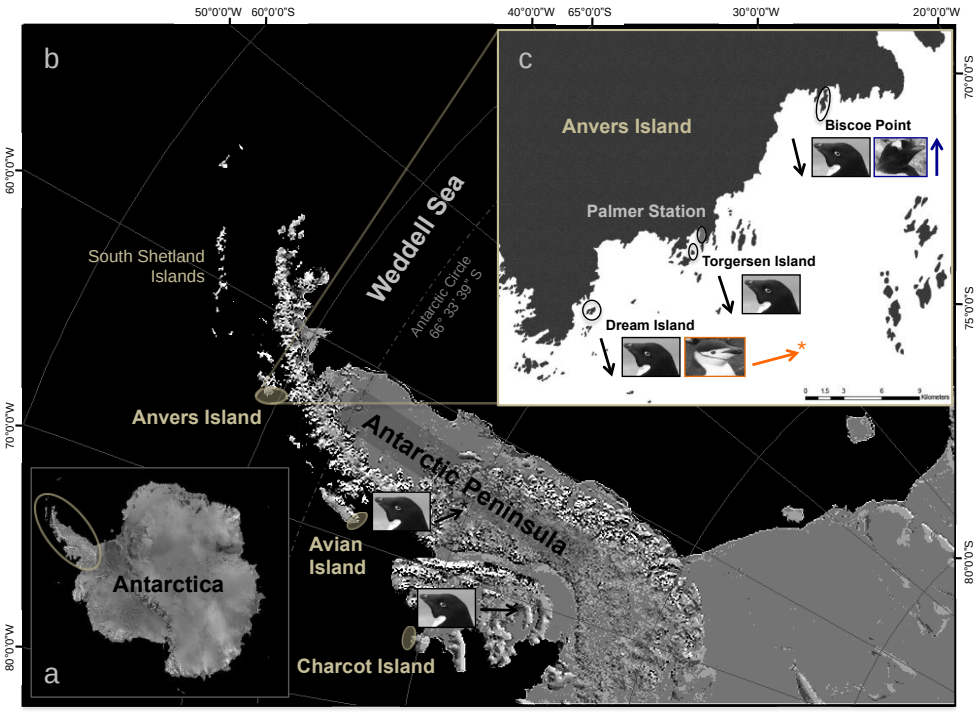

Figure 2.1. The marine ecosystem west of the Antarctic Peninsula (a) extends from northern Alexander Island (approximately Charcot Island) to the South Shetland Islands (b). The Scotia Sea extends northeast from the South Shetland Islands (b) to include islands throughout the Scotia Arc (not pictured here). Fieldwork for the present study was conducted at major penguin rookeries within the Palmer Archipelago near Anvers Island and Palmer Station, a United States supported research base, as well as at Avian and Charcot Islands (b, c). Northern penguin rookeries were located at Dream (Adélie [black] and chinstrap [orange]) and Torgersen (Adélie only), and Biscoe (Adélie [black] and gentoo [blue]) Islands (c). Southern Adélie penguin rookeries were located approximately 400 and 700 kilometers south of Anvers Island at Avian and Charcot Islands, respectively. Arrows color coded for each species at breeding rookeries indicate associated breeding population trajectory (i.e., declining [↓], moderately increasing [→*,→*], rapidly increasing [↑], or stable [→]). Image generated from base maps provided by the National Snow and Ice Data Center's map server A-CAP (http://nsidc.org/agdc/acap/).

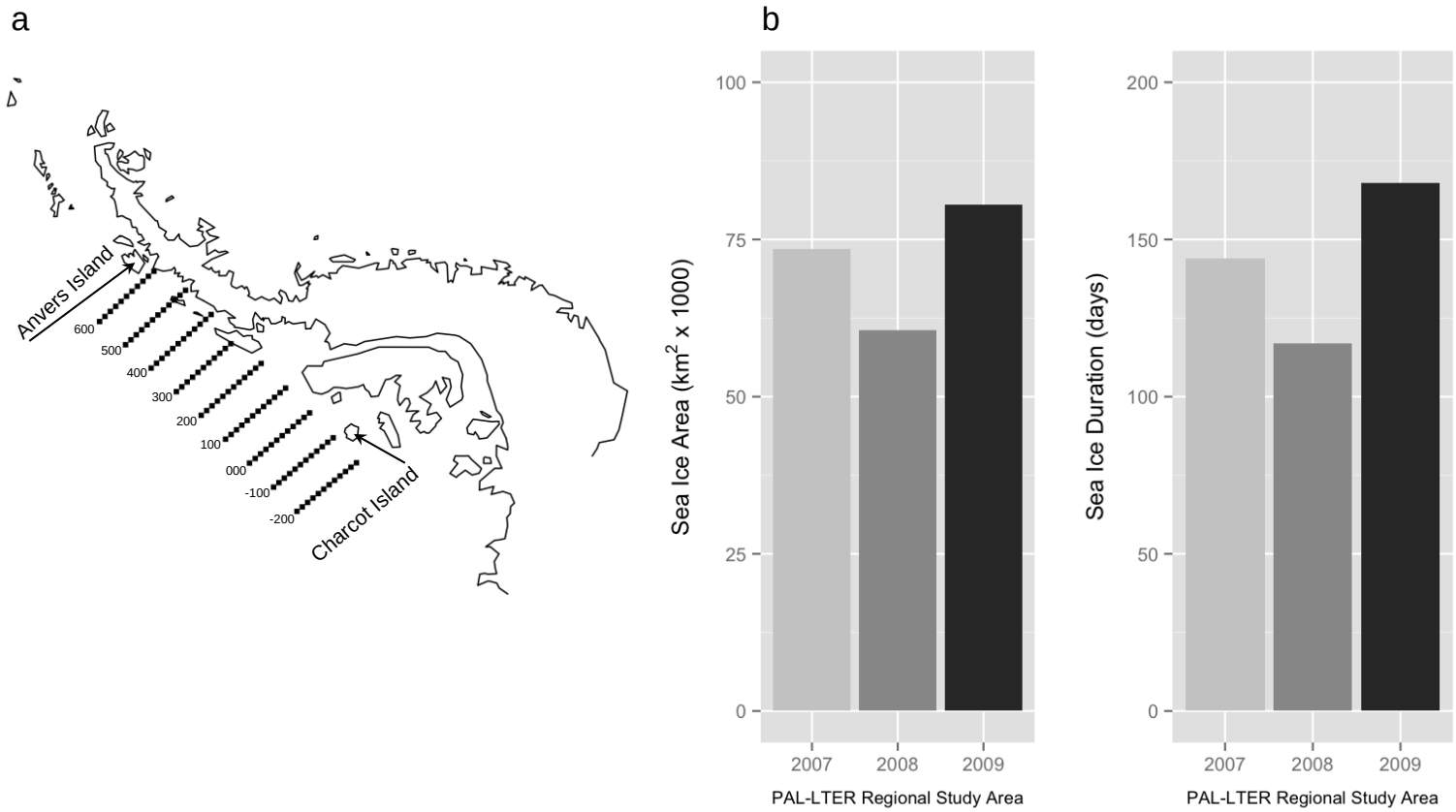

Figure 2.2. Palmer Station, Antarctica, Long Term Ecological Research (PAL-LTER) Program's regional study area west of the Antarctic Peninsula, extending 800 km from Anvers Island (600 line) to just south of Charcot Island (-200 line) and 220 km inshore to offshore (a). Study area grid lines encompass the entire area over which sea ice metrics were calculated. Winter average sea ice area (km^2) and sea ice duration (days) within the PAL-LTER regional study area for each year of the present study (b).

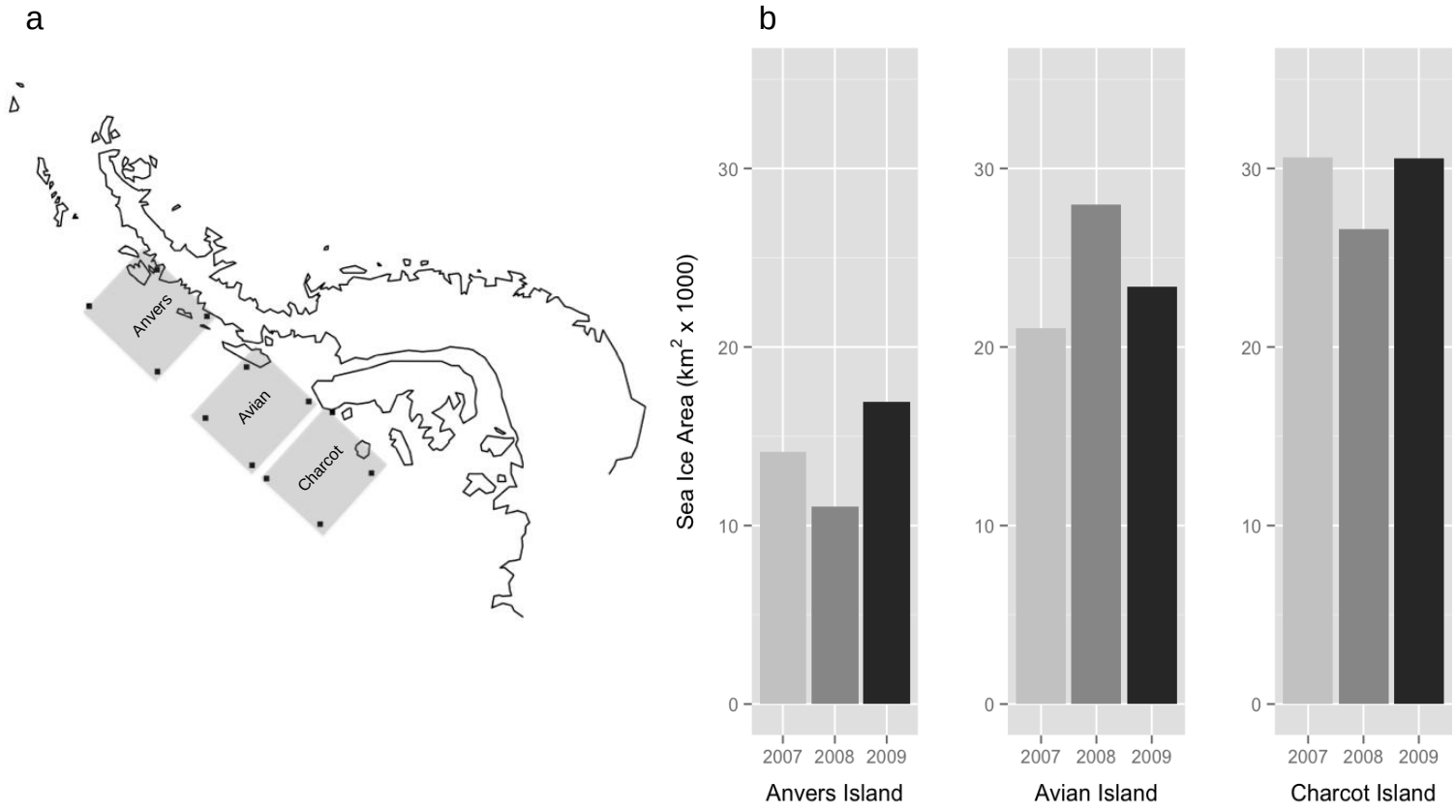

Figure 2.3. Penguin foraging grids (200 km^2) off of Anvers, Avian and Charcot Islands west of the Antarctic Peninsula (a). Winter average sea ice area (km^2) within regional penguin foraging grids for each year of the present study (b).

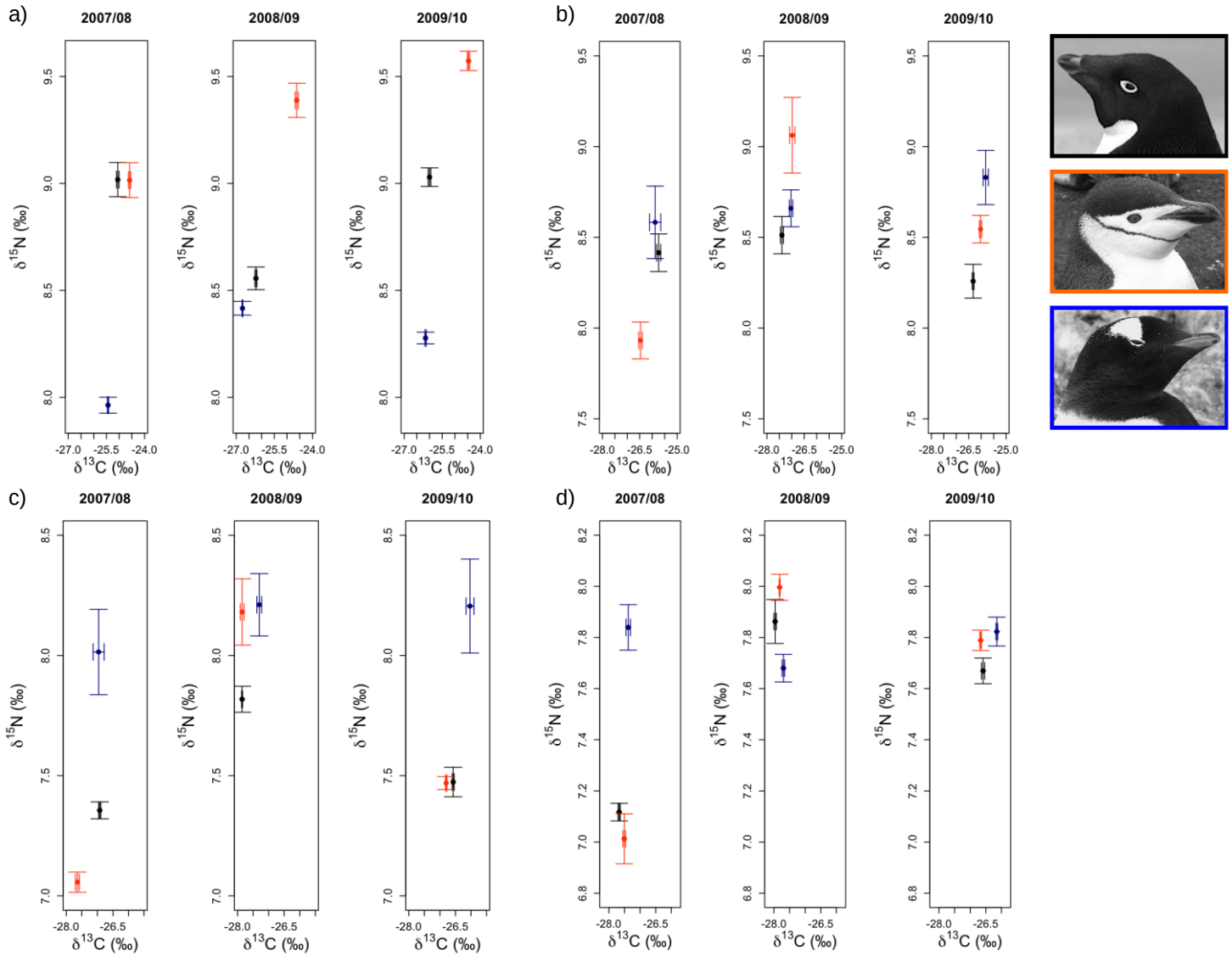
a)
2007/08
2008/09
2009/10
b)
2007/08
2008/09
2009/10
c)
2007/08
2008/09
2009/10
d)
2007/08
2008/09
2009/10
δ15N (‰)
δ13C (‰)

Figure 2.4. Variation in $\delta^{13}C$ and $\delta^{15}N$ stable isotope signatures of Adélie (●), chinstrap (●), and gentoo (●) penguin red blood cells for (a) adults at the one-egg stage, and their young chicks at (b) five and (c) 15 days of age, as well as (d) older chicks at a population level age of five weeks at Anvers Island. Average isotope signatures (± 1 SE) for each species are presented for each year based on sample sizes exactly following analyses previously described.

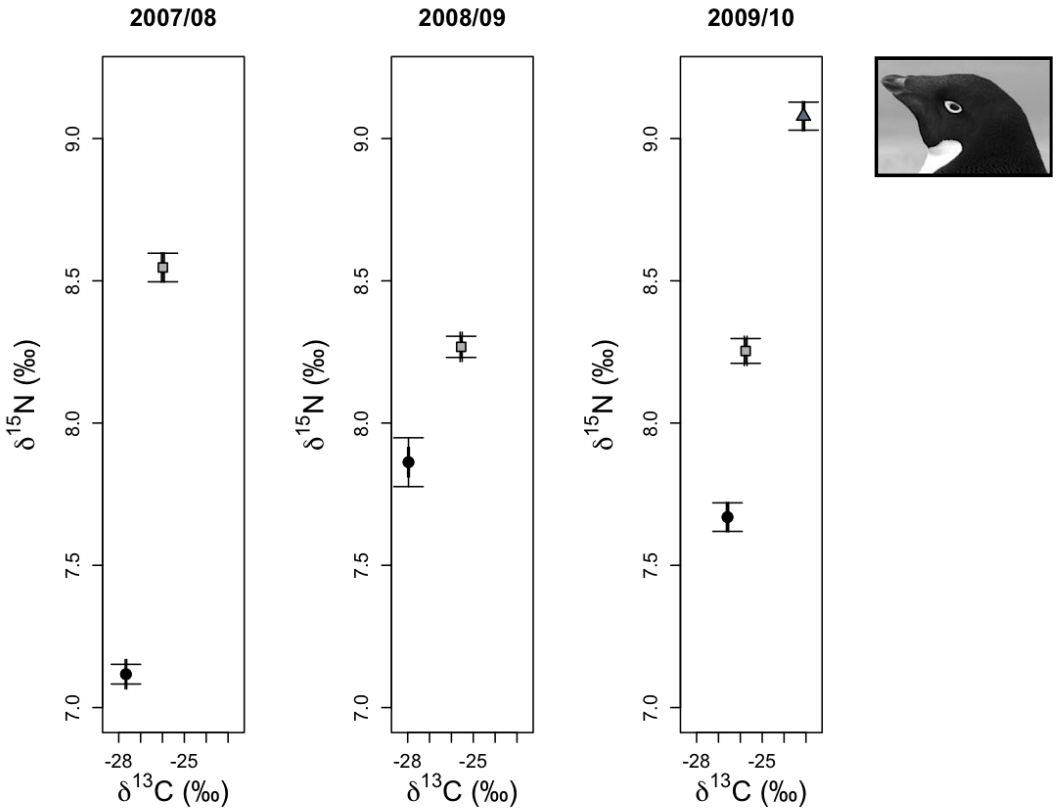

Figure 2.5. Variation in Adélie penguin $\delta^{13}C$ and $\delta^{15}N$ stable isotope signatures for older chicks at a population level age of five weeks from Anvers (●), Avian (□), and Charcot (△) Islands. Average isotope signatures (± 1 SE) for each rookery location are presented for each year based on sample sizes exactly following analyses previously described.

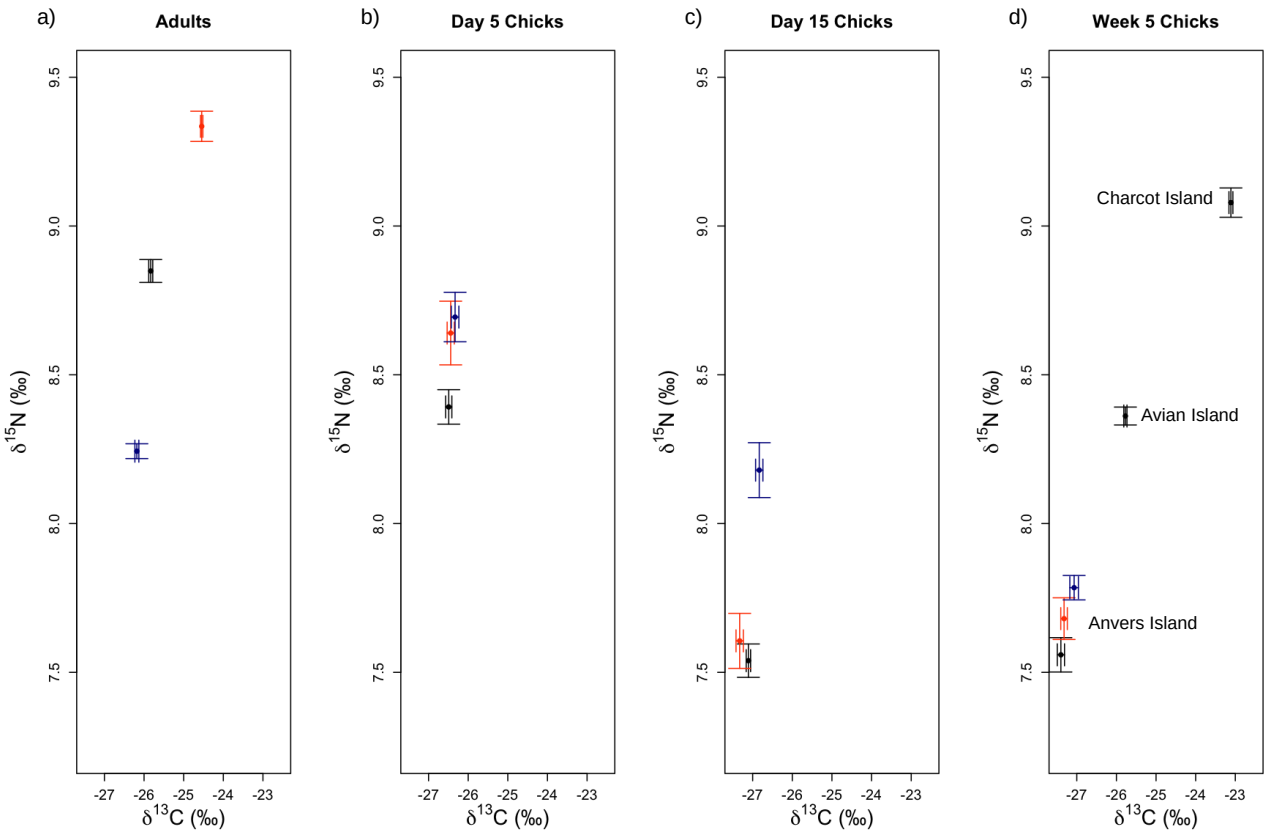

Figure 2.6. Variation in $\delta^{13}C$ and $\delta^{15}N$ stable isotope signatures of Adélie (●), chinstrap (●), and gentoo (●) penguin red blood cells for (a) adults at the one-egg stage, and their young chicks at (b) five and (c) 15 days of age, as well as (d) older chicks at a population level age of five weeks at regional rookeries. Isotope signatures (± 1 SE), averaged across years, are presented for each reproductive stage based on sample sizes exactly following analyses previously described.

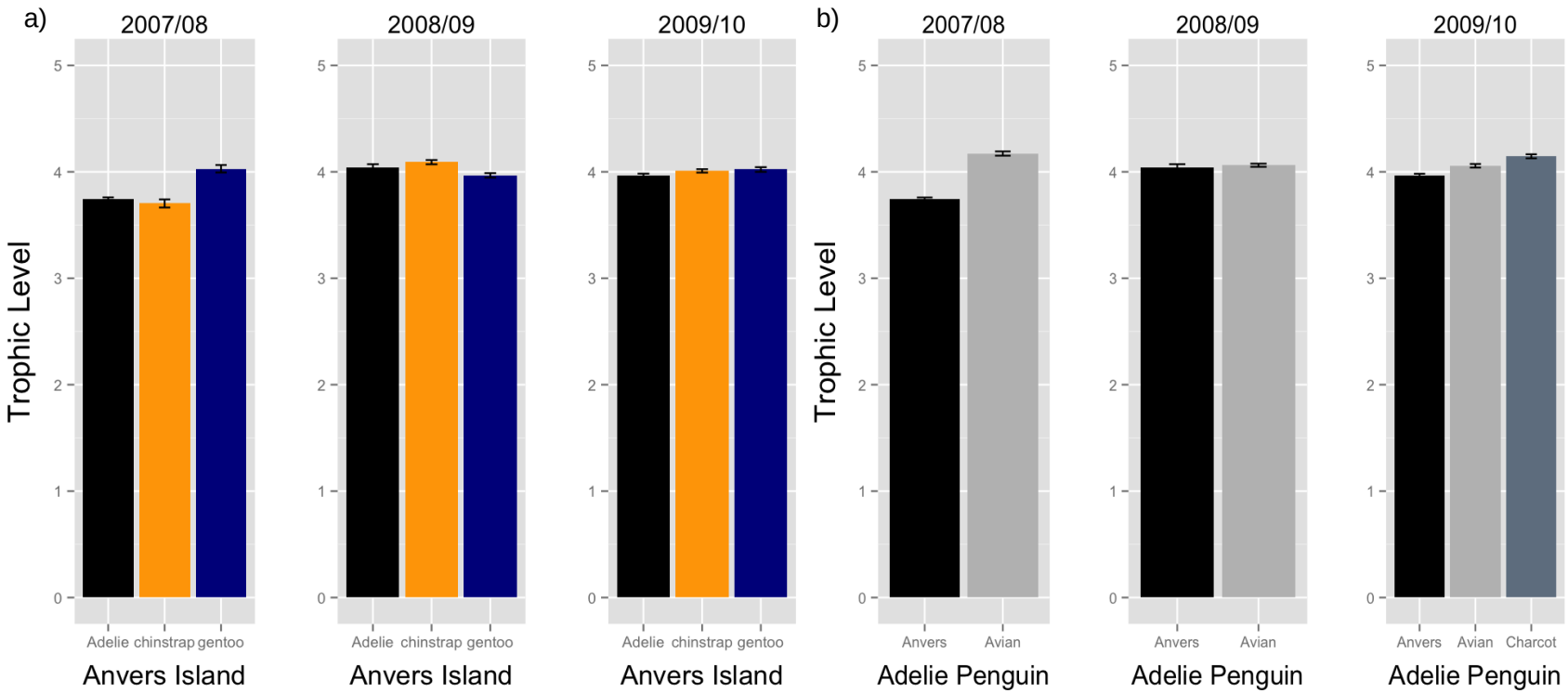

Figure 2.7. Variation in trophic level of (a) Adélie (■), chinstrap (■), and gentoo (■) penguins at a population level age of five weeks at Anvers Island and (b) Adélie penguins at a population level age of five weeks at Anvers (■), Avian (■), and Charcot (■) Islands. Average isotope signatures (± 1 SE) for each species and rookery location are presented for each year based on sample sizes exactly following analyses previously described.

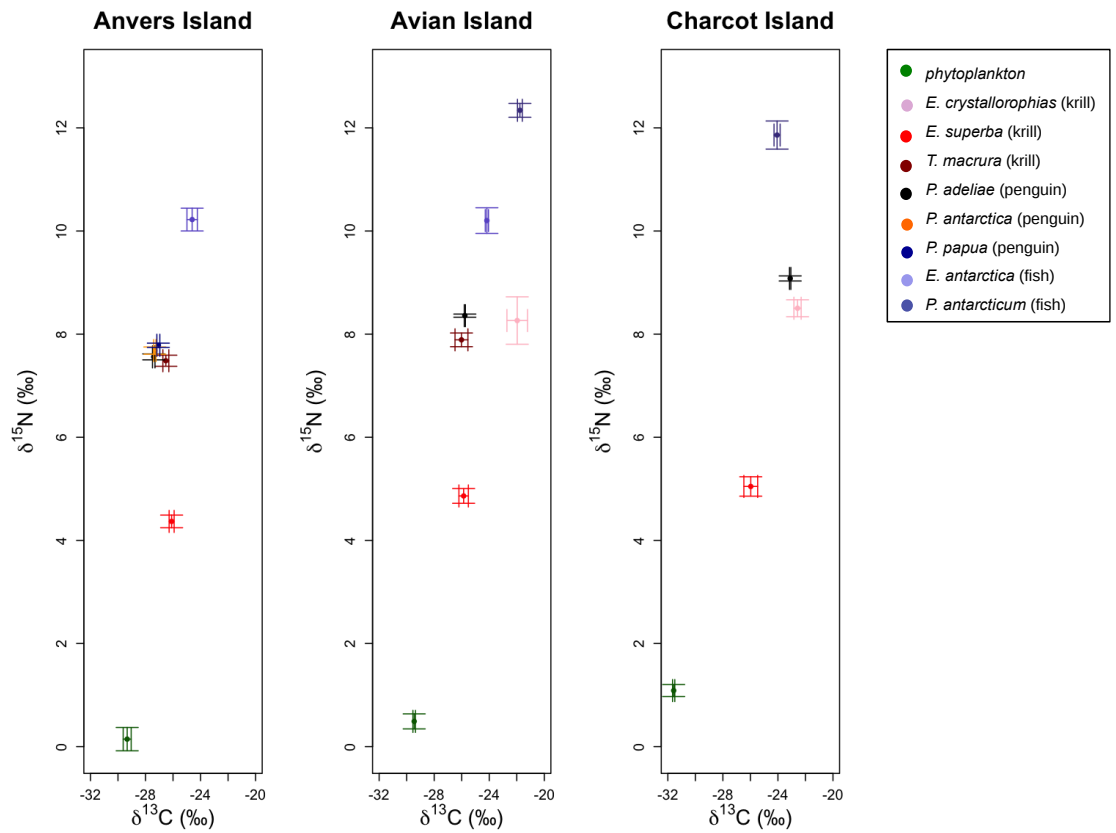

Figure 2.8. Variation in $\delta^{13}C$ and $\delta^{15}N$ stable isotope signatures of marine food-web components including *Pygoscelis* penguin chicks at a population level age of five weeks, their major prey items, and phytoplankton only. Average isotope signatures (± 1 SE) for each food-web component and rookery location are presented based on sample sizes reported in Table 1.

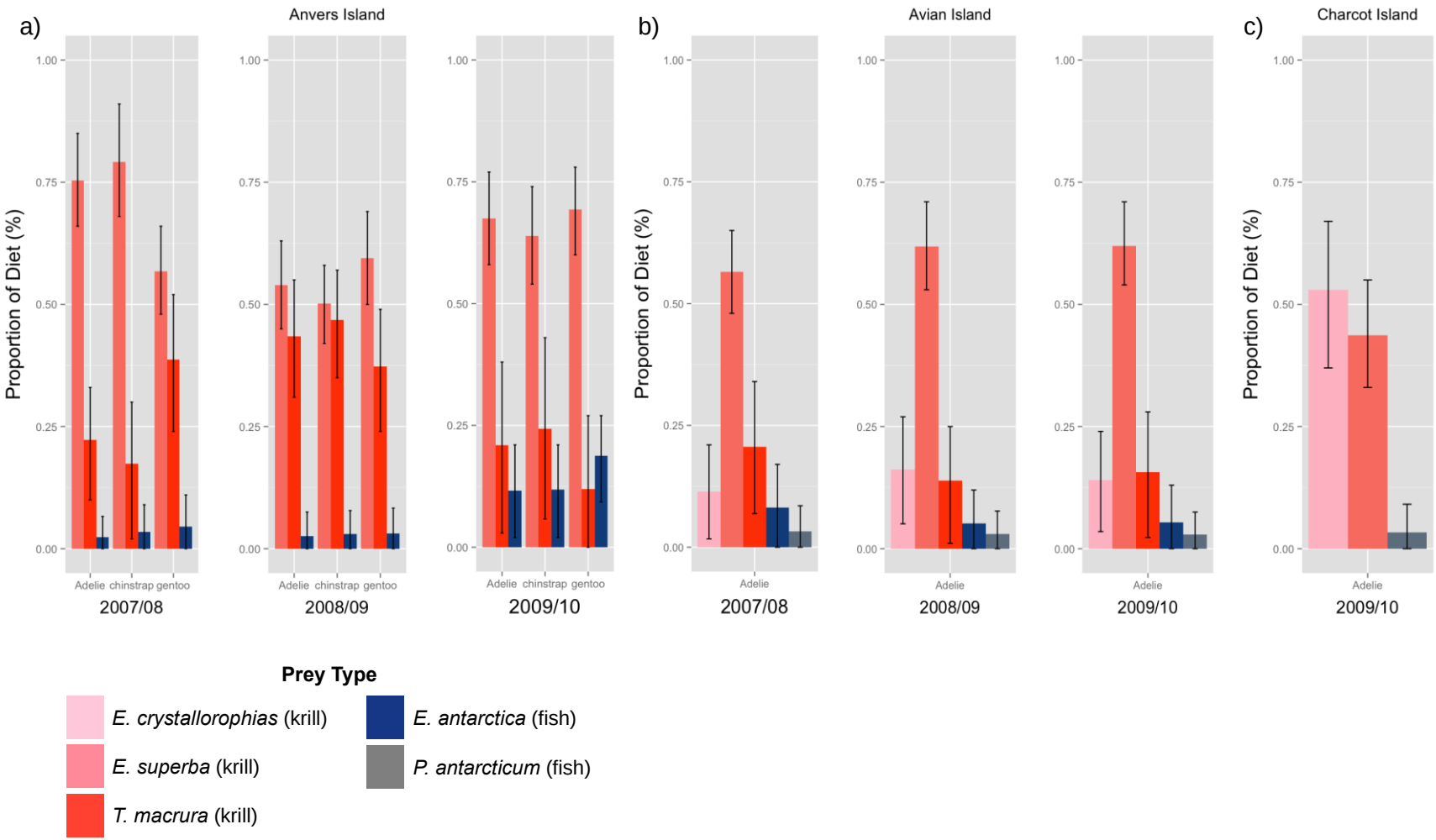
a)
Anvers Island
b)
Avian Island
c)
Charcot Island
Proportion of Diet (%)
1.00
0.75
0.50
0.25
0.00
Adelie
chinstrap
gentoo
2007/08
2008/09
2009/10
Prey Type
E. crystallorophias (krill)
E. superba (krill)
T. macrura (krill)
E. antarctica (fish)
P. antarcticum (fish)

Figure 2.9. Variation in proportion of dietary items provisioned by *Pygoscelis* penguin adults to rear (a) Adélie, chinstrap, and gentoo penguin chicks at Anvers Island, (b) Adélie penguin chicks at Avian Island, and (c) Adélie penguin chicks at Charcot Island all at a population level age of five weeks. Average dietary proportions (± 95% credibility intervals) are presented based on Bayesian isotope mixing models of penguin consumers and their prey. All prey types considered in mixing models for each rookery location are shown in results. Sample sizes exactly follow analyses previously described.

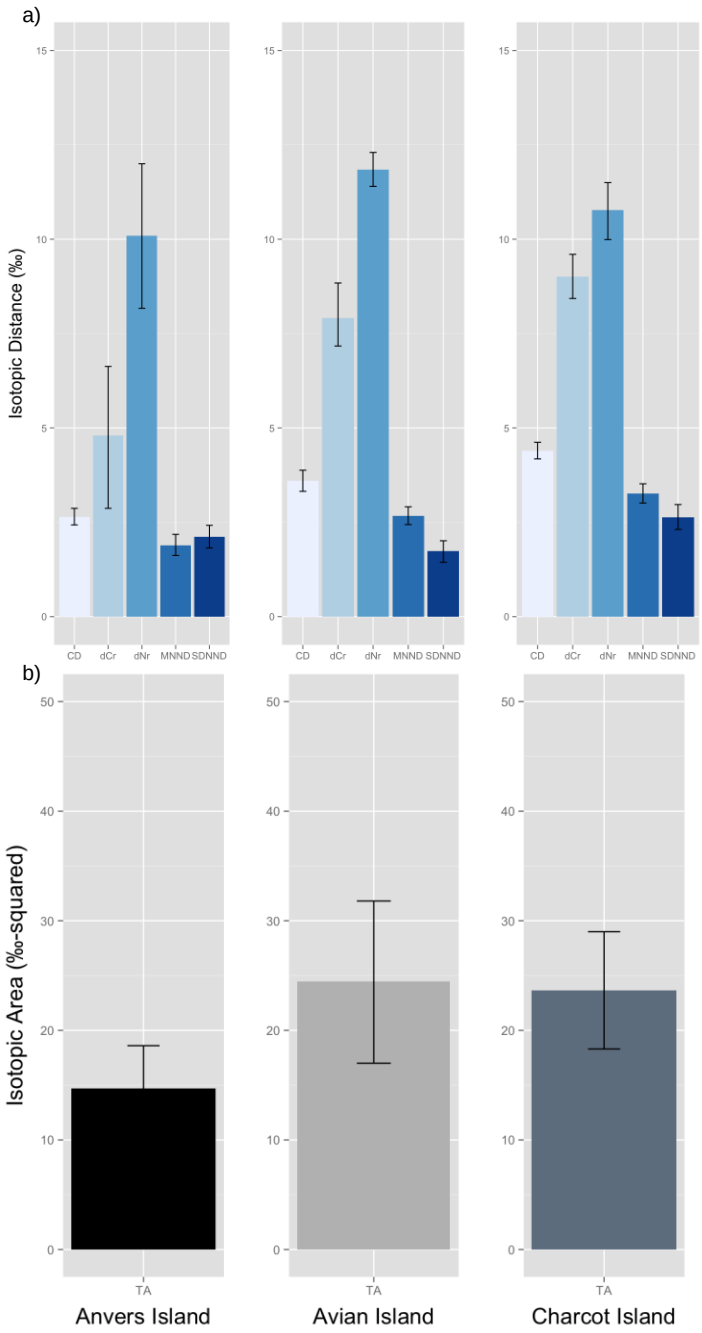

Figure 2.10. Variation in six Bayesian-derived metrics for isotopic niche structure and width of regional penguin food-webs west of the Antarctic Peninsula. (a) Isotopic distance as described by mean distance to centroid (CD), $\delta^{13}C$ range (dCr), $\delta^{15}N$ range (dNr), mean nearest neighbor distance (MNND), and standard deviation of nearest neighbor distance (SDNND). (b) Isotopic area (i.e., Total Area ‰^{2}) based on the convex hull of each regional penguin food-web.

Chapter 3.

Ecological Sexual Dimorphism and Environmental Variability within a Community of Antarctic Penguins (genus *Pygoscelis*)

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Abstract

Sexual segregation in vertebrate foraging niche is often associated with sexual size dimorphism (SSD), i.e., *ecological sexual dimorphism*. Although foraging behavior of male and female seabirds can vary markedly, differences in isotopic (carbon, $\delta^{13}C$ and nitrogen, $\delta^{15}N$) foraging niche are generally more pronounced within sexually dimorphic species and during phases when competition for food is greater. We examined ecological sexual dimorphism among sympatric nesting *Pygoscelis* penguins asking whether environmental variability is associated with differences in male and female pre-breeding foraging niche. We predicted that all *Pygoscelis* species would forage sex-specifically, and that higher quality winter habitat, i.e., higher or lower sea ice coverage for a given species, would be associated with a more similar foraging niche among the sexes.

P2/P8 primers reliably amplified DNA of all species. On average, male *Pygoscelis* penguins are structurally larger than female conspecifics. However, chinstrap penguins were more sexually dimorphic in culmen and flipper features than Adélie and gentoo penguins. Adélies and gentoos were more sexually dimorphic in body mass than chinstraps. Only male and female chinstraps and gentoos occupied separate $\delta^{15}N$ foraging niches. Strong year effects in $\delta^{15}N$ signatures were documented for all three species, however, only for Adélies, did yearly variation in $\delta^{15}N$ signatures tightly correlate with winter sea ice conditions. There was no evidence that variation in sex-specific foraging niche interacted with yearly winter habitat quality.

Chinstraps were most sexually size dimorphic followed by gentoos and Adélies. Pre-breeding sex-specific foraging niche was associated with overall SSD indices across

species; male chinstrap and gentoo penguins were enriched in $\delta^{15}N$ relative to females. Our results highlight previously unknown trophic pathways that link *Pygoscelis* penguins with variation in Southern Ocean sea ice suggesting that each sex within a species should respond similarly in pre-breeding trophic foraging to changes in future winter habitat.

Introduction

Intra-population variation in ecological niche, sensu Hutchinson (1957), is widespread in nature (Smith and Skulason 1996, Bolnick et al. 2003) however, the ecological and evolutionary causes and consequences of such individual variability remain poorly understood (Dall et al. 2012). Sexual segregation in foraging niche of vertebrates is a relatively well-studied example of intra-population ecological differences (Ruckstuhl and Neuhaus 2005) that is often considered to be mediated by sexual size dimorphism (SSD, Fairbairn 1997, Székely et al. 2007). In theory, the evolution and maintenance of SSD reflects sexual variance in the adaptive process in which fitness of adult males and females is maximized at differing body sizes (Fairbairn 1997), with major functional hypotheses including sexual and/or fecundity selection (Darwin 1871, Lande 1980, Andersson 1994). Ecological niche divergence (Slatkin 1984, Shine 1989) is an alternative hypothesis, generally considered secondary, which evolves often as a consequence of existing SSD (Andersson 1994, Fairbairn 1997), i.e., *ecological sexual dimorphism* (Dall et al. 2012).

Studies of marine birds have documented differences in foraging behavior, i.e., spatial and temporal variation in activity budgets of males and females within SSD-associated systems (e.g., Phillips et al. 2004, Weimerskirch et al. 2009). However, sexually monomorphic species also exhibit sex-specific foraging (e.g., Lewis et al. 2002, Pinet et al. 2012), raising questions about the importance of structural size in mediating sexual differences in foraging behavior, see Lewis et al. (2002) for review. Recent studies have advanced the issue using naturally occurring ratios of carbon ($^{13}C/^{12}C$, $\delta^{13}C$) and nitrogen ($^{15}N/^{14}N$, $\delta^{15}N$) stable isotope (SI) signatures to more rigorously assess whether

males and females occupy different foraging niches (Forero et al. 2005, Bearhop et al. 2006, Phillips et al. 2011) based on a time-integrated, biogeochemical parameter that specifically reflects assimilated prey (Deniro and Epstein 1978, 1981, Peterson and Fry 1987). In a recent review, Phillips et al. (2011) concluded that a) isotopic differences between male and female seabirds were extremely rare in sexually monomorphic species, strengthening the idea that sexual segregation in foraging niche is importantly mediated by SSD, and b) sex differences in SI signatures were much more common during the pre-laying and breeding season, when competition is greater due to reduced foraging ranges, than during the non-breeding season. The latter finding lends support to an important hypothesis of sexual segregation in animals: dominant individuals, i.e., larger individuals, often males, out-compete subordinate conspecifics, i.e., smaller or less experienced individuals, often females or juveniles, for high quality habitat (Gauthreaux 1978, Conradt 2005).

Pygoscelis penguins, see Baker et al. (2006) for Spheniscidae phylogeny, occurring throughout the marine ecosystem west of the Antarctic Peninsula (AP, Fig. 3.1a, b) provide a unique system for testing the hypothesis that ecological sexual dimorphism increases or decreases depending on levels of competition for nutritional resources, following Phillips et al. (2011). Changes in environmental conditions that affect the quality and/or quantity of prey might be an important driver of variation in foraging competition between males and females, however such variability has not been extensively examined in the context of ecological sexual dimorphism among penguins, but see (Dehnhard et al. 2011). We explore this issue within an interesting community context in which winter environmental conditions would be expected to result in differing

inter-specific levels of foraging competition between males and females given generally opposing life history affinities among species for Southern Ocean sea ice, a key physical parameter structuring polar marine food-webs (Smetacek and Nicol 2005, Moline et al. 2008).

Sea ice is a critical ecological feature structuring habitat use by *Pygoscelis* penguins. Adélie penguins (*P. adeliae*) are associated with pack ice habitat during winter (Ainley 2002, Clarke et al. 2003, Ballard et al. 2010, Dunn et al. 2011), while chinstrap penguins (*P. antarctica*) are noted to winter off-shore, in sea ice-free waters predominantly throughout the northwestern AP and Scotia Sea (Fraser et al. 1992, Trivelpiece et al. 2007). Gentoo penguins are noted to inhabit near shore, open water wintering areas close to breeding colonies (Tanton et al. 2004). During the summer breeding season, Adélie penguins hold a circumpolar distribution at high southern latitudes, nesting within terrestrial rookeries located in close proximity to marine foraging areas generally characterized by persistent summer sea ice (Ainley 2002). Only along the northwestern AP region do Adélie penguins breed in sympatry with their sea ice-intolerant congeners. Although Adélie penguins initiate nesting earlier here (Ducklow et al. 2007), all three species typically forage in sea ice-free waters during summer as a result of marked ocean-climate warming (Vaughan et al. 2003, Ducklow et al. 2007) and associated reductions in annual sea ice coverage (Stammerjohn et al. 2008, Stammerjohn et al. 2012), particularly within the northern seasonal sea ice zone (Jacobs and Comiso 1997), see also Chapman et al. (2010, Fig. 3.1). Adélie penguins breeding within the northwestern AP are considered mismatched with current environmental conditions given notable population declines (Ducklow et al. 2007, Forcada and Trathan 2009,

Bestelmeyer et al. 2011, Trivelpiece et al. 2011, Lynch et al. 2012). Therefore, *Pygoscelis* penguins appear to hold opposing life-history affinities for the presence of sea ice (Fraser et al. 1992, Ainley 2002, Tanton et al. 2004, Trivelpiece et al. 2007, Forcada and Trathan 2009) that have been shaped over evolutionary time scales (Baker et al. 2006). However, associated trophic pathways critical to each species, and especially each sex within species, have not been well resolved.

Here, we examined ecological sexual dimorphism among adult *Pygoscelis* penguins nesting within the northwestern AP, along the Palmer Archipelago located near Anvers Island (Fig. 3.1b, c). Specifically, we considered variation in $\delta^{13}C$ and $\delta^{15}N$ SI signatures of blood tissue, obtained during egg laying, as a biogeochemical proxy of pre-breeding trophic foraging given that these isotopes are known to reliably reflect trophic position (Kelly 2000, Inger and Bearhop 2008) by integrating dietary information over approximately the previous 30 to 60 days given allometric turn-over rates for the cellular fraction of blood (Hobson and Clark 1992, Bearhop et al. 2002, Bearhop et al. 2006, Cherel et al. 2007). We predicted that males and females of all three species should show sex-specific foraging given that male and female gentoo penguins nesting at Bird Island, South Georgia, have been shown to differ in their breeding foraging niche (Bearhop et al. 2006) and hold generally similar, moderate levels of SSD as Adélie and chinstrap penguins (Fairbairn and Shine 1993, Williams 1995). We also predicted that in years of relatively higher quality winter habitat, i.e., greater sea ice coverage for Adélie penguins and lower coverage for chinstrap and gentoo penguins, males and females would be more similar in their pre-breeding foraging niche given reduced competition for prey as a test of the conclusion by Phillips et al. (2011).

We first (1) validated molecular primers for universal sex determination of all three *Pygoscelis* species. In birds, the chromobox-helicase-DNA-binding (CHD) gene CHD-Z occurs in both males (ZZ) and females (ZW), while CHD-W only occurs in females. By amplifying primers specific to both genes from a sample of genomic DNA, gel electrophoresis is expected to reveal two bands in females and one band in males. Thus, we tested two sets of primers, P2/P8 and 2550F/2718R, for consistent amplification of CHD-Z and CHD-W regions (Griffiths et al. 1998, Fridolfsson and Ellegren 1999) using mated adults at the one-egg stage where each pair is expected to include one male and one female. Based on our molecular sexing data, we (2) quantified levels of adult SSD within each species by developing predictive models of sex based on field-measured morphometrics of breeding adults, and then tested the predictive utility of best supported morphometric models for determining sex, using an independent dataset. We (3) calculated a commonly used index of SSD following Lovich and Gibbons (1992) to quantify structural size for each species based on morphometric parameters considered in previous analyses. Lastly, we (4) asked whether SSD-associated sex (as defined previously), or structural size independent of sex, are better predictors of pre-breeding isotopic foraging niche while also assessing support for the hypothesis that yearly environmental variability, discussed within the context of winter sea ice conditions, interacts with sex- or size-specific foraging.

Materials and Methods

Ethics statement

Research was conducted in accordance with an Antarctic Conservation Act permit to WRF (2008-020), in addition to Canadian Committee on Animal Care guidelines (Simon Fraser University, SFU, Animal Care Permit 890B-08 to KBG and TDW).

Field methods

Field research was conducted on *Pygoscelis* penguins nesting on several islands within the Palmer Archipelago west of the AP near Anvers Island (64°46'S, 64°03'W, Fig. 3.1a-c), during the austral summers of 2007/08, 2008/09, and 2009/10. Specifically, study nests were located on Biscoe (64°48'S, 63°46'W), Torgersen (64°46'S, 64°04'W), and Dream (64°43'S, 64°13'W) Islands (Fig. 3.1c). Each study season, Adélie penguin study nests (n = 30) were distributed equally between the three study islands, with 10 nests located on each island. Gentoo penguin study nests (n = 30) were all located on Biscoe Island, while chinstrap penguin study nests (n = 15) were all located on Dream Island (Fig. 3.1c). The reduced sample size for chinstraps was due to the overall smaller number of individuals breeding at rookeries on Dream Island.

Each season, study nests, where pairs of adults were present, were individually marked and chosen before the onset of egg laying, and consistently monitored. When study nests were found at the one-egg stage, both adults were captured to obtain blood samples used for molecular sexing and SI analyses, and measurements of structural size and body mass. At the time of capture, each adult penguin was quickly blood sampled (~1 ml) from the brachial vein using a sterile 3 ml syringe and heparinized infusion

needle. Collected blood was stored in 1.5 ml micro-centrifuge tubes that were kept cool. In the field, a small amount of whole blood was smeared on clean filter paper stored in a 1.5 ml micro-centrifuge tube for molecular sexing. Measurements of culmen length and depth (using dial calipers ± 0.1 mm), right flipper (using a ruler ± 1 mm), and body mass (using 5 kg ± 25 g or 10 kg ± 50 g Pesola spring scales and a weigh bag) were obtained to quantify body size variation. After handling, individuals at study nests were further monitored to ensure the pair reached clutch completion, i.e., two eggs.

Sea ice data were based on satellite estimates of sea ice concentration (SIC) within the Palmer Station, Antarctica, Long-Term Ecological Research (PAL-LTER) Program's regional study grid (Figs. 3.1, 3.2a). Daily and monthly time series of SIC were used to calculate two metrics, 1-average winter sea ice area (km^2) representing the yearly average area covered by winter sea ice, and 2-duration (days) representing the yearly total length of the winter sea ice season, both within the PAL-LTER grid, using time series based on Version 2 of the Goddard Space Flight Center (GSFC) Bootstrap algorithm (Comiso 1999) provided by the National Snow and Ice Data Center (NSIDC; www.nsidc.org). These sea ice metrics were derived based on 1-monthly averages between the winter months of sea ice advance and retreat, and 2-the day of first and last appearance of sea ice within the same region and averaged for the PAL-LTER grid. Both metrics are useful for describing both the winter spatial and temporal variability of Southern Ocean sea ice (Stammerjohn et al. 2008).

Laboratory methods

Within 12 hours (hrs) of field collection, tubes containing whole blood were centrifuged to separate plasma and red blood cell (RBC) fractions, which were stored separately and frozen at -80 degrees Celsius (ºC). Tubes containing whole blood smears on filter paper were allowed to dry in a desiccator. After drying, tubes were sealed and frozen at -80 ºC.

Tubes containing RBCs were first allowed to dry to a consistent mass in a drying oven at 60 ºC. Using a mortar and pestle lined with clean weighing paper, dried RBC pellets were homogenized into a powder. Each mortar and pestle was washed and dried in between sample processing. Aliquots of powdered samples were transferred to 8 x 5 mm pressed tin capsules (Elemental Microanalysis) and weighed (~2 mg) using an analytical balance. Samples were organized in 96-microwell plates and analyzed for $\delta^{13}C$ and $\delta^{15}N$ SI signatures using an elemental analyzer interfaced with an isotope ratio mass spectrometer at the Stable Isotope Facility, University of California (UC) - Davis. Data expressed as $\delta^{13}C$ or $\delta^{15}N$ were calculated using the following equation: $\delta^{13}C$ or $\delta^{15}N = ([R_{sample}/R_{standard}]-1) \times 1000$, where R_{sample} is the ratio of the heavy to light isotope for either $^{13}C/^{12}C$ or $^{15}N/^{14}N$, and $R_{standard}$ is the heavy to light isotope ratios for international standards - Vienna PeeDee Belemnite for carbon, and atmospheric N_2 (Air) for nitrogen.

Whole blood smears were allowed to dry a second time in a desiccator for at least 24 hrs prior to analysis. Sex of adult *Pygoscelis* penguins was determined molecularly using PCR amplification as outlined by Griffiths et al. (1998), as well as Fridolfsson and Ellegren (1999). See Supporting Information Text S1 for specific details regarding PCR methods including extraction, amplification, and gel electrophoresis.

Statistical methods

Percent of mated adult pairs correctly identified by the primers P2/P8 and 2550F/2718R was calculated for a subset of individuals. Correct identification was defined as gel electrophoresis revealing one female and one male, per pair. DNA from five adult pairs per species (n = 30 individuals total) for each primer set was used in the analysis.

Final sample sizes for study nests / individual adults of each species were n = 76 / 152 for Adélie, n = 34 / 68 for chinstrap, and n = 62 / 124 for gentoo penguins over the three years of study. These sample sizes are reduced in comparison with the original number of study nests marked and monitored per species as at times weather conditions hindered rookery access resulting in some study nests not being sampled if the pair had already reached clutch completion. In addition, of those adults sampled, some pairs were excluded from statistical analyses because a final egg was never observed at the nest, likely due to brown skua (*Stercorarius lonnbergi*) depredation. Therefore, it was unknown whether these individuals represented adults exactly at the one-egg stage. All statistical analyses were performed in the R language environment (R Core Team 2012).

To assess the explanatory value of various structural parameters for discerning sex of adult penguins, logistic regression was employed using generalized linear models (GLM, family = binomial) to account for a response where males were coded as 0 and females coded as 1, in relation to four continuous structural size parameters including 1-culmen depth, 2-culmen length, 3-flipper length, and 4-body mass. Information-theoretic methods, performed in R, were used to direct model selection and parameter estimation following Burnham and Anderson (2002). An a priori set of 15 candidate models consisted of an equal-means (null) model and all combinations of culmen depth and

length, flipper length, and body mass as main effects (14 models). This same candidate model set was evaluated for all three species, separately, using a truncated dataset (2/3^{rds}) of randomly chosen individuals (Adélie n = 88, chinstrap n = 36, gentoo n = 74). For each species' analysis, we first assessed overdispersion ($\hat{c}$) in the most parameterized model using the following equation: $\hat{c}$ = residual deviance / residual degrees of freedom, see Burnham and Anderson (2002, p. 68). In the event $\hat{c}$ was found to be greater than 1, quasi Akaike's Information Criterion (QAICc) values, which include a correction for small sample size, would be calculated for each model to correct for overdispersion. Where $\hat{c}$ was found to be less than 1, Akaike's Information Criterion (AICc) values, again corrected for small sample size, would be calculated for each model. In addition, ΔAICc and Akaike weight (w) values were calculated and used to compare candidate models (Burnham and Anderson 2002). For each candidate model, a pseudo r^2mf (McFadden) value was calculated to provide a measure of fit for each model, where r^2mf = 1-(logLikelihood(model)/logLikelihood(null model)) (Hosmer and Lemeshow 2000). Inference was based on the relative support for parameters across all models and weighted parameter estimates. Parameter estimation included calculation of model-averaged parameter estimates based on w values for all candidate models. Standard errors (SE) for parameter estimates were based on unconditional variances calculated across the same models. Parameter likelihood values were evaluated by summing w values across all models that included each parameter under consideration (Burnham and Anderson 2002).

To assess the predictive utility of these same structural measurements to determine sex of adult penguins, we calculated the probability of individuals being

female (1), using the *predict* function in R, with a dataset (1/3rd) comprised of individuals (Adélie n = 44, chinstrap n = 18, gentoo n = 38) not included in developing most parsimonious structural size models as described above. Models used to predict sex were those best supported, in analyses described above, which received ΔAICc values ≤ 2.

To quantify levels of SSD in adults of each species, we calculated a standard index of size dimorphism (SDI) following Lovich and Gibbons (1992), where SDI = ((mean size of larger sex / mean size of smaller sex) - 1) with the resulting value arbitrarily defined as positive when females are larger and negative when males are larger (Adélie n = 137, chinstrap n = 54, gentoo n = 116). An SDI was calculated for all structural parameters considered as best predictors of adult penguin sex for each species. In addition, two overall average SDIs were calculated based on SDIs of 1-all four structural parameters, and 2-culmen depth, length and flipper length SDIs only.

Least-squares general linear models (LM) were used to examine continuous variation in $\delta^{13}C$ and $\delta^{15}N$ SI signatures of adult penguin RBCs in relation to three parameters treated as main effects including 1-sex, as determined by molecular data and treated categorically, 2-overall size using a principal components score (PC1) based on culmen depth and length, and flipper length and treated continuously, and 3-year, treated categorically. Individual scores for PC1 were calculated using the *prcomp* function in R for datasets consisting of both males and females, but calculated separately for each species. An a priori set of eight candidate models consisted of an equal-means model, each predictor variable as a main effect (three models), additive models for sex or size with year, defined so that sex and size were never included in the same model (two models), and interaction models for these same additive models where an interaction was

included for each parameter considered as a main effect in the model (two models). This same candidate model set was evaluated for each isotope separately using datasets for each species (Adélie n = 127 for $\delta^{13}C$ and n = 128 for $\delta^{15}N$ due to the exclusion of one data point as a $\delta^{13}C$ outlier based on residual plots for normality, chinstrap n = 53, gentoo n = 115). For each candidate model, AICc, ΔAICc and w values were calculated and used to compare models (Burnham and Anderson 2002), as well as an r^2 value, defined as the fraction of the total variance explained by the model and given by the Multiple R-squared calculation in R, see Crawley (2007, p. 399), to provide a general measure of fit for each model. Inference was based on model averaging with parameter estimation following that described above for logistic regression analyses.

Data management

Data reported here are publicly available within the PAL-LTER data system (datasets #219, 220, and 221): http://oceaninformatics.ucsd.edu/datazoo/data/pallter/datasets. These data are additionally archived within the United States (US) LTER Network's Information System Data Portal: https://portal.lternet.edu/. Individuals interested in using these data are therefore expected to follow the US LTER Network's Data Access Policy, Requirements and Use Agreement: http://www.lternet.edu/policies/data-access.

Results

Primer validation

P2/P8 primers correctly identified 100% of each species' pairs, while 2550F/2718R primers correctly identified 60% of Adélie and gentoo penguin pairs and 0% of chinstrap penguin pairs. See Supporting Information Figure S1 for an image of PCR bands from both primer sets and Supporting Information Text S2 for details on PCR optimization with P2/P8 primers. Of the entire dataset for each species, P2/P8 primers failed to amplify in one of 147 adult Adélie penguins and four of 123 adult gentoo penguins. P2/P8 primers amplified in all 68 adult chinstrap penguins.

Quantifying SSD

Overdispersion ($\hat{c}$) was found to be less than 1 for each species analysis (Adélie $\hat{c} = 0.37$, chinstrap $\hat{c} = 0.44$, gentoo $\hat{c} = 0.23$), precluding the need to calculate QAICc values for each model. For Adélie penguins, three models received ΔAICc values ≤ 2 for predicting sex from structural parameters. The most parsimonious model received a slightly higher *w* value (0.39) than the second and third best supported model, which received similar values for *w* (0.31 and 0.30, respectively). All supported models received high r^2mf values (> 70%) with the best supported model including terms for culmen length and depth, as well as body mass. The second supported model included only terms for culmen length and body mass. However, the global model including all parameters was the third best supported model (Table 3.1). Parameter likelihood values indicated very strong support for culmen length and body mass, moderately strong support for culmen depth,

but much lower support for flipper length given the data and candidate model set (Table 3.2).

For chinstrap penguins, one model received ΔAICc values ≤ 2. The most parsimonious model received a higher *w* value (0.37) than the second ranked model (*w* = 0.12), a high r^2mf value (70%), and included terms for culmen length and depth (Table 3.1). Parameter likelihood values indicated strong support for culmen length and depth, but weaker support for flipper length and body mass given the data and candidate model set (Table 3.2).

For gentoo penguins, one model received ΔAICc values ≤ 2. The most parsimonious model received a higher *w* value than the next best supported model (0.53 and 0.17, respectively) and a high r^2mf value (85%). The best supported model included terms for culmen length and depth, and body mass (Table 3.1). Parameter likelihood values indicated very strong support for culmen depth and body mass, and moderately strong support for culmen length, but only weak support for flipper length given the data and candidate model set (Table 3.2).

Probability estimates of adult Adélie penguin sex, based on models receiving ΔAICc values ≤ 2 as reported above and tested on independent datasets, suggested that when using 0.50 as a probability threshold (i.e., >0.50 = female, <0.50 = male), all supported models accurately predicted sex for 39 out of 44 individuals (88.64%, Table 3.1). For chinstrap penguins, the most parsimonious model accurately classified 94.44% individuals (Table 3.1). For gentoo penguins, the most parsimonious model accurately predicted sex for 34 out of 38 individuals (89.47%, Table 3.1). See Supporting Information Text S3 for details on probability estimates and misclassification.

As expected, *Pygoscelis* penguin SDIs were larger for morphological parameters that better predicted sex of adult penguins (Table 3.2). Negative SDI values indicate that for each structural parameter male *Pygoscelis* penguins were, on average, larger than females.

Environmental variability and foraging niche

Sea ice metrics indicated that the austral winters preceding each study year were generally characterized by lower than average sea ice coverage, with winter 2008 being the lowest sea ice season in the PAL-LTER record since 1979 (Stammerjohn et al. 2008), see also sea ice datasets within the PAL-LTER data system. Relative yearly variability in sea ice (Fig. 3.2b) over the course of the study suggested that 2007 was an intermediate sea ice season relative to 2008 (low) and 2009 (high). Results for inter-annual variability in foraging niche of *Pygoscelis* penguins are discussed within the context of these qualitative differences in each year's sea ice season.

Scores for PC1 explained 56%, 71% and 78% of the total variance in body size for Adélie, chinstrap and gentoo penguins, respectively. Adélie penguin analyses resulted in two models receiving ΔAICc values ≤ 2 for predicting variation in $\delta^{13}C$ SI signatures. The most parsimonious model, which included the year term only, received a considerably higher *w* value (0.53) than that for the next best supported model (*w* = 0.21), which included terms for sex and year. Both models had high r^2 values (0.71) (Table 3.3). Parameter likelihoods indicated strong support for the year term, but only weak support for the sex term given the data and the candidate model set (Table 3.4, Fig. 3.3). PC1 and the sex term received similar parameter likelihoods as the model including both PC1 and

Year was the third ranked model. However, both models with either sex or PC1 terms only were ranked lower than the equal-means model, suggesting that these parameters provided little explanatory value. No other explanatory variables, such as any of the interaction terms, were strongly supported by the data (Table 3.4). Inter-annual variability in $\delta^{13}C$ SI signatures of Adélie penguins did not associate with qualitative variation in winter sea ice conditions given that weighted parameter estimates for both 2008 (-1.17 ± 0.14 95% confidence interval, CI = parameter estimate SE*1.96) and 2009 (-0.94 ± 0.14 CI), were depleted in years with the lowest and highest sea ice conditions during the study (Table 3.4, Fig. 3.2b). Two models also received ΔAICc values ≤ 2 for predicting variation in Adélie penguin $\delta^{15}N$ SI signatures. The most parsimonious model, which included terms for sex and year, received a higher *w* value (0.53) then that for the next best supported model (*w* = 0.25), which included these same parameters as main effects and an interaction for sex*year. Both models had similar r^2 values (0.30 and 0.32, respectively) (Table 3.3). Parameter likelihoods suggested stronger support for sex and year terms, however, the interaction term for sex*year received less support given the data and the candidate model set (Table 3.4, Fig. 3.3). Weighted parameter estimates for the year term indicated that Adélie penguin $\delta^{15}N$ SI signatures were depleted in 2008 (-0.43 ± 0.21 CI) relative to higher sea ice years in 2007 and 2009 (0.02 ± 0.19 CI; Table 3.4, Fig. 3.3). Weighted parameter estimates suggested that female Adélie penguin $\delta^{15}N$ SI signatures were not depleted relative to males (-0.08 ± 0.17 CI). Confidence intervals overlapped 0 indicating that sex was not important in accounting for variation in Adélie $\delta^{15}N$ SI signatures (Table, 3.4, Fig. 3.3). Weighted parameter estimates for the interaction between sex*year suggested a possibly larger effect in 2008, the year with low sea ice (-

0.07 ± 0.13 CI), than in 2009 (-0.03 ± 0.09 CI), however confidence intervals for both years overlapped 0, indicating that this interaction also was not important in accounting for variation in Adélie δ^{15}N SI signatures (Table, 3.4, Fig. 3.3).

Chinstrap penguin analyses resulted in four models receiving ΔAICc values ≤ 2 for predicting variation in δ^{13}C SI signatures, however, the best supported model was the equal-means model (Table 3.3). Parameter likelihoods indicated little support for all explanatory parameters including interaction terms (Table 3.4). Variation in chinstrap δ^{13}C SI signatures was not qualitatively associated with winter sea ice conditions (Fig. 3.4). One model received ΔAICc values ≤ 2 for predicting variation in chinstrap penguin δ^{15}N SI signatures, which included terms for sex and year as main effects only. This model received a high *w* value (0.88) and a moderately high r^2 value (0.53) (Table 3.3). Accordingly, sex and year terms received strong support based on parameter likelihoods, while all other parameters received essentially no support given the data and candidate model set (Table 3.4, Fig. 3.4). Weighted parameter estimates for the year term indicated that chinstrap penguin δ^{15}N SI signatures were enriched in 2008 (0.38 ± 0.19 CI) and 2009 (0.57 ± 0.17 CI) suggesting no obvious relationship with variation in winter sea ice conditions (Table 3.4, Fig. 3.4). Weighted parameter estimates for the sex term indicated that female chinstrap penguins (-0.22 ± 0.15 CI) were depleted in δ^{15}N relative to males (Table 3.4, Fig. 3.4).

Gentoo penguin analyses resulted in three models receiving ΔAICc values ≤ 2 for predicting variation in δ^{13}C SI signatures. The most parsimonious model included terms for sex and year, which received a higher *w* value (0.43) then the next best supported

model that included terms for PC1 and year (w = 0.27). However, both models had very high r^2 values (0.94). The third ranked model included the year term only, and received a low w value (0.19), but also a very high r^2 value (0.94, Table 3.3). Parameter likelihoods indicated strong support for the year term, and moderate support for the sex term given the data and the candidate model set (Table 3.4, Fig. 3.5). The PC1 term received a slightly lower parameter likelihood than the sex term as the model including both PC1 and year was the second ranked model. However, both models with either sex or PC1 terms only were ranked lower than the equal-means model, suggesting that these parameters provided little explanatory value. No other explanatory variables, such as any of the interaction terms, were strongly supported by the data (Table 3.4). Weighted parameter estimates for the year term indicated that gentoo penguin δ^{13}C SI signatures were depleted in 2008 (-1.33 ± 0.07 CI) and 2009 (-0.72 ± 0.07 CI), the years with lowest and highest sea ice conditions, respectively (Table 3.4, Fig. 3.5). Weighted parameter estimates for sex (-0.03 ± 0.04 CI) and PC1 score (0.004 ± 0.009 CI) both overlapped 0 indicating these variables were not important in explaining variation in δ^{13}C SI signatures of gentoo penguins (Table 3.4, Fig. 3.5). Two models received ΔAICc values ≤ 2 for predicting variation in gentoo penguin δ^{15}N SI signatures. The most parsimonious model, which included terms for sex and year, received a higher w value (0.58) than that for the next best supported model (w = 0.30), which included these same parameters as main effects and an interaction for sex*year. Both models had similar r^2 values (0.50 and 0.51, respectively) (Table 3.3). Parameter likelihoods suggested stronger support for sex and year terms, however, the interaction term for sex*year received less support given the data and the candidate model set (Table 3.4, Fig. 3.5). Weighted parameter estimates for

the year term indicated that gentoo penguin $\delta^{15}N$ SI signatures were most enriched in 2008 (0.43 ± 0.12 CI) and slightly less enriched in 2009 (0.30 ± 0.10 CI), years with the greatest difference in sea ice conditions (Table 3.4, Fig. 3.5). Weighted parameter estimates for the sex term indicated that gentoo penguin $\delta^{15}N$ SI signatures were depleted in females (-0.11 ± 0.09 CI) relative to males (Table 3.4, Fig. 3.5). Lastly, weighted parameter estimates for the interaction between sex*year (2008: 0.04 ± 0.08 CI, 2009: 0.01 ± 0.06 CI) suggested that this interaction was not important in accounting for variation in gentoo $\delta^{15}N$ SI signatures as confidence intervals for both years overlapped 0 (Table 3.4, Fig. 3.5).

Discussion

Our primer validation adds to a growing body of literature that has used molecular techniques for gender determination of penguins (Bertellotti et al. 2002, Fargallo et al. 2004, Costantini et al. 2008, Hart et al. 2009, Reis et al. 2011, Polito et al. 2012), however, much of this work was aimed at developing morphometric methodologies for sex determination. We used molecular sexing data to quantify SSD specifically among *Pygoscelis* penguins nesting along the Palmer Archipelago given that structural size of penguins can vary regionally (Williams 1995), and therefore, SSD indices developed from other populations may not be readily applicable to our own study system. We further demonstrated that overall larger size dimorphism indices between species were associated with greater differences in sex-specific pre-breeding foraging niche based on $\delta^{15}N$ SI signatures of RBCs. However, there was no strong evidence for the hypothesis

that ecological sexual dimorphism increased, or decreased, in association with higher or lower quality winter habitat that might induce variation in foraging competition between males and females.

The molecular primers P2/P8 were more reliable genetic markers for determining sex of all three *Pygoscelis* species than 2550F/2718R primers; P2/P8 successfully amplified in 99.3% of Adélie penguins, 100% of chinstrap penguins, and 96.8% of gentoo penguins sampled from our study population. Hart et al. (2009) showed P2/P8 primers to have a much higher rate of successful amplification than 2550/2718 primers in macaroni penguins (*Eudyptes chrysolophus*). A recent paper by Polito et al. (2012) used P0/P2/P8 primers to successfully sex 94% of *Pygoscelis* penguins, i.e., 97 of 103 individuals tested, nesting at Admiralty Bay, King George Island, Antarctica. Our slightly higher success rate of P2/P8 amplification in *Pygoscelis* penguins may be due to either a) differing quality of genomic material offered by whole blood versus tissue from the feather calamus, or b) the different thermal profiles used by each study, compared to Polito et al. (2012).

Estimates of penguin SSD have generally relied on one or only a few morphological features. Williams (1990) used bill length and depth to assess structural size differences between male and female gentoo penguins. Fairbairn and Shine (1993) relied on published estimates of body mass for 17 species of Spheniscidae in their meta-analysis of seabird SSD, while Polito et al. (2012) considered measurements of bill features only for developing SSD indices for *Pygoscelis* penguins. The evolution of SSD is primarily considered a result of sexual and/or fecundity selection (Fairbairn 1997), therefore, it is highly likely that several traits are under selection that result in overall

body size differences between the sexes. To this end, we considered a suite of morphological features in our analyses similar to Bertellotti et al. (2002), to more broadly assess SSD in *Pygoscelis* penguins. We recognize that body mass is a plastic trait that can vary over the annual cycle, therefore, our body mass results are truly only relevant for individuals during the egg laying period and should not be considered representative of individuals during other seasonal phases such as chick rearing or outside the breeding season.

Our results demonstrate inter-specific differences in best morphological predictors of sex among *Pygoscelis* penguins. Adélie penguin body mass and culmen length were the strongest predictors of sex, while body mass and culmen depth were best predictors of male and female gentoo penguins. For chinstrap penguins, body mass was the least predictive structural feature, while culmen length and depth were similarly strong predictors of sex. Species-specific models based on these best morphological predictors correctly classified a high percentage of individuals from independent datasets (i.e., 89-94%). Interestingly, flipper length was not a strong predictor of sex for any of the three species. Culmen features and body mass are structures important during penguin courtship (Williams 1995), and therefore, likely targets of sexual selection, which may be why these parameters are strong predictors of sex across *Pygoscelis* species. Similar results were found by Bertellotti et al. (2002) who included bill depth and length, but not flipper length, of Magellanic penguins (*Spheniscus magellanicus*) in a discriminant function that correctly classified 97% of adults.

Variation in our calculated size dimorphism indices generally reflected differences in best morphological predictors of adult penguin sex discussed above, which

would be expected. Size dimorphism indices for Adélie penguin body mass and culmen length were the largest of those calculated for the species. Similarly, SDIs for gentoo penguin body mass and culmen depth were the largest calculated. Culmen length and depth of chinstrap penguins were equally strong predictors of sex, but associated SDIs were similar to the index for body mass, which was the least predictive structural feature for male and female chinstrap penguins. The overall average SDI, based on all four parameters, indicated that Adélie and gentoo penguins hold the same levels of SSD, while chinstrap penguins are slightly less dimorphic. However, when excluding body mass from these calculations by considering only culmen and flipper features, the overall average SDI suggested that chinstrap penguins were most sexually dimorphic, followed by gentoo penguins, with Adélie penguins being the least dimorphic of all three species. Body mass variation is closely coupled with the seasonal energetic requirements of migration, reproduction, and molt, therefore, the overall average SDI calculated that excludes body mass is a more robust estimate of SSD that is relevant throughout the annual cycle. Within this context, although based on a different SDI, our results corroborate those by Polito et al. (2012) suggesting that chinstrap penguins are the most sexually size dimorphic *Pygoscelis* species based on bill features only.

Ecological niche divergence by the sexes is thought to evolve often as a consequence of existing SSD (Fairbairn 1997), which might facilitate sexual segregation particularly when resources are limited (Conradt 2005, Phillips et al. 2011). Within this context, and following our more conservative and robust overall average SDI, we would expect chinstrap penguins to show the greatest difference, and Adélie penguins the least difference, in sex-specific foraging niche. Our trophic foraging analyses, based on $\delta^{15}N$

signatures of penguin RBCs follow this prediction. Male and female chinstrap penguins showed the greatest difference in pre-breeding trophic niche (~ 0.2‰), male and female gentoo penguins foraged at a slightly more similar trophic niche (~ 0.1‰), while there was no difference in pre-breeding trophic niche of male and female Adélie penguins. Signatures of $\delta^{15}N$ were depleted in female chinstrap and gentoo penguins in comparison with their male counterparts, indicating that females foraged at a slightly lower trophic level during the 30 - 60 days prior to egg laying. Female chinstrap and gentoo penguins were only very slightly depleted in $\delta^{15}N$, which may reflect females relying on a slightly higher percentage of certain prey items such as Antarctic krill (*Euphausia superba*) that are relatively depleted in $\delta^{15}N$ in comparison with many Antarctic fishes (Polito et al. 2011b) and even other species of Southern Ocean krill (K. B. Gorman this thesis Chapter 2). In terms of $\delta^{13}C$ foraging niche, there was no evidence that males and females foraged differently as the only parameter that accounted for variation in $\delta^{13}C$ signatures of penguin RBCs at the one-egg stage was the year term, which accounted for a high percentage of variation in Adélie and gentoo penguins only. Studies examining isotopic differences between male and female penguins is a growing body of literature (Forero et al. 2002, Bearhop et al. 2006, Cherel et al. 2007, Bost et al. 2009, Dehnhard et al. 2011, Thiebot et al. 2011, 2012). Dehnhard et al. (2011) sampled southern rockhopper penguins (*Eudyptes chrysocome chrysocome*) during a similar time period to capture pre-breeding foraging niche as in our study, in addition to work on macaroni penguins (Cherel et al. 2007, Bost et al. 2009, Thiebot et al. 2011) as well as eastern (*E. filholi*) and northern (*E. moseleyi*) rockhopper penguins (Thiebot et al. 2012). Studies have shown male Magellanic, gentoo, and southern rockhopper penguins to be enriched in $\delta^{15}N$ relative to

female conspecifics, similar to our own results. One other study examined pre-breeding foraging niche of western AP Adélie and gentoo penguins, however, the study was based on isotopic signatures of eggshells, and therefore, could not address differences between males and females of these species (Polito et al. 2011a).

Our study revealed important variation in pre-breeding $\delta^{15}N$ foraging niche across years, both within and among species. However, we did not detect strong support for the hypothesis that sex-specific trophic foraging interacted with yearly environmental conditions. Pre-breeding trophic foraging by male and female Adélie penguins was similarly enriched in years of relatively higher sea ice conditions, and relatively depleted in 2008, the year characterized by the lowest sea ice conditions. This result suggests that Adélie penguins were foraging at a lower trophic level, possibly including a slightly higher percentage of prey items that are relatively depleted in $\delta^{15}N$ such as Antarctic krill (Polito et al. 2011b, K. B. Gorman this thesis Chapter 2), in association with lower winter average sea ice conditions. This result supports the idea that Adélie penguins inhabiting the western AP region of Antarctica are truly sea ice obligate, and from the perspective of foraging, are relying on prey items linked to the seasonal variability of winter sea ice. Conversely, in the first two years of study, gentoo penguins were relatively more enriched in $\delta^{15}N$ following the 2008 winter of extremely low average winter sea ice, which suggests their pre-breeding trophic response to winter environmental variability is opposite that of Adélie penguins. However, although gentoo penguins were relatively depleted in the third year of the study characterized by the highest average sea ice conditions, individuals were not as greatly depleted as in year one, suggesting that their pre-breeding trophic foraging response was not as tightly coupled across years to the

seasonal variability of winter sea ice as was detected for Adélie penguins. Even more interesting, yearly variability in chinstrap penguin pre-breeding $\delta^{15}N$ foraging niche appeared entirely independent of winter sea ice conditions as males and females were increasingly enriched in $\delta^{15}N$ across years. *Pygoscelis* penguins within our study population varied in their nest initiation dates both among species and years during the course of our study (see Supporting Information Table S1). This aspect of *Pygoscelis* penguin breeding ecology suggests that variation in pre-breeding $\delta^{15}N$ foraging niche may have reflected, to some degree, the various conditions experienced during the differing time periods at which the 30 – 60 days prior to breeding occurred across species and years. However, the fact that each species winters and subsequently migrates to breeding rookeries from disparate areas along the western AP is likely a stronger determinant of variation in pre-breeding $\delta^{15}N$ foraging niche than temporal variation in arrival and timing of nest initiation.

We have demonstrated key trophic pathways linking *Pygoscelis* penguins with environmental variability in the form of winter sea ice where Adélie penguin trophic foraging is most tightly coupled, and that of chinstrap penguins least tightly coupled, with winter environmental conditions likely reflecting the affinity each species holds during late winter and spring for sea ice habitats (Fraser et al. 1992, Williams 1995, Ainley 2002, Tanton et al. 2004, Trivelpiece et al. 2007). Furthermore, pre-breeding trophic foraging by Adélie and gentoo penguins appears generally divergent in response to winter environmental variability, which is interesting within the context that these two species have shown the greatest breeding population change, characterized by opposing trajectories, among our study populations of the Palmer Archipelago (Ducklow et al.

2007), see also Ducklow et al. (2013, Fig. 7). Although male and female chinstrap and gentoo penguins were sexually segregated in their pre-breeding trophic foraging niche, the absolute difference was not large enough to represent an entire trophic level, i.e., 3-4‰ for $\delta^{15}N$ (Deniro and Epstein 1981, Peterson and Fry 1987), suggesting that males and females within a species should respond similarly in terms of trophic foraging to changes in future winter environmental conditions.

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Table 3.1. Candidate models for predicting sex of *Pygoscelis* penguins. Models presented are those determined to be most parsimonious, as well as all models receiving ΔAICc values ≤ 2. Percent of individuals from independent datasets correctly classified for sex based on probability estimates using supported models.

Species	Response variable	Model number	Explanatory variable	Number of parameters	ΔAICc	*w*	r^2mf	% correctly classified
Adélie penguin	Sex	1	Culmen length + Culmen depth + Body mass	4	0.000	0.392	0.73	88.64%
		2	Culmen length + Body mass	3	0.480	0.308	0.71	88.64%
		3	Culmen length + Culmen depth + Flipper length + Body mass	5	0.539	0.299	0.75	88.64%
Chinstrap penguin	Sex	1	Culmen length + Culmen depth	3	0.000	0.37	0.70	94.44%
Gentoo penguin	Sex	1	Culmen length + Culmen depth + Body mass	4	0.000	0.528	0.85	89.47%

Abbreviations: ΔAICc = Akaike's Information Criterion corrected for small sample size, *w* = Akaike weight, r^2mf = pseudo r^2 McFadden.

Table 3.2. Parameter estimates and likelihoods from candidate models for predicting sex of *Pygoscelis* penguins. Parameter estimates (±1 SE) are weighted averages, and standard errors are based on unconditional variances. Parameter likelihoods are Akaike weight (*w*) values summed across all models that include the variable. Size dimorphism indices are also reported for each structural parameter.

Species	Response variable	Explanatory variable	Parameter likelihood	Parameter estimate (±1 SE)	Size Dimorphism Index
Adélie penguin	Sex	Intercept	1.000	77.00 ± 23.38	
		Culmen length	0.999	-1.04 ± 0.34	-0.09
		Culmen depth	0.691	-0.58 ± 0.41	-0.08
		Flipper length	0.299	0.03 ± 0.03	-0.02
		Body mass	0.999	-0.009 ± 0.003	-0.20
					AVE -0.10 (Overall)
					AVE -0.06 (-Body mass)
Chinstrap penguin	Sex	Intercept	1.000	88.86 ± 37.16	
		Culmen length	0.816	-0.687 ± 0.37	-0.11
		Culmen depth	0.841	-2.01 ± 1.07	-0.10
		Flipper length	0.407	-0.12 ± 0.13	-0.05
		Body mass	0.328	0.002 ± 0.002	-0.10
					AVE -0.09 (Overall)
					AVE -0.09 (-Body mass)
Gentoo penguin	Sex	Intercept	1.000	138.78 ± 57.35	
		Culmen length	0.739	-0.72 ± 0.46	-0.08
		Culmen depth	0.954	-3.05 ± 1.50	-0.10
		Flipper length	0.299	-0.02 ± 0.08	-0.04
		Body mass	0.999	-0.01 ± 0.004	-0.17
					AVE -0.10 (Overall)
					AVE -0.07 (-Body mass)

Abbreviations: ± 1SE = plus or minus 1 standard error, AVE = average.

Table 3.3. Candidate models for describing variation in foraging niche of *Pygoscelis* penguins assessed by $\delta^{13}C$ and $\delta^{15}N$ stable isotope signatures. Models presented are those determined to be most parsimonious, as well as all models receiving ΔAICc values ≤ 2.

Species	Response variable	Model number	Explanatory variable	Number of parameters	ΔAICc	*w*	r^2
Adélie penguin	$\delta^{13}C$	1	Year	4	0.000	0.530	0.71
		2	Sex + Year	5	1.858	0.209	0.71
Chinstrap penguin	$\delta^{13}C$	1	Equal-means model	2	0.000	0.286	0.00
		2	Year	4	0.484	0.225	0.08
		3	PC1	3	1.379	0.144	0.02
		4	Sex	3	1.553	0.132	0.01
Gentoo penguin	$\delta^{13}C$	1	Sex + Year	5	0.000	0.428	0.94
		2	PC1 + Year	5	0.903	0.272	0.94
		3	Year	4	1.665	0.186	0.94
Adélie penguin	$\delta^{15}N$	1	Sex + Year	5	0.000	0.528	0.30
		2	Sex + Year + Sex*Year	7	1.489	0.251	0.32
Chinstrap penguin	$\delta^{15}N$		Sex + Year	5	0.000	0.884	0.53
Gentoo penguin	$\delta^{15}N$	1	Sex + Year	5	0.000	0.578	0.50
		2	Sex + Year + Sex*Year	7	1.346	0.295	0.51

Abbreviations: ΔAICc = Akaike's Information Criterion corrected for small sample size, *w* = Akaike weight.

Table 3.4. Parameter estimates and likelihoods from candidate models for describing variation in foraging niche of *Pygoscelis* penguins assessed by $\delta^{13}C$ and $\delta^{15}N$ stable isotope signatures. Parameter estimates (±1 SE) are weighted averages, and standard errors are based on unconditional variances. Parameter likelihoods are Akaike weight (*w*) values summed across all models that include the variable.

Species	Response variable	Explanatory variable	Parameter likelihood		Parameter estimate ±1 SE	
			$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
Adélie penguin	$\delta^{13}C$ or $\delta^{15}N$	Intercept	1.000	1.000	-25.05 ± 0.06	9.06 ± 0.08
		Sex	0.245	0.778	-0.008 ± 0.02	-0.08 ± 0.09
		PC1	0.227	0.077	0.0005 ± 0.006	-0.002 ± 0.004
		Year 2008	0.999	0.999	-1.17 ± 0.07	-0.43 ± 0.11
		Year 2009	0.999	0.999	-0.95 ± 0.07	0.02 ± 0.01
		Sex*Year 2008	0.037	0.251	-0.002 ± 0.006	-0.07 ± 0.07
		Sex*Year 2009	0.037	0.251	0.003 ± 0.006	-0.03 ± 0.05
		PC1*Year 2008	0.048	0.027	0.0006 ± 0.003	0.003 ± 0.003
		PC1*Year 2009	0.048	0.027	-0.003 ± 0.004	0.002 ± 0.003
Chinstrap penguin	$\delta^{13}C$ or $\delta^{15}N$	Intercept	1.000	1.000	-24.57 ± 0.04	9.12 ± 0.08
		Sex	0.243	0.963	-0.01 ± 0.02	-0.22 ± 0.08
		PC1	0.247	0.017	0.004 ± 0.006	0.0006 ± 0.0008
		Year 2008	0.439	0.999	-0.01 ± 0.03	0.38 ± 0.10
		Year 2009	0.439	0.999	0.04 ± 0.04	0.57 ± 0.09
		Sex*Year 2008	0.0104	0.078	-0.0003 ± 0.002	-0.008 ± 0.02
		Sex*Year 2009	0.010	0.078	0.0008 ± 0.002	-0.004 ± 0.01
		PC1*Year 2008	0.011	0.002	-0.0004 ± 0.0007	5.83E-05 ± 0.0001
		PC1*Year 2009	0.011	0.002	-0.0002 ± 0.0006	8.76E-05 ± 0.0001
Gentoo penguin	$\delta^{13}C$ or $\delta^{15}N$	Intercept	1.000	1.000	-25.42 ± 0.03	8.02 ± 0.05
		Sex	0.493	0.872	-0.03 ± 0.02	-0.11 ± 0.05
		PC1	0.321	0.107	0.004 ± 0.005	0.003 ± 0.003

		Year 2008	0.999	0.999	-1.32 ± 0.03	0.43 ± 0.06
		Year 2009	0.999	0.999	-0.72 ± 0.03	0.30 ± 0.05
		Sex*Year 2008	0.066	0.295	0.003 ± 0.005	0.04 ± 0.04
		Sex*Year 2009	0.066	0.295	-0.0003 ± 0.004	0.01 ± 0.03
		PC1*Year 2008	0.049	0.014	0.0006 ± 0.001	-0.0004 ± 0.0006
		PC1*Year 2009	0.049	0.014	0.001 ± 0.002	-0.0001 ± 0.0005

Abbreviations: ± 1SE = plus or minus 1 standard error.

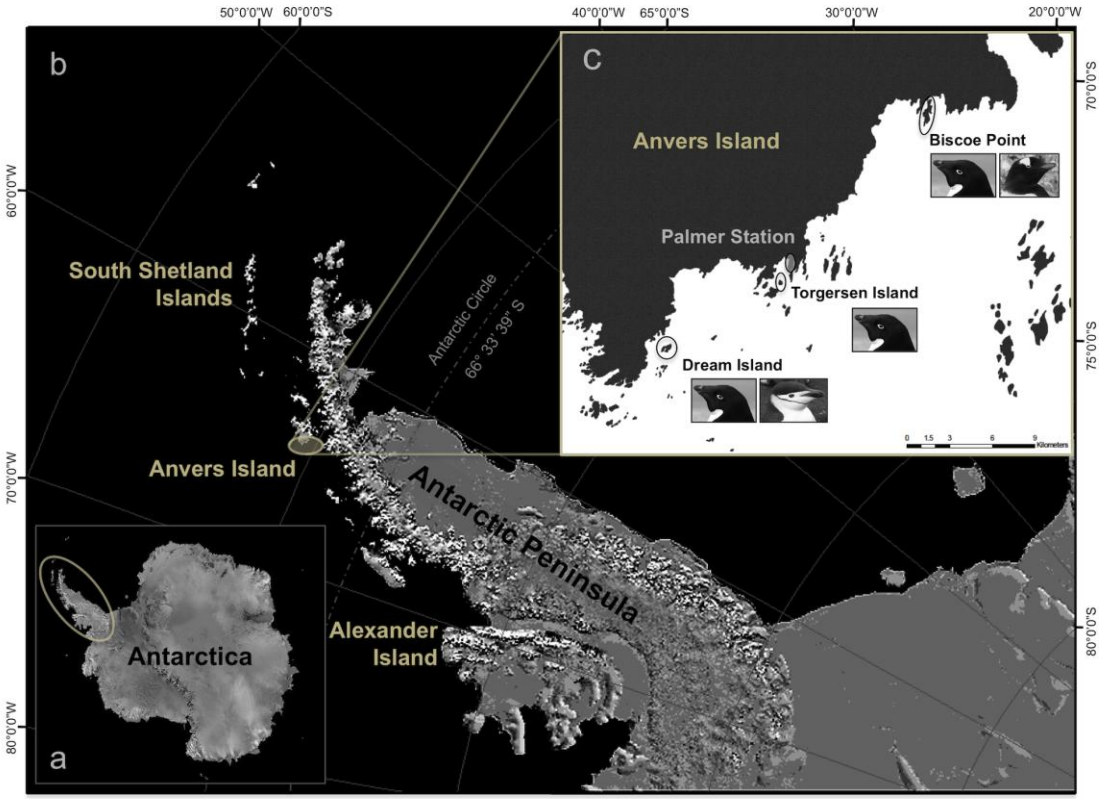


Figure 3.1. The marine ecosystem west of the Antarctic Peninsula (a) extends from northern Alexander Island to the South Shetland Islands (b). Fieldwork for the present study took place within the Palmer Archipelago near Anvers Island and Palmer Station, a United States supported research base (b, c). Penguin rookeries were located at Dream [Adélie and chinstrap], Torgersen [Adélie only], and Biscoe [Adélie and gentoo] Islands (c). Image generated from base maps provided by the National Snow and Ice Data Center's map server A-CAP (http://nsidc.org/agdc/acap/).

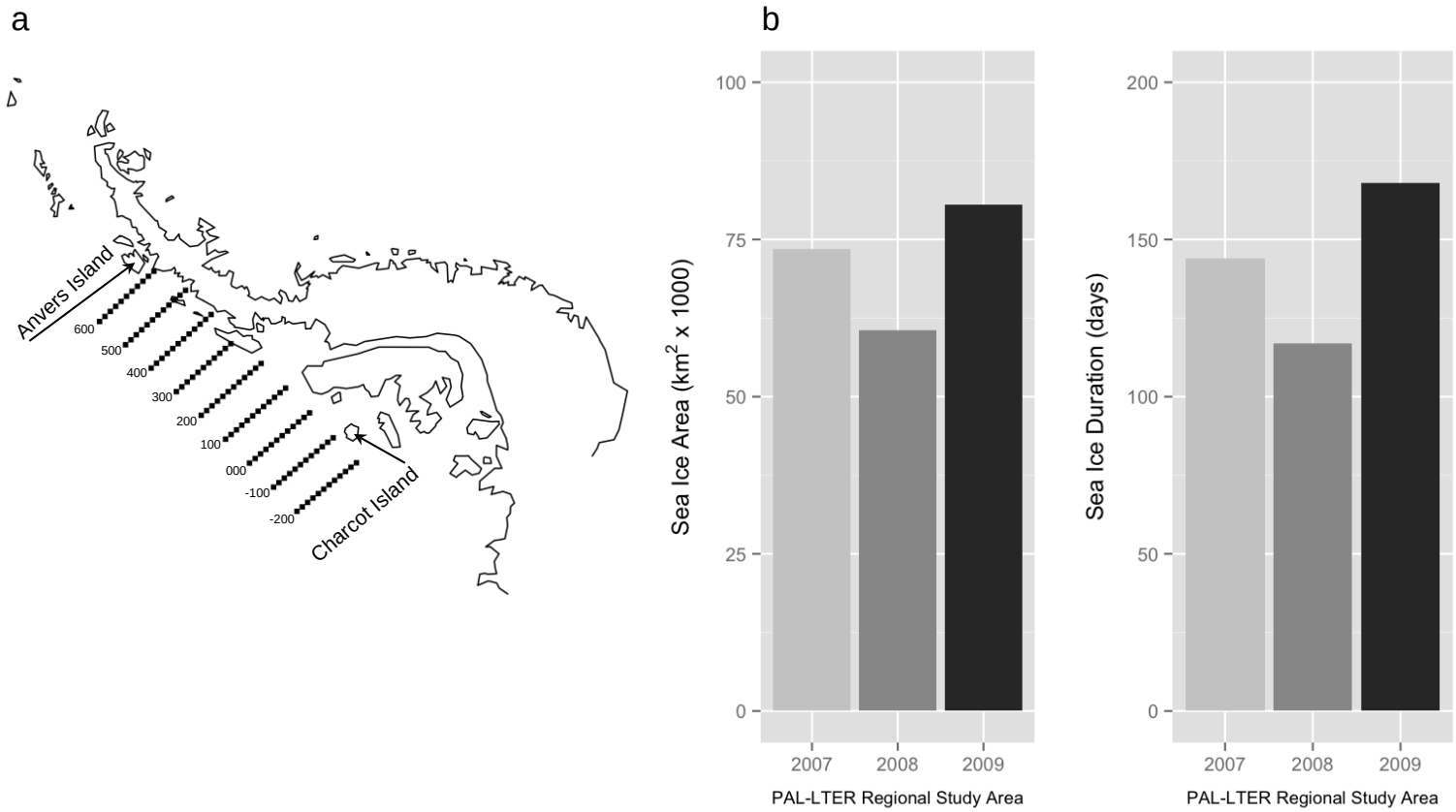


Figure 3.2. Palmer Station, Antarctica, Long Term Ecological Research (PAL-LTER) Program's regional study area west of the Antarctic Peninsula, extending 800 km from Anvers Island (600 line) to just south of Charcot Island (-200 line) and 220 km inshore to offshore (a). Study area grid lines encompass the entire area over which sea ice metrics were calculated. Winter average sea ice area (km^2) and sea ice duration (days) within the PAL-LTER regional study area for each year of the present study (b).

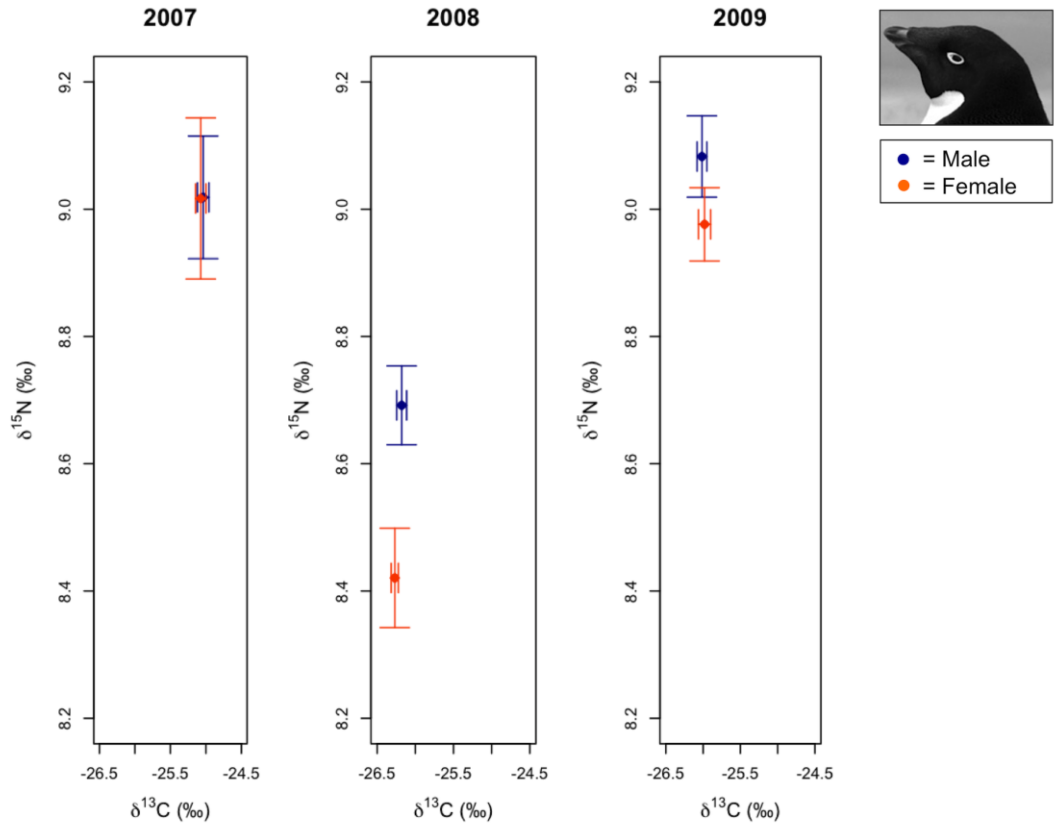


Figure 3.3. Variation in Adélie penguin $\delta^{13}C$ and $\delta^{15}N$ stable isotope signatures. Average male and female isotope signatures (± 1 SE) are presented for each year based on sample sizes exactly following analyses (*n* = 127 for $\delta^{13}C$ and *n* = 128 for $\delta^{15}N$).

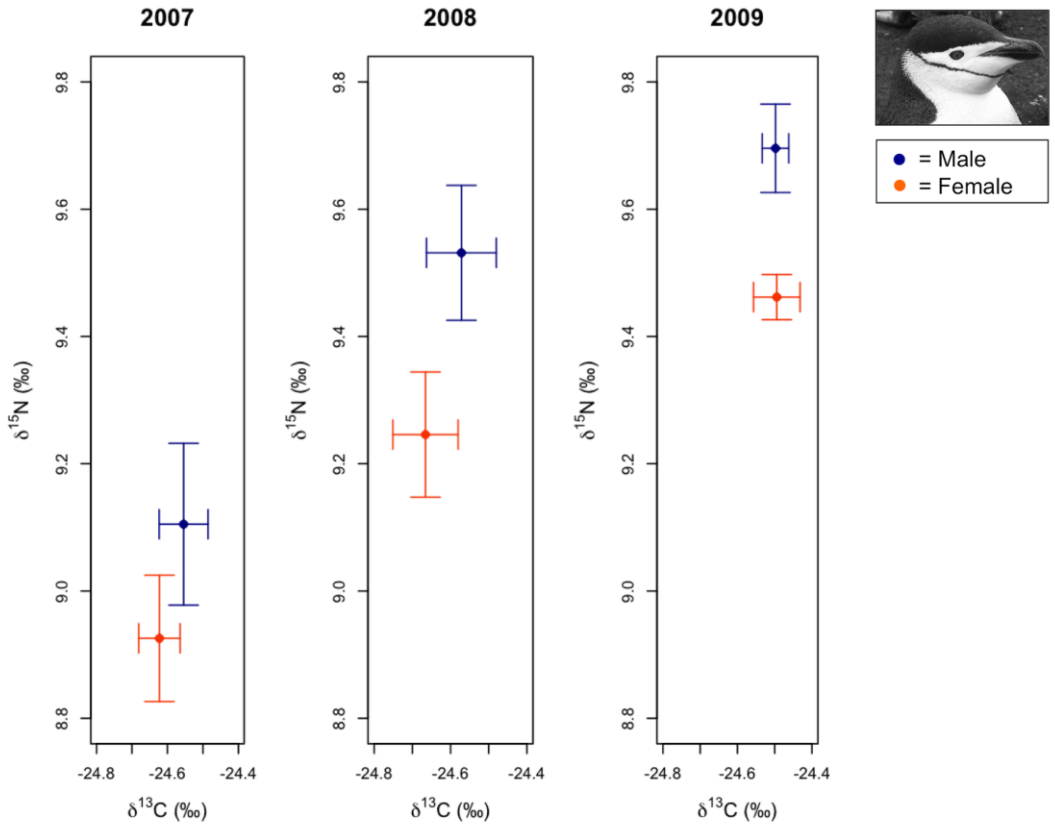


Figure 3.4. Variation in chinstrap penguin $\delta^{13}C$ and $\delta^{15}N$ stable isotope signatures. Average male and female isotope signatures (± 1 SE) are presented for each year based on sample sizes exactly following analyses (*n* = 53).

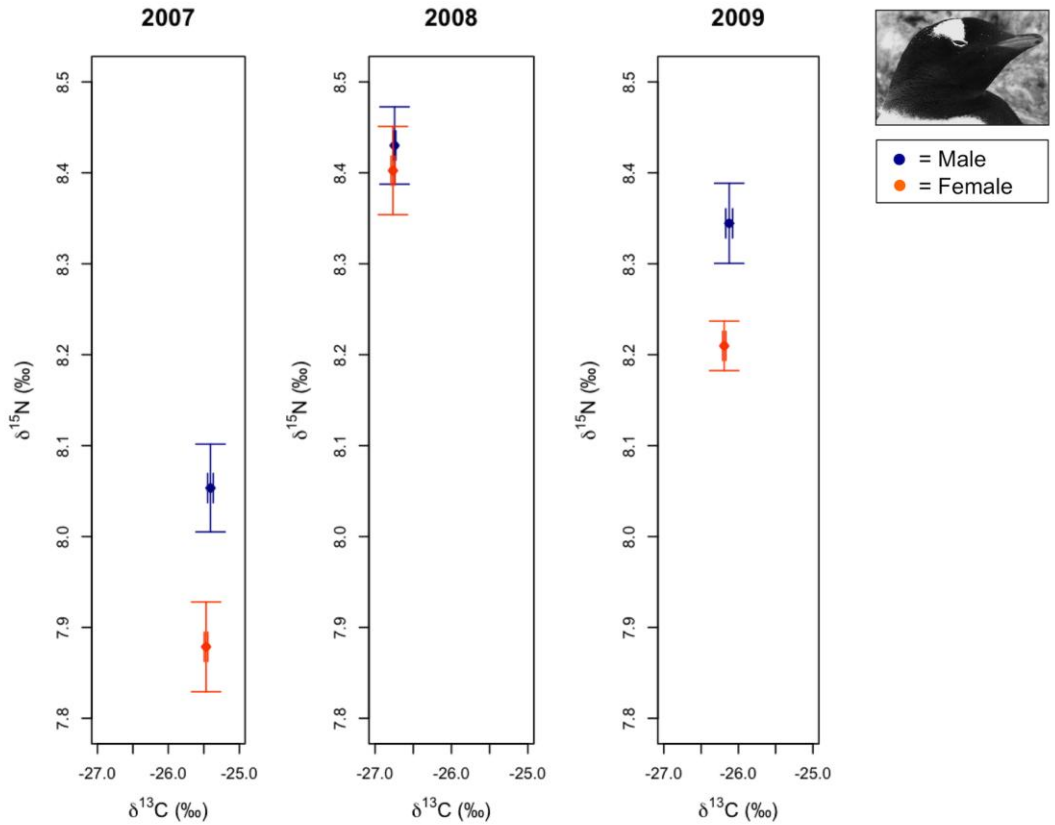


Figure 3.5. Variation in gentoo penguin $\delta^{13}C$ and $\delta^{15}N$ stable isotope signatures. Average male and female isotope signatures (± 1 SE) are presented for each year based on sample sizes exactly following analyses (*n* = 115).

Supporting Information

Text S1. PCR Methods.

Genomic DNA was extracted from collected samples of whole blood smeared on filter paper using Insta-Gene Matrix (Bio-Rad, Hercules, CA) following standard protocols. The PCR primers P2 (5'-TCTGCATCGCTAAATCCTTT-3') and P8 (5'-CTCCCAAGGATGAGRAAYTG-3', where R=A/G, Y=T/C), as well as 2550F (5'-GTTACTGATTCGTCTACGAGA-3') and 2718R (5'-ATTGAAATGATCCAGTGCTTG-3') were both used to amplify regions of the CHD-Z and CHD-W genes. PCR was carried out using a total volume of 25 µl (15 µl PCR mix added to 10 µl genomic DNA) where PCR mix consisted of 10x PCR reaction buffer minus Mg (55 µl), 50 mM MgCl2 (44 µl), Taq polymerase (5.5 µl, 5 units/µl, invitrogen), dNTP mix (11 µl, 10 mM each, Fermentas), P2/P8 or 2550F/2718R primers (44 µl of 20 µM working stock, invitrogen), and autoclaved distilled water (156.5 µl). Using an Eppendorf Thermal Cycler (Mastercycler personal), the following thermal profile was used for P2/P8 primers as outlined by Griffiths et al. (1998) including a slight modification for an initial hot start where the lid and thermoblock were pre-heated to 105 and 85 ºC, respectively, before loading samples; an initial denaturing step at 94 ºC for 1 minute (min) 30 seconds (sec) was followed by 30 cycles of 48 ºC for 45 sec, 72 ºC for 45 sec, and 94 ºC for 30 sec, followed by a final cycle of 48 ºC for 1 min and 72 ºC for 5 min. The thermal profile for 2550F/2718R followed Fridolfsson and Ellegren (1999) and was modified as described above to include an hot start; the initial denaturing step at 94

ºC for 2 min was followed by a touch-down method where the annealing temperature starting at 60 ºC was lowered 1 ºC per cycle until a temperature of 50 ºC was reached. Denaturation began at 94 ºC for 30 sec, followed by annealing for 30 sec, and extension at 72ºC for 35 sec. Twenty-nine additional cycles were run after reaching the final cycle at the 50 ºC annealing temperature (i.e., 30 cycles total of 94 ºC for 30 sec, 50 ºC for 30 sec, and 72ºC for 35 sec). A final extension step included holding at 72 ºC for 5 min. After both P2/P8 and 2550F/2718R thermal profiles were completed, samples were held at 4 ºC. Loading buffer (2.5 µl) was added to PCR products, with 10 µl then separated by electrophoresis for 120 min, at 90 V and 200 mA, using a 3% agarose gel stained with ethidium bromide, and visualized under ultra-violet light.

Text S2. PCR optimization with P2/P8 primers.

During PCR optimization with P2/P8 primers, we found that for Adélie and gentoo samples the volume of *Taq* polymerase within the PCR mix could be reduced to 1.8 *u*l without any effect to the quality of DNA amplification, but for chinstraps it was best to use the original volume of 5.5 *u*l. In addition, for chinstrap samples, the PCR thermal profile for P2/P8 primers was modified to increase the time of the initial denaturing step at 94 ºC from 1 min 30 sec to 5 min, followed by 40, instead of 30 cycles, of 48 ºC for 45 sec, 72 ºC for 45 sec, and 94 ºC for 30 sec.

Text S3. Misclassification details for probability estimates of adult *Pygoscelis* penguin sex.

Of the five Adélie penguin individuals misclassified, four were females based on molecular data that were classified as males based on structural data for the first and third supported models. For the second best-supported model, three females and two males were incorrectly classified. The one chinstrap penguin individual misclassified was a male. The four gentoo penguin individuals misclassified were two males and two females.

Table S1. Average nest initiation dates of *Pygoscelis* penguins relative to November 1 for each study year.

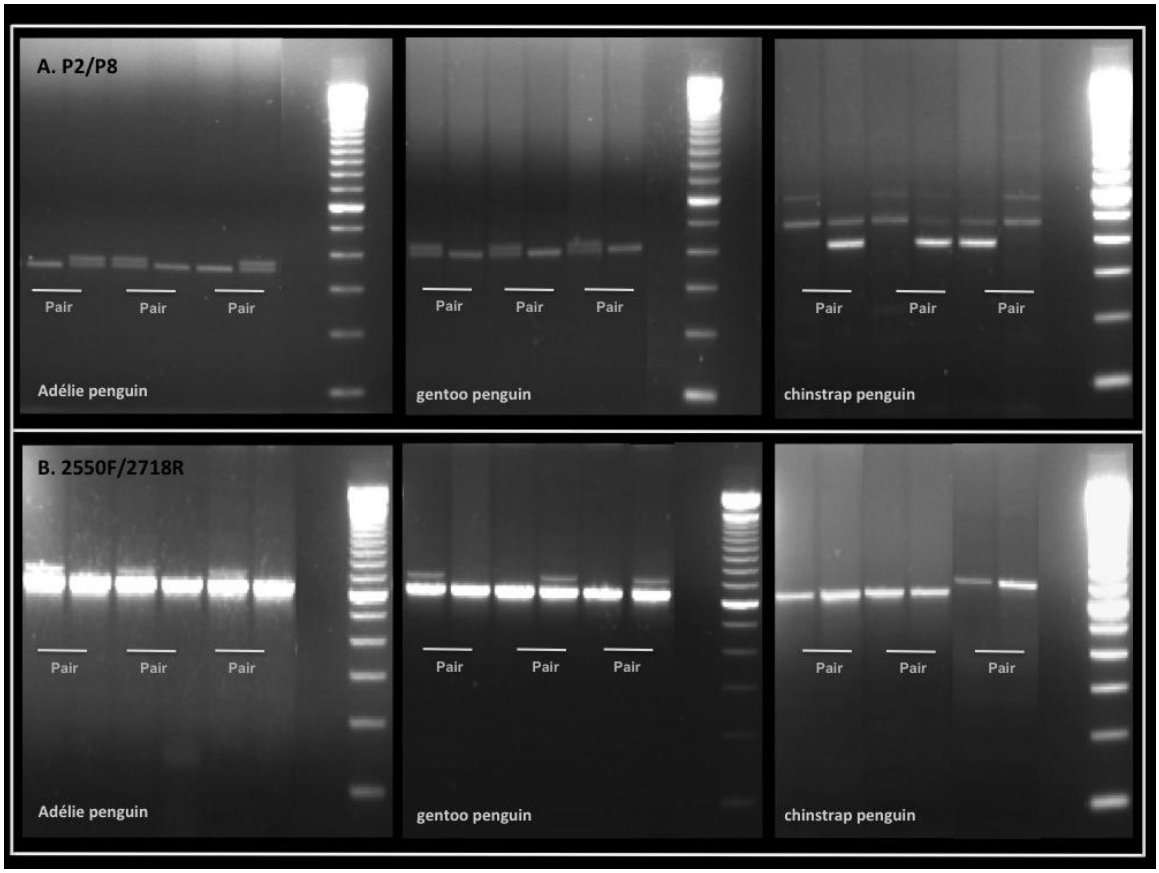
Date paired adult penguins with a full clutch (two eggs) were observed with the first egg.

Species	Study year	Average nest initiation date	± 1SE	*n*
Adélie penguin	2007/08	13	0.45	44
	2008/09	10	0.57	48
	2009/10	16	0.46	46
chinstrap penguin	2007/08	26	0.95	18
	2008/09	20	1.41	14
	2009/10	22	0.70	22
gentoo penguin	2007/08	28	0.57	32
	2008/09	6	0.56	42
	2009/10	22	0.93	42

Abbreviations: ± 1SE = plus or minus 1 standard error, *n* = sample size.

Figure S1. Examples of PCR bands from both P2/P8 and 2550F/2718R primers for three mated adult pairs of *Pygoscelis* penguins.

P2/P8 primers correctly identified 100% of each species' pairs, while 2550F/2718R primers correctly identified 60% of Adélie and gentoo penguin pairs and 0% of chinstrap penguin pairs. The light upper P2/P8 band for chinstrap penguins was considered non-specific, as it was present in both male and female samples as shown above. Adult pairs per species shown here are not the same between primer sets.



Chapter 4.

Trophic Interactions and Individual Variation in Reproductive Performance among *Pygoscelis* Penguins at Opposing Range Margins

This article was prepared as a manuscript with the following authorship:

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Abstract

Pygoscelis penguins of the western Antarctic Peninsula are demonstrating pole-ward breeding range shifts that generally correlate with long-term environmental warming trends throughout the region. Mechanistic relationships that link environmental variability with demographic parameters that drive population changes remain a topic of considerable debate. Here, we assess individual-level indices of trophic foraging and influences on breeding performance parameters across all three species of *Pygoscelis* penguins. At more northern rookeries where sea ice loss has been most prominent, we detected little evidence of an effect of trophic foraging on body and egg mass of adult penguins, as well as body mass of chicks. However, regional analyses of Adélie penguin chick body mass variation suggested that a provisioning diet enriched in $\delta^{15}N$, which is found at a southern rookery characterized by greater over-winter sea ice conditions, resulted in heavier five-week-old chicks. We interpret our results as being important in developing the empirical evidence for how climate-driven changes in sea ice-associated food-webs may in fact influence fledge mass of Adélie penguin chicks, and possibly their survival and recruitment probability that would shape differences in regional breeding population trajectories.

Key words: Biogeographic range shift; breeding performance; $\delta^{13}C$ and $\delta^{15}N$; foraging niche; information-theoretic methods; Pygoscelis penguins; sea ice; stable isotopes; western Antarctic Peninsula

Introduction

Contemporary climate-induced shifts in bio-geographic range are an important ecological issue relevant to a great diversity of the world's species (Parmesan and Yohe 2003, Root et al. 2003, Perry et al. 2005). Bio-geographic range shifts generally result from a change in the strength or direction of natural selection at opposing range margins that alter individual reproductive survival, or a change in patterns of immigration or emigration (Sexton et al. 2009). In the case of environmental warming, the progressively higher-latitudinal or -elevational displacement of individuals is expected (Parmesan 2006), which can be mediated by spatial variability in the environment having divergent effects on fitness of conspecifics at opposing range margins (Fig. 4.1a, b modified from Hewitt and Nichols 2005). Furthermore, directional climate-induced selection in one geographic area may lower mean-fitness for individuals of a species at its contracting range margin, while not affecting or increasing mean-fitness of sympatric species at their expanding range margin, driving population dynamics between species to differ that result in climate-induced shifts in local community composition (Fig. 4.1c, d modified from Hewitt and Nichols 2005, Parmesan 2006).

Antarctic penguins, particularly those within the genus *Pygoscelis*, breeding throughout the Scotia Arc and west of the Antarctic Peninsula (AP, Fig. 4.2a, b), are currently undergoing pole-ward range shifts in response to ocean-climate warming within this region of the Southern Ocean (Forcada and Trathan 2009, Trivelpiece et al. 2011, Lynch et al. 2012, Ducklow et al. 2013). Within the context of the conceptual model described above, northern breeding ranges in this region for Adélie (*P. adeliae*) and emperor (*Aptenodytes forsteri*) penguins, the only two sea ice-obligate species among the

Spheniscidae with the most southerly distribution, have contracted as evidenced by declines of 80% from historical numbers at Adélie penguin rookeries located near Anvers Island (Fig. 4.2b, c Ducklow et al. 2007, Ducklow et al. 2013), in addition to the extirpation of emperor penguins from the Dion Islands near Avian Island (Fig. 4.2b, Trathan et al. 2011). Conversely, chinstrap (*P. antarctica*) and gentoo (*P. papua*) penguins, sea ice-intolerant congeners of the Adélie penguin that hold a more low- to sub-Antarctic distribution, have shown stable or rapid increases in numbers of breeding adults at their regional southern range margin near Anvers Island (Fig. 4.2b, c Ducklow et al. 2007, Ducklow et al. 2013). Similarly, Adélie penguin breeding rookeries within the core of the species range along the western AP at Avian Island (Fig. 4.2b) have increased (Sailley et al. 2013, W. R. Fraser unpubl. data), which contrasts with the previously described trends for emperor penguins within this same region. Divergent breeding rookery responses by *Pygocelis* and *Aptenodytes* penguins throughout the western AP have occurred in association with significant, long-term reductions in annual sea ice coverage within the northern seasonal sea ice zone along the western AP (Jacobs and Comiso 1997, Ducklow et al. 2007, Stammerjohn et al. 2008, see Chapman et al. 2010 Fig. 1, Stammerjohn et al. 2012, Ducklow et al. 2013). However, annual sea ice coverage remains more prominent physical oceanographic feature within the southwestern AP (see Ducklow et al. 2013 Fig. 2).

From an ecosystem perspective, climate-driven change in sea ice dynamics is considered a key natural selective factor influencing regional variability in penguin reproductive survival and dispersal (Forcada and Trathan 2009) – demographic factors that determine bio-geographic ranges. Reductions in sea ice influence critical habitat,

both in the form of sea ice as a platform for resting or breeding, as well as a determinant of marine trophic structure that Antarctic penguins depend upon to sustain the energetic demands of their specific life histories (Moline et al. 2008). Within this context, juvenile survival and recruitment to breeding populations has been identified as a potentially significant demographic parameter contributing to population changes for Antarctic penguins of the western AP (Hinke et al. 2007, Trivelpiece et al. 2011), which is to be expected when adult survival is relatively stable (Croxall and Rothery 1991, Morris and Doak 2002, Lee et al. 2008). As with many seabirds, body condition of pre-fledge penguin chicks is positively correlated with survival and recruitment to adulthood (e.g., Saraux et al. 2011) as greater nutrient reserves are thought to provide individuals with an energetic safeguard while learning to self-feed after leaving natal colonies. To date, research has tended to focus on relating oceanographic variability with population-level indices of foraging and breeding performance (i.e., provisioning diet composition, foraging trip duration, or fledgling mass), as well as subsequent recruitment to better understand the mechanisms by which marine ecosystem variability influence penguin demography (e.g., Fraser and Hofmann 2003, Hinke et al. 2007, Trivelpiece et al. 2011). No empirical penguin studies have related variability in provisioning diet, as a function of oceanographic conditions, with chick quality at the individual level. Studies have traditionally relied on diet sampling techniques, such as stomach lavage (e.g., Wilson 1984), which directly bias the amount of food parents deliver to their individual chicks, and therefore, the chick's energetic state. Importantly, advances in stable isotope methods to infer animal trophic ecology (see Inger and Bearhop 2008 for review) now circumvent many of the difficulties of traditional diet sampling by allowing for dietary inference at

the individual level and during early stages of reproductive investment (e.g., Sorensen et al. 2009).

Chapman et al. (2011) developed an individual-based bioenergetics model to examine Adélie penguin chick growth and fledgling mass as a function of varying dietary proportions of Antarctic krill (*Euphausia superba*) and Antarctic silverfish (*Pleuragramma antarcticum*). Antarctic krill are a dominant summer prey item of Adélie penguins within the northwestern AP and the South Shetland Islands (Fraser and Hofmann 2003, Hinke et al. 2007, Trivelpiece et al. 2011), and stocks have declined in association with reductions in sea ice (Loeb et al. 1997, Atkinson et al. 2004, Ducklow et al. 2007). Antarctic silverfish hold an early life history tightly associated with the presence of sea ice (Vacchi et al. 2004), and therefore, are an important species in energy transfer to upper trophic levels in high Antarctic food-webs (La Mesa et al. 2004). However, unlike Antarctic krill, silverfish appear to be no longer available to *Pygoscelis* penguins nesting within the northwestern AP, but remain present in the diet of Adélie penguins nesting at southwestern AP rookeries (W. R. Fraser unpubl. data) presumably due to climate-driven changes in annual sea ice coverage and local hydrographic processes within this region of the western AP (Kellerman 1996, Donnelly and Torres 2008). Model simulations by Chapman et al. (2011) indicated that chicks provisioned a krill-based diet supplemented with 17% Antarctic silverfish of age class 3 increased fledge mass by 5%, and more importantly, above a body mass threshold associated with recruitment given equal provisioning rates as Antarctic silverfish are more energy dense than krill. Importantly, these results suggest a possible nutritional mechanism by which reductions in sea ice might result in breeding range contractions due to climate-driven

changes in the availability of a critical forage fish that shapes the quality, survival and recruitment probability of young Adélie penguins.

We conducted a field study to empirically test the conclusions by Chapman et al. (2011) by examining relationships between individual adult *Pygoscelis* penguin trophic foraging and breeding performance based on body and egg mass variation. We rely on signatures of naturally occurring carbon ($^{13}C/^{12}C$, $\delta^{13}C$) and nitrogen ($^{15}N/^{14}N$, $\delta^{15}N$) stable isotopes (SI) of blood tissue as proxies of penguin trophic foraging. Signatures of $\delta^{13}C$ and $\delta^{15}N$ SIs are time-integrated, biogeochemical parameters that specifically reflect assimilated prey (Deniro and Epstein 1978, 1981, Peterson and Fry 1987) over approximately the previous 30 – 60 days given allometric turn-over rates for the cellular fraction of blood (Hobson and Clark 1992, Bearhop et al. 2002, Bearhop et al. 2006, Cherel et al. 2007). Our analyses of individuals all *Pygoscelis* species breeding at northern rookeries located near Anvers Island (Fig. 4.2b, c) included four reproductive stages – adults at the one-egg stage, young chicks at five and 15 days of age, and pre-fledge crèched chicks at five-weeks of age. Analyses of individuals at regional rookeries included Adélie penguin pre-fledge crèched chicks at five-weeks of age only. Key forage fishes are isotopically enriched relative to Antarctic krill (Polito et al. 2011, K. B. Gorman this thesis Chapter 2). Therefore, throughout our analyses we predicted that (1) adults and chicks at Anvers Island with enriched $\delta^{13}C$ and $\delta^{15}N$ blood SI signatures would be heavier after accounting for other parameters that we expected would influence body mass such as sex given sexual size dimorphism (Gorman et al. 2014), structural size within sexes, as well as the seasonal timing of breeding. Similarly, we predicted that (2) females with enriched $\delta^{13}C$ and $\delta^{15}N$ blood SI signatures would have larger egg masses.

At the regional level, we predicted (3) that Adélie penguin chicks reared at Avian Island would be heavier as we have previously shown these individuals to be isotopically enriched (K. B. Gorman this thesis Chapter 2). Lastly, we qualitatively considered the influence of over-winter sea ice coverage on body and egg mass variation modeled as a year effect where we expected differing inter-specific body mass responses, at all reproductive stages, to over-winter sea ice coverage given generally opposing life history affinities for sea ice among *Pygoscelis* penguins (Fraser et al. 1992, Ainley 2002, Forcada and Trathan 2009). Specifically, we predicted that (4) Adélie penguins and their eggs should be heavier following years of higher winter sea ice coverage, while chinstrap and gentoo penguins and their eggs should be lighter. Similarly, (5) Adélie penguin chicks should be heavier in regions with higher winter sea ice coverage.

Materials and Methods

Field methods

Pygoscelis penguins nesting on several islands within the Palmer Archipelago west of the AP near Anvers Island (64°46’S, 64°03’W, Fig. 4.2a-c) were studied during the austral summers of 2007/08, 2008/09, and 2009/10. Specifically, study nests were located on Biscoe Point (64°48’S, 63°46’W), Torgersen (64°46’S, 64°04’W) and Dream (64°43’S, 64°13’W) Islands (Fig. 4.2c). Each study season, Adélie penguin study nests (n = 30) were distributed equally between the three study islands, with 10 nests located on each island. Gentoo penguin study nests (n = 30) were all located on Biscoe Point, while chinstrap penguin study nests (n = 15) were all located on Dream Island (Fig. 4.2c). The

reduced sample size for chinstraps was due to the overall smaller number of individuals breeding at rookeries on Dream Island. Final sample sizes of individuals used in analyses differ from the number of original study nests reported here as at times weather conditions hindered rookery access resulting in some adults reaching clutch completion before sampling, and therefore, these study nests were not sampled and excluded from subsequent monitoring. In addition, some study chicks were never sampled due to brown skua (*Stercorarius lonnbergi*) depredation. During annual oceanographic research cruises aboard the A.R.S.V. *Laurence M. Gould*, as part of the Palmer Station, Antarctica, Long-Term Ecological Research Program (PAL-LTER), in January of each study year, we further sampled Adélie penguin chicks from rookeries at Avian Island (n = 25, Fig. 4.2b).

Each season, study nests, where pairs of adults were present, were individually marked and chosen before the onset of egg laying, and monitored consistently. When study nests were found at the one-egg stage, both adults were captured to obtain blood samples used for SI analyses, and measurements of structural size and body mass. At the time of capture, each adult penguin was quickly blood sampled (~1 ml) from the brachial vein using a sterile 3 ml syringe and heparinized infusion needle. Measurements of culmen length and depth (using dial calipers ± 0.1 mm), right flipper (using a ruler ± 1 mm), and body mass (using 5 kg ± 25 g or 10 kg ± 50 g Pesola spring scales and a weigh bag) were obtained to quantify body size variation. After handling, individuals at study nests were further monitored to ensure the pair reached clutch completion, i.e., two eggs. Study nests were monitored for hatch of the first egg. At day six and 16 post-hatch for first chicks, i.e., resulting in an approximate average nest age of five and 15 days given hatching asynchrony, offspring from study nests were captured and quickly blood

sampled (≤ ~500 μl for day five chicks, and ≤ ~1 ml for day 15 chicks) from the tarsus vein using a sterile needle and heparinized capillary tubes for day five chicks, and a sterile 3 ml syringe and heparinized infusion needle for day 15 chicks, again to obtain blood tissue for SI analyses. Measurements of culmen length and depth (using dial calipers ± 0.1 mm), right flipper (using a ruler ± 1 mm), and body mass (using 600 g ± 5 g, 1000 g ± 10 g or 2000 g ± 20 g Pesola or Homs spring scales and a weigh bag) were obtained to quantify body size variation. Study nests were monitored for chick survival to 25 days, prior to crèche. At five weeks into chick-rearing, based on a seasonal population average hatch date for each species, older crèched chicks of all three species were captured and quickly blood sampled from study rookeries near Anvers Island. Handling of crèched chicks occurred over a one or two day period, which varied seasonally and by species depending on nest initiation dates. Adélie penguin chicks at Avian Island were sampled on the same day Anvers Island Adélie penguin chicks were sampled. Blood samples from crèched chicks (~1 ml) were taken from the brachial vein using a sterile 3 ml syringe and heparinized infusion needle following sampling procedures used for adult penguins, again to obtain blood tissue for SI analyses. Measurements of culmen length and depth (using dial calipers ± 0.1 mm), right flipper (using a ruler ± 1 mm), and body mass (using 5 kg ± 25 g or 10 kg ± 50 g Pesola spring scales and a weigh bag) were obtained to quantify body size variation. At the time of blood sampling for each reproductive stage, a small amount of whole blood was smeared on clean filter paper and stored in a micro-centrifuge tube for molecular sexing. All collected blood was stored in 1.5 ml microcentrifuge tubes and kept cool in the field.

Sea ice data were based on satellite estimates of sea ice concentration (SIC) within two regions, 1-the PAL-LTER regional study grid (Fig. 4.2a) and 2-two 200 km^2 penguin foraging grids associated with major penguin rookeries at Anvers and Avian Islands (Fig. 4.3a). Daily and monthly time series of SIC were used to calculate two metrics within the PAL-LTER regional grid, 1-average winter sea ice area (km^2) representing the yearly average area of the ocean covered by winter sea ice, and 2-duration (days) representing the yearly total length of the winter sea ice season. For the 200 km^2 penguin foraging grids, the only sea ice metric calculated was 1-average winter sea ice area within each foraging grid. Sea ice metrics were calculated using time series based on Version 2 of the Goddard Space Flight Center (GSFC) Bootstrap algorithm (Comiso 1999) provided by the National Snow and Ice Data Center (NSIDC; www.nsidc.org), and derived based on 1-monthly averages between the winter months of sea ice advance and retreat, and 2-the day of first and last appearance of sea ice within the same region and averaged for the PAL-LTER grid. These metrics are useful for describing both the winter spatial and temporal variability of Southern Ocean sea ice (Stammerjohn et al. 2008).

Laboratory methods

Within 12 hrs of field collection, tubes containing whole blood were centrifuged to separate plasma and red blood cell (RBC) fractions, which were stored separately and frozen at -80 ºC. Tubes containing whole blood smears on filter paper were allowed to dry in a desiccator. After drying, tubes were sealed and frozen at -80 ºC.

Tubes containing RBCs were first allowed to dry to a consistent mass in a drying oven at 60 ºC. Using a mortar and pestle lined with clean weighing paper, dried RBC pellets were homogenized into a powder. Each mortar and pestle was washed and dried in between sample processing. Aliquots of powdered samples were transferred to 8 x 5 mm pressed tin capsules (Elemental Microanalysis) and weighed (~2 mg) using an analytical balance. Samples were organized in 96-microwell plates and analyzed for $\delta^{13}C$ and $\delta^{15}N$ SI signatures using an elemental analyzer interfaced with an isotope ratio mass spectrometer at the Stable Isotope Facility, University of California (UC) - Davis. Data expressed as $\delta^{13}C$ or $\delta^{15}N$ were calculated using the following equation: $\delta^{13}C$ or $\delta^{15}N = ([R_{sample}/R_{standard}]-1) \times 1000$, where R_{sample} is the ratio of the heavy to light isotope for either $^{13}C/^{12}C$ or $^{15}N/^{14}N$, and $R_{standard}$ is the heavy to light isotope ratios for international standards - Vienna PeeDee Belemnite for carbon, and atmospheric N_2 (Air) for nitrogen. Sex of all sampled individuals (adults and chicks) was determined using molecular genetic methods outlined by Gorman et al. (2014, see also this thesis Chapter 3).

Statistical methods

Final sample sizes for study nests / individual adults / day five chicks / day 15 chicks / five week chicks of each species were for Adélie penguins: *n* = 76 / 152 / 102 / 99 / 49 (Anvers Island) and 71 (Avian Island), chinstrap penguins: *n* = 34 / 68 / 40 / 35 / 41, and gentoo penguins: *n* = 62 / 124 / 100 / 92 / 46 over the three years of study. Final sample sizes used in analyses differ from those reported here as some individuals were excluded. For example, occasionally adult pairs were never observed having reached clutch completion, i.e., two eggs, likely due to brown skua depredation, and therefore we could

not be certain those individuals represented adults exactly at the one egg stage. Furthermore, at times weather conditions hindered rookery access resulting in some chicks being sampled at the incorrect age. All statistical analyses were performed in the R language environment (2012).

Analysis 1 – adult body mass

Least-squares general linear models (LM) were used to examine continuous variation in body mass of adult male and female *Pygoscelis* penguins in relation to five parameters treated as main effects including 1-year (categorical variable), 2-overall structural size using a principal components score (PC1) based on culmen depth and length, and flipper length (continuous variable), 3-nest initiation date defined as the date adult penguins were sampled when study nests were found at the one-egg stage (continuous variable), 4-δ^{13}C SI signatures of adult penguin RBCs (continuous variable), and 5-δ^{15}N RBC signatures (continuous variable). Individual scores for PC1 were calculated using the *prcomp* function in R for male and female datasets, separately, for each species. Nest initiation dates for males and females of each study nest was calculated in relation to the earliest nest initiation date for each species across all years, which was set at 0 (Adélie penguins = 02 November 2008, chinstrap penguins = 14 November 2008, and gentoo penguins = 02 November 2008). An a priori set of 21 candidate models consisted of an equal-means (null) model; each predictor variable as a main effect (five models); a simple multiple predictor model for both δ^{13}C and δ^{15}N SI signatures of RBCs (one model); more complex multiple predictor models for PC1 score, or nest initiation date, with both δ^{13}C and δ^{15}N SI signatures of RBCs (two models); as well as a model for PC1 score, nest initiation date, and both δ^{13}C and δ^{15}N SI signatures (one model). All these

models were further evaluated including a year term (eight models). Three additional interaction models were included: nest initiation date and year were considered as main effects and their interaction; both $\delta^{13}C$ and $\delta^{15}N$ SI signatures and nest initiation date were considered as main effects and the interaction between each SI and nest initiation date; as well as both $\delta^{13}C$ and $\delta^{15}N$ SI signatures and year considered as main effects and the interaction between each SI and year. See Appendix 4.1 for details regarding candidate models considered in adult penguin body mass analyses. This same candidate model set was evaluated separately for both sexes within each species (Adélie females n = 65 and males n = 63, chinstrap females n = 27 and males n = 26, and gentoo females n = 58 and males n = 57).

Analysis 2 – adult female egg mass

Continuous variation in egg mass of adult female *Pygoscelis* penguins was examined using LM in relation to six parameters including those five considered in adult body mass analyses described above (1-year, 2-PC1 score, 3-nest initiation date, 4-$\delta^{13}C$ and 5-$\delta^{15}N$ SI signatures of RBCs), in addition to 6-female body mass (continuous variable). Individual scores for PC1 were calculated separately for each species' female dataset. An a priori set of 17 candidate models consisted of an equal-means (null) model; year and nest initiation date as main effect models (two models); simple multiple predictor models for female body mass and PC1 score, as well as $\delta^{13}C$ and $\delta^{15}N$ SI signatures of RBCs (two models); more complex multiple predictor models where female body mass and PC1 score, or both $\delta^{13}C$ and $\delta^{15}N$ SI signatures, were considered with nest initiation date (two models); a model including female body mass and PC1 score with both $\delta^{13}C$ and $\delta^{15}N$ SI

signatures (one model); as well as a model including all five parameters excluding year (one model). All these models were further evaluated including a year term (seven models). One interaction model was included where female body mass, PC1 score, and year where considered as main effects and the interaction between female body mass and year (one model). See Appendix 4.2 for details regarding candidate models considered in female penguin egg mass analyses. This same candidate model set was evaluated for females of each species, separately (Adélie females n = 65, chinstrap females n = 27, and gentoo females n = 57). Sample sizes follow female body mass analyses, however, for gentoo penguins one egg mass data point was excluded as an extreme outlier based on residual plots for normality.

Analyses 3 and 4 – chick body mass at five and 15 days

Linear mixed effects models (hereafter LME, nlme package R Core Team 2012) were used to examine continuous variation in body mass of five-day-old and 15-day-old *Pygoscelis* penguin chicks. Males and females were not analyzed separately, unlike adult body mass analyses. Variation in five and 15-day-old chick body mass was considered in relation to six parameters treated as fixed main effects including 1-year, 2-sex (categorical variable), 3-PC1 score, 4-hatch date (continuous variable), 5-δ^{13}C and 6-δ^{15}N SI signatures of RBCs. Individual scores for PC1 were calculated separately for each species' five- and 15-day-old chick dataset. Hatch date of each study nest was calculated in relation to the earliest hatch date for each species across all years, which was set at 0 (Adélie penguins = 10 December 2008, chinstrap penguins = 21 December 2008, and gentoo penguins = 07 December 2008). An a priori set of 24 candidate models consisted of an equal-means (null) model; each predictor variable as a fixed main effect (six

models); simple multiple predictor models that included sex and PC1 scores, or both $\delta^{13}C$ and $\delta^{15}N$ SI signatures (two models); more complex multiple predictor models where sex and PC1 score, or both $\delta^{13}C$ and $\delta^{15}N$ SI signatures, were considered with hatch date (two models); a model that included all five parameters excluding year (one model). All these models were further evaluated including a year term (10 models). Two additional interaction models were included: hatch date and year were considered as fixed main effects and their interaction; as well as both $\delta^{13}C$ and $\delta^{15}N$ SI signatures and year considered as fixed main effects and the interaction between each SI and year. See Appendix 4.3 for details regarding candidate models considered in five- and 15-day-old chick body mass analyses. This same candidate model set was evaluated for chicks of each species, separately (five-day-old chicks: Adélie n = 94, chinstrap n = 33, and gentoo n = 96; 15-day-old chicks: Adélie n = 98, chinstrap n = 31, and gentoo n = 73). A random effect of nest on intercept (random = ~1|nest) was included in all candidate LME models to control for non-independence of data given that sibling chicks were sampled.

Analysis 5 – chick body mass at 5 weeks (Anvers Island)

Continuous variation in body mass of *Pygoscelis* penguin chicks at five weeks of age reared near Anvers Island was examined using LM in relation to five parameters treated as main effects including 1-year, 2-sex, 3-PC1 score, 4-$\delta^{13}C$ and 5-$\delta^{15}N$ SI signatures of RBCs. Males and females were not analyzed separately. Individual scores for PC1 were calculated separately for each species' five-week-old chick dataset. An a priori set of 18 candidate models consisted of an equal-means (null) model; each predictor variable as a main effect (five models); simple multiple predictor models that included sex and PC1

score, or both $\delta^{13}C$ and $\delta^{15}N$ SI signatures (two models); a more complex multiple predictor model including sex, PC1 score, and both $\delta^{13}C$ and $\delta^{15}N$ SI signatures (one model). All these models were further evaluated including a year term (seven models). Two interaction models were included: both $\delta^{13}C$ and $\delta^{15}N$ SI signatures and year were considered as main effects and the interaction between each SI and year; as well as both $\delta^{13}C$ and $\delta^{15}N$ SI signatures and sex considered as main effects and the interaction between each SI and sex. See Appendix 4.4 for details regarding candidate models considered in body mass analyses of five-week-old penguin chicks reared near Anvers Island. This same candidate model set was evaluated for each species, separately (Adélie n = 47, chinstrap n = 41, and gentoo n = 44).

Analysis 6 - chick body mass at 5 weeks (regional islands)

Continuous variation in body mass of Adélie penguin chicks at five weeks of age reared at regionals rookeries (i.e., Anvers and Avian Islands) was examined using LM in relation to six parameters treated as main effects including 1-year, 2-sex, 3-PC1 score, 4-rookery location (categorical variable), 5-$\delta^{13}C$ and 6-$\delta^{15}N$ SI signatures of RBCs. Individual scores for PC1 were calculated specifically for the regional five-week-old Adélie penguin chick dataset. An a priori set of 24 candidate models consisted of an equal-means (null) model; each predictor variable as a main effect (six models); simple multiple predictor models that included PC1 score and sex, or both $\delta^{13}C$ and $\delta^{15}N$ SI signatures (two models); more complex multiple predictor models where PC1 score and sex, or both $\delta^{13}C$ and $\delta^{15}N$ SI signatures, were considered with location (two models); as well as a model including all five parameters excluding year (one model). All these

models were further evaluated including a year term (10 models). Two additional interaction models were included where location and year were considered as main effects and their interaction; as well as both $\delta^{13}C$ and $\delta^{15}N$ SI signatures and location considered as main effects and the interaction between each SI and location. See Appendix 4.5 for details regarding candidate models considered in body mass analyses of five-week-old Adélie penguin chicks reared at regional rookeries (n = 118).

For each of the six analyses, information-theoretic methods were used to direct model selection and parameter estimation (Burnham and Anderson 2002). For each candidate model set described, Akaike's Information Criterion (AICc), corrected for small sample size, in addition to ΔAICc and Akaike weight (w) values were calculated and used to compare models (Burnham and Anderson 2002). An r^2 value, defined as the fraction of the total variance explained by the model and given by the Multiple R-squared calculation in R (see Crawley 2007 p. 399), was calculated as a general measure of fit for each model. Inference was based on the relative support for parameters across all models and weighted parameter estimates. Parameter estimation included calculation of model-averaged parameter estimates based on w values for all candidate models. Standard errors (SE) for parameter estimates were based on unconditional variances calculated across the same models. Parameter likelihood values were evaluated by summing w values across all models that included each parameter under consideration (Burnham and Anderson 2002).

Data management

Data reported here will be made publicly available within the PAL-LTER data system (dataset numbers forthcoming): http://oceaninformatics.ucsd.edu/datazoo/data/pallter/datasets. These data will be additionally archived within the US LTER Network's Information System Data Portal: https://portal.lternet.edu/. Individuals interested in using these data once available are therefore expected to follow the US LTER Network's Data Access Policy, Requirements and Use Agreement: http://www.lternet.edu/policies/data-access.

Results

Sea ice variability

Sea ice metrics indicated that the austral winters preceding each study year were generally characterized by lower than average sea ice coverage, with winter 2008 being the lowest sea ice season in the Palmer LTER record since 1979 (Stammerjohn et al. 2008, see also sea ice datasets within http://pal.lternet.edu/data/). Relative yearly variability in sea ice area and duration (Fig. 4.3b) over the course of the study suggested that 2007 was an intermediate sea ice season relative to 2008 (low) and 2009 (high). Results for inter-annual variability in body and egg mass of adult *Pygoscelis* penguins at the one-egg stage are discussed within the context of these qualitative differences in each year's winter sea ice season over the entire PAL-LTER regional study grid (Fig. 4.3a).

Variation in average winter sea ice area across the 200 km^2 penguin foraging grids (Fig. 4.4a) confirmed that in general sea ice area increased to the south during the

austral winters preceding each study year (Fig. 4.4b). Yearly variation in average winter sea ice area within Anvers Island penguin foraging grid confirmed our previous finding based on sea ice metrics calculated over the entire PAL-LTER regional study grid (Fig. 4.3a, b) that 2007 was an intermediate winter sea ice season relative to 2008 (low) and 2009 (high). This trend was not apparent within the Avian Island penguin foraging grid (Fig. 4.4b) where 2009 was an intermediate winter sea ice season relative to 2007 (low) and 2008 (high). Results for inter-annual variability in body mass of *Pygoscelis* penguin chicks at all stages and locations are discussed within the context of these qualitative differences in each foraging grid's winter sea ice season (Fig. 4.4a).

Adult body mass

Analyses of variation in body mass of adult female Adélie penguins resulted in only one model receiving a ΔAICc value ≤ 2. The most parsimonious model included terms for PC1 score, nest initiation date, as well as SI signatures of both $\delta^{13}C$ and $\delta^{15}N$ (Table 4.1). This model received moderate *w* and r^2 values (0.57 and 0.35, respectively, Table 4.1). All other models in the candidate set received no support. Parameter likelihoods indicated strong support for PC1 score (1.00) in addition to $\delta^{13}C$ and $\delta^{15}N$ SI signatures (0.85 for each). However, nest initiation date received more moderate support (0.65, Table 4.2). Weighted parameter estimates and associated 95% confidence intervals (CI = parameter estimate SE*1.96) indicated that structurally larger female Adélie penguins were heavier (115.38 ± 52.75 CI, Table 4.2). Importantly, 95% CIs overlapped 0 for all other parameters, indicating that nest initiation date (-10.81 ± 12.48 CI) and both SI signatures ($\delta^{13}C$: 28.13 ± 101.23 CI, $\delta^{15}N$: -101.18 ± 141.17 CI) were not important in predicting

variation in body mass of female Adélie penguins (Table 4.2). Furthermore, there was no qualitative relationship between yearly sea ice conditions and body mass variation of female Adélie penguins as the year term received little support (parameter likelihood = 0.13 for both references years, Table 4.2). Two models received ΔAICc values ≤ 2 for describing variation in body mass of adult male Adélie penguins. Both models included terms for PC1 score, nest initiation date, as well as SI signatures of both $\delta^{13}C$ and $\delta^{15}N$ (Table 4.1). However, the second best supported model also included the year term (Table 4.1). Both supported models received moderate *w* (0.54 and 0.45, respectively) and r^2 (0.39 and 0.43, respectively) values (Table 4.1). Parameter likelihoods indicated strong support for PC1 score (1.00), nest initiation date (0.99) and $\delta^{13}C$ and $\delta^{15}N$ SI signatures (0.99 for each), while the year term received weaker support (0.45, Table 4.2). Weighted parameter estimates and associated 95% CIs indicated that structurally larger male Adélie penguins were heavier (168.89 ± 61.25 CI, Table 4.2), and that males that initiated nesting earlier were heavier (-38.76 ± 23.24 CI, Table 4.2). There was no evidence that blood SI signatures accounted for variation in body mass of adult male Adélies as 95% CIs overlapped 0 ($\delta^{13}C$: 167.98 ± 220.21 CI, $\delta^{15}N$: -69.40 ± 254.40 CI, Table 4.2). Similar to female Adélies, there was no qualitative relationship between yearly sea ice conditions and body mass variation of males as the year term received weaker support (Table 4.2).

The best-supported model for describing variation in body mass of adult female chinstrap penguins included terms for PC1 score and year only, and received moderate *w* (0.43) and r^2 (0.48) values (Table 4.1). The second best-supported model included terms for PC1 score and signatures of both $\delta^{13}C$ and $\delta^{15}N$ SIs, and also received moderate *w*

(0.31) and r^2 (0.46) values (Table 4.1). No other models received a ΔAICc value ≤ 2 (Table 4.1). Parameter likelihoods indicated strong support for PC1 score (0.93), but only moderate support for year (0.46) and SI signatures of both $\delta^{13}C$ (0.42) and $\delta^{15}N$ (0.45, Table 4.2). Weighted parameter estimates and associated 95% CIs suggested that structurally larger female chinstrap penguins were heavier (102.22 ± 61.69 CI), while 95% CIs overlapped 0 for all year (2008: -27.40 ± 88.51 CI, 2009: -106.21 ± 140.54 CI) and isotope ($\delta^{13}C$: -3.88 ± 190.03 CI, $\delta^{15}N$: -144.36 ± 193.92 CI) parameters (Table 4.2). Analyses of variation in body mass of adult male chinstrap penguins resulted in only one model receiving a ΔAICc value ≤ 2, which included a term for PC1 score only (Table 4.1). This model received a high *w* value (0.72), but a weaker r^2 value (0.29, Table 4.1). Parameter likelihoods indicated stronger support for PC1 score (0.83), while all other parameters received only weak support (Table 4.2). Weighted parameter estimates and 95% CIs indicated that structurally larger male chinstrap penguins were heavier (128.82 ± 93.49 CI), however, 95% CIs overlapped 0 for all other parameters (Table 4.2). For adult chinstrap penguins there was no qualitative relationship between yearly sea ice conditions and body mass variation as the year term for both females and males received weaker support (0.46 and 0.07, respectively, for both reference years, Table 4.2).

One model received a ΔAICc value ≤ 2 for describing variation in body mass of adult female gentoo penguins, which included a term for PC1 score only (Table 4.1). This model received moderate *w* (0.55) and r^2 (0.21) values (Table 4.1). Parameter likelihoods indicated strong support for the PC1 term (0.98), while all other parameters received only weak support (Table 4.2). Weighted parameter estimates and 95% CIs indicated that structurally larger female gentoo penguins were heavier (92.03 ± 51.51 CI), however,

95% CIs overlapped 0 for all other parameters (Table 4.2). Model uncertainty was evident for analyses describing variation in body mass of adult male gentoo penguins as indicated by four models receiving ΔAICc values ≤ 2 associated with lower *w* (0.116 – 0.298) and r^2 (0.17 – 0.26) values (Table 4.1). Parameter likelihoods indicated strong support for PC1 score (0.93), moderately strong support for signatures of both SIs ($\delta^{13}C$ = 0.52, $\delta^{15}N$ = 0.52), but weaker support for the year term (0.40 for both references years, Table 4.2). Weighted parameter estimates indicated that structurally larger male gentoo penguins were heavier (97.61 ± 58.00 CI), while 95% CIs overlapped 0 for all other parameters (Table 4.2). Similar to adult analyses for the other species, there was no qualitative relationship between yearly sea ice conditions and body mass variation of adult gentoo penguins as the year term received weaker support (Table 4.2).

Adult female egg mass

Two models received ΔAICc values ≤ 2 for describing variation in female Adélie penguin egg mass. The most parsimonious model included terms for PC1 score and body mass and received a moderately high *w* (0.52) value, but a lower r^2 (0.18) value (Table 4.3). The second best-supported model included terms for PC1 score, nest initiation date and body mass, but received half the support (*w*) of the best supported-model (*w* = 0.21, r^2 = 0.19, Table 4.3). Parameter likelihoods indicated strong support for both PC1 score (0.99) and body mass (0.99). However, nest initiation date received only weak support (0.28, Table 4.4). No other parameters were supported by the data (Table 4.4). Weighted parameter estimates and associated 95% CIs indicated that heavier female Adélie penguins had larger egg masses (0.02 ± 0.01 CI, Table 4.4). However, structurally larger

female Adélies did not have larger egg masses as 95% CIs overlapped 0 (-0.34 ± 2.81 CI, Table 4.4). Importantly, there was no evidence that yearly sea ice conditions influenced female Adélie penguin egg mass as the year term received only weak support (parameter likelihood = 0.188 for both references years, Table 4.4). The best-supported model for describing variation in egg mass of adult female chinstrap penguins also included terms for PC1 score and body mass (Table 4.3), and received a lower *w* (0.33) value and r^2 (0.18) value. The second best-supported model was the equal-means model, which received a similar *w* (0.30) value as the best-supported model (Table 4.3). Parameter likelihoods suggested only moderate support for PC1 score and body mass parameters (0.45 for both, Table 4.4). Associated 95% CIs for both these parameters overlapped 0 (PC1 score: -0.17 ± 1.69 CI, body mass: 0.01 ± 0.01 CI) indicating that egg mass of adult female chinstrap penguins was independent of structural size and body mass (Table 4.4). Again, there was no evidence that yearly sea ice conditions influenced female chinstrap penguin egg mass as the year term received only weak support (parameter likelihood = 0.10 for both references years, Table 4.4). Lastly, seven models received ΔAICc values ≤ 2 for describing variation in female gentoo penguin egg mass. A high degree of model uncertainty was evident as both *w* and r^2 values were low across all supported models (*w*: 0.064 – 0.172, r^2: ≤ 0.11, Table 4.3). The most parsimonious model included the year term only, while the second best-supported model included terms for PC1 and body mass (Table 4.3). The third best-supported model was the equal-means model (Table 4.3). Other supported models included terms for nest initiation date, signatures of each SI, and year (Table 4.3). Parameter likelihoods suggested only moderate support for PC1 score and body mass parameters (0.43 for both, Table 4.4). Terms for year and nest initiation

date also received moderate support (parameter likelihoods = 0.35 [both references years] and 0.32, respectively, Table 4.4). Weighted parameter estimates and associated 95% CIs indicated that structurally larger (0.45 ± 0.95 CI) and heavier (0.002 ± 0.004 CI) female gentoo penguins did not have larger egg masses as 95% CIs overlapped 0 (Table 4.4). Again, there was no evidence that yearly sea ice conditions influenced female gentoo penguin egg mass as the year term received only weak support (Table 4.4).

Chick body mass at day five

Analyses of variation in body mass of five-day-old Adélie penguin chicks resulted in only one model receiving a ΔAICc value ≤ 2. This best-supported model received a moderately high *w* value (0.56) and included terms for PC1 score, hatch date, signatures of both $\delta^{13}C$ and $\delta^{15}N$ SIs and sex (Table 4.5). Parameter likelihoods indicated strong support for PC1 score (0.99), moderately strong support for sex (0.76), hatch date (0.68), and signatures of both $\delta^{13}C$ (0.63) and $\delta^{15}N$ (0.63) SIs (Table 4.6). Weighted parameter estimates and associated 95% CIs indicated that structurally larger five-day-old Adélie penguin chicks were heavier (61.29 ± 7.92 CI). For all other parameters, 95% CIs overlapped 0 (Table 4.6). Only one model received a ΔAICc value ≤ 2 for describing variation in body mass of five-day-old chinstrap penguin chicks, which included a term for PC1 score only and received a moderately high *w* value (0.67, Table 4.5). Parameter likelihoods indicated strong support for PC1 score (1.00), however all other parameters received only weak support (Table 4.6). Weighted parameter estimates and associated 95% CIs indicated that structurally larger five-day-old chinstrap chicks were heavier (46.31 ± 8.19 CI). For all other parameters, 95% CIs overlapped 0 (Table 4.6). Three

models received ΔAICc value ≤ 2 for describing variation in body mass of five-day-old gentoo penguin chicks. This best supported model included terms for PC1 score and sex and received twice the support (*w* = 0.42) of the next best model that included terms for PC1 score, hatch date, signatures of both $\delta^{13}C$ and $\delta^{15}N$ SIs and sex (*w* = 0.21, Table 4.5). The third best-supported model received a similar *w* value as the second best-supported model and included terms for PC1 score, hatch date and sex only (*w* = 0.21, Table 4.5). Parameter likelihoods indicated strong support for PC1 score (1.00) and sex (1.00, Table 4.6). Hatch date also received a moderately strong parameter likelihood (0.53), however all other parameters received only weak support (Table 4.6). Weighted parameter estimates and associated 95% CIs indicated that structurally larger five-day-old gentoo chicks were heavier (36.60 ± 5.05 CI), and that female five-day-old gentoo chicks were heavier (27.82 ± 14.21 CI, Table 4.6). There was no evidence that yearly sea ice conditions influenced five-day-old penguin chick body mass of any species as the year term received only weak support for all analyses (Table 4.6).

Chick body mass at day 15

Four models received ΔAICc values ≤ 2 for describing variation in body mass of 15-day-old Adélie penguin chicks. The most parsimonious model, which included the PC1 score term only, and the second best-supported model including terms for PC1 score and year, both received similar *w* values (0.29, Table 4.7). The third and fourth best-supported models received approximately half the support of the first and second models (*w* = 0.16 and 0.15, respectively) and included terms for PC1 score and sex, or both these terms and year (Table 4.7). Parameter likelihoods indicated strong support for PC1 score (1.00), and

weaker support for the sex (0.42) and year terms (0.49 for both reference years, Table 4.8). Weighted parameter estimates indicated that structurally larger 15-day-old Adélie penguin chicks were heavier (127.42 ± 14.49 CI). However, 95% CIs for all other parameters overlapped 0 (Table 4.8). Only one model received a ΔAICc value ≤ 2 for describing variation in body mass of 15-day-old chinstrap penguin chicks. The most parsimonious model included a term for PC1 score only and received a high *w* value (0.73, Table 4.7). Parameter likelihoods indicated strong support for PC1 score (1.00), while all other parameters received only weak support (Table 4.8). Weighted parameter estimates indicated that structurally larger 15-day-old chinstrap penguin chicks were heavier (88.47 ± 26.42 CI). However, 95% CIs for all other parameters overlapped 0 (Table 4.8). Two models received ΔAICc values ≤ 2 for describing variation in body mass of 15-day-old gentoo penguin chicks. The most parsimonious model, which included terms for PC1 score only, received approximately four times the support (w = 0.46) of the next best model that included terms for PC1 score and sex (w = 0.17, Table 4.7). Parameter likelihoods indicated strong support for PC1 score (1.00), while the sex term received more moderate support (0.48, Table 4.8). All other parameters received weak support (Table 4.8). Weighted parameter estimates indicated that structurally larger 15-day-old gentoo penguin chicks were heavier (118.74 ± 15.67 CI). However, 95% CIs for all other parameters overlapped 0 (Table 4.8). There was no evidence that yearly sea ice conditions influenced 15-day-old penguin chick body mass of any species as the year term received only weak support for all analyses (Table 4.8).

Chick body mass at 5 weeks (Anvers Island)

One model received a ΔAICc value ≤ 2 for describing variation in body mass of five-week-old Adélie penguin chicks, which included terms for PC1 score and year (Table 4.9). This best-supported model received moderately high *w* and r^2 values (0.63 and 0.64, respectively, Table 4.9). Parameter likelihoods indicated strong support for PC1 score (1.00) and year (0.84 for both reference years), while all other parameters received weaker support (Table 4.10). Weighted parameter estimates and associated 95% CIs suggested that structurally larger five-week-old Adélie penguin chicks were heavier (203.73 ± 54.98 CI), and that chicks reared during the second year of the study following the winter of low sea ice conditions were heavier (228.29 ± 175.10 CI). For all other parameters, 95% CIs overlapped 0 (Table 4.10). Body mass analyses of five-week-old chinstrap penguins resulted in three models received ΔAICc values ≤ 2. The most parsimonious model received approximately two times the support (*w* = 0.45) of the next best model, and included terms for PC1 score, sex and year (Table 4.9). The second and third best-supported models received similar *w* values (0.23 and 0.20, respectively) and included terms for PC1 score and year or PC1 score, sex and signatures of both $\delta^{13}C$ and $\delta^{15}N$ SIs (Table 4.9). Parameter likelihoods suggested strong support for PC1 score (1.00), as well as sex (0.76) and year (0.71 for both reference years, Table 4.10). Weighted parameter estimated and associated 95% CIs indicated that structurally larger five-week-old chinstrap penguins were heavier (260.33 ± 62.26 CI), in addition chicks reared in year three of the study following the winter of higher sea ice coverage were lighter (-198.29 ± 190.93, Table 4.10). For all other parameters, 95% CIs overlapped 0 (Table 4.10). Only one model received a ΔAICc value ≤ 2 for describing variation in

body mass of five-week-old gentoo penguin chicks, which included a term for PC1 score only (Table 4.9). This best-supported model had a moderately high *w* (0.56) and higher r^2 (0.77) values (Table 4.9). Parameter likelihoods indicated strong support for PC1 score (1.00) and only weaker support for all other parameters (Table 4.10). Weighted parameter estimated and associated 95% CIs indicated that structurally larger five-week-old gentoo penguins were heavier (449.93 ± 78.55 CI, Table 4.10). Unlike the other two species, there was no evidence that yearly sea ice conditions influenced five-week-old gentoo penguin chick body mass as the year term received only weak support (Table 4.10).

Chick body mass at 5 weeks (regional islands)

Only one model received a ΔAICc value ≤ 2 for describing variation in body mass of five-week-old Adélie penguin chicks at the regional scale, which included terms for PC1 score, sex, signatures of both $\delta^{13}C$ and $\delta^{15}N$ SIs and year (Table 4.11). This best-supported model received very high *w* value (0.99) and higher r^2 value (0.62). Parameter likelihoods indicated strong support for all these parameters and weak support for all remaining parameters (Table 4.12). Weighted parameter estimates and associated 95% CIs suggested that structurally larger five-week-old Adélie penguins were heavier (192.99 ± 45.97 CI) and chicks enriched in $\delta^{15}N$ were heavier (247.96 ± 170.59 CI, Fig. 4.5). Further, chicks were lighter in both the second (-175.92 ± 145.07 CI) and particularly the third (-512.37 ± 155.61 CI) year of the study (Table 4.12). For all other parameters, 95% CIs overlapped 0 (Table 4.12).

Discussion

Regional breeding population changes among *Pygoscelis* penguins throughout the western AP are key examples of climate-driven shifts in bio-geographic range (Parmesan 2006, Forcada and Trathan 2009). Here, decreases in numbers of breeding penguins (i.e., range contractions) have occurred at species' relative northern range margins, while increases in numbers of breeding penguins (i.e., range expansions) have occurred at species' relative southern range margins, resulting in the overall pole-ward displacement of individuals (Fig. 4.1). Although the issue has been well documented (Ducklow et al. 2007, Trivelpiece et al. 2011, Lynch et al. 2012), the mechanistic relationships that link environmental variability with demographic parameters that drive population changes remain a topic of debate (Trivelpiece et al. 2011, W. R. Fraser unpubl. data, Fraser et al. 2013). To date, empirical studies have almost entirely focused on *population-level* indices of forging and relationships with variability in breeding effort during later chick-rearing phases, as well as survival (Fraser and Hofmann 2003, Hinke et al. 2007, Trivelpiece et al. 2011). Only Chapman *et al*. (2011) considered this issue at the individual-level, but within the context of a modeling study. The current study using SI methodology expands this body of literature by specifically evaluating the effects of *individual-level* foraging, from a trophic perspective, on *individual-level* variability in breeding performance in terms of body and egg mass. Further, our use of blood $\delta^{13}C$ and $\delta^{15}N$ signatures as a proxy of trophic foraging allowed for assessing dietary relationships with breeding performance during early stages of reproductive investment (i.e., egg

production and very early chick-rearing) unlike other studies based on traditional diet sampling that is difficult outside of the later chick-rearing period.

We predicted that adult *Pygoscelis* penguins nesting at Anvers Island with enriched $\delta^{13}C$ and $\delta^{15}N$ blood SI signatures would be heavier after accounting for other parameters that we expected would influence body mass such as structural size within sexes, nest initiation date and year. Our data did not support this hypothesis. There was no evidence that blood $\delta^{13}C$ and $\delta^{15}N$ SI signatures accounted for any variation in body mass of males or females of all three species. The only important parameter for predicting body mass variation was PC1 score for structural size, which would be expected. Blood $\delta^{13}C$ and $\delta^{15}N$ SI signatures of adults at the one-egg stage reflect trophic foraging by each individual over the previous several weeks leading up to the arrival at breeding rookeries. Previously, we argued that adult male and female chinstrap and Adélie penguins may be relying on a fish based diet prior to egg production in association with off-shore winter habitats in sea ice-free or sea ice-associated regions, respectively. However, gentoo penguins may rely primarily on krill while wintering near-shore close to breeding colonies (Williams 1991, K. B. Gorman this dissertation Chapter 2). The occupied dietary niches of adult *Pygoscelis* penguins show little trophic variability within species, and thus, do not account for variation in body mass at the one-egg stage. Possibly, individuals that do return to breed in a given season may have in fact reached some critical threshold of body condition that allows for successful migration and investment in early reproduction, which is reflected in very consistent blood SI signatures. Alternatively, it is possible that trophic variability prior to breeding results in not reaching a critical threshold of body condition in order to return to breed, which is not

captured by our data. We did detect a negative effect of nest initiation date on body mass of male Adélie penguins. However, this relationship was not evident for females. Male Adélie penguins are known to fast while defending territories at rookeries prior to the arrival of females (Ainley 2002). Thus, a decline in body mass with nest initiation date is understandable and likely reflects capital breeding by this species. Further, male Adélies take the first incubation shift after females complete the clutch (Ainley 2002). Therefore, male body reserves are key to successful early reproductive investment by Adélies. However, our data suggest that a narrow dietary niche is what drives this successful early investment in egg production (see Gorman et al. 2014 Fig. 3, K. B. Gorman this dissertation Chapter 2 Fig. 2.6a). Interestingly, we did not detect an effect of nest initiation date on body mass of either male chinstrap or gentoo penguins. Nesting male gentoo penguins at Anvers Island have been observed to leave the nest during egg laying (K. B. Gorman pers. obs.). Thus, they appear to be foraging during this phase of breeding and not investing body reserves in early reproduction, unlike Adélie penguins. Chinstrap penguins, like gentoos, nest seasonally later than Adélies at Anvers Island (Ducklow et al. 2007). Although chinstraps were never observed actively foraging during egg laying, they may have a shorter duration between arrival and egg production, which would account for a lack of investment of body reserves by males during this phase of breeding.

Similarly, our data did not support a hypothesis for trophic foraging as an important predictor of variation in egg mass among females of all three species. Interestingly, heavier female Adélies had larger eggs, however, structurally larger females did not. Similar relationships were not found for female chinstrap or gentoo penguins. Again, these results are similar to those for adult males in suggesting that

Adélie penguins rely on body reserves for investment in early reproduction, whereas chinstrap and gentoo penguins do not. These are fundamental differences between species in their reproductive energetic strategies and likely reflect each species adaptations to either sea ice-dominated or sea ice-free habitats.

Body mass of five- and 15-day-old, as well as five-week-old *Pygoscelis* penguin chicks reared at Anvers Island was independent of parental trophic foraging. The only important predictor of body mass variation of these younger chicks was structural size, as would be expected. These results suggest again that provisioning parents fed their young chicks consistent diets that resulted in little trophic variation to influence body mass within the foraging range of breeding rookeries. Previous analyses indicated these diets were characterized primarily by Antarctic krill at the five-week stage (see K. B. Gorman this dissertation Chapter 2 Fig. 2.9a), which is known as a dominant prey item within northern regions of the western AP (Fraser and Hofmann 2003, Trivelpiece et al. 2011). Interestingly, five-week-old Adélie penguins were heavier during the second year of the study following a winter of extremely low sea ice conditions (Fig. 4.4b). Provisioning diet during this year was previously shown to have a larger proportion of the krill *Thysanoessa macrura* (see K. B. Gorman this dissertation Chapter 2 Fig. 2.9a), which may have resulted in enhanced body mass during this year as Ruck et al. (2014) showed that *T. macrura* holds the highest prey quality in terms of lipid content and energy density of other dominant krill species such as Antarctic krill and crystal krill (*Euphausia crystallorophias*). Chinstrap penguin chicks were lighter in the third year of the study following the winter of higher sea ice coverage (Fig. 4.4b), which generally follows our predictions for the species response to sea ice conditions. However, previous analyses

indicated that the main difference in diet during year three for chinstrap chicks was the greater proportion of lanternfish (see K. B. Gorman this dissertation Chapter 2 Fig. 2.9a). Why this might result in lighter chicks for chinstrap penguins is unclear because lanternfish is known to have a high energy density (Ruck et al. 2014). We detected an effect of sex on body mass of five-day-old gentoo penguins chicks were females were heavier than males, however this relationship was not apparent for older 15-day-old chicks.

Our most compelling result for an effect of trophic foraging on body mass of five-week-old chicks was detected within regional analyses of Adélie penguins. We have previously shown that chicks reared at Avian Island, a higher latitude rookery that experiences greater over-winter sea ice coverage (Fig. 4.4), are enriched in blood $\delta^{15}N$ signatures (see K. B. Gorman this dissertation Chapter 2 Figs. 2.5, 2.6d) and also are heavier (Fig. 4.5). Isotopic enrichment at Avian Island appears to be due to the inclusion of dietary items that are more characteristic of higher latitude Southern Ocean food-webs such crystal krill (see K. B. Gorman this dissertation Chapter 2 Fig. 2.9a, b). However, the dominant prey item at Avian is Antarctic krill similar to Anvers Island in year one and three of the study. Ruck et al. (2014) showed that Antarctic krill of the southwestern AP had approximately 20% higher lipid content than those found in the northwestern AP. Thus, regional differences in prey quality and composition are likely drivers of enhanced body mass of Adélie penguins at southern rookeries.

An important issue in the interpretation of the data presented here is whether or not the spatial isotopic variability detected among five-week-old Adélie penguin chicks reflects actual differences in their diet (as has been interpreted here) versus simply

reflecting variability in isotopic baselines of the marine ecosystem (i.e., signal to noise ratio). As has been shown previously (Chapter 2), isotopic baselines vary slightly among phytoplankton, and vary little among Antarctic krill, across the western AP (Table 2.1 and Figure 2.8). Thus, isotopic baseline variability in phytoplankton and krill is much smaller than the observed differences in penguin isotope signatures, which would indicate that diet is in fact driving the differences in body mass of five-week-old Adélie penguin chicks. However, isotopic baseline variability undoubtedly drives to some degree the variability in penguin predator isotope signatures, the question is how much? It would have been useful to quantify baseline isotopic variability at least across the years of study during the summer months and future studies would benefit from such an approach.

Our study was unable to assess an effect on chick body mass variation due to foraging on fish that occur at higher trophic levels (following Chapman et al. 2011, see also K. B. Gorman this dissertation Chapter 2 Table 2.1, Fig. 2.8) as the fish component of penguin diets was very small over the course of the study. However, our results do suggest that the inclusion of dietary items that are more characteristic of higher latitude Southern Ocean food-webs, which include Antarctic silverfish, result in greater body mass among five-week-old chicks. Thus, we interpret our results as being important in developing the empirical evidence for how changes in sea ice-associated food-webs may in fact influence fledge mass of Adélie penguin chicks and possibly their survival and recruitment probability that would shape differences in regional populations trajectories.

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Table 4.1. Candidate models describing variation in body mass of adult *Pygoscelis* penguins breeding near Anvers Island. Models presented are those determined to be most parsimonious, as well as all models receiving ΔAICc values ≤ 2.

Species	Response variable	Model number	Explanatory variable	Number of parameters	ΔAICc	*w*	r^2
Adélie penguin	F-body mass	10	PC1 + NestInit + $\delta^{13}C$ + $\delta^{15}N$	6	0.000	0.569	0.35
	M-body mass	10	PC1 + NestInit + $\delta^{13}C$ + $\delta^{15}N$	6	0.000	0.537	0.39
		18	PC1 + NestInit + $\delta^{13}C$ + $\delta^{15}N$ + Year	8	0.339	0.453	0.43
Chinstrap penguin	F-body mass	11	PC1 + Year	5	0.000	0.431	0.48
		8	PC1 + $\delta^{13}C$ + $\delta^{15}N$	5	0.664	0.309	0.46
	M-body mass	3	PC1	3	0.000	0.723	0.29
Gentoo penguin	F-body mass	3	PC1	3	0.000	0.548	0.21
	M-body mass	8	PC1 + $\delta^{13}C$ + $\delta^{15}N$	5	0.000	0.298	0.26
		11	PC1 + Year	5	0.072	0.288	0.26
		3	PC1	3	1.557	0.137	0.17
		10	PC1 + NestInit + $\delta^{13}C$ + $\delta^{15}N$	6	1.898	0.116	0.26

Abbreviations: ΔAICc = Akaike's Information Criterion corrected for small sample size, *w* = Akaike weight, F-body mass = female body mass, M-body mass = male body mass, PC1 = principal components score, NestInit = nest initiation date, $\delta^{13}C$ = carbon stable isotope signature, $\delta^{15}N$ = nitrogen stable isotope signature.

Table 4.2. Parameter estimates and likelihoods from candidate models for describing variation in body mass of adult *Pygoscelis* penguins breeding near Anvers Island. Parameter estimates (±1 SE) are weighted averages, and standard errors are based on unconditional variances. Parameter likelihoods are Akaike weight (*w*) values summed across all models that include the variable.

Response variable	Explanatory variable	Parameter likelihoods			Parameter estimates ± 1 SE		
		Adélie	Chinstrap	Gentoo	Adélie	Chinstrap	Gentoo
F-body mass	Intercept	1.000	1.000	1.000	5102.86 ± 1984.65	4883.30 ± 3226.01	5849.41 ± 2967.94
	PC1	0.998	0.933	0.975	115.38 ± 26.91	102.22 ± 31.47	92.03 ± 26.28
	NestInit	0.651	0.084	0.084	-10.81 ± 6.37	-62.53 ± 69.86	-0.24 ± 0.64
	$\delta^{13}C$	0.854	0.422	0.352	28.13 ± 51.65	-3.88 ± 96.95	15.81 ± 92.47
	$\delta^{15}N$	0.854	0.449	0.352	-101.18 ± 72.02	-144.36 ± 98.94	-97.07 ± 88.09
	Year2008	0.128	0.461	0.216	2.83 ± 15.17	-27.40 ± 45.16	83.09 ± 94.66
	Year2009	0.128	0.461	0.216	6.04 ± 14.48	-106.21 ± 71.70	61.78 ± 66.87
	NestInit*Year2008	0.000007	0.00009	0.0001	0.0001 ± 0.0002	0.003 ± 0.004	-0.0007 ± 0.003
	NestInit*Year2009	0.000007	0.00009	0.0001	-0.00004 ± 0.0002	0.003 ± 0.004	-0.0006 ± 0.003
	$\delta^{13}C$*Year2008	0.000024	0.000004	0.00006	-0.00003 ± 0.009	-0.005 ± 0.006	-0.05 ± 0.07
	$\delta^{13}C$*Year2009	0.000024	0.000004	0.00006	-0.01 ± 0.01	-0.0009 ± 0.003	-0.07 ± 0.09
	$\delta^{15}N$*Year2008	0.000024	0.000004	0.00006	-0.007 ± 0.009	0.0007 ± 0.002	-0.006 ± 0.02
	$\delta^{15}N$*Year2009	0.000024	0.000004	0.00006	-0.005 ± 0.008	-0.001 ± 0.003	0.0003 ± 0.03
	$\delta^{13}C$*NestInit	0.00014	-2.088	0.00009	-0.002 ± 0.002	-2.09 ± 2.23	-0.0005 ± 0.001
	$\delta^{15}N$*NestInit	0.00014	1.177	0.00009	-0.002 ± 0.003	1.18 ± 1.25	0.002 ± 0.003
M-body mass	Intercept	1.000	1.000	1.000	9397.34 ± 3700.00	3864.12 ± 978.07	7200.22 ± 3198.27
	PC1	0.999	0.827	0.932	168.89 ± 31.25	128.82 ± 47.70	97.60 ± 29.59
	NestInit	0.989	0.119	0.213	-38.76 ± 11.86	-4.72 ± 5.22	2.44 ± 2.97
	$\delta^{13}C$	0.993	0.080	0.523	167.98 ± 112.35	-7.95 ± 34.04	79.14 ± 89.44

	$\delta^{15}N$	0.993	0.079	0.520	-69.40 ± 129.80	-8.38 ± 17.47	44.39 ± 101.12
	Year2008	0.454	0.074	0.396	13.27 ± 69.19	11.59 ± 18.76	-55.52 ± 105.63
	Year2009	0.454	0.074	0.396	104.19 ± 93.51	2.11 ± 15.24	-68.31 ± 80.84
	NestInit*Year2008	0.00001	0.0003	0.002	-0.0008 ± 0.0009	-0.008 ± 0.01	0.005 ± 0.07
	NestInit*Year2008	0.00001	0.0003	0.002	-0.001 ± 0.001	-0.01 ± 0.02	-0.05 ± 0.08
	$\delta^{13}C$*Year2008	0.0000004	0.00004	0.0000007	-0.0004 ± 0.0004	-0.02 ± 0.04	0.003 ± 0.0009
	$\delta^{13}C$*Year2008	0.0000004	0.00004	0.0000007	-0.0003 ± 0.0003	-0.05 ± 0.08	0.003 ± 0.0006
	$\delta^{15}N$*Year2009	0.0000004	0.00004	0.0000007	-0.00002 ± 0.0002	-0.06 ± 0.06	0.003 ± 0.0005
	$\delta^{15}N$*Year2009	0.0000004	0.00004	0.0000007	-0.0001 ± 0.0002	-0.05 ± 0.06	0.002 ± 0.0006
	$\delta^{13}C$*NestInit	0.0002	0.0006	0.001	0.01 ± 0.01	-0.03 ± 0.06	0.01 ± 0.02
	$\delta^{15}N$*NestInit	0.0002	0.0006	0.001	-0.008 ± -0.009	0.03 ± 0.05	-0.02 ± 0.03

Abbreviations: ± 1SE = plus or minus 1 standard error, F-body mass = female body mass, M-body mass = male body mass, PC1 = principal components score, NestInit = nest initiation date, $\delta^{13}C$ = carbon stable isotope signature, $\delta^{15}N$ = nitrogen stable isotope signature.

Table 4.3. Candidate models describing variation in egg mass of female *Pygoscelis* penguins breeding near Anvers Island. Models presented are those determined to be most parsimonious, as well as all models receiving ΔAICc values ≤ 2.

Species	Response variable	Model number	Explanatory variable	Number of parameters	ΔAICc	*w*	r^2
Adélie penguin	Egg mass	4	PC1 + F-Body mass	4	0.000	0.523	0.18
		6	PC1 + NestInit + F-Body mass	5	1.821	0.211	0.19
Chinstrap penguin	Egg mass	4	PC1 + F-Body mass	3	0.000	0.328	0.18
		1	Equal-means model	2	0.180	0.299	NA
Gentoo penguin	Egg mass	2	Year	4	0.000	0.172	0.09
		4	PC1 + F-Body mass	4	0.127	0.162	0.09
		1	Equal-means model	2	0.625	0.126	NA
		6	PC1 + NestInit + F–Body mass	5	0.978	0.106	0.11
		5	$\delta^{13}C + \delta^{15}N$	4	1.667	0.075	0.06
		3	NestInit	3	1.714	0.073	0.02
		10	NestInit + Year	5	1.970	0.064	0.09

Abbreviations: ΔAICc = Akaike's Information Criterion corrected for small sample size, *w* = Akaike weight, F-body mass = female body mass, PC1 = principal components score, NestInit = nest initiation date, $\delta^{13}C$ = carbon stable isotope signature, $\delta^{15}N$ = nitrogen stable isotope signature, NA = r^2 value not available.

Table 4.4. Parameter estimates and likelihoods from candidate models for describing variation in egg mass of female *Pygoscelis* penguins breeding near Anvers Island. Parameter estimates (±1 SE) are weighted averages, and standard errors are based on unconditional variances. Parameter likelihoods are Akaike weight (*w*) values summed across all models that include the variable.

Response variable	Explanatory variable	Parameter likelihoods			Parameter estimates ± 1 SE		
		Adélie	Chinstrap	Gentoo	Adélie	Chinstrap	Gentoo
Egg mass	Intercept	1.000	1.000	1.000	51.69 ± 31.35	93.90 ± 70.01	95.35 ± 47.51
	PC1	0.986	0.450	0.431	-0.34 ± 1.43	-0.16 ± 0.86	0.45 ± 0.48
	NestInit	0.281	0.237	0.321	0.07 ± 0.12	-0.08 ± 0.13	-0.002 ± 0.06
	$\delta^{13}C$	0.098	0.092	0.211	-0.11 ± 0.37	0.64 ± 1.12	-0.65 ± 1.14
	$\delta^{15}N$	0.098	0.092	0.211	-0.04 ± 0.39	-0.54 ± 0.84	0.14 ± 1.22
	Year2008	0.188	0.104	0.348	-0.64 ± 1.53	-0.77 ± 0.97	2.18 ± 2.20
	Year2009	0.188	0.104	0.348	-0.63 ± 1.45	-0.48 ± 0.77	1.96 ± 1.95
	F-Body Mass	0.986	0.450	0.431	0.02 ± 0.006	0.01 ± 0.01	0.002 ± 0.002
	F-Body Mass*Year2008	0.010	0.001	0.006	-0.00007 ± 0.0002	0.00001 ± 0.00002	-0.00003 ± 0.00006
	F-Body Mass*Year2009	0.010	0.001	0.006	-0.00006 ± 0.0002	0.00001 ± 0.00002	-0.00004 ± 0.00008

Abbreviations: ± 1SE = plus or minus 1 standard error, F-body mass = female body mass, PC1 = principal components score, NestInit = nest initiation date, $\delta^{13}C$ = carbon stable isotope signature, $\delta^{15}N$ = nitrogen stable isotope signature.

Table 4.5. Candidate models describing variation in body mass of five-day-old *Pygoscelis* penguin chicks reared near Anvers Island. Models presented are those determined to be most parsimonious, as well as all models receiving ΔAICc values ≤ 2.

Species	Response variable	Model number	Explanatory variable	Number of parameters	ΔAICc	*w*	r^2
Adélie penguin	Chick mass at day 5	12	PC1 + HatchDate + δ^{13}C + δ15N + Sex, random = ~1|Nest	8	0.000	0.558	NA
Chinstrap penguin	Chick mass at day 5	4	PC1, random = ~1|Nest	4	0.000	0.670	NA
Gentoo penguin	Chick mass at day 5	8	PC1 + Sex, random = ~1|Nest	5	0.000	0.423	NA
		12	PC1 + HatchDate + δ^{13}C + δ15N + Sex, random = ~1|Nest	8	1.370	0.213	NA
		10	PC1 + HatchDate + Sex, random = ~1|Nest	6	1.433	0.207	NA

Abbreviations: ΔAICc = Akaike's Information Criterion corrected for small sample size, *w* = Akaike weight, PC1 = principle components score, δ^{13}C = carbon stable isotope signature, δ^{15}N = nitrogen stable isotope signature, NA = r^2 value not available for linear mixed effects models.

Table 4.6. Parameter estimates and likelihoods from candidate models for describing variation in body mass of five-day-old *Pygoscelis* penguin chicks reared near Anvers Island. Parameter estimates (±1 SE) are weighted averages, and standard errors are based on unconditional variances. Parameter likelihoods are Akaike weight (*w*) values summed across all models that include the variable.

Response variable	Explanatory variable	Parameter likelihoods			Parameter estimates ± 1 SE		
		Adélie	Chinstrap	Gentoo	Adélie	Chinstrap	Gentoo
Chick mass at day 5	Intercept	1.000	1.000	1.000	-3331.30 ± 542.48	308.48 ± 11.68	393.05 ± 207.77
	PC1	0.999	0.999	0.999	61.29 ± 4.04	46.31 ± 4.18	36.60 ± 2.58
	Hatch date	0.679	0.044	0.527	1.81 ± 1.96	-0.01 ± 0.11	-0.49 ± 0.66
	$\delta^{13}C$	0.634	0.002	0.246	-18.10 ± 15.73	-0.02 ± 0.06	3.86 ± 6.40
	$\delta^{15}N$	0.634	0.002	0.246	18.65 ± 17.07	-0.02 ± 0.06	-2.22 ± 4.18
	Sex	0.763	0.231	0.999	2.18 ± 6.87	0.11± 3.02	27.82 ± 7.25
	Year2008/09	0.331	0.124	0.157	7.46 ± 17.08	-2.83 ± 5.96	-2.98 ± 6.84
	Year2009/10	0.331	0.124	0.157	5.77 ± 10.70	-3.83 ± 7.50	-1.78 ± 3.93
	Hatch date*Year2008/09	1.475E-32	1.11E-13	5.02E-26	6.38E-32 ± 2.20E-31	-5.63E-13 ± 2.46E-12	-1.58E-25 ± 4.70E-25
	Hatch date*Year2009/10	1.475E-32	1.11E-13	5.02E-26	1.51E-31 ± 3.55E-31	-6.16E-13 ± 2.49E-12	-7.62E-26 ± 3.48E-25
	$\delta^{13}C$*Year2008/09	5.127E-27	3.04E-12	6.89E-27	-1.143E-24 ± 2.37E-24	-4.34E-10 ± 1.31E-09	-3.38E-25 ± 7.85E-25
	$\delta^{13}C$*Year2009/10	5.127E-27	3.04E-12	6.89E-27	-8.28E-25 ± 1.82E-24	2.91E-10 ± 1.16E-09	-1.53E-25 ± 4.92E-25
	$\delta^{15}N$*Year2008/09	5.127E-27	3.04E-12	6.89E-27	4.20E-25 ± 9.36E-25	5.17E-10 ± 1.25E-09	1.41E-25 ± 4.34E-25
	$\delta^{15}N$*Year2009/10	5.127E-27	3.04E-12	6.89E-27	3.12E-25 ± 7.25E-25	9.71E-11 ± 6.71E-10	2.76E-25 ± 6.59E-25

Abbreviations: ± 1SE = plus or minus 1 standard error, PC1 = principal components score, $\delta^{13}C$ = carbon stable isotope signature, $\delta^{15}N$ = nitrogen stable isotope signature.

Table 4.7. Candidate models describing variation in body mass of 15-day-old *Pygoscelis* penguin chicks reared near Anvers Island. Models presented are those determined to be most parsimonious, as well as all models receiving ΔAICc values ≤ 2.

Species	Response variable	Model number	Explanatory variable	Number of parameters	ΔAICc	*w*	r^2
Adélie penguin	Chick mass at day 15	4	PC1, random = ~1|Nest	4	0.000	0.291	NA
		14	PC1 + Year, random = ~1|Nest	6	0.002	0.291	NA
		8	PC1 + Sex, random = ~1|Nest	5	1.225	0.158	NA
		18	PC1 + Sex + Year, random = ~1|Nest	7	1.306	0.151	NA
Chinstrap penguin	Chick mass at day 15	4	PC1, random = ~1|Nest	4	0.000	0.733	NA
Gentoo penguin	Chick mass at day 15	4	PC1, random = ~1|Nest	4	0.000	0.462	NA
		8	PC1 + Sex, random = ~1|Nest	5	1.993	0.171	NA

Abbreviations: ΔAICc = Akaike's Information Criterion corrected for small sample size, *w* = Akaike weight, PC1 = principle components score, NA = r^2 value not available for linear mixed effects models.

Table 4.8. Parameter estimates and likelihoods from candidate models for describing variation in body mass of 15-day-old *Pygoscelis* penguin chicks reared near Anvers Island. Parameter estimates (±1 SE) are weighted averages, and standard errors are based on unconditional variances. Parameter likelihoods are Akaike weight (*w*) values summed across all models that include the variable.

Response variable	Explanatory variable	Parameter likelihoods			Parameter estimates ± 1 SE		
		Adélie	Chinstrap	Gentoo	Adélie	Chinstrap	Gentoo
Chick mass at day 15	Intercept	1.000	1.00	1.0000	1189.77 ± 39.86	1039.89 ± 29.71	1441.14 ± 738.01
	PC1	0.999	0.999	0.999	127.42 ± 7.39	88.47 ± 13.48	118.74 ± 7.99
	Hatch date	0.110	0.040	0.294	-0.07 ± 0.49	-0.01 ± 0.34	-1.51 ± 2.44
	$\delta^{13}C$	0.011	0.002	0.146	0.06 ± 0.60	-0.06 ± 0.18	12.55 ± 23.15
	$\delta^{15}N$	0.011	0.002	0.146	0.027 ± 0.79	0.03 ± 0.13	-5.39 ± 11.05
	Sex	0.419	0.226	0.483	-8.15 ± 14.36	1.48 ± 10.88	-4.00 ± 12.83
	Year2008/09	0.493	0.050	0.239	19.47± 27.82	0.36± 3.47	-22.34 ± 42.00
	Year2009/10	0.493	0.050	0.239	30.08 ± 36.39	-0.70 ± 3.78	-12.92 ± 28.78
	Hatch date*Year2008/09	4.74E-32	2.77E-09	1.23E-23	-7.76E-31 ± 1.98E-30	2.06E-08 ± 1.26E-07	1.02E-22 ± 3.43E-22
	Hatch date*Year2009/10	4.74E-32	2.77E-09	1.23E-23	-5.68E-31 ± 1.63E-30	-1.12E-08 ± 1.35E-07	9.08E-23 ± 3.20E-22
	$\delta^{13}C$*Year2008/09	3.01E-33	1.30E-09	9.67E-23	1.24E-30 ± 3.18E-30	-1.98E-06 ± 4.17E-06	-6.50E-21 ± 3.33E-20
	$\delta^{13}C$*Year2009/10	3.01E-33	1.30E-09	9.67E-23	5.71E-31 ± 1.63E-30	-1.80E-06 ± 3.82E-06	-3.80E-20 ± 8.17E-20
	$\delta^{15}N$*Year2008/09	3.01E-33	1.30E-09	9.67E-23	-8.55E-31 ± 1.95E-30	8.10E-07 ± 2.00E-06	2.56E-20 ± 5.88E-20
	$\delta^{15}N$*Year2009/10	3.01E-33	1.30E-09	9.67E-23	-3.00E-31 ± 1.02E-30	1.79E-06 ± 3.85E-06	5.39E-20 ± 1.11E-19

Abbreviations: ± 1SE = plus or minus 1 standard error, PC1 = principal components score, $\delta^{13}C$ = carbon stable isotope signature, $\delta^{15}N$ = nitrogen stable isotope signature.

Table 4.9. Candidate models describing variation in body mass of 5-week-old *Pygoscelis* penguin chicks reared near Anvers Island. Models presented are those determined to be most parsimonious, as well as all models receiving ΔAICc values ≤ 2.

Species	Response variable	Model number	Explanatory variable	Number of parameters	ΔAICc	*w*	r^2
Adélie penguin	Chick mass at week 5	11	PC1 + Year	5	0.000	0.626	0.64
Chinstrap penguin	Chick mass at week 5	14	PC1 + Sex + Year	6	0.000	0.446	0.78
		11	PC1 + Year	5	1.286	0.234	0.76
		9	PC1 + Sex + $\delta^{13}C$ +$\delta^{15}N$	6	1.597	0.201	0.78
Gentoo penguin	Chick mass at week 5	4	PC1	3	0.000	0.562	0.77

Abbreviations: ΔAICc = Akaike's Information Criterion corrected for small sample size, *w* = Akaike weight, PC1 = principle components score.

Table 4.10. Parameter estimates and likelihoods from candidate models for describing variation in body mass of 5-week-old *Pygoscelis* penguin chicks reared near Anvers Island. Parameter estimates (±1 SE) are weighted averages, and standard errors are based on unconditional variances. Parameter likelihoods are Akaike weight (*w*) values summed across all models that include the variable.

Response variable	Explanatory variable	Parameter likelihoods			Parameter estimates ± 1 SE		
		Adélie	Chinstrap	Gentoo	Adélie	Chinstrap	Gentoo
Chick mass at 5 weeks	Intercept	1.000	1.000	1.000	2062.96 ± 1712.15	2568.06 ± 1755.79	3507.81 ± 911.28
	PC1	0.999	0.999	0.999	203.73 ± 28.05	260.33 ± 31.77	449.93 ± 40.08
	$\delta^{13}C$	0.158	0.226	0.075	-24.20 ± 27.16	-32.95 ± 44.07	10.71 ± 13.61
	$\delta^{15}N$	0.158	0.226	0.075	34.18 ± 34.14	-14.63 ± 26.65	-18.06 ± 20.32
	Sex	0.365	0.761	0.301	-17.37 ± 31.69	118.36 ± 68.60	-15.84 ± 39.82
	Year2008/09	0.842	0.706	0.184	228.29 ± 89.34	-50.38 ± 73.04	-27.04 ± 33.88
	Year2009/10	0.842	0.706	0.184	-35.13 ± 84.68	-198.29 ± 97.41	-0.46 ± 31.65
	$\delta^{13}C$*Year2008/09	5.81E-12	7.89E-13	2.82E-18	5.50E-09 ± 8.98E-09	-3.63E-10 ± 1.45E-09	1.34E-16 ± 8.77E-15
	$\delta^{13}C$*Year2009/10	5.81E-12	7.89E-13	2.82E-18	3.61E-09 ± 7.46E-09	-1.32E-09 ± 1.66E-09	-5.65E-15 ± 9.56E-15
	$\delta^{15}N$*Year2008/09	5.81E-12	7.89E-13	2.82E-18	1.45E-09 ± 6.11E-09	3.20E-10 ± 5.94E-10	-5.67E-16 ± 6.31E-15
	$\delta^{15}N$*Year2009/10	5.81E-12	7.89E-13	2.82E-18	4.69E-10 ± 6.43E-09	-1.97E-10 ± 6.43E-10	-1.48E-16 ± 4.55E-15
	$\delta^{13}C$*Sex	9.09E-10	4.94E-12	4.59E-15	-4.56E-17 ± 2.78E-07	1.78E-09 ± 2.16E-09	-1.22E-12 ± 1.99E-12
	$\delta^{15}N$*Sex	9.09E-10	4.94E-12	4.59E-15	1.64E-08 ± 2.78E-07	-3.60E-10 ± 1.48E-09	7.34E-13 ± 2.56E-12

Abbreviations: ± 1SE = plus or minus 1 standard error, PC1 = principal components score, $\delta^{13}C$ = carbon stable isotope signature, $\delta^{15}N$ = nitrogen stable isotope signature.

Table 4.11. Candidate models describing variation in body mass of 5-week-old Adélie penguin chicks reared at regional rookeries along the western Antarctic Peninsula. Models presented are those determined to be most parsimonious, as well as all models receiving ΔAICc values ≤ 2.

Species	Response variable	Model number	Explanatory variable	Number of parameters	ΔAICc	*w*	r^2
Adélie penguin	Chick mass at week 5	22	Sex + PC1 + $\delta^{13}C$ + $\delta^{15}N$ + Year	8	0.000	0.987	0.62

Abbreviations: ΔAICc = Akaike's Information Criterion corrected for small sample size, *w* = Akaike weight, PC1 = principle components score, $\delta^{13}C$ = carbon stable isotope signature, $\delta^{15}N$ = nitrogen stable isotope signature.

Table 4.12. Parameter estimates and likelihoods from candidate models for describing variation in body mass of 5-week-old Adélie penguin chicks reared at regional rookeries along the western Antarctic Peninsula. Parameter estimates (±1 SE) are weighted averages, and standard errors are based on unconditional variances. Parameter likelihoods are Akaike weight (*w*) values summed across all models that include the variable.

Response variable	Explanatory variable	Parameter likelihoods	Parameter estimates ± 1 SE
Chick mass at 5 weeks	Intercept	1.000	1305.95 ± 1926.42
	Year2008/09	0.999	-175.92 ± 74.02
	Year2009/10	0.999	-512.37 ± 79.39
	PC1	0.999	192.99 ±23.45
	Sex	0.995	-27.94 ± 66.16
	LocationANVERS	0.006	-0.78 ± 0.86
	$\delta^{13}C$	0.987	-2.84 ± 49.45
	$\delta^{15}N$	0.987	247.96 ± 87.04
	LocationANVERS*Year.22	2.65E-13	1.23E-10 ± 1.33E-10
	LocationANVERS*Year.23	2.65E-13	1.67E-10 ± 1.75E-10
	LocationANVERS*$\delta^{13}C$	2.77E-21	-7.46E-19 ± 9.19E-19
	LocationANVERS*$\delta^{15}N$	2.77E-21	-1.47E-18 ± 1.68E-18

Abbreviations: ± 1SE = plus or minus 1 standard error, PC1 = principal components score, $\delta^{13}C$ = carbon stable isotope signature, $\delta^{15}N$ = nitrogen stable isotope signature.

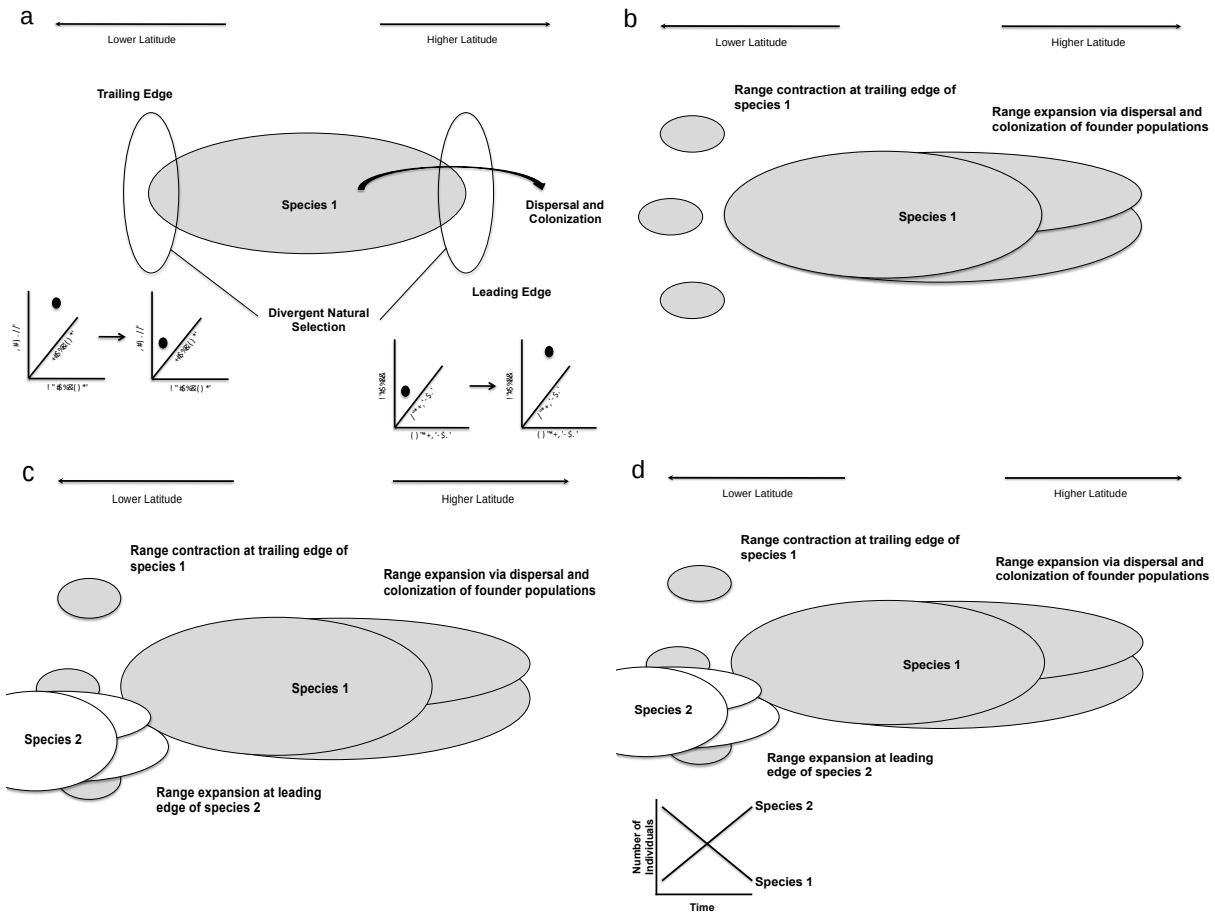

Figure 4.1. Conceptual model for bio-geographic range shifts. Spatial variability in the environment can have divergent effects on fitness of conspecifics at opposing range margins (a), which over time results in contracting and expanding range margins (b). Where expanding and contracting range margins occur among sympatric species, over time changes in community composition can result (c, d).

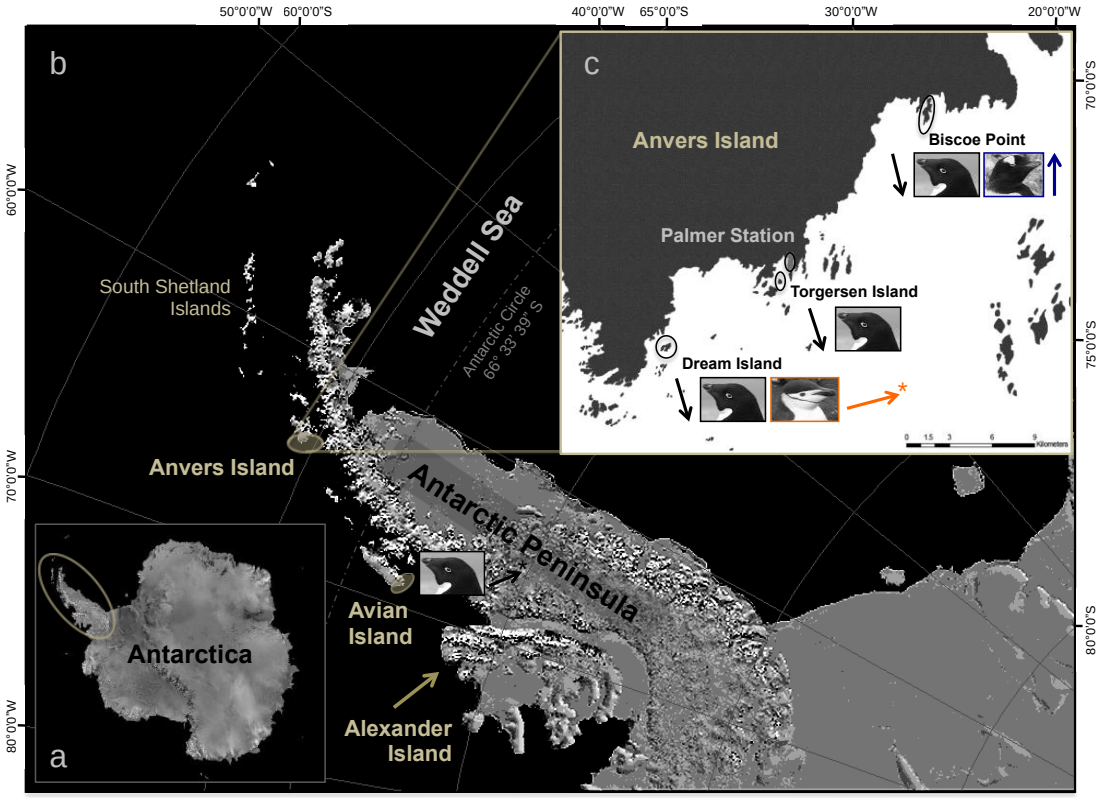

Figure 4.2. The marine ecosystem west of the Antarctic Peninsula (a) extends from northern Alexander Island to the South Shetland Islands (b). The Scotia Sea extends northeast from the South Shetland Islands (b) to include islands throughout the Scotia Arc (not pictured here). Fieldwork for the present study was conducted at major penguin rookeries within the Palmer Archipelago near Anvers Island and Palmer Station, a United States supported research base, as well as at Avian Island (b, c). Northern penguin rookeries were located at Dream (Adélie [black] and chinstrap [orange]) and Torgersen (Adélie only) Islands, as well as Biscoe Point (Adélie [black] and gentoo [blue]) (c). Southern Adélie penguin rookeries at Avian Island were located approximately 400 kilometers south of Anvers Island. Arrows color coded for each species at breeding rookeries indicate associated breeding population trajectory (i.e., declining [↓], moderately increasing [→*,→*], rapidly increasing [↑], or stable [→]). Image generated from base maps provided by the National Snow and Ice Data Center's map server A-CAP (http://nsidc.org/agdc/acap/).

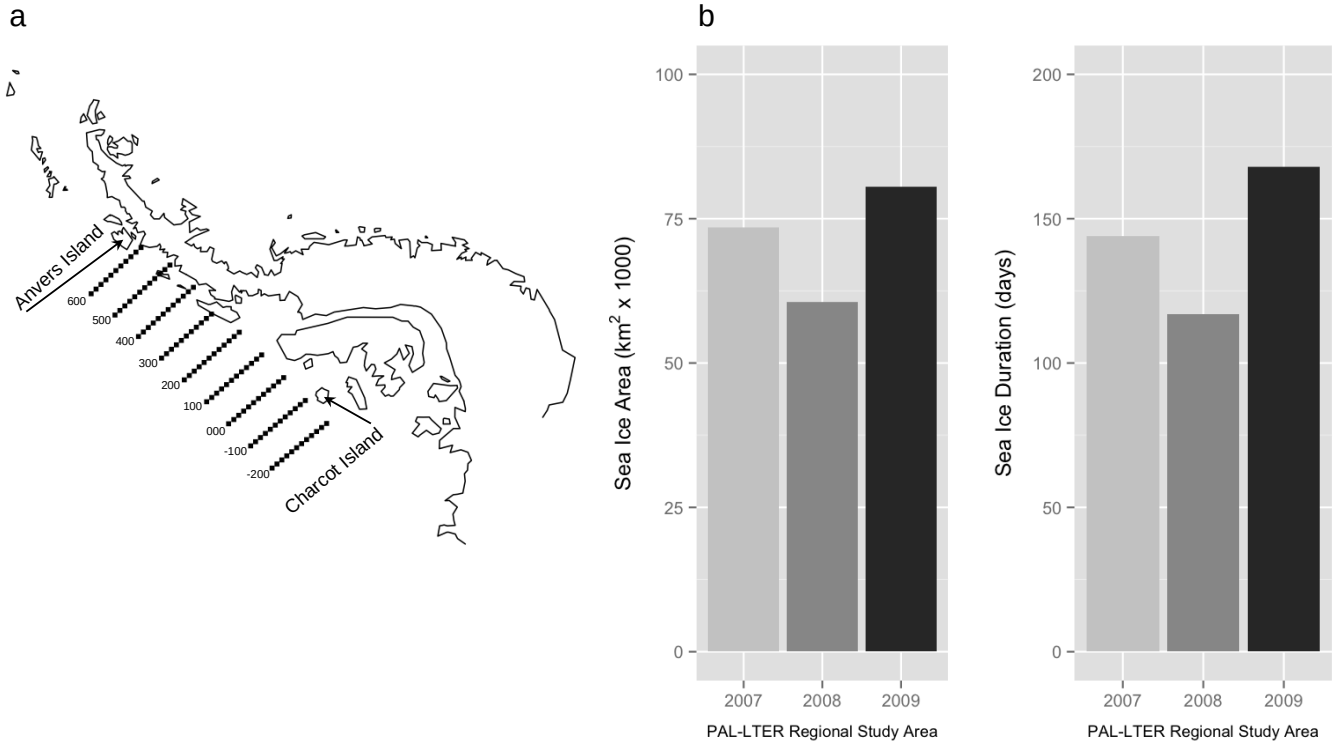

Figure 4.3. Palmer Station, Antarctica, Long Term Ecological Research (PAL-LTER) Program's regional study area west of the Antarctic Peninsula, extending 800 km from Anvers Island (600 line) to just south of Charcot Island (-200 line) and 220 km inshore to offshore (a). Study area grid lines encompass the entire area over which sea ice metrics were calculated. Winter average sea ice area (km2) and sea ice duration (days) within the PAL-LTER regional study area for each year of the present study (b).

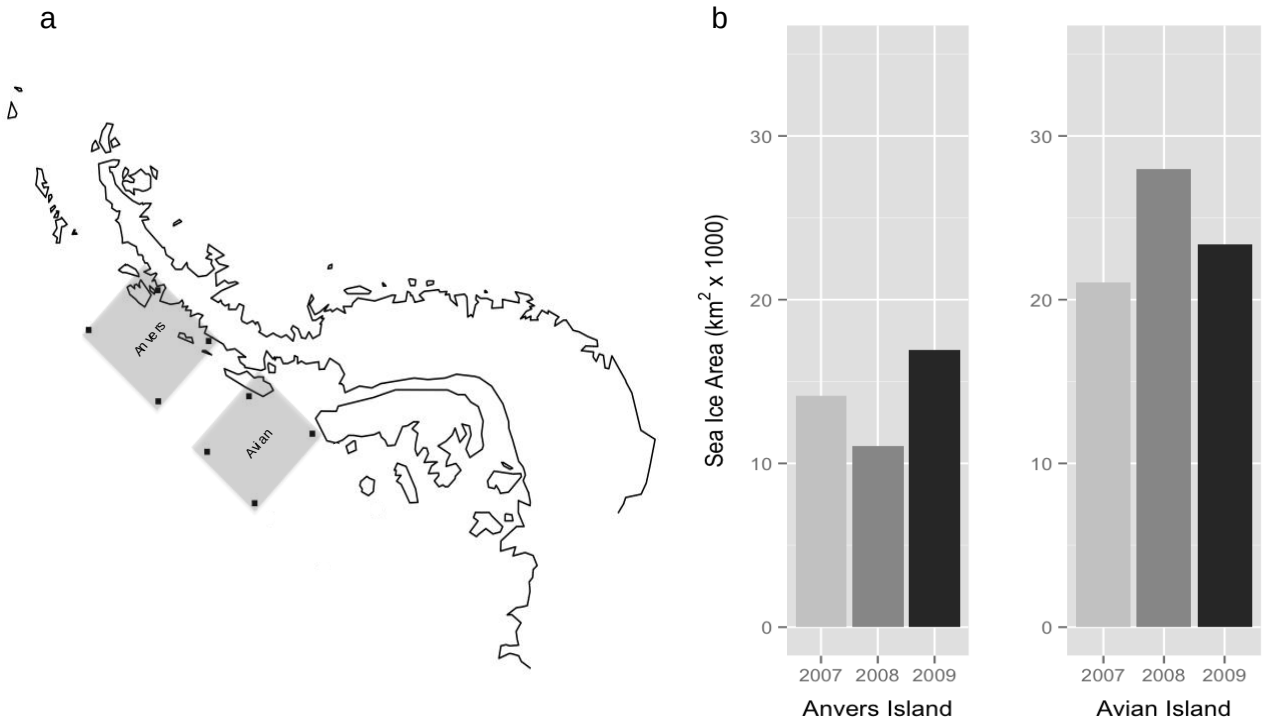

Figure 4.4. Penguin foraging grids (200 km^2) off of Anvers and Avian Islands west of the Antarctic Peninsula (a). Winter average sea ice area (km^2) within regional penguin foraging grids for each year of the present study (b).

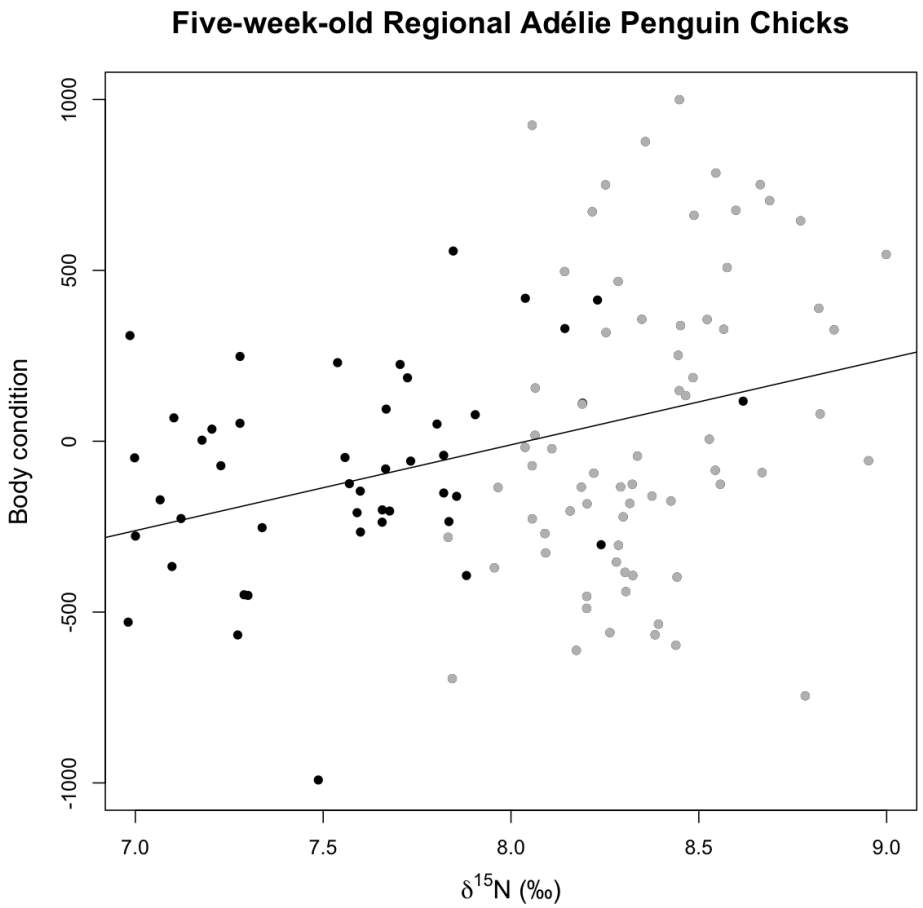

Figure 4.5. Variation in body condition of five-week-old Adélie penguins at the regional scale in relation to $\delta^{15}N$ signatures of blood tissue. Chicks from Anvers Island are noted in black, chicks from Avian Island are noted in grey. Body condition is presented as residual values from the relationship between body mass and PC1 score for structural size.

Appendix 4.1. Candidate models describing variation in body mass of adult *Pygoscelis* penguins. Females and males of each species were considered separately.

Model number	Parameters included	Model type
1	Mass.g~1	Null
2	Mass.g~Year	Main effect
3	Mass.g~PC1	Main effect
4	Mass.g~NestInit	Main effect
5	Mass.g~delta.13.C	Main effect
6	Mass.g~delta.15.N	Main effect
7	Mass.g~delta.13.C + delta.15.N	Multiple predictor
8	Mass.g~PC1 + delta.13.C + delta.15.N	Multiple predictor
9	Mass.g~NestInit + delta.13.C + delta.15.N	Multiple predictor
10	Mass.g~PC1 + NestInit + delta.13.C + delta.15.N	Multiple predictor
11	Mass.g~PC1 + Year	Multiple predictor with year
12	Mass.g~NestInit + Year	Multiple predictor with year
13	Mass.g~delta.13.C + Year	Multiple predictor with year
14	Mass.g~delta.15.N + Year	Multiple predictor with year
15	Mass.g~delta.13.C + delta.15.N + Year	Multiple predictor with year
16	Mass.g~PC1 + delta.13.C + delta.15.N + Year	Multiple predictor with year
17	Mass.g~NestInit + delta.13.C + delta.15.N + Year	Multiple predictor with year
18	Mass.g~PC1 + NestInit + delta.13.C + delta.15.N + Year	Global
19	Mass.g~NestInit + Year + NestInit*Year	Interaction
20	Mass.g~delta.13.C + delta.15.N + NestInit + delta.13.C*NestInit + delta.15.N*NestInit	Interaction
21	Mass.g~delta.13.C + delta.15.N + Year + delta.13.C*Year + delta.15.N*Year	Interaction

Abbreviations: Mass.g = adult penguin body mass (grams), PC1 = principal components score, NestInit = nest initiation date, delta.13.C = $\delta^{13}C$, delta.15.N = $\delta^{15}N$.

Appendix 4.2. Candidate models describing variation in egg mass of adult female *Pygoscelis* penguins. Each species was considered separately.

Model number	Parameters included	Model type
1	Egg.Mass.g~1	Null
2	Egg.Mass.g~Year	Main effect
3	Egg.Mass.g~NestInit	Main effect
4	Egg.Mass.g~Mass.g + PC1	Multiple predictor
5	Egg.Mass.g~delta.13.C + delta.15.N	Multiple predictor
6	Egg.Mass.g~Mass.g + PC1 + NestInit	Multiple predictor
7	Egg.Mass.g~NestInit + delta.13.C + delta.15.N	Multiple predictor
8	Egg.Mass.g~Mass.g + PC1 + delta.13.C + delta.15.N	Multiple predictor
9	Egg.Mass.g~Mass.g + PC1 + NestInit + delta.13.C + delta.15.N	Multiple predictor
10	Egg.Mass.g~NestInit.0 + Year	Multiple predictor with year
11	Egg.Mass.g~Mass.g + PC1 + Year	Multiple predictor with year
12	Egg.Mass.g~delta.13.C + delta.15.N + Year	Multiple predictor with year
13	Egg.Mass.g~Mass.g + PC1 + NestInit + Year	Multiple predictor with year
14	Egg.Mass.g~NestInit + delta.13.C + delta.15.N + Year	Multiple predictor with year
15	Egg.Mass.g~Mass.g + PC1 + delta.13.C + delta.15.N + Year	Multiple predictor with year
16	Egg.Mass.g~Mass.g + PC1 + NestInit + delta.13.C + delta.15.N + Year	Global
17	Egg.Mass.g~Mass.g + PC1 + Year.2 + Mass.g*Year	Interaction

Abbreviations: Egg.Mass.g = female penguin egg mass (grams), Mass.g = adult penguin body mass (grams), PC1 = principal components score, NestInit = nest initiation date, delta.13.C = $\delta^{13}C$, delta.15.N = $\delta^{15}N$.

Appendix 4.3. Candidate models describing variation in body mass of male and female *Pygoscelis* penguin chicks at five- and 15-days-old. Each species was considered separately.

Model number	Parameters included	Model type
1	Mass.g~1, random=~1|Nest	Null
2	Mass.g~Year, random=~1|Nest	Main effect
3	Mass.g~Sex, random=~1|Nest	Main effect
4	Mass.g~PC1, random=~1|Nest	Main effect
5	Mass.g~HatchDate, random=~1|Nest	Main effect
6	Mass.g~delta.13.C, random=~1|Nest	Main effect
7	Mass.g~delta.15.N, random=~1|Nest	Main effect
8	Mass.g~Sex + PC1, random=~1|Nest	Multiple predictor
9	Mass.g~delta.13.C + delta.15.N, random=~1|Nest	Multiple predictor
10	Mass.g~Sex + PC1 + HatchDate.0, random=~1|Nest	Multiple predictor
11	Mass.g~HatchDate + delta.13.C + delta.15.N, random=~1|Nest	Multiple predictor
12	Mass.g~Sex + PC1 + HatchDate + delta.13.C + delta.15.N, random=~1|Nest	Multiple predictor
13	Mass.g~Sex + Year, random=~1|Nest	Multiple predictor with year
14	Mass.g~PC1 + Year, random=~1|Nest	Multiple predictor with year
15	Mass.g~HatchDate + Year, random=~1|Nest	Multiple predictor with year
16	Mass.g~delta.13.C + Year, random=~1|Nest	Multiple predictor with year
17	Mass.g~delta.15.N + Year, random=~1|Nest	Multiple predictor with year
18	Mass.g~Sex + PC1 + Year, random=~1|Nest	Multiple predictor with year
19	Mass.g~delta.13.C + delta.15.N + Year, random=~1|Nest	Multiple predictor with year
20	Mass.g~Sex + PC1 + HatchDate + Year, random=~1|Nest	Multiple predictor with year
21	Mass.g~HatchDate + delta.13.C + delta.15.N + Year, random=~1|Nest	Multiple predictor with year
22	Mass.g~Sex + PC1 + HatchDate + delta.13.C + delta.15.N + Year, random=~1|Nest	Global

23	Mass.g~HatchDate + Year + HatchDate*Year, random=~1|Nest	Interaction
24	Mass.g~delta.13.C + delta.15.N + Year + delta.13.C*Year + delta.15.N*Year, random=~1|Nest	Interaction

Abbreviations: Mass.g = chick penguin body mass (grams), PC1 = principal components score, HatchDate = date of hatch, delta.13.C = $\delta^{13}C$, delta.15.N = $\delta^{15}N$.

Appendix 4.4. Candidate models describing variation in body mass of male and female five-week-old *Pygoscelis* penguin chicks reared near Anvers Island.

Model number	Parameters included	Model type
1	Mass.g~1	Null
2	Mass.g~Year	Main effect
3	Mass.g~Sex	Main effect
4	Mass.g~PC1	Main effect
5	Mass.g~delta.13.C	Main effect
6	Mass.g~delta.15.N	Main effect
7	Mass.g~Sex + PC1	Multiple predictor
8	Mass.g~delta.13.C + delta.15.N	Multiple predictor
9	Mass.g~Sex + PC1 + delta.13.C + delta.15.N	Multiple predictor
10	Mass.g~Sex + Year	Multiple predictor with year
11	Mass.g~PC1 + Year	Multiple predictor with year
12	Mass.g~delta.13.C + Year	Multiple predictor with year
13	Mass.g~delta.15.N + Year	Multiple predictor with year
14	Mass.g~Sex + PC1 + Year	Multiple predictor with year
15	Mass.g~delta.13.C + delta.15.N + Year	Multiple predictor with year
16	Mass.g~Sex + PC1 + delta.13.C + delta.15.N + Year	Global
17	Mass.g~delta.13.C + delta.15.N + Year + delta.13.C*Year + delta.15.N*Year	Interaction
18	Mass.g~delta.13.C + delta.15.N + Sex + delta.13.C*Sex + delta.15.N*Sex	Interaction

Abbreviations: Mass.g = adult penguin body mass (grams), PC1 = principal components score, delta.13.C = $\delta^{13}C$, delta.15.N = $\delta^{15}N$.

Appendix 4.5. Candidate models describing variation in body mass of male and female five-week-old Adélie penguin chicks reared along the western Antarctic Peninsula.

Model number	Parameters included	Model type
1	Mass.g~1	Null
2	Mass.g~Year	Main effect
3	Mass.g~Sex	Main effect
4	Mass.g~PC1	Main effect
5	Mass.g~Location	Main effect
6	Mass.g~delta.13.C	Main effect
7	Mass.g~delta.15.N	Main effect
8	Mass.g~Sex + PC1	Multiple predictor
9	Mass.g~delta.13.C + delta.15.N	Multiple predictor
10	Mass.g~Sex + PC1 + Location	Multiple predictor
11	Mass.g~Location + delta.13.C + delta.15.N	Multiple predictor
12	Mass.g~Sex + PC1 + Location + delta.13.C + delta.15.N	Multiple predictor
13	Mass.g~Sex + Year	Multiple predictor with year
14	Mass.g~PC1 + Year	Multiple predictor with year
15	Mass.g~Location + Year	Multiple predictor with year
16	Mass.g~delta.13.C + Year	Multiple predictor with year
17	Mass.g~delta.15.N + Year	Multiple predictor with year
18	Mass.g~Sex + PC1 + Year	Multiple predictor with year
19	Mass.g~delta.13.C + delta.15.N + Year	Multiple predictor with year
20	Mass.g~Sex. + PC1 + Location + Year	Multiple predictor with year
21	Mass.g~Location + delta.13.C + delta.15.N + Year	Multiple predictor with year
22	Mass.g~Sex + PC1 + delta.13.C + delta.15.N + Year	Global

23	Mass.g~Location + Year + Location*Year	Interaction
24	Mass.g~Location + delta.13.C + delta.15.N + Location*delta.13.C + Location*delta.15.N	Interaction

Abbreviations: Mass.g = adult penguin body mass (grams), PC1 = principal components score, delta.13.C = $\delta^{13}C$, delta.15.N = $\delta^{15}N$.

Chapter 5.

Limited Evidence for Baseline Corticosterone Modulation of Individual Variation in Reproductive Performance among *Pygoscelis* Penguins at Opposing Range Margins

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Abstract

Climate-driven changes in sea ice-associated marine food-webs of the Southern Ocean are hypothesized to influence breeding population dynamics of penguin predators through energetic effects on young chicks that differentially influence their survival and recruitment probability as breeding adults, especially at breeding range margins. Although this issue has been studied by several long-term research programs along the western Antarctic Peninsula (AP), where changes in both sea ice and the population dynamics of *Pygoscelis* penguins have been most marked, no studies to date have examined physiological parameters intimately tied with energy balance such as glucocorticoids. Here, we focus on baseline corticosterone, a primary glucocorticoid involved in homeostatic energetic balance, as an index of physiological quality among all three species of *Pygoscelis* penguins and its role in mediating body and egg mass variation across four breeding stages and two regional rookeries. We found limited evidence for corticosterone modulation of individual variation in breeding performance across all three species. Corticosterone levels were an important predictor of body condition among 15-day-old chinstrap penguin (*P. antarctica*) chicks only. For all other analyses, there was no hormonal control of body or egg mass variation detected. We interpret these results to indicate that breeding *Pygoscelis* penguins of the western AP, and their chicks, are of adequate physiological quality so as to not require the up-regulation of corticosterone that is typically associated with improved body condition via increased foraging effort, or reproductive abandonment. Our results provide a novel perspective on this system by indicating that breeding populations with differing trajectories are comprised of individuals that are of sufficient energetic quality, despite

our previous results demonstrating considerable variation in the isotopic niche occupied by these populations that at the regional level does have an important influence on body condition of 5-week-old Adélie penguin chicks.

Key words: Biogeographic range shift; breeding performance; corticosterone; information-theoretic methods; Pygoscelis penguins; sea ice; western Antarctic Peninsula

Introduction

The vertebrate stress-response is a neuroendocrine pathway critical in maintaining homeostatic energetic balance (Dallman et al. 1993). In avian systems, corticosterone is the primary glucocorticoid produced during stimulation of the hypothalamic-pituitary-adrenal axis by environmental stressors such as the presence of natural enemies or a reduced energetic state (Sapolsky et al. 2000). During the stress-response, a cascade of biochemical events results in the mobilization of energy via gluconeogenesis to assist in fight or flight behavior or to aid in the acquisition of nutritional resources (Sapolsky et al. 2000, Landys et al. 2006). Thus, the stress hormone corticosterone is considered important in mediating life-history trade-offs resulting from the allocation of limited energetic resources (Stearns 1992), as for example between survival and reproduction (Wingfield and Sapolsky 2003, Bokony et al. 2009). Many examples exist, particularly among birds, which demonstrate elevated baseline corticosterone levels associated with reduced reproductive output (Williams et al. 2008) or skewed investment decisions (Love et al. 2005). Therefore, baseline corticosterone profiles can provide a physiological perspective on the degree to which an individual might be trading-off between survival and reproduction, and has particular utility for better understanding proximate mechanisms involved in ecological scenarios where inter-individual variation in this trade-off is of interest.

An increasingly important challenge facing ecologists and conservation biologists is to understand proximate mechanisms ultimately contributing to shifting bio-geographic ranges and altered patterns of biodiversity in response to climate variability (Chown and Gaston 2008, Portner and Farrell 2008, Wingfield 2008). As the geographic range of a

species is determined by spatial variation in population-demographic processes including individual survival, reproduction, immigration and emigration (Sexton et al. 2009), the physiology underlying these facets of demography is of particular interest to both pure and applied research in biogeography, population and global change biology. Given stress hormone's role in mediating survival versus reproduction trade-offs, studies of baseline corticosterone within systems currently shifting in response to climate change offer unique opportunities to investigate the mechanistic basis of a critical ecological issue.

Population responses to ocean-climate warming are now occurring in the Southern Ocean where seven of the world's 18 extant species of penguin reside (Williams 1995, Baker et al. 2006). Of these, all three species within the genus *Pygoscelis*, and the sea-ice obligate emperor penguin (*Aptenodytes forsteri*), are demonstrating climate-induced shifts in abundance and distribution (Forcada and Trathan 2009, Jenouvrier et al. 2009, Trathan et al. 2011). However, population responses differ among rookeries of *Pygoscelis* penguins located west of the Antarctic Peninsula (AP, Trivelpiece et al. 2011, Lynch et al. 2012), particularly along the Palmer Archipelago, near Anvers Island (Ducklow et al. 2007, Ducklow et al. 2013). Here, breeding population declines and northern range contractions have occurred for the sea-ice obligate Adélie penguin (*P. adeliae*) as numbers of breeding adults have declined by 80% from historical numbers (Fig. 5.2b, c Ducklow et al. 2007, Ducklow et al. 2013). Conversely, chinstrap (*P. antarctica*) and gentoo (*P. papua*) penguins, sea ice-intolerant congeners of the Adélie penguin that hold a more low- to sub-Antarctic distribution, have shown stable or rapid population increases associated with southern range expansions

that has included the establishment of nesting colonies in 1976 and 1994, respectively (Fig. 5.2b, c Ducklow et al. 2007, Ducklow et al. 2013). Similarly, Adélie penguin breeding rookeries within the core of the species range along the western AP at Avian Island (Fig. 5.2b) have increased (Sailley et al. 2013, W. R. Fraser unpubl. data).

Winter temperature of the western AP has increased almost 6°C in the last half century in association with a reduction in the extent and duration of annual sea-ice formation (Vaughan et al. 2003, Ducklow et al. 2007), a phenomenon driven by physical oceanographic forcing of relatively warm water derived from the Antarctic Circumpolar Current (Ducklow et al. 2007, Martinson et al. 2008) in association with climate phases such as El Niño-Southern Oscillation and the Southern Annular Mode (Stammerjohn et al. 2008b). Forcada and Trathan (2009) reviewed penguin response to climate change in the Southern Ocean, emphasizing the need to better understand the role of climate-induced change in sea-ice dynamics and oceanographic conditions in affecting individual nutritional status, breeding performance and survival. However, to date, no studies have taken an endocrine perspective to this issue, despite wide recognition of the need for better integration of ecological, evolutionary and mechanistic studies of why and how animals respond to environmental variability (Portner and Farrell 2008, Wingfield 2008).

Here, we test the general hypothesis that baseline corticosterone is a key physiological mediator of individual reproductive performance among *Pygoscelis* penguins breeding at rookeries that represent each species' relative contracting or expanding range margin along the western AP. We interpret circulating baseline levels of corticosterone as a physiological index of individual quality. Our analyses of penguins breeding at northern rookeries located near Anvers Island (Fig. 5.1b, c) included four

reproductive stages assessed for all *Pygoscelis* species where data for males and females were pooled – adults at the one-egg stage, young chicks at five and 15 days of age, and prefledge crèched chicks at five-weeks of age. Analyses of individuals at regional rookeries included Adélie penguin pre-fledge crèched chicks at five-weeks of age only. Throughout our analyses we predicted that (1) adults and chicks at Anvers Island with lower levels of baseline corticosterone would have larger body masses after accounting for other parameters that we expected would influence body mass such as structural size and the seasonal timing of breeding. Similarly, we predicted that (2) females with lower levels of baseline corticosterone would have larger egg masses. Lastly, we considered the influence of over-winter sea ice coverage on body mass variation modeled as a year effect where we expected differing inter-specific body mass responses, at all reproductive stages, to over-winter sea ice coverage given generally opposing life history affinities for sea ice among *Pygoscelis* penguins (Fraser et al. 1992, Ainley 2002, Forcada and Trathan 2009). Specifically, we predicted that (3) Adélie penguins should be heavier and associated with lower baseline corticosterone levels following years of higher winter sea ice coverage, while chinstrap and gentoo penguins should be lighter and associated with higher baseline levels of corticosterone. At the regional level, we predicted (4) that Adélie penguin chicks reared at Anvers Island, where reductions in winter sea ice have been most pronounced, would have higher baseline corticosterone levels as compared with their more southern conspecifics reared at Avian Island where winter sea ice coverage is greater as we have previously shown that chicks reared at Avian Island are not only isotopically enriched, but also heavier (K. B. Gorman this thesis Chapters 2 and 4).

Materials and Methods

Field methods

Pygoscelis penguins nesting on several islands within the Palmer Archipelago west of the AP near Anvers Island (64°46'S, 64°03'W, Fig. 5.1a-c) were studied during the austral summers of 2007/08, 2008/09, and 2009/10. Specifically, study nests were located on Biscoe Point (64°48'S, 63°46'W), Torgersen (64°46'S, 64°04'W) and Dream (64°43'S, 64°13'W) Islands (Fig. 5.1c). Each study season, Adélie penguin study nests (n = 30) were distributed equally between the three study islands, with 10 nests located on each island. Gentoo penguin study nests (n = 30) were all located on Biscoe Point, while chinstrap penguin study nests (n = 15) were all located on Dream Island (Fig. 5.1c). The reduced sample size for chinstraps was due to the overall smaller number of individuals breeding at rookeries on Dream Island. Final sample sizes of individuals used in analyses differ from the number of original study nests reported here as at times weather conditions hindered rookery access resulting in some adults reaching clutch completion before sampling, and therefore, these study nests were not sampled and excluded from subsequent monitoring. In addition, some study chicks were never sampled due to brown skua (*Stercorarius lonnbergi*) depredation. During annual oceanographic research cruises aboard the A.R.S.V. *Laurence M. Gould*, as part of the Palmer Station, Antarctica, Long-Term Ecological Research Program (PAL-LTER), in January of each study year, we further sampled Adélie penguin chicks from rookeries at Avian Island (n = 25, Fig. 5.1b).

Each season, study nests, where pairs of adults were present, were individually marked and chosen before the onset of egg laying, and monitored consistently. When

study nests were found at the one-egg stage, both adults were captured to obtain blood samples used for baseline corticosterone analysis, and measurements of structural size and body mass. At the time of capture, each adult penguin was quickly blood sampled (~1 ml) from the brachial vein using a sterile 3 ml syringe and heparinized infusion needle. Measurements of culmen length and depth (using dial calipers ± 0.1 mm), right flipper (using a ruler ± 1 mm), and body mass (using 5 kg ± 25 g or 10 kg ± 50 g Pesola spring scales and a weigh bag) were obtained to quantify body size variation. After handling, individuals at study nests were further monitored to ensure the pair reached clutch completion, i.e., two eggs. Study nests were monitored for hatch of the first egg. At day six and 16 post-hatch for first chicks, i.e., resulting in an approximate average nest age of five and 15 days given hatching asynchrony, offspring from study nests were captured and quickly blood sampled (≤ ~500 μl for day five chicks, and ≤ ~1 ml for day 15 chicks) from the tarsus vein using a sterile needle and heparinized capillary tubes for day five chicks, and a sterile 3 ml syringe and heparinized infusion needle for day 15 chicks, again to obtain blood tissue for baseline corticosterone analysis. Measurements of culmen length and depth (using dial calipers ± 0.1 mm), right flipper (using a ruler ± 1 mm), and body mass (using 600 g ± 5 g, 1000 g ± 10 g or 2000 g ± 20 g Pesola or Homs spring scales and a weigh bag) were obtained to quantify body size variation. Study nests were monitored for chick survival to 25 days, prior to crèche. At five weeks into chick-rearing, based on a seasonal population average hatch date for each species, older crèched chicks of all three species were captured and quickly blood sampled from study rookeries near Anvers Island. Handling of crèched chicks occurred over a one or two day period, which varied seasonally and by species depending on nest initiation dates. Adélie

penguin chicks at Avian Island were sampled on the same day Anvers Island Adélie penguin chicks were sampled. Blood samples from crèched chicks (~1 ml) were taken from the brachial vein using a sterile 3 ml syringe and heparinized infusion needle following sampling procedures used for adult penguins, again to obtain blood tissue for baseline corticosterone analysis. Measurements of culmen length and depth (using dial calipers ± 0.1 mm), right flipper (using a ruler ± 1 mm), and body mass (using 5 kg ± 25 g or 10 kg ± 50 g Pesola spring scales and a weigh bag) were obtained to quantify body size variation. All collected blood was stored in 1.5 ml microcentrifuge tubes and kept cool in the field.

Circulating levels of corticosterone increase in association with handling stress in birds (Wingfield et al. 1992, Wingfield 1994). Therefore, we adopted several specific field protocols to ensure we obtained blood from birds that did not reflect any sampling disturbance. First, study nests were distributed at a distance from one another so that when study nests were sampled for baseline corticosterone, the sampling procedure at one study nest would not influence any subsequent study nests that might also be sampled that same day. Study nests were monitored by visual observation from a distance in order to assess nesting status prior to capture, keeping disturbance to a minimum prior to sampling. We recorded the time (± 1 sec) needed to obtain blood samples by conservatively starting our timing before approaching any study nest and ended our timing once all blood had been obtained.

Sea ice data were based on satellite estimates of sea ice concentration (SIC) within two regions, 1-the PAL-LTER regional study grid (Fig. 5.2a) and 2-three 200 km^2 penguin foraging grids associated with major penguin rookeries at Anvers and Avian

Islands (Fig. 5.3a). Daily and monthly time series of SIC were used to calculate two metrics within the PAL-LTER regional grid, 1-average winter sea ice area (km^2) representing the yearly average area of the ocean covered by winter sea ice, and 2-duration (days) representing the yearly total length of the winter sea ice season. For the 200 km^2 penguin foraging grids, the only sea ice metric calculated was 1-average winter sea ice area within each foraging grid. Sea ice metrics were calculated using time series based on Version 2 of the Goddard Space Flight Center (GSFC) Bootstrap algorithm (Comiso 1999) provided by the National Snow and Ice Data Center (NSIDC; www.nsidc.org), and derived based on 1-monthly averages between the winter months of sea ice advance and retreat, and 2-the day of first and last appearance of sea ice within the same region and averaged for the PAL-LTER grid. These metrics are useful for describing both the winter spatial and temporal variability of Southern Ocean sea ice (Stammerjohn et al. 2008a).

Laboratory methods

Within 12 hrs of field collection, tubes containing whole blood were centrifuged to separate plasma and red blood cell (RBC) fractions, which were stored separately and frozen at -80 ºC.

Circulating levels of baseline corticosterone in blood plasma were determined by double antibody radioimmunoassay (125 I-RIA, MP Biomedicals, 07-120103) with modifications validated for several avian species (Washburn et al. 2002, Newman et al. 2008, Schmidt and Soma 2008). Standard curves were fit using a cubic polynomial function in the R language environment (2012). The assay detection limit was 3.13 pg

corticosterone per tube (i.e., the lowest corticosterone standard, 12.5 ng/mL, using a 50 μL assay volume). The low corticosterone control and a consistent native plasma sample were analyzed in each assay to determine an inter-assay coefficient of variation (6.75%). Intra-assay coefficient of variation was 8.16%, calculated from variation in a native plasma sample assayed 8 times within a single assay. Serially diluted native plasma samples were parallel to the corticosterone standard curve as coefficients of variation for final concentrations were 6.32% for Adélie penguins (n = 6), 7.58% for chinstrap penguins (n = 5) and 8.11% for gentoo penguins (n = 6) following methods outlined by Plikaytis et al. (1994). All samples were assayed in duplicate.

Statistical methods

Final sample sizes for study nests / individual adults / day five chicks / day 15 chicks / five week chicks of each species were for Adélie penguins: n = 76 / 152 / 102 / 99 / 49 (Anvers Island) and 71 (Avian Island), chinstrap penguins: n = 34 / 68 / 40 / 35 / 41, and gentoo penguins: n = 62 / 124 / 100 / 92 / 46 over the three years of study. Final sample sizes used in analyses differ from those reported here as some individuals were excluded. For example, occasionally adult pairs were never observed having reached clutch completion, i.e., two eggs, likely due to brown skua depredation, and therefore we could not be certain those individuals represented adults exactly at the one egg stage. Furthermore, at times weather conditions hindered rookery access resulting in some chicks being sampled at the incorrect age. All analyses were restricted to using individuals with bleed times of 3 minutes or less, which further restricted the sample size for each analysis. Other researchers have shown no relationship between blood sampling

within 5 minutes of capture and corticosterone levels among Adélie penguins (Vleck and Vleck 2002). However, blood collected from individuals within 3 minutes following capture is standard protocol for assessing baseline corticosterone (Wingfield et al. 1982, Romero and Reed 2005). Our final datatsets indicated no relationship between bleed time within 3 minutes and corticosterone levels for each species and stage. All statistical analyses were performed in the R language environment (2012).

Analysis 1 – adult body mass

Linear mixed effects models (LME, nlme package R Core Team 2012) were used to examine continuous variation in body mass of adult Adélie and gentoo penguins. A random effect of nest on intercept (random = ~1|nest) was included in all candidate LME models to control for non-independence of data given that paired adults comprised these datasets. Least-squares general linear models (LM) were used to examine continuous variation in body mass of adult chinstrap penguins. The final chinstrap dataset did not include any paired adults due the elimination of data points for reasons previously identified. For all species, male and female adults were pooled for analyses. For each species, variation in adult body mass was considered in relation to four parameters treated as main effects including 1-year (categorical variable), 2-overall structural size using a principal components score (PC1) based on culmen depth and length, and flipper length (continuous variable), 3-circulating levels of corticosterone (continuous variable), and 4-nest initiation date defined as the date adult penguins were sampled when study nests were found at the one-egg stage (continuous variable). Individual scores for PC1 were calculated using the *prcomp* function in R separately for each species dataset. Nest initiation dates for males and females of each study nest was calculated in relation to the

earliest nest initiation date for each species across all years, which was set at 0 (Adélie penguins = 05 November 2008, chinstrap penguins = 14 November 2008, and gentoo penguins = 02 November 2008). An a priori set of 18 candidate models consisted of an equal-means (null) model; each predictor variable as a main effect (four models); simple multiple predictor models for PC1 score and corticosterone level, or PC1 score and nest initiation date, or corticosterone level and nest initiation date (three models); a more complex multiple predictor model that included PC1 score, corticosterone level, and nest initiation date (one model). All these models were further evaluated including a year term (seven models). Two additional interaction models were included: all four main predictor variables were considered as main effects and the interaction between corticosterone level and year, as well as all four main predictor variables and the interaction between corticosterone level and nest initiation date. See Appendix 5.1 for details regarding candidate models considered in adult penguin body mass analyses. This same candidate model set was evaluated separately for each species (Adélies n = 53, chinstraps n = 22, and gentoos n = 65).

Analysis 2 – adult female egg mass

Continuous variation in egg mass of adult female *Pygoscelis* penguins was examined using LM in relation to five parameters including those four considered in adult body mass analyses described above (1-year, 2-PC1 score, 3-circulating corticosterone level, and 4-nest initiation date), in addition to 5-female body mass (continuous variable). Individual scores for PC1 were calculated separately for each species' female only dataset. Nest initiation dates for females were calculated as previously described. An a priori set of 18 candidate models consisted of an equal-means (null) model; year,

corticosterone level, and nest initiation date as main effect models (three models); simple multiple predictor models for female body mass and PC1 score, or corticosterone level and nest initiation date (two models); more complex multiple predictor models where female body mass and PC1 score were considered with corticosterone level or nest initiation date (two models); as well as a model including all four parameters excluding year (one model). All these models were further evaluated including a year term (seven models). Two additional interaction models were included: all five main predictor variables were considered as main effects and the interaction between corticosterone level and year, as well as all five main predictor variables and the interaction between corticosterone level and nest initiation date. See Appendix 5.2 for details regarding candidate models considered in female penguin egg mass analyses. This same candidate model set was evaluated for females of each species, separately (Adélie females n = 41, chinstrap females n = 14, and gentoo females n = 49).

Analyses 3 and 4 – chick body mass at five and 15 days

Continuous variation in body mass of five- and 15-day-old Adélie and gentoo penguin chicks was examined using LME models. A random effect of nest on intercept (random = ~1|nest) was included in all candidate LME models to control for non-independence of data given that sibling chicks were sampled. Least-squares general linear models (LM) were used to examine continuous variation in body mass of five- and 15-day-old chinstrap penguin chicks. The final chinstrap datasets did not include any sibling chicks due the elimination of data points for reasons previously identified. For all species, male and female chicks were pooled for analyses. Variation in five- and 15-day-old chick body mass was considered in relation to four parameters including 1-year, 2-PC1 score, 3-

corticosterone level, and 4- hatch date (continuous variable). Individual scores for PC1 were calculated separately for each species' five- and 15-day-old chick dataset. Hatch date of each study nest was calculated in relation to the earliest hatch date for each species across all years, which was set at 0 (Adélie penguins = 10 December 2008, chinstrap penguins = 21 December 2008, and gentoo penguins = 07 December 2008). An a priori set of 18 candidate models consisted of an equal-means (null) model; each predictor variable as a fixed main effect (four models); simple multiple predictor models for PC1 score and corticosterone level, or PC1 score and hatch date, or corticosterone level and hatch date (three models); a more complex multiple predictor model that included PC1 score, corticosterone level, and hatch date (one model). All these models were further evaluated including a year term (seven models). Two additional interaction models were included: all four main predictor variables were considered as main effects and the interaction between corticosterone level and year, as well as all four main predictor variables and the interaction between corticosterone level and hatch date. See Appendix 5.3 for details regarding candidate models considered in five- and 15-day-old *Pygoscelis* penguin chick body mass analyses. This same candidate model set was evaluated separately for each species (five-day-old chicks: Adélie n = 60, chinstrap n = 23, and gentoo n = 59; 15-day-old chicks: Adélie n = 56, chinstrap n = 16, and gentoo n = 51).

Analysis 5 – chick body mass at 5 weeks (Anvers Island)

Continuous variation in body mass of *Pygoscelis* penguin chicks at five weeks of age reared near Anvers Island was examined using LM in relation to three parameters treated as main effects including 1-year, 2-PC1 score, and 3-corticosterone level. Individual

scores for PC1 were calculated separately for each species' five-week-old chick dataset. An a priori set of 9 candidate models consisted of an equal-means (null) model; each predictor variable as a main effect (three models); and a simple multiple predictor model that included PC1 score and corticosterone levels (one model). All these models were further evaluated including a year term (three models). One interaction model was included: all three main predictor variables were considered as main effects and the interaction between corticosterone level and year. See Appendix 5.4 for details regarding candidate models considered in body mass analyses of five-week-old penguin chicks reared near Anvers Island. This same candidate model set was evaluated for each species, separately (Adélie n = 39, chinstrap n = 30, and gentoo n = 36).

Analysis 6 - chick body mass at 5 weeks (regional islands)

Continuous variation in body mass of Adélie penguin chicks at five weeks of age reared at regionals rookeries (i.e., Anvers and Avian Islands) was examined using LM in relation to four parameters treated as main effects including 1-year, 2-PC1 score; 3-corticosterone level, and 4-rookery location (categorical variable). Individual scores for PC1 were calculated specifically for the regional five-week-old Adélie penguin chick dataset. An a priori set of 18 candidate models consisted of an equal-means (null) model; each predictor variable as a main effect (four models); simple multiple predictor models that included PC1 score and corticosterone level, or PC1 score and location, or corticosterone level and location (three models); a more complex multiple predictor model that included PC1 score, corticosterone level, and location (one model). All these models were further evaluated including a year term (seven models). Two additional interaction models were included: all four main predictor variables were considered as

main effects and the interaction between corticosterone level and location, as well as all four main predictor variables and the interaction between corticosterone level and year. See Appendix 5.5 for details regarding candidate models considered in body mass analyses of five-week-old Adélie penguin chicks reared at regional rookeries. This candidate model set was only evaluated for this one dataset (n = 100).

For each of the six analyses, information-theoretic methods were used to direct model selection and parameter estimation (Burnham and Anderson 2002). For each candidate model described in the previous 6 analyses, Akaike's Information Criterion (AICc), corrected for small sample size, in addition to ΔAICc and Akaike weight (w) values were calculated and used to compare models (Burnham and Anderson 2002). An r^2 value, defined as the fraction of the total variance explained by the model and given by the Multiple R-squared calculation in R (see Crawley 2007 p. 399), was calculated as a general measure of fit for each model. Inference was based on the relative support for parameters across all models and weighted parameter estimates. Parameter estimation included calculation of model-averaged parameter estimates based on w values for all candidate models. Standard errors (SE) for parameter estimates were based on unconditional variances calculated across the same models. Parameter likelihood values were evaluated by summing w values across all models that included each parameter under consideration (Burnham and Anderson 2002).

Data management

Data reported here will be made publicly available within the PAL-LTER data system (dataset numbers forthcoming):

http://oceaninformatics.ucsd.edu/datazoo/data/pallter/datasets. These data will be additionally archived within the US LTER Network's Information System Data Portal: https://portal.lternet.edu/. Individuals interested in using these data are therefore expected to follow the US LTER Network's Data Access Policy, Requirements and Use Agreement: http://www.lternet.edu/policies/data-access.

Results

Sea ice variability

Sea ice metrics indicated that the austral winters preceding each study year were generally characterized by lower than average sea ice coverage, with winter 2008 being the lowest sea ice season in the Palmer LTER record since 1979 (Stammerjohn et al. 2008a, see also sea ice datasets within http://pal.lternet.edu/data/). Relative yearly variability in sea ice area and duration (Fig. 5.2b) over the course of the study suggested that 2007 was an intermediate sea ice season relative to 2008 (low) and 2009 (high). Results for inter-annual variability in body and egg mass of adult *Pygoscelis* penguins at the one-egg stage are discussed within the context of these qualitative differences in each year's winter sea ice season over the entire PAL-LTER regional study grid (Fig. 5.2a).

Variation in average winter sea ice area across the 200 km^2 penguin foraging grids (Fig. 5.3a) confirmed that in general sea ice area increased to the south during the austral winters preceding each study year (Fig. 5.3b). Yearly variation in average winter sea ice area within the Anvers Island penguin foraging grid generally confirmed our previous finding based on sea ice metrics calculated over the entire PAL-LTER regional

study grid (Fig. 5.2a, b) that 2007 was an intermediate winter sea ice season relative to 2008 (low) and 2009 (high). This trend was not apparent within the Avian Island penguin foraging grid (Fig. 5.3b) where 2009 was an intermediate winter sea ice season relative to 2007 (low) and 2008 (high). Results for inter-annual variability in body mass of *Pygoscelis* penguin chicks at all stages and locations are discussed within the context of these qualitative differences in each foraging grid's winter sea ice season (Fig. 5.3a).

Adult body mass

Analyses of variation in body mass of adult Adélie penguins resulted in two models receiving a ΔAICc value ≤ 2. The most parsimonious model included terms for PC1 score, corticosterone level, as well as nest initiation date (Table 5.1). These models received moderate *w* values (0.48 and 0.38, respectively, Table 5.1). All other models in the candidate set received no support. Parameter likelihoods indicated strong support for PC1 score (0.99) in addition to nest initiation date (0.97). However, corticosterone levels received more moderate support (0.59, Table 5.2). Weighted parameter estimates and associated 95% confidence intervals (CI = parameter estimate SE*1.96) indicated that structurally larger female Adélie penguins were heavier (217.26 ± 69.92 CI, Table 5.2). Further, Adélie penguins initiating nesting earlier were heavier (-33.31 ± 22.47 CI, Table 5.2). Importantly, 95% CIs overlapped 0 for all other parameters, indicating that corticosterone level (-24.31 ± 48.45 CI) was not important in predicting variation in body mass of adult Adélie penguins (Table 5.2). The best-supported model for describing variation in body mass of adult chinstrap penguins included the PC1 score term only, and received moderately strong *w* (0.63) and r^2 (0.52) values (Table 5.1). No other models

received a ΔAICc value ≤ 2 (Table 5.1). Parameter likelihoods indicated strong support for PC1 score (0.99), but only weak support for all other parameters (Table 5.2). Weighted parameter estimates and associated 95% CIs suggested that structurally larger adult chinstrap penguins were heavier (116.62 ± 49.88 CI), while 95% CIs overlapped 0 for all other parameters (Table 5.2). Thus, corticosterone level (-0.99 ± 7.96 CI) was not important in predicting variation in body mass of adult chinstrap penguins (Table 5.2). Two models received a ΔAICc value ≤ 2 for describing variation in body mass of adult gentoo penguins. The most parsimonious model included a term for PC1 score only and received a moderate *w* value (0.45, Table 5.1). The second best-supported model included terms for PC1 score and corticosterone level, however, this model received much weaker support (*w* = *0*.18, Table 5.1). Parameter likelihoods indicated strong support for the PC1 term (0.99), while all other parameters received only weak support (Table 5.2). Weighted parameter estimates and 95% CIs indicated that structurally larger adult gentoo penguins were heavier (291.62 ± 62.11 CI), however, 95% CIs overlapped 0 for all other parameters (Table 5.2). For each species' analysis, there was no qualitative relationship between body mass variation and corticosterone level or yearly sea ice conditions as the year, and corticosterone by year interaction, terms received only weaker support (Table 5.2).

Adult female egg mass

One model received ΔAICc values ≤ 2 for describing variation in female Adélie penguin egg mass. The most parsimonious model included terms for PC1 score, body mass, and nest initiation date and received a moderately high *w* (0.50) value, but a lower r^2 (0.28)

value (Table 5.3). No other models received a ΔAICc value ≤ 2 (Table 5.3). Parameter likelihoods indicated strong support for PC1 score (0.94), body mass (0.94), and nest initiation date (0.79). No other parameters were supported by the data including corticosterone level (Table 5.4). Weighted parameter estimates and associated 95% CIs indicated that heavier female Adélie penguins had larger egg masses (0.03 ± 0.02 CI, Table 5.4). For all other parameters, 95% CIs overlapped 0 (Table 5.4). The best-supported model for describing variation in egg mass of adult female chinstrap penguins also included terms for body mass and PC1 score (Table 5.3), and received a moderately high *w* (0.63) value and r^2 (0.52) value. Parameter likelihoods suggested moderately strong support for PC1 score and body mass parameters (0.78 for both, Table 5.4). Associated 95% CIs for both these parameters overlapped 0 (PC1 score: -0.85 ± 3.77 CI, body mass: 0.03 ± 0.03 CI) indicating that egg mass of adult female chinstrap penguins was independent of structural size and body mass (Table 5.4). Lastly, three models received ΔAICc values ≤ 2 for describing variation in female gentoo penguin egg mass. A higher degree of model uncertainty was evident as both *w* and r^2 values were low across all supported models (*w*: 0.11 – 0.24, r^2: ≤ 0.16, Table 5.3). The most parsimonious model included terms for PC1 score and body mass, while the second best-supported model included terms for PC1, body mass, and corticosterone levels (Table 5.3). The third best-supported model included terms for PC1, body mass, and nest initiation date (Table 5.3). Parameter likelihoods suggested only moderate support for PC1 score and body mass parameters (0.64 for both, Table 5.4). Terms for corticosterone level and nest initiation date received weaker support (parameter likelihoods = 0.40 and 0.30, respectively, Table 5.4). Weighted parameter estimates and associated 95% CIs

indicated that structurally larger (1.19 ± 1.53 CI) and heavier (0.002 ± 0.006 CI) female gentoo penguins did not have larger egg masses as 95% CIs overlapped 0 (Table 5.4). Again, for each species' analysis, there was no evidence that yearly sea ice conditions or the interaction with corticosterone level influenced adult female penguin egg mass as the year terms and corticosterone level by year interaction term received essentially no support (Table 5.4).

Chick body mass at day five

Analyses of variation in body mass of five-day-old Adélie penguin chicks resulted in two models receiving a ΔAICc value ≤ 2. The best-supported model received a weaker *w* value (0.36) and included terms for PC1 score and year (Table 5.5). The next best-supported model included a term for PC1 score only and received approximately three times less support than the best-supported model (*w* = 0.15, Table 5.5). Parameter likelihoods indicated strong support for PC1 score (0.99) and moderately strong support for year (0.71 for both references years, Table 5.6). However, corticosterone levels and hatch date received only weak support (0.31 and 0.31, respectively, Table 5.6). Weighted parameter estimates and associated 95% CIs indicated that structurally larger five-day-old Adélie penguin chicks were heavier (54.58 ± 8.63 CI). For all other parameters, 95% CIs overlapped 0 (Table 5.6). Only one model received a ΔAICc value ≤ 2 for describing variation in body mass of five-day-old chinstrap penguin chicks, which included a term for PC1 score only and received a moderately high *w* value (0.61, Table 5.5). Parameter likelihoods indicated strong support for PC1 score (0.99) however all other parameters received only weak support (Table 5.6). Weighted parameter estimates and associated

95% CIs indicated that structurally larger five-day-old chinstrap chicks were heavier (40.02 ± 9.49 CI). For all other parameters, 95% CIs overlapped 0 (Table 5.6). Eight models received ΔAICc value ≤ 2 for describing variation in body mass of five-day-old gentoo penguin chicks. A high degree of model uncertainty was evident as both *w* values were low across all supported models (*w*: 0.08 – 0.17, Table 5.5). Parameters included in supported models were PC1 score, corticosterone level, hatch date, year, and the interactions between corticosterone level and year or hatch date (Table 5.5). Parameter likelihoods indicated strong support for PC1 score (0.99) and moderately strong support for corticosterone level (0.56), hatch date (0.51), and year (0.56 for both reference years) terms (Table 5.6). Weighted parameter estimates and associated 95% CIs indicated that structurally larger five-day-old gentoo chicks were heavier (36.89 ± 6.39 CI). However, for all other parameters, 95% CIs overlapped 0 (Table 5.6). There was no evidence that yearly sea ice conditions or the interaction with corticosterone level influenced 5-day-old penguin chick body mass of any species as the year terms and corticosterone level by year interaction term received only weak support (Table 5.6).

Chick body mass at day 15

Two models received ΔAICc values ≤ 2 for describing variation in body mass of 15-day-old Adélie penguin chicks. The most parsimonious model included terms for PC1 score and year, while the second best-supported model included the PC1 score term only. Both supported models received similar *w* values (0.30, Table 5.7). Parameter likelihoods indicated strong support for PC1 score (0.99), and moderate support for the year term (0.51 for both references years, Table 5.8). All other parameters received only weak

support (Table 5.8). Weighted parameter estimates indicated that structurally larger 15-day-old Adélie penguin chicks were heavier (84.49 ± 18.60 CI). However, 95% CIs for all other parameters overlapped 0 (Table 5.8). Two models received ΔAICc value ≤ 2 for describing variation in body mass of 15-day-old chinstrap penguin chicks. The most parsimonious model included a term for PC1 score and corticosterone level and received a moderately weak *w* value (0.37), but a stronger r^2 value (0.57, Table 5.7). The second best-supported model included a term for corticosterone level only and received similar *w* and r^2 values as the best-supported model (0.34 and 0.46, respectively, Table 5.7). Parameter likelihoods indicated moderately strong support for PC1 score (0.51), but surprisingly strong support for corticosterone level (0.95, Fig. 5.4, Table 5.8). All other parameters received essentially no support (Table 5.8). Weighted parameter estimates indicated that structurally larger 15-day-old chinstrap penguin chicks were not heavier (16.40 ± 23.53 CI) and that 15-day-old chinstrap penguin chicks with higher corticosterone levels were lighter (-27.75 ± 17.43 CI). For all other parameters, 95% CIs overlapped 0 (Table 5.8). Three models received ΔAICc values ≤ 2 for describing variation in body mass of 15-day-old gentoo penguin chicks. The most parsimonious model, which included terms for PC1 score only, received approximately 1.5 times the support (*w* = 0.30) of the next best model that included terms for PC1 score, hatch date and year (*w* = 0.20, Table 5.7). The third best-supported model included terms for PC1 score and corticosterone levels (*w* = 0.12). Parameter likelihoods indicated strong support for PC1 score (0.99), while hatch date, year and corticosterone level all received moderate to weaker support (*w* = 0.53, 0.45 for both reference years, and 0.36, respectively, Table 5.8). Weighted parameter estimates indicated that structurally larger

15-day-old gentoo penguin chicks were heavier (120.90 ± 18.79 CI). However, 95% CIs for all other parameters overlapped 0 (Table 5.8). There was no evidence that yearly sea ice conditions or the interaction with corticosterone level influenced 15-day-old penguin chick body mass of any species as the year terms and corticosterone level by year interaction term received only weak support for all analyses (Table 5.8).

Chick body mass at week 5 (Anvers Island)

Two models received ΔAICc values ≤ 2 for describing variation in body mass of five-week-old Adélie penguin chicks. The best-supported model included terms for PC1 score and year (w = 0.48, r^2 = 0.67), while the second best-supported model included terms for PC1 score, corticosterone level, year, and the interaction between corticosterone level and year (w = 0.33, r^2 = 0.74, Table 5.9). Parameter likelihoods indicated strong support for PC1 score (0.99) and year (0.98 for both reference years), while corticosterone level received more moderate support (0.50, Table 5.10). All other parameters received weaker support (Table 5.10). Weighted parameter estimates and associated 95% CIs suggested that structurally larger five-week-old Adélie penguin chicks were heavier (214.17 ± 55.17 CI), and that chicks reared during the second year of the study following the winter of low sea ice conditions were heavier (357.37 ± 323.86 CI). For all other parameters, 95% CIs overlapped 0 (Table 5.10). Body mass analyses of five-week-old chinstrap penguins resulted in two models received ΔAICc values ≤ 2. The most parsimonious model received approximately two times the support (w = 0.60, r^2 = 0.77) of the next best model, and included terms for PC1 score and year (Table 5.9). The second best-supported model included terms for PC1 score, corticosterone level, and year, and received a

weaker *w* value (0.28), but similar r^2 value (0.78, Table 5.9). Parameter likelihoods suggested strong support for PC1 score (0.99) and year (0.89 for both reference years), but weaker support for corticosterone level (0.33, Table 5.10). Weighted parameter estimated and associated 95% CIs indicated that structurally larger five-week-old chinstrap penguins were heavier (223.11 ± 67.23 CI, Table 5.10). In addition, chicks reared in year three of the study following the winter of higher sea ice coverage were lighter (-275.01 ± 186.56, Table 5.10). For all other parameters, 95% CIs overlapped 0 (Table 5.10). Only one model received a ΔAICc value ≤ 2 for describing variation in body mass of five-week-old gentoo penguin chicks, which included a term for PC1 score only (Table 5.9). This best-supported model had a moderately high *w* (0.58) and higher r^2 (0.78) values (Table 5.9). Parameter likelihoods indicated strong support for PC1 score (0.99) and only weaker support for all other parameters (Table 5.10). Weighted parameter estimated and associated 95% CIs indicated that structurally larger five-week-old gentoo penguins were heavier (460.84 ± 84.62 CI, Table 5.10). For all other parameters, 95% confidence intervals overlapped 0 (Table 5.10). There was no evidence that yearly sea ice conditions or the interaction with corticosterone level influenced 5-week-old penguin chick body mass of any species as the year terms and corticosterone level by year interaction term received only weak support (Table 5.10).

Chick body mass at week 5 (regional islands)

Two models received a ΔAICc value ≤ 2 for describing variation in body mass of five-week-old Adélie penguin chicks at the regional scale. The most parsimonious model included terms for PC1 score, location, and year (Table 5.11). This best-supported model

received moderate *w* value (0.48) and r^2 (0.57) values. The second best-supported model included terms for PC1 score and year only and received approximately half the support (w = 0.22, r^2 = 0.59) of the best-supported model (Table 5.11). Parameter likelihoods indicated strong support for all these parameters (parameter likelihoods: 0.71 – 0.99) and weak support for all remaining parameters (Table 5.12). Weighted parameter estimates and associated 95% CIs suggested that structurally larger five-week-old Adélie penguins were heavier (203.29 ± 47.41 CI). Further, chicks were lighter in the third (-477.18 ± 177.24 CI) year of the study (Table 5.12). For all other parameters, 95% CIs overlapped 0 (Table 5.12).

Discussion

Our study tested the general hypothesis that corticosterone is a primary hormonal modulator of reproductive performance among *Pygoscelis* penguins breeding west of the AP. This is a particularly interesting system in which to examine the physiology of energy management and associations with breeding performance as climate-driven changes in marine food-webs are thought to influence the demography of these populations through energetic effects on young chicks that influence their subsequent survival and recruitment as breeding adults (e.g., Forcada and Trathan 2009, Chapman et al. 2011, Trivelpiece et al. 2011). Despite energetic influences being a central component of how regional changes in sea ice cascade to higher trophic levels, no penguin studies to date have taken an endocrine perspective to this issue and have typically relied on field–based measurements of body mass only as a proxy of quality or energetic status. Although previous studies have indicated that climate and food-web variability have

important influences on penguin chick fledge mass (e.g., Hinke et al. 2007, Trivelpiece et al. 2011, Cimino et al. 2014), in addition to our own data that suggest a provisioning diet enriched in $\delta^{15}N$ resulted in heavier five-week-old Adélie penguin chicks at the regional scale (K. B. Gorman this thesis Chapter 4), it has remained unknown whether this variation in fledge mass is at all associated with actual physiological differences in energy management. We found limited evidence for corticosterone in mediating individual variation in breeding performance across all three *Pygoscelis* species, which indicates that individuals from our study populations were generally of adequate physiological quality so as to not require the up-regulation of corticosterone.

For all analyses, with the exception of 15-day-old chinstrap penguins, there was no hormonal control of body or egg mass variation detected. We feel this conclusion is biologically real and not a reflection of the quality of the radioimmunoassay or sampling procedure employed. First, our assay validation, as reported by our intra- and inter-assay CVs, as well as parallelism CVs, indicated that the radioimmunoassay used worked reliably. Further, although we assayed very low levels of baseline corticosterone in our plasma samples, our data were within the range of levels detected by other studies of Adélie penguins. For example, Beaulieu et al. (2010) detected on average 1.5 – 3.5 ng/ml corticosterone in breeding Adélie penguins of eastern Antarctica. Vleck and Vleck (2002) also detected levels generally below 5 ng/ml for Adélie penguins nesting at Torgersen Island – one of our own study sites. Other studies also indicate low baseline corticosterone in Adélie penguins from the Ross Sea (Cockrem et al. 2006). To our knowledge, plasma corticosterone has not been previously examined in gentoo or chinstrap penguins at southern breeding colonies along the western AP (see Holberton et

al. 1996 for wintering gentoo penguins at South Georgia, see Barbosa et al. 2013 for feather corticosterone in breeding gentoo penguins at the South Shetland Islands). However, low levels similar to those detected for Adélie penguins by our study and others were also noted for chinstrap and gentoo penguins. Lastly, baseline corticosterone levels in our study, were in fact, an important predictor of body condition among 15-day-old chinstrap penguin chicks, where individuals in better condition had lower levels of corticosterone, as would be expected (Fig. 5.5). Thus, our analysis did detect the predicted relationship, but only for one breeding stage within one study species.

The study of corticosterone as a physiological mechanism linking food abundance and population processes has a well-developed history among northern seabird systems of the Gulf of Alaska. Here, studies of kittiwakes (*Rissa brevirostris* and *R. tridactyla*), puffins (*Fratercula cirrhata*) and murres (*Uria aalge*) provided much of the initial evidence that developed corticosterone as an important hormonal mediator linking food availability in the marine environment with population processes in seabirds (Kitaysky et al. 1999, Kitaysky et al. 2006, Kitaysky et al. 2007, Williams et al. 2008). Kitaysky et al. (2007) showed that as food supply diminished for common murres, circulating corticosterone profiles increased, which resulted in a decreased reproductive output. Further, at a declining colony of murres, baseline corticosterone predicted the disappearance of individuals, but not at an increasing colony (Kitaysky et al. 2007). The *Pygoscelis* penguin system of the western AP is ideal for testing similar ideas in the Southern Ocean, however, our study revealed that corticosterone is largely independent of reproductive performance in terms of body and egg mass variation. Other researchers have suggested that high latitude breeding birds circumvent trade-offs between self-

maintenance and reproduction by accumulating large fat reserves that allow for reproduction under unpredictable environmental conditions (Wingfield et al. 1994). *Pygoscelis* penguins of the western AP may be an extreme example where the accumulation of fat reserves renders the adrenocortical response unnecessary in the trade-off between self-maintenance and breeding. Along these lines, Vleck and Vleck (2000, 2002) documented no increase in corticosterone levels with fasting among Adélie penguins. Furthermore, Beaulieu et al. (2010) also detected constant corticosterone levels across breeding stages associated with variation in body mass. However, Cockrem et al. (2006) report initial corticosterone concentrations were inversely related to body mass of departing Adélie penguins lighter than 3.5 kg, unlike other studies.

Our results suggest that other physiological parameters might play an important role in mediating food-web variation, body condition, and reproductive trade-offs among *Pygoscelis* penguins of the western AP. Possible candidates are oxidative stress and immune function (K. B. Gorman unpubl. data). A growing body of literature implicates oxidative stress as being associated with costs to life span and reproduction, as well immune function or aerobic capacity (Costantini 2008). Because oxidative stress is related to aerobic metabolism, antioxidants play an important role for species that experience large variations in oxygen availability, which would be particularly true for diving seabirds (Corsolini et al. 2001). Where changes in marine food-webs cause increases in foraging effort, either by provisioning adults or newly fledged chicks, oxidative stress may be enhanced having implications for life history costs noted previously.

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Table 5.1. Candidate models describing variation in body mass of adult *Pygoscelis* penguins breeding near Anvers Island. Models presented are those determined to be most parsimonious, as well as all models receiving ΔAICc values ≤ 2.

Species	Response variable	Model number	Explanatory variable	Number of parameters	ΔAICc	*w*	r^2
Adélie penguin	Body mass	9	PC1 + Cort + NestInit, random = ~1|Nest	6	0.000	0.476	NA
		7	PC1 + NestInit, random = ~1|Nest	5	0.455	0.379	NA
Chinstrap penguin	Body mass	3	PC1	3	0.000	0.628	0.52
Gentoo penguin	Body mass	3	PC1, random = ~1|Nest	4	0.000	0.451	NA
		6	PC1 + Cort, random = ~1|Nest	5	1.874	0.177	NA

Abbreviations: ΔAICc = Akaike's Information Criterion corrected for small sample size, *w* = Akaike weight, PC1 = principal components score, Cort = corticosterone, NestInit = nest initiation date, NA = r^2 value not available for linear mixed effects models.

Table 5.2. Parameter estimates and likelihoods from candidate models for describing variation in body mass of adult *Pygoscelis* penguins breeding near Anvers Island. Parameter estimates (±1 SE) are weighted averages, and standard errors are based on unconditional variances. Parameter likelihoods are Akaike weight (*w*) values summed across all models that include the variable.

Response variable	Explanatory variable	Parameter likelihoods			Parameter estimates ± 1 SE		
		Adélie	Chinstrap	Gentoo	Adélie	Chinstrap	Gentoo
Body mass	Intercept	1.000	1.000	1.000	3975.75 ± 120.10	3705.72 ± 62.43	5052.20 ± 81.37
	PC1	0.999	0.999	0.999	217.26 ± 35.67	116.62 ± 25.45	291.62 ± 31.69
	Cort	0.586	0.180	0.279	-24.31 ± 24.72	-0.99 ± 4.06	3.18 ± 7.67
	NestInit	0.972	0.181	0.262	-33.31 ± 11.47	-0.49 ± 1.72	0.18 ± 1.52
	Year2008	0.123	0.053	0.154	-17.33 ± 39.72	-1.16 ± 5.69	-20.06 ± 42.00
	Year2009	0.123	0.053	0.154	-18.03 ± 41.44	-4.26 ± 6.60	-15.36 ± 33.16
	Cort*Year2008	0.044	4.05E-06	0.0008	3.44 ± 6.80	-0.0003 ± 0.0006	0.01 ± 0.05
	Cort*Year2009	0.044	4.05E-06	0.0008	4.05 ± 8.27	-0.0001 ± 0.0003	0.01 ± 0.05
	Cort*NestInit	0.009	6.16E-05	0.003	0.02 ± 0.07	-9.30E-05 ± 0.0005	0.001 ± 0.007

Abbreviations: ± 1SE = plus or minus 1 standard error, PC1 = principal components score, Cort = corticosterone, NestInit = nest initiation date.

Table 5.3. Candidate models describing variation in egg mass of female *Pygoscelis* penguins breeding near Anvers Island. Models presented are those determined to be most parsimonious, as well as all models receiving ΔAICc values ≤ 2.

Species	Response variable	Model number	Explanatory variable	Number of parameters	ΔAICc	*w*	r^2
Adélie penguin	Egg mass	7	Mass + PC1 + NestInit	5	0.000	0.501	0.28
Chinstrap penguin	Egg mass	3	Mass + PC1	4	0.000	0.633	0.52
Gentoo penguin	Egg mass	3	Mass + PC1	4	0.000	0.241	0.14
		6	Mass + PC1 + Cort	5	1.281	0.127	0.16
		7	Mass + PC1 + NestInit	5	1.635	0.106	0.15

Abbreviations: ΔAICc = Akaike's Information Criterion corrected for small sample size, *w* = Akaike weight, F-body mass = female body mass, PC1 = principal components score, NestInit = nest initiation date.

Table 5.4. Parameter estimates and likelihoods from candidate models for describing variation in egg mass of female *Pygoscelis* penguins breeding near Anvers Island. Parameter estimates (±1 SE) are weighted averages, and standard errors are based on unconditional variances. Parameter likelihoods are Akaike weight (*w*) values summed across all models that include the variable.

Response variable	Explanatory variable	Parameter likelihoods			Parameter estimates ± 1 SE		
		Adélie	Chinstrap	Gentoo	Adélie	Chinstrap	Gentoo
Egg mass	Intercept	1.000	1.000	1.000	32.13 ± 33.88	-4.99 ± 74.02	113.35 ± 17.47
	PC1	0.937	0.778	0.636	-2.57 ± 1.65	-0.85 ± 1.92	1.19 ± 0.78
	F-body mass	0.937	0.778	0.636	0.03 ± 0.008	0.03 ± 0.01	0.002 ± 0.003
	Cort	0.243	0.126	0.402	0.05 ± 0.23	-0.17 ± 0.27	0.38 ± 0.38
	NestInit	0.794	0.098	0.300	0.83 ± 0.45	0.003 ± 0.06	-0.02 ± 0.05
	Year2008	0.207	0.011	0.251	-1.40 ± 1.44	-0.07 ± 0.11	1.26 ± 1.34
	Year2009	0.207	0.011	0.251	-0.53 ± 0.97	-0.02 ± 0.08	1.32 ± 1.31
	Cort*Year2008	0.003	4.91E-17	0.008	0.003 ± 0.006	3.03E-16 ± 4.43E-16	-0.04 ± 0.04
	Cort*Year2009	0.003	4.91E-17	0.008	0.009 ± 0.01	-9.81E-17 ± 2.80E-16	-0.04 ± 0.04
	Cort*NestInit	0.019	1.30E-09	0.001	0.006 ± 0.007	-8.66E-10 ± 9.63E-10	7.50E-05 ± 0.0001

Abbreviations: ± 1SE = plus or minus 1 standard error, PC1 = principal components score, F-body mass = female body mass, Cort = corticosterone, NestInit = nest initiation date.

Table 5.5. Candidate models describing variation in body mass of five-day-old *Pygoscelis* penguin chicks reared near Anvers Island. Models presented are those determined to be most parsimonious, as well as all models receiving ΔAICc values ≤ 2.

Species	Response variable	Model number	Explanatory variable	Number of parameters	ΔAICc	*w*	r^2
Adélie penguin	Chick mass at day 5	10	PC1 + Year, random = ~1|Nest	6	0.000	0.364	NA
		3	PC1, random = ~1|Nest	4	1.763	0.151	NA
Chinstrap penguin	Chick mass at day 5	3	PC1	3	0.000	0.606	0.77
Gentoo penguin	Chick mass at day 5	3	PC1, random = ~1|Nest	4	0.000	0.169	NA
		6	PC1 + Cort, random = ~1|Nest	5	0.229	0.150	NA
		14	PC1 + HatchDate + Year, random = ~1|Nest	7	0.686	0.120	NA
		17	PC1 + Cort + HatchDate + Year + Cort*Year, random = ~1|Nest	10	0.932	0.106	NA
		10	PC1 + Year, random = ~1|Nest	6	1.113	0.096	NA
		18	PC1 + Cort + HatchDate + Year + Cort*HatchDate, random = ~1|Nest	9	1.232	0.091	NA
		16	PC1 + Cort + HatchDate + Year, random = ~1|Nest	8	1.278	0.089	NA
		13	PC1 + Cort + Year, random = ~1|Nest	7	1.573	0.077	NA

Abbreviations: ΔAICc = Akaike's Information Criterion corrected for small sample size, *w* = Akaike weight, PC1 = principal components score, Cort = corticosterone, HatchDate = date of hatch, NA = r^2 value not available for linear mixed effects models.

Table 5.6. Parameter estimates and likelihoods from candidate models for describing variation in body mass of five-day-old *Pygoscelis* penguin chicks reared near Anvers Island. Parameter estimates (±1 SE) are weighted averages, and standard errors are based on unconditional variances. Parameter likelihoods are Akaike weight (*w*) values summed across all models that include the variable.

Response variable	Explanatory variable	Parameter likelihoods			Parameter estimates ± 1 SE		
		Adélie	Chinstrap	Gentoo	Adélie	Chinstrap	Gentoo
Chick mass at day 5	Intercept	1.000	1.000	1.000	340.22 ± 21.50	334.61 ± 11.54	321.05 ± 32.17
	PC1	0.999	0.999	0.999	54.58 ± 4.40	40.02 ± 4.84	36.89 ± 3.26
	Cort	0.310	0.187	0.559	0.03 ± 1.07	0.14 ± 0.49	1.49 ± 2.37
	HatchDate	0.313	0.180	0.508	0.32 ± 1.10	-0.02 ± 0.40	-0.58 ± 0.97
	Year2008	0.705	0.081	0.579	28.29 ± 26.52	-1.87 ± 2.36	-28.60 ± 31.68
	Year2009	0.705	0.081	0.579	19.05 ± 20.21	-1.57 ± 2.25	-14.82 ± 17.50
	Cort*Year2008	0.063	2.22E-05	0.106	0.67 ± 1.30	1.42E-05 ± 0.0003	0.82 ± 1.53
	Cort*Year2009	0.063	2.22E-05	0.106	0.30 ± 0.73	0.0002 ± 0.0003	-0.03 ± 0.65
	Cort*HatchDate	0.017	0.0005	0.091	-0.01 ± 0.03	0.0008 ± 0.001	-0.02 ± 0.05

Abbreviations: ± 1SE = plus or minus 1 standard error, PC1 = principal components score, Cort = corticosterone, HatchDate = date of hatch.

Table 5.7. Candidate models describing variation in body mass of 15-day-old *Pygoscelis* penguin chicks reared near Anvers Island. Models presented are those determined to be most parsimonious, as well as all models receiving ΔAICc values ≤ 2.

Species	Response variable	Model number	Explanatory variable	Number of parameters	ΔAICc	*w*	r^2
Adélie penguin	Chick mass at day 15	10	PC1 + Year, random = ~1|Nest	6	0.000	0.300	NA
		3	PC1, random = ~1|Nest	4	0.041	0.290	NA
Chinstrap penguin	Chick mass at day 15	6	PC1 + Cort	4	0.000	0.373	0.57
		4	Cort	3	0.191	0.339	0.46
Gentoo penguin	Chick mass at day 15	3	PC1, random = ~1|Nest	4	0.000	0.302	NA
		14	PC1 + HatchDate + Year, random = ~1|Nest	7	0.813	0.201	NA
		6	PC1 + Cort, random = ~1|Nest	5	1.902	0.117	NA

Abbreviations: ΔAICc = Akaike's Information Criterion corrected for small sample size, *w* = Akaike weight, PC1 = principal components score, Cort = corticosterone, HatchDate = date of hatch, NA = r^2 value not available for linear mixed effects models.

Table 5.8. Parameter estimates and likelihoods from candidate models for describing variation in body mass of 15-day-old *Pygoscelis* penguin chicks reared near Anvers Island. Parameter estimates (±1 SE) are weighted averages, and standard errors are based on unconditional variances. Parameter likelihoods are Akaike weight (*w*) values summed across all models that include the variable.

Response variable	Explanatory variable	Parameter likelihoods			Parameter estimates ± 1 SE		
		Adélie	Chinstrap	Gentoo	Adélie	Chinstrap	Gentoo
Chick mass at day 15	Intercept	1.000	1.000	1.000	1251.26 ± 30.35	1234.76 ± 53.76	1124.20 ± 113.70
	PC1	0.999	0.508	0.999	84.49 ± 9.49	16.40 ± 12.00	120.90 ± 9.59
	Cort	0.233	0.954	0.362	-0.22 ± 1.48	-27.75 ± 8.89	2.56 ± 4.90
	HatchDate	0.246	0.217	0.530	-0.29 ± 1.58	1.76 ± 2.31	-3.56 ± 4.61
	Year2008	0.506	0.039	0.449	0.005 ± 19.51	-0.82 ± 2.41	-67.07 ± 93.41
	Year2009	0.506	0.039	0.449	42.32 ± 50.30	-3.19 ± 4.05	7.73 ± 43.21
	Cort*Year2008	0.007	2.74E-07	0.083	-0.03 ± 0.12	-8.81E-06 ± 2.02E-05	-1.52 ± 2.98
	Cort*Year2009	0.007	2.74E-07	0.083	-0.25 ± 0.54	-6.49E-06 ± 8.84E-06	-6.42 ± 12.69
	Cort*HatchDate	0.007	5.16E-05	0.048	0.006 ± 0.018	0.0003 ± 0.0003	0.03 ± 0.07

Abbreviations: ± 1SE = plus or minus 1 standard error, PC1 = principal components score, Cort = corticosterone, HatchDate = date of hatch.

Table 5.9. Candidate models describing variation in body mass of 5-week-old *Pygoscelis* penguin chicks reared near Anvers Island. Models presented are those determined to be most parsimonious, as well as all models receiving ΔAICc values ≤ 2.

Species	Response variable	Model number	Explanatory variable	Number of parameters	ΔAICc	*w*	r^2
Adélie penguin	Chick mass at week 5	6	PC1 + Year	5	0.000	0.481	0.67
		9	PC1 + Cort + Year + Cort*Year	8	0.752	0.330	0.74
Chinstrap penguin	Chick mass at week 5	6	PC1 + Year	5	0.000	0.600	0.77
		8	PC1 + Cort + Year	6	1.500	0.282	0.78
Gentoo penguin	Chick mass at week 5	3	PC1	3	0.000	0.583	0.78

Abbreviations: ΔAICc = Akaike's Information Criterion corrected for small sample size, *w* = Akaike weight, PC1 = principal components score, Cort = corticosterone.

Table 5.10. Parameter estimates and likelihoods from candidate models for describing variation in body mass of 5-week-old *Pygoscelis* penguin chicks reared near Anvers Island. Parameter estimates (±1 SE) are weighted averages, and standard errors are based on unconditional variances. Parameter likelihoods are Akaike weight (*w*) values summed across all models that include the variable.

Response variable	Explanatory variable	Parameter likelihoods			Parameter estimates ± 1 SE		
		Adélie	Chinstrap	Gentoo	Adélie	Chinstrap	Gentoo
Chick mass at 5 weeks	Intercept	1.000	1.000	1.000	2912.58 ± 107.55	3492.63 ± 106.89	3097.29 ± 103.79
	PC1	0.999	0.999	0.999	214.17 ± 28.15	223.11 ± 34.30	460.84 ± 43.18
	Cort	0.500	0.327	0.236	9.38 ± 14.55	-16.63 ± 18.13	-7.71 ± 17.18
	Year2008	0.975	0.887	0.235	357.37 ± 165.24	-128.68 ± 88.43	-38.89 ± 46.67
	Year2009	0.975	0.887	0.235	-64.15 ± 147.01	-275.01 ± 95.18	22.57 ± 44.71
	Cort*Year2008	0.330	0.010	0.002	-30.54 ± 26.51	0.62 ± 1.17	0.16 ± 0.49
	Cort*Year2009	0.330	0.010	0.002	14.94 ± 17.86	0.26 ± 0.93	0.16 ± 0.42

Abbreviations: ± 1SE = plus or minus 1 standard error, PC1 = principal components score, Cort = corticosterone.

Table 5.11. Candidate models describing variation in body mass of 5-week-old Adélie penguin chicks reared at regional rookeries along the western Antarctic Peninsula. Models presented are those determined to be most parsimonious, as well as all models receiving ΔAICc values ≤ 2.

Species	Response variable	Model number	Explanatory variable	Number of parameters	ΔAICc	*w*	r^2
Adélie penguin	Chick mass at week 5	14	PC1 + Location + Year	5	0.000	0.483	0.57
		10	PC1 + Year	6	1.600	0.217	0.59

Abbreviations: ΔAICc = Akaike's Information Criterion corrected for small sample size, *w* = Akaike weight, PC1 = principal components score.

Table 5.12. Parameter estimates and likelihoods from candidate models for describing variation in body mass of 5-week-old Adélie penguin chicks reared at regional rookeries along the western Antarctic Peninsula. Parameter estimates (±1 SE) are weighted averages, and standard errors are based on unconditional variances. Parameter likelihoods are Akaike weight (*w*) values summed across all models that include the variable.

Response variable	Explanatory variable	Parameter likelihoods	Parameter estimates ± 1 SE
Chick mass at 5 weeks	Intercept	1.000	3340.79 ±79.47
	PC1	0.999	203.29 ± 24.19
	Cort	0.300	0.24 ± 5.46
	LocationANVERS	0.712	-92.39 ± 61.38
	Year2008	0.999	-111.62 ± 83.90
	Year2009	0.999	-477.18 ± 90.43
	Cort*LocationANVERS	0.054	-0.99 ± 2.08
	Cort*Year2008	0.022	0.39 ± 0.94
	Cort*Year2009	0.022	0.94 ± 1.35

Abbreviations: ± 1SE = plus or minus 1 standard error, PC1 = principal components score, Cort = corticosterone.

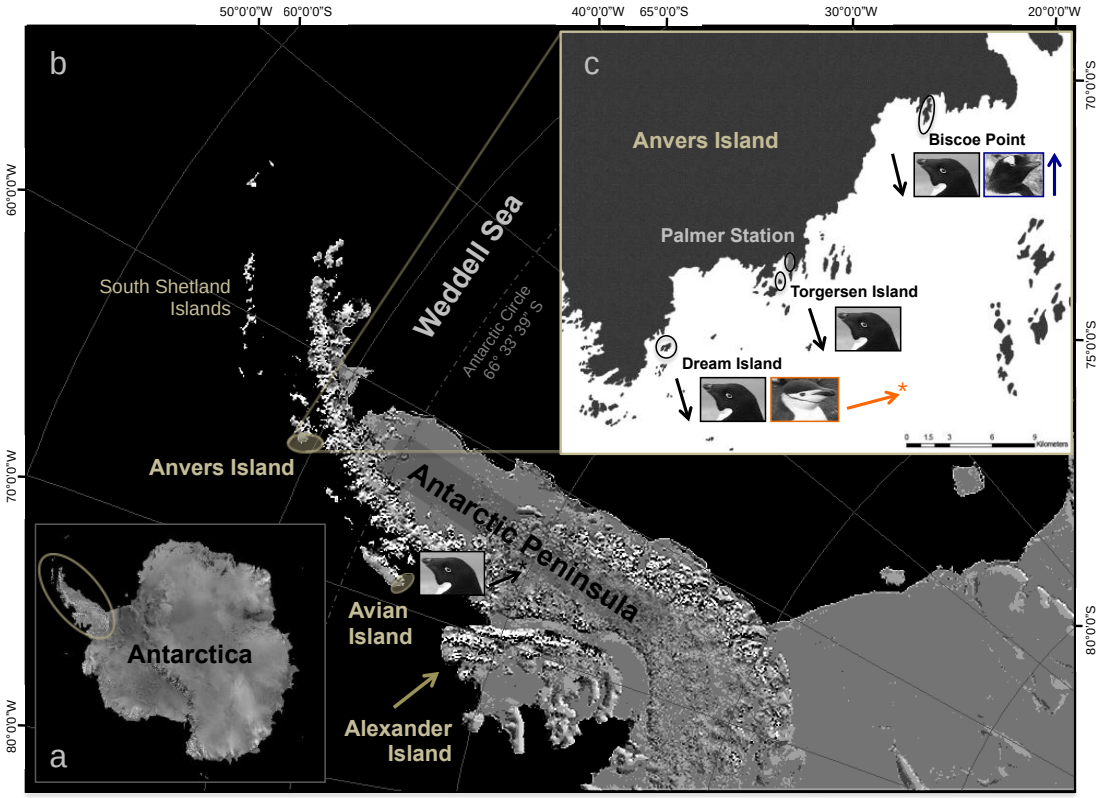

Figure 5.1. The marine ecosystem west of the Antarctic Peninsula (a) extends from northern Alexander Island to the South Shetland Islands (b). The Scotia Sea extends northeast from the South Shetland Islands (b) to include islands throughout the Scotia Arc (not pictured here). Fieldwork for the present study was conducted at major penguin rookeries within the Palmer Archipelago near Anvers Island and Palmer Station, a United States supported research base, as well as at Avian Island (b, c). Northern penguin rookeries were located at Dream (Adélie [black] and chinstrap [orange]) and Torgersen (Adélie only) Islands, as well as Biscoe Point (Adélie [black] and gentoo [blue]) (c). Southern Adélie penguin rookeries at Avian Island were located approximately 400 kilometers south of Anvers Island. Arrows color coded for each species at breeding rookeries indicate associated breeding population trajectory (i.e., declining [↓], moderately increasing [→*,→*], rapidly increasing [↑], or stable [→]). Image generated from base maps provided by the National Snow and Ice Data Center's map server A-CAP (http://nsidc.org/agdc/acap/).

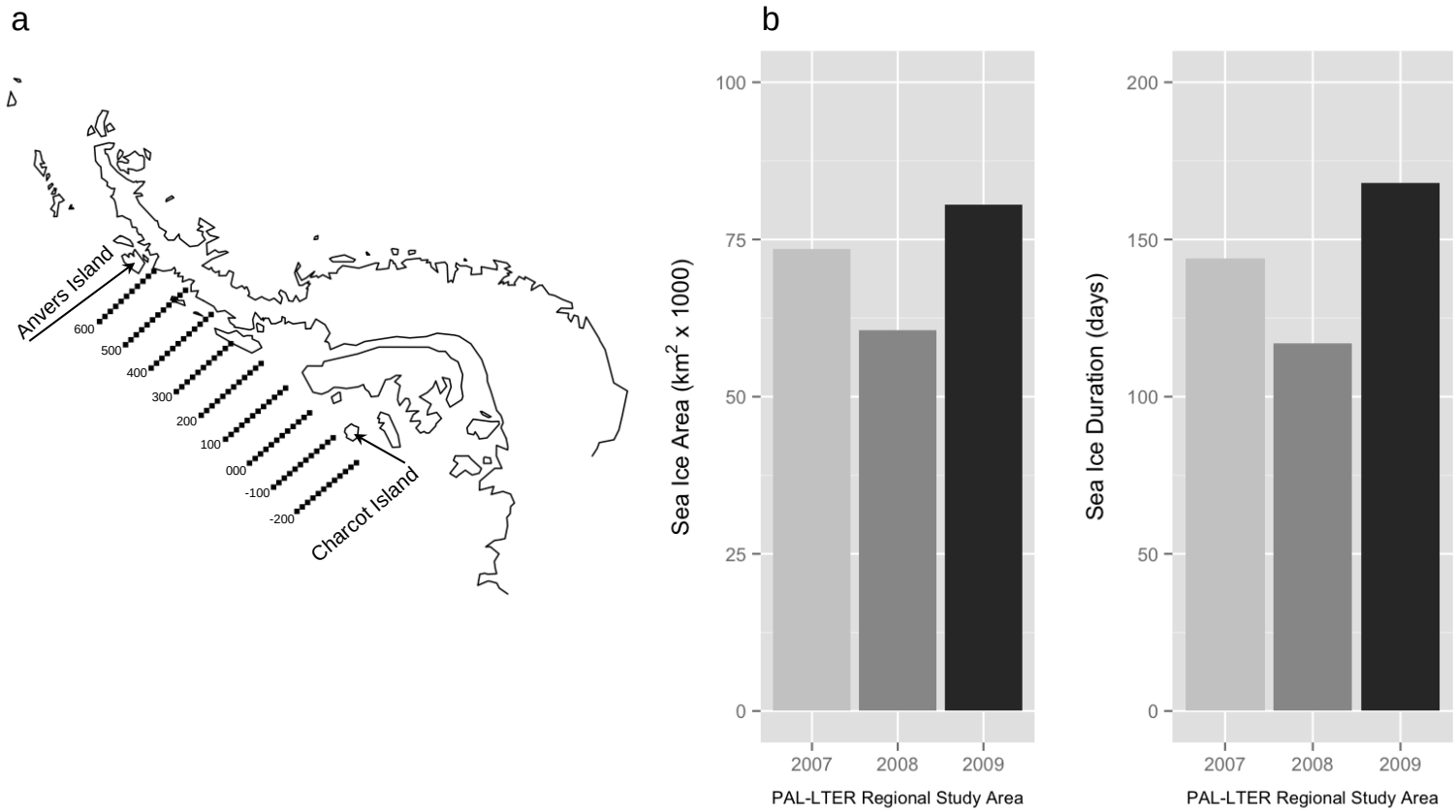

Figure 5.2. Palmer Long Term Ecological Research (PAL-LTER) program's regional study area west of the Antarctic Peninsula, extending 800 km from Anvers Island (600 line) to just south of Charcot Island (-200 line) and 220 km inshore to offshore (a). Study area grid lines encompass the entire area over which sea ice metrics were calculated. Winter average sea ice area (km2) and sea ice duration (days) within the PAL-LTER regional study area for each year of the present study (b).

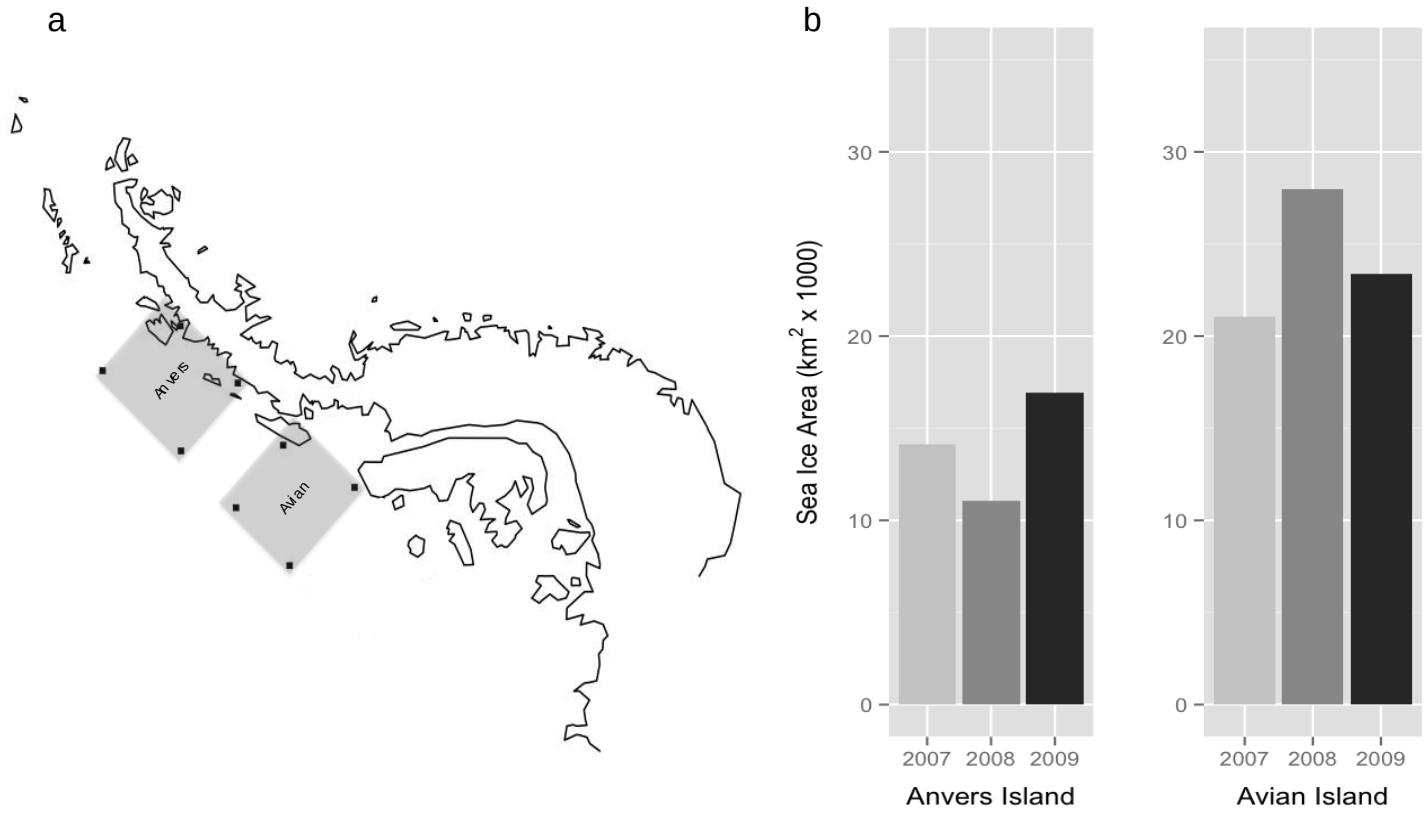

Figure 5.3. Penguin foraging grids (200 km^2) off of Anvers and Avian Islands west of the Antarctic Peninsula (a). Winter average sea ice area (km^2) within regional penguin foraging grids for each year of the present study (b).

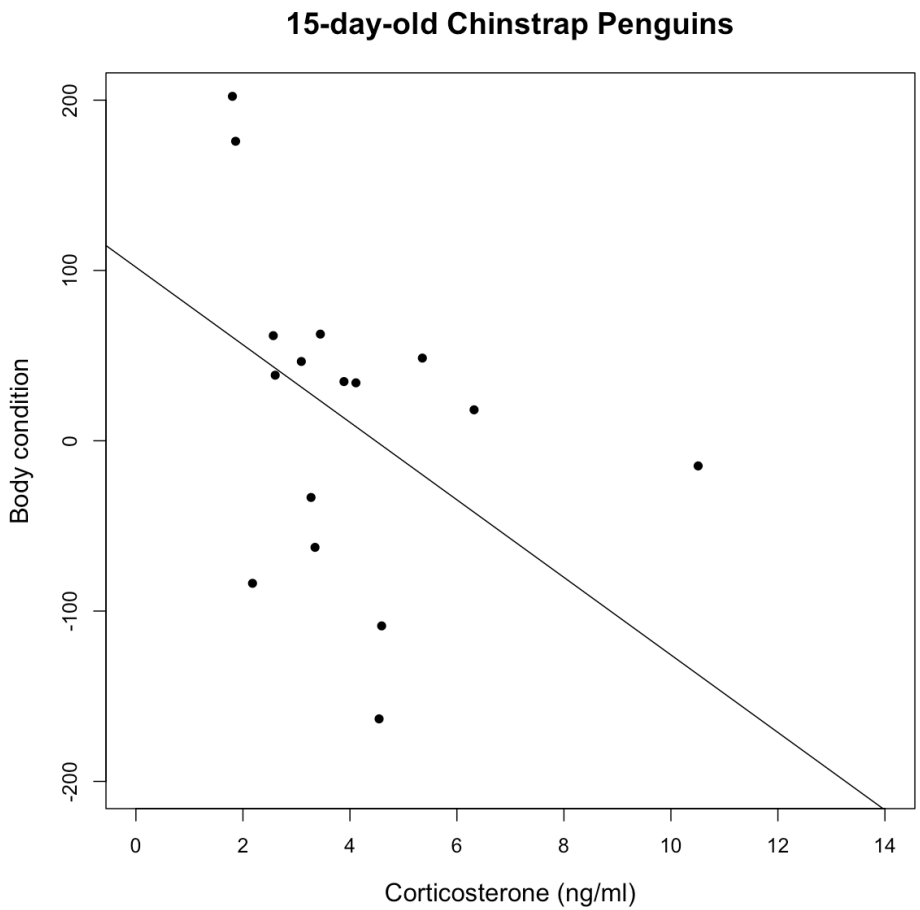

Figure 5.4. Variation in body condition of 15-day-old chinstrap penguins in relation to circulating levels of baseline corticosterone. Body condition is presented as residual values from the relationship between body mass and PC1 score for structural size for each individual.

Appendix 5.1. Candidate models describing variation in body mass of adult male and female *Pygoscelis* penguins. Each species was considered separately.

Model number	Parameters included	Model type
1	Mass.g~1[1]	Null
2	Mass.g~Year[1]	Main effect
3	Mass.g~PC1[1]	Main effect
4	Mass.g~Cort[1]	Main effect
5	Mass.g~NestInit[1]	Main effect
6	Mass.g~PC1 + Cort[1]	Multiple predictor
7	Mass.g~PC1 + NestInit[1]	Multiple predictor
8	Mass.g~Cort + NestInit[1]	Multiple predictor
9	Mass.g~PC1 + Cort + NestInit[1]	Multiple predictor
10	Mass.g~PC1 + Year[1]	Multiple predictor with year
11	Mass.g~Cort + Year[1]	Multiple predictor with year
12	Mass.g~NestInit + Year[1]	Multiple predictor with year
13	Mass.g~PC1 + Cort + Year[1]	Multiple predictor with year
14	Mass.g~PC1 + NestInit + Year[1]	Multiple predictor with year
15	Mass.g~Cort + NestInit + Year[1]	Multiple predictor with year
16	Mass.g~PC1 + Cort + NestInit + Year[1]	Global
17	Mass.g~PC1 + Cort + NestInit + Year + Cort*Year[1]	Interaction
18	Mass.g~PC1 + Cort + NestInit + Year + Cort*NestInit[1]	Interaction

Abbreviations: Mass.g = adult penguin body mass (grams), PC1 = principal components score, Cort = corticosterone level, NestInit = nest initiation date.

[1]Candidate linear mixed effects models for Adélie and gentoo penguins also included a random effect of nest on intercept (random = ~1|nest).

Appendix 5.2. Candidate models describing variation in egg mass of adult female *Pygoscelis* penguins. Each species was considered separately.

Model number	Parameters included	Model type
1	Egg.Mass.g~1	Null
2	Egg.Mass.g~Year	Main effect
3	Egg.Mass.g~Mass.g + PC1	Multiple predictor
4	Egg.Mass.g~Cort	Main effect
5	Egg.Mass.g~NestInit	Main effect
6	Egg.Mass.g~Mass.g + PC1 + Cort	Multiple predictor
7	Egg.Mass.g~Mass.g + PC1 + NestInit	Multiple predictor
8	Egg.Mass.g~Cort + NestInit	Multiple predictor
9	Egg.Mass.g~Mass.g + PC1 + Cort + NestInit	Multiple predictor
10	Egg.Mass.g~Mass.g + PC1 + Year	Multiple predictor with year
11	Egg.Mass.g~Cort + Year	Multiple predictor with year
12	Egg.Mass.g~NestInit + Year	Multiple predictor with year
13	Egg.Mass.g~Mass.g + PC1 + Cort + Year	Multiple predictor with year
14	Egg.Mass.g~Mass.g + PC1 + NestInit + Year	Multiple predictor with year
15	Egg.Mass.g~Cort + NestInit + Year	Multiple predictor with year
16	Egg.Mass.g~Mass.g + PC1 + Cort + NestInit + Year	Global
17	Egg.Mass.g~Mass.g + PC1 + Cort + NestInit + Year + Cort*Year	Interaction
18	Egg.Mass.g~Mass.g + PC1 + Cort + NestInit + Year + Cort*NestInit	Interaction

Abbreviations: Egg.Mass.g = female penguin egg mass (grams), Mass.g = adult penguin body mass (grams), PC1 = principal components score, Cort = corticosterone level, NestInit = nest initiation date.

Appendix 5.3. Candidate models describing variation in body mass of male and female *Pygoscelis* penguin chicks at five- and 15-days-old. Each species was considered separately.

Model number	Parameters included	Model type
1	Mass.g~1[1]	Null
2	Mass.g~Year[1]	Main effect
3	Mass.g~PC1[1]	Main effect
4	Mass.g~Cort[1]	Main effect
5	Mass.g~HatchDate[1]	Main effect
6	Mass.g~PC1 + Cort[1]	Multiple predictor
7	Mass.g~PC1 + HatchDate[1]	Multiple predictor
8	Mass.g~Cort + HatchDate[1]	Multiple predictor
9	Mass.g~PC1 + Cort + HatchDate[1]	Multiple predictor
10	Mass.g~PC1 + Year[1]	Multiple predictor with year
11	Mass.g~Cort + Year[1]	Multiple predictor with year
12	Mass.g~HatchDate + Year[1]	Multiple predictor with year
13	Mass.g~PC1 + Cort + Year[1]	Multiple predictor with year
14	Mass.g~PC1 + HatchDate + Year[1]	Multiple predictor with year
15	Mass.g~Cort + HatchDate + Year[1]	Multiple predictor with year
16	Mass.g~PC1 + Cort + HatchDate + Year[1]	Multiple predictor with year
17	Mass.g~PC1 + Cort + HatchDate + Year + Cort*Year[1]	Interaction
18	Mass.g~PC1 + Cort + HatchDate + Year + Cort*HatchDate[1]	Interaction

Abbreviations: Mass.g = chick penguin body mass (grams), PC1 = principal components score, Cort = corticosterone level, HatchDate = date of hatch.

[1]Candidate linear mixed effects models for Adélie and gentoo penguins also included a random effect of nest on intercept (random = ~1|nest).

Appendix 5.4. Candidate models describing variation in body mass of male and female five-week-old *Pygoscelis* penguin chicks reared near Anvers Island.

Model number	Parameters included	Model type
1	Mass.g~1	Null
2	Mass.g~Year	Main effect
3	Mass.g~PC1	Main effect
4	Mass.g~Cort	Main effect
5	Mass.g~PC1 + Cort	Multiple predictor
6	Mass.g~PC1 + Year	Multiple predictor with year
7	Mass.g~Cort + Year	Multiple predictor with year
8	Mass.g~PC1 + Cort + Year	Multiple predictor with year
9	Mass.g~PC1 + Cort + Year + Cort*Year	Interaction

Abbreviations: Mass.g = adult penguin body mass (grams), PC1 = principal components score, Cort = corticosterone level.

Appendix 5.5. Candidate models describing variation in body mass of male and female five-week-old Adélie penguin chicks reared along the western Antarctic Peninsula.

Model number	Parameters included	Model type
1	Mass.g~1	Null
2	Mass.g~Year	Main effect
3	Mass.g~PC1	Main effect
4	Mass.g~Cort	Main effect
5	Mass.g~Location	Main effect
6	Mass.g~PC1 + Cort	Multiple predictor
7	Mass.g~PC1 + Location	Multiple predictor
8	Mass.g~Cort + Location	Multiple predictor
9	Mass.g~PC1 + Cort + Location	Multiple predictor
10	Mass.g~PC1 + Year	Multiple predictor with year
11	Mass.g~Cort + Year	Multiple predictor with year
12	Mass.g~Location + Year	Multiple predictor with year
13	Mass.g~PC1 + Cort + Year	Multiple predictor with year
14	Mass.g~PC1 + Location + Year	Multiple predictor with year
15	Mass.g~Cort + Location Year	Multiple predictor with year
16	Mass.g~PC1 + Cort + Location + Year	Multiple predictor with year
17	Mass.g~PC1 + Cort + Location + Year + Cort*Location	Interaction
18	Mass.g~PC1 + Cort + Location + Year + Cort*Year	Interaction

Abbreviations: Mass.g = adult penguin body mass (grams), PC1 = principal components score, Cort = corticosterone level.

Chapter 6.

Population Genetic Structure and Gene Flow of Adélie Penguins (*Pygoscelis adeliae*) Breeding West of the Antarctic Peninsula

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Abstract

Adélie penguins are responding to ocean-climate variability west of the Antarctic Peninsula (AP) where some breeding rookeries have declined by 80% from historical numbers. We used both nuclear and mitochondrial DNA (mtDNA) markers to better understand historical population genetic structure and gene flow given relatively recent and ongoing reductions in sea ice habitats associated with changes in the number of breeding adults at rookeries in this region. Specifically, we estimated genetic diversity, spatial genetic structure, population demography and gene flow among four regional Adélie penguin rookeries. Our analyses indicated little genetic structure overall based on bi-parentally inherited microsatellite markers (θ_{ST} = –0.006–0.004). Similarly, we observed no significant variance in overall haplotype frequency (mtDNA Φ_{ST} = 0.017; P = 0.112). However, some comparisons with Charcot Island were significant and suggestive of female-biased philopatry. Estimates of gene flow based on a two population coalescent model were asymmetrical from the species regional core rookery at Avian Island to its northern range rookery at Anvers Island. We conclude that Adélie penguins breeding throughout the western AP are a panmictic population and appear to hold adequate genetic diversity and dispersal capacity to be resilient to environmental change. However, sex-biased philopatry/dispersal may be an underappreciated feature of the Adélie penguin system that has been decoupled where environmental conditions are less stable such as the northwestern AP. Demographic structuring, despite the lack of genetic structure reported here, may characterize these populations and should not be overlooked.

Key words: Adélie penguin, breeding population, gene flow, genetic structure, microsatellite, mitochondrial DNA, Pygoscelis adeliae, sea ice, western Antarctic Peninsula

Introduction

The penguins (Spheniscidae) include 18 extant species that diversified in association with a northward dispersal out of Antarctica, and subsequent isolation and adaptation to island environments of the sub-Antarctic and continents at lower southern latitudes, as a result of historical global cooling events that started during the Eocene (~50 million years ago [mya]) and magnified between 34 – 25 and 14 – 12 mya (Baker et al. 2006). Only two species, the emperor penguin (*Aptenodytes forsteri*) - a member of the basal lineage that diverged 40 mya, and the Adélie penguin (*Pygoscelis adeliae*) - a member of the second oldest lineage that diverged 38 mya, remained in Antarctica and adapted to the cold, sea ice environment of the Southern Ocean where they currently hold circumpolar distributions at the highest coastal southern latitudes (Williams 1995, Ainley 2002, Baker et al. 2006). The nature of the Spheniscidae diversification out of Antarctica has resulted in both the *Aptenodytes* and *Pygoscelis* genera representing the greatest evolutionary history of the family (see Baker et al. 2006 Figs. 2 and 3), defined as species' branch lengths within a phylogenetic tree representing time since divergence from the last common ancestor where longer branch lengths assume deeper evolutionary history (Crozier 1997, Nee and May 1997, Heard and Mooers 2000).

Although *Aptenodytes* and *Pygoscelis* penguins are currently ranked by the International Union for Conservation of Nature (IUCN) as species of lower conservation concern (i.e., least concern and near threatened), which contrasts with the vulnerable or endangered listings of the great majority of other penguin species (IUCN 2013), breeding rookeries of *Aptenodytes* and *Pygoscelis* penguins are responding to ocean-climate variability in the Southern Ocean (Forcada and Trathan 2009) particularly throughout the

Scotia Sea and marine ecosystem west of the Antarctic Peninsula (AP, Fig. 6.1a-c, Ducklow et al. 2007, Trivelpiece et al. 2011, Lynch et al. 2012, Ducklow et al. 2013). Here, environmental warming has been especially marked and associated with considerable reductions in over-winter sea ice formation (Stammerjohn et al. 2008a, b), particularly in comparison with other regions of the Antarctic (Stammerjohn et al. 2012). As for example, the most northern breeding rookery of emperor penguins along the western AP located at the Dion Islands in Marguerite Bay (Fig. 6.1b), which once included 150 breeding pairs in the mid-1900's, is now extirpated (Trathan et al. 2011). Similarly, breeding rookeries of Adélie penguins located near Anvers Island in the northwestern AP (Fig. 6.1b, c) have declined by 80% from historical numbers (Ducklow et al. 2007, Ducklow et al. 2013). In addition, widespread rookery declines, of up to 50%, have been noted among nesting Adélie and chinstrap (*P. antarctica*) penguins of the western AP and the South Shetland Islands over approximately the last 30 years (Trivelpiece et al. 2011, Lynch et al. 2012). Because *Aptenodytes* and *Pygoscelis* penguins represent the greatest evolutionary history of the Spheniscidae, current changes in the number of adults breeding at rookeries occurring throughout the Scotia Sea and western AP have conservation importance. A better understanding of the spatial genetic structure of breeding rookeries and gene flow among them would help evaluate these populations' vulnerability to current and future environmental change, particularly their adaptive capacity to deal with environmental perturbations in terms of genetic diversity and dispersal capacity (e.g., Foden et al. 2013).

To this end, we studied the population genetics of Adélie penguins breeding throughout four major rookeries located west of the AP as a model system for better

understanding historical genetic structuring and gene flow given relatively recent and ongoing reductions in sea ice habitats associated with changes in rookery trends within this region of the Southern Ocean. Adélie penguins are known to be philopatric to their natal breeding colonies (Ainley et al. 1983), which is expected based on the species' life histories of high adult survivorship, delayed onset of reproductive maturity and low annual reproductive output (Williams 1995, Ainley 2002). Since philopatry reduces the likelihood an individual disperses to a new breeding area, this behavior can create fine-scale patterns of spatial genetic structure across populations in contrast to dispersal behavior that generally acts as a homogenizing force on genetic variability (Avise 2000). Within this context, breeding Adélie penguins are expected to show significant genetic structuring, however, of the few studies that have examined this question for the species, a lack of genetic differentiation based on bi-parentally inherited nuclear markers was observed at the circumpolar level among rookeries of eastern Antarctica, the Victoria Land coast of the Ross Sea, and the Antarctic Peninsula (Roeder et al. 2001). However, a study of the ancient and modern mitochondrial DNA (mtDNA) genome of Adélie penguins collected from around the continent, which unlike nuclear markers is only maternally inherited, revealed two distinct lineages – one present in the Ross Sea only, while the other was found in all other regions of Antarctica including the Ross Sea (Ritchie et al. 2004). There is therefore some evidence that nuclear versus mtDNA genetic markers, that differ in modes of inheritance, in Adélie penguins show contrasting patterns of population structure, which is generally consistent with sex-biased dispersal tendencies (Avise 2000). To date, no study has concurrently evaluated both nuclear and mtDNA genomes of Adélie penguins to assess sex-biased patterns of breeding population

genetic structure, particularly at the regional scale within Antarctica (but see Clucas et al. 2014 regarding Scotia Arc and western AP regional variability in Adélie penguin mtDNA markers only).

Here, we apply a series of population genetic analyses to both the nuclear and mtDNA genomes to develop estimates of genetic diversity, spatial genetic structure, population demography and gene flow at the regional scale among Adélie penguins breeding along the western AP. A population genetics approach will provide a better understanding of the species' ability to respond to future environmental changes in terms of impacts to genetic diversity and a possible role for sex-biased dispersal - issues highly relevant to a number of Antarctic penguins within the *Aptenodytes*, *Pygoscelis*, and *Eudyptes* genera in this region of the Southern Ocean (Forcada and Trathan 2009, Clucas et al. 2014). Study rookeries encompass 800 kms (Fig. 6.1b, c), which captures a latitudinal gradient in annual sea ice coverage (see Ducklow et al. 2013 Fig. 2) and change in rookery trajectory. Along the Palmer Archipelago near Anvers Island, Adélie penguins occur at the margin of their northern breeding range along the western AP (Fraser et al. 1992, Polito et al. 2011), which is contracting given extensive population declines of 80% from historical numbers (Ducklow et al. 2007, see Ducklow et al. 2013 Fig. 7) and reflects trends for the species throughout other rookeries of the region (Trivelpiece et al. 2011, Lynch et al. 2012). Similarly, Adélie penguins nesting at Prospect Point have also declined (W. R. Fraser unpubl. data). These breeding rookery declines have occurred in association with significant, long-term reductions in annual sea ice coverage within the northern seasonal sea ice zone along the western AP (Jacobs and Comiso 1997, Ducklow et al. 2007, Stammerjohn et al. 2008a, see Chapman et al. 2010

Fig. 1, Stammerjohn et al. 2012, Ducklow et al. 2013). However, breeding rookeries of Adélie penguins at Avian and Charcot Islands, within the species core to most southern distribution along the western AP, respectively, have increased (Avian Island, Sailley et al. 2013) or appear stable based on currently available census data (Charcot Island, Henderson 1976, W. R. Fraser unpubl. data). At these southern breeding rookeries, over-winter sea ice coverage remains a prominent physical oceanographic feature (Ducklow et al. 2013).

Materials and Methods

Field methods

Adélie penguins nesting on several islands within the Palmer Archipelago west of the AP near Anvers Island (64°46'S, 64°03'W, Fig. 6.1a-c) were studied during the austral summers of 2008/09 and 2009/10. Specifically, study nests were located on Biscoe (64°48'S, 63°46'W), Torgersen (64°46'S, 64°04'W) and Dream (64°43'S, 64°13'W) Islands (Fig. 6.1c). Each study season, Adélie penguin study nests (n = 30) were distributed equally between the three study islands, with 10 nests located on each island. Each season, study nests, where pairs of adults were present, were individually marked and chosen before the onset of egg laying, and monitored consistently. When study nests were found at the one-egg stage, both adults were captured to obtain blood samples used for genetic analyses. At the time of capture, each adult penguin was quickly blood sampled (~1 ml) from the brachial vein using a sterile 3 ml syringe and non-heparinized infusion needle. After handling, individuals at study nests were further monitored to

ensure the pair reached clutch completion, i.e., two eggs. Final sample sizes of adults used in analyses (*n* = 100) differ from the number of original study nests reported here as at times weather conditions hindered rookery access resulting in some adults reaching clutch completion before sampling, and therefore, these study nests were not sampled and excluded from subsequent monitoring. During annual oceanographic research cruises aboard the A.R.S.V. *Laurence M. Gould*, as part of the Palmer Station, Antarctica, Long-Term Ecological Research Program (PAL-LTER), in January of 2009 - 2011, we further blood sampled five-week-old crèched Adélie penguin chicks for genetic analyses from rookeries at Prospect Point (*n* = 15 in January 2011), Avian (*n* = 46 total between January 2009 and 2010) and Charcot (*n* = 25 total between January 2010 and 2011) Islands (Fig. 6.1b). Blood samples from crèched chicks (~1 ml) were taken from the brachial vein using a sterile 3 ml syringe and non-heparinized infusion needle following sampling procedures used for adult penguins. All collected blood was stored in 1.5 ml microcentrifuge tubes containing 1 ml of Longmire blood preservation buffer (Longmire et al. 1988). Within 12 hours of field collection, tubes containing whole blood in buffer were stored frozen at -80 ºC.

Laboratory methods – genotyping and sequencing

Blood samples in preservation buffer were extracted according to Medrano et al. (1990) using modifications reported in Sonsthagen et al. (2004). Sex of all sampled individuals was determined genetically using methods outlined by Gorman et al. (2014). Genotype data from a total of 186 individuals were collected for 10 polymorphic microsatellite loci using primers selected from the published literature (AM12, AM13, RM3, RM6 after

Roeder et al. 2001, Emm1, Emm4 after Billing et al. 2007, Ech008, Ech060, Ech065, Ech091 after Ahmed et al. 2009). Primer sequences are identified in Table 6.1, including those redesigned for this study. Polymerase chain reaction amplification of microsatellite loci followed multiplex procedures similar to those described by Handel et al. (2006) and Cronin et al. (2009). To obtain size standards for each locus, six individuals were scored against a fluorescently labeled M13 sequence ladder of known size. Based on these standards, genotypes for each individual were determined using GeneImagIR™ 4.05 software (Scanalytics, Inc.). For quality control purposes, DNA from a minimum of 10% of the individuals representing each designated subpopulation was re-extracted, amplified and sequenced or genotyped again. Level of error due to allelic dropout, null alleles, or scoring error was assessed for microsatellite loci using MICRO-CHECKER (van Oosterhout et al. 2004).

A fragment of approximately 467 nucleotide-pairs (nt) comprising a portion of domain I of the penguin mitochondrial DNA control region was amplified using the primers CR122 (5'- CAAGTACATTTAATGTACG-3'), and CR576 (5'-GAATGGTTGAATGTTGGGC-3'). PCR products were purified using Exo-SAP-IT® PCR Product Cleanup (Affymetrics, Inc., Cleveland, OH). Purified products were cycle-sequenced via simultaneous bidirectional sequencing (LI-COR 1999) using a commercial kit (Sequitherm LCII 2.0®: Epicentre Technologies, Madison, WI) following protocols described in Sonsthagen et al. (2004). Comparison of sequences with homologous sequences archived in GenBank (Ritchie et al. 2004) verified that the sequences generated were likely of mitochondrial in origin, rather than from a nuclear pseudogene (Sorenson and Fleischer 1996). For quality control purposes, DNA from two to five

individuals representing each sampling rookery were extracted, amplified, and sequenced in duplicate. MtDNA sequences were analyzed using eSeq™ ver. 2.0 (LI-COR Inc., Lincoln, Nebraska) imaging software and aligned using AlignIR ver. 2.0™. Haplotype sequence data were archived in GenBank (accession numbers forthcoming).

Statistical methods

Estimation of genetic diversity

We calculated number of alleles (A) and private alleles (PA) using Microsatellite Toolkit (Park 2001). Allelic richness and inbreeding coefficient (F_{IS}) were calculated using FSTAT version 2.9.3 (Goudet 1995). Observed and expected heterozygosities, Hardy-Weinberg equilibrium (HWE), and linkage disequilibrium were calculated for each microsatellite locus in GENEPOP 3.1 (Raymond and Rousset 1995). Estimates of average relatedness (*rxy*) were calculated using INDENTIX (Belkhir et al. 2002).

MtDNA control region haplotypes were assigned based on at least a single nucleotide substitution (no insertions or deletions were observed) found within the segment sequenced. We used ARLEQUIN 3.11 (Excoffier et al. 2005) to estimate haplotype (h) and nucleotide (π) diversity (Nei 1987, Eq. 8.4 and 10.6, respectively). We tested the hypothesis of selective neutrality for mtDNA control region sequence data, and for historical fluctuations in population demography, using Fu's F_S (Fu 1997) and Tajima's D (Tajima 1989), implemented in ARLEQUIN. We applied critical significance values of 5%, which requires a P-value of below 0.02 for Fu's F_S (Fu 1997). To place the individual assayed from each of the four sampling rookeries (i.e., Anvers, Prospect Point, Avian, and Charcot Islands) within a larger context, we also generated a minimum

evolution (ME) phylogenetic tree to show relationships based on our data relative to 105 haplotypes reported in Richie et al. (2004), retrieved from GenBank. The ME tree was generated using MEGA (Tamura et al. 2013), under the Kimura two parameter distance model (Kimura 1980).

Estimation of spatial genetic structure

Spatial variance in allelic and haplotypic frequencies, overall and between populations, was calculated (θ_{ST}, R_{ST} and Φ_{ST}, respectively) using ARLEQUIN. Φ_{ST} values were calculated using the Kimura two parameter model provided in ARLEQUIN. Because samples sizes varied among populations (Goudet et al. 1996), population differentiation based on χ^2 distributions of alleles and haplotypes was also determined using GENEPOP, applying Bonferroni correction ($\alpha = 0.05$) for microsatellite data.

We used the Bayesian clustering program, STRUCTURE 2.3.3, to assign breeding individuals to clusters based on their microsatellite allelic frequencies to infer the occurrence of population structure (Pritchard et al. 2000, Hubisz et al. 2009). Data were analyzed using an admixture model assuming correlated frequencies and assisted by sample group information with the number of possible populations (K) ranging from 1 to 5 (search strategy: 50,000 burn-in period, 500,000 Markov chain Monte Carlo iterations); the analysis was repeated five times.

Estimation of population demography

Evidence for fluctuations in recent population demography was evaluated for all microsatellite loci using Bottleneck 1.2.02 (Cornuet and Luikart 1996). We conducted Bottleneck analyses under the infinite allele model (IAM, Maruyama and Fuerst 1985), stepwise mutation model (SMM, Ohta and Kimura 1973), and a two-phase model of

mutation (TPM, Di Rienzo et al. 1994). Parameters for the TPM were set at 80% SMM with a variance of 9% (Piry et al. 1999, Garza and Williamson 2001); 1000 simulations were performed for each population. Significance was assessed using a Wilcoxon sign-rank test, which determines if the average of standardized differences between observed and expected heterozygosities is significantly different from zero (Cornuet and Luikart 1996). Significant heterozygote deficiency values relative to the number of alleles indicate recent population growth, whereas heterozygote excess relative to the number of alleles indicates a recent population bottleneck (Cornuet and Luikart 1996). BOTTLENECK compares heterozygote deficiency and excess relative to genetic diversity, not to Hardy-Weinberg equilibrium expectation (Cornuet and Luikart 1996). For Anvers Island Adélie penguins, we performed BOTTLENECK analyses using only the seven loci that conformed to HWE.

We tested for historical fluctuations in population demography, using Fu's F_S (Fu 1997) and Tajima's *D* (Tajima 1989), described above. We also generated mismatch distributions (Rogers and Harpending 1992) using ARLEQUIN, to test whether individuals from in each breeding rookery deviated from the sudden expansion model.

Estimation of gene flow

Estimates of gene flow between the Anvers Island and Avian Island breeding rookeries were calculated in BayesAss version 3.0.1 (Wilson and Rannala 2003) and MIGRATE version 3.0.3 (Beerli and Felsenstein 1999, 2001), as Avian Island is the largest breeding rookery of Adélie penguins in the region (Poncet and Poncet 1987) and is therefore considered a possible source for other regional rookeries. These programs use different models to estimate rates of gene flow. BayesAss uses an assignment method and does not

incorporate genealogy, whereas MIGRATE uses a coalescent model of population differentiation that incorporates 2 parameters scaled to the mutation rate (μ): θ, the effective population size parameter ($4N_e\mu$), and M, the rate of gene flow (m/μ). Estimates of gene flow can thus be interpreted differently and at different temporal scales. BayesAss reflects gene flow that occurred only in the past several generations, whereas in MIGRATE gene flow estimates are averaged over the past *n* generations, where *n* equals the number of generations the populations have been at mutation-drift equilibrium.

BayesAss was conducted with the microsatellite dataset using the default delta values for allelic frequency, migration rate, and inbreeding. Subsequent analyses incorporated different delta values to ensure that proposed changes between chains at the end of the run were between 20 - 40% of the total chain length. Once delta values (ΔA = 0.50, Δm = 0.10, and ΔF = 0.80) were within the accepted proportion of proposed changes (a = 20%, m = 38%, and f = 27%), analyses were conducted 3 additional times (10,000,000 iterations, 1,000,000 burn-in, and sampling frequency of 200) with different random seeds, and parameter estimates converged.

MIGRATE analyses was also conducted with microsatellite data included a full migration model for which θ, the effective population size parameter ($4N_e\mu$), and M, the rate of gene flow (m/μ), were estimated individually from the data. The two population migration model was compared to a restricted model ([1] θ was averaged and M was symmetrical between the two populations) to test for asymmetry in gene flow between rookeries. MIGRATE was run using maximum likelihood search parameters; 10 short chains (1000 used trees out of 250,000 sampled), five long chains (7,500 used trees out 1,875,000 sampled), and five adaptively heated chains (start temperatures: 1, 1.5, 3, 6,

and 12; swapping interval = 1). Models were run five times, and parameters converged. We evaluated the alternative model for goodness-of-fit given the data using a log-likelihood ratio test (Beerli and Felsenstein 2001).

Data management

Microsatellite data reported here are publicly available within the PAL-LTER data system (dataset #239): http://oceaninformatics.ucsd.edu/datazoo/data/pallter/datasets. These data are additionally archived within the US LTER Network's Information System Data Portal: https://portal.lternet.edu/. Individuals interested in using these data are therefore expected to follow the US LTER Network's Data Access Policy, Requirements and Use Agreement: http://www.lternet.edu/policies/data-access, in addition to performing any necessary laboratory cross-validation (contact SLT: stalbot@usgs.gov).

Results

Genetic diversity

Unique genotypes were observed for all individuals in the study (n = 186) based on data collected from 10 polymorphic microsatellite loci. Average number of alleles (A) and allelic richness (AR) per microsatellite locus ranged from 3 to 19 with an average of 9.8 alleles per locus, and 2.08 to 10.77 with an average of 5.38 per locus, respectively (Table 6.1). For each breeding population, A and AR ranged from 49 to 90 and 4.9 to 5.52, respectively (Table 6.2). Alleles only observed within a single population (i.e., PA) occurred within each breeding population and ranged from 1 to 16, with the Anvers

Island breeding population holding the greatest number (Table 6.2). Observed heterozygosity for each breeding population ranged from 56% to 63% with an average of 60% for all populations. Average relatedness (*rxy*) ranged from -0.011 to -0.064 (Table 6.2).

All loci and populations were in Hardy-Weinberg equilibrium (HWE) and linkage equilibrium (χ^2 values ranged from 1.29 to 15.66, $P > 0.0125$) with one exception: Anvers Island deviated overall from HW expectations ($\chi^2 = 45.67$, df = 20, $P_{(\alpha:\ 0.005)} = 0.0009$) due to significant nonconformities at three loci (Ech060: $P_{(\alpha:\ 0.0125)} = 0.011$; Emm1: $P_{(\alpha:\ 0.0125)} = 0.012$; and RM3: $P_{(\alpha:\ 0.0125)} = 0.003$). Among these three loci, potential nulls were identified by MICROCHECKER (Ech060, $P_{(\alpha:\ 0.05)} < 0.01$). Inbreeding coefficient (F_{IS}) ranged from -0.029 to 0.067 (Table 6.2) and was not significantly different from zero overall ($F_{IS} = 0.015$, 95% confidence intervals [CI] = -0.009, 0.040).

Fifty-two mtDNA control region haplotypes characterized by 79 polymorphic sites (all transitions) across a total of 415 nt consistently retrieved across all samples were identified among 54 penguins from across the four sampling rookeries (Anvers Island n = 17, Prospect Point n = 11, Avian Island n = 16, and Charcot Island n = 10). No mtDNA haplotypes were shared among locales, with 1 exception: haplotype 11 was shared between Prospect Point and Avian Island. Haplotype diversity (h) was very high, ranging from 0.993 (Anvers) to 1.00 (Prospect Point, Avian, and Charcot Islands), and nucleotide diversity (π) ranged from 0.016 to 0.021 (Table 6.2). Fu's F_S and Tajima's D were not significantly positive (Table 6.2), suggesting the portion of the mitochondrial control region assayed is selectively neutral.

Phylogenetic analyses of our 52 haplotypes and sequences archived in GenBank (Fig. 6.2) demonstrated that all haplotypes assayed from all four regional rookeries belonged to the Antarctic lineage (A), one of the two monophyletic lineages of Adélie penguins in Antarctica observed by Ritchie et al. (2004).

Spatial genetic structure

Spatial genetic structure based on overall (θ_{ST} = 0.00; 95% CI:-0.003, 0.004) and population pairwise θ_{ST} values was not observed based on microsatellite markers (Table 6.3). Our analyses of microsatellite data in STRUCTURE further confirmed a lack of breeding population differentiation based in microsatellite loci as the likelihood was maximized when *K* equaled 1 (LnP = -5034.73; population prior assigned) and sample group information was not informative (r >8.5). Similarly, we observed no significant variance in mitochondrial haplotype frequency overall; only 1.67% of the variance was partitioned among populations (Φ_{ST} = 0.017; P = 0.112). However, some population pairwise Φ_{ST} values, which ranged from -0.017 to 0.066, were significant: Prospect Point was significantly differentiated from Charcot Island, and Avian Island was significantly differentiated from Charcot Island (Table 6.3). χ^2 values for microsatellites ranged from 11.33 to 29.79 and were non-significant (Table 6.3), while those for mtDNA all equaled infinity and were significant (∞, Table 6.3).

Population demography

Evidence for recent fluctuations in population demography was not detected for Prospect Point, Avian or Charcot Island rookeries based on the IAM, SMM and TPM mutation

models for all 10 microsatellite loci ($P_{(\alpha:0.005)}$ = 0.065 – 0.98). For Anvers Island, we removed the three loci previously identified as deviating from HWE for this population from analyses and again did not detect any evidence for changes in population demography ($P_{(\alpha:0.007)}$ = 0.34 – 0.99). Therefore, we conclude there is no strong evidence for recent (within the last several to hundreds of generations) fluctuations in population demography among study rookeries. Mismatch distributions for mitochondrial DNA failed to deviate from the signal of sudden population expansion (mean SSD = 0.011, sd = 0.003 P = 0.209) and were not significantly ragged (mean raggedness index = 0.028, sd = 0.006, P = 0.563), and neutrality tests (Fu's F_S and Tajima's D, Table 6.2) were significantly negative, consistent with a more historical signal of population expansion.

Gene flow

Asymmetry in gene-flow estimates was observed based on results from the two population MIGRATE model (Table 6.4), where number of immigrants (Nem) to the Anvers Island rookery (8.9, 95% CI = 7.8, 10.2) was significantly greater than the Nem from this population (6.2, 95% CI = 5.4, 7.1). Similar gene-flow asymmetry was not observed based on results from the BayesAss model where 95% CIs for immigration and emigration overlapped (Table 6.4). We note that gene flow estimated in BayesAss differs from those estimated in MIGRATE; BayesAss analyses are frequency-based, and results only reflect the last few generations (up to 10 generations), and the values reported (m) represent the proportion of the population identified as dispersing from the other populations. By contrast, MIGRATE analyses are coalescent-based, and Nem values reflect the average number of migrants per generation, across hundreds of generations.

Discussion

Our analyses contribute to a growing interest in better understanding regional breeding population genetic structure of *Pygoscelis* penguins given ongoing climate-driven alterations in regional sea ice dynamics associated with breeding population changes among Antarctic penguins of the Scotia Sea and western AP (e.g., Clucas et al. 2014). Central to these current ecological issues, and especially to predicting the response of populations to future environmental change, is discerning population units that reflect meaningful genetic and/or demographic independence. Demographic studies of Antarctic penguins have been conducted based on banded individuals to assess apparent survival, breeding success and movement (e.g., Dugger et al. 2010, Trivelpiece et al. 2011). However, researchers have noted significant survival differences in banded versus un-banded penguins, particularly so within some years (Gauthier-Clerc et al. 2004, Dugger et al. 2006), which has raised questions regarding the ethics and biases introduced by these techniques. In some cases where breeding rookeries are in severe decline, researchers have entirely forfeited penguin-banding efforts given the apparent negative consequences (W. R. Fraser, pers. comm.). Furthermore, banding studies are difficult to conduct over the large spatial scales typically needed to assess demographic variability relevant to the population structure of highly mobile wildlife such as penguins, especially in remote regions such as Antarctica. Given the limitations of traditional demographic studies, we chose to tackle the issue of population structure from a molecular evolutionary perspective. Although there is considerable debate regarding the criteria used for defining population and sub-population units important to conservation (Moritz 1994, Esler et al. 2006, Pearce and Talbot 2006), genetic distinctiveness is widely

appreciated as a critical component of population delineation as nuclear and mtDNA signatures ultimately reflect variation in the demographic processes of reproductive survival and dispersal at various temporal scales related to coalescence as a function of effective population size (Zink and Barrowclough 2008, Allendorf et al. 2013).

We detected similar levels of genetic diversity at nuclear loci (H_O: 56% - 63%) in comparison with many avian taxa considered at lower extinction risk (Evans and Sheldon 2008), in addition to high mtDNA diversity (*h*: 0.99 – 1.0, π = 0.016 - 0.021) that corroborates recent findings by Clucas et al. (2014). Our estimates of higher genetic diversity across all breeding rookeries accord with the lack of any recent fluctuations in population demography as indicated by our data; had we detected any notable population bottleneck among our study rookeries, we would have expected to observe reduced diversity indices. The high genetic diversity observed across sampling rookeries is consistent with Ritchie et al. (2004), who analyzed mtDNA control region diversity (based on 594 nt) in 557 Adélie penguins from 16 breeding areas representing three regions in Antarctica, including the western AP, as well as fossil bones from 17 locations along the coast of the Ross Sea. Ritchie et al. (2004) reported haplotype diversity ranges in modern Adelie penguins from 0.957 to 1.000, and nucleotide diversity from 0.021 to 0.060, similar to the results we report here. Two monophyletic lineages (clades) of Adélie penguins in Antarctica were observed (Ritchie et al. 2004). One lineage was recorded mostly in the Ross Sea (Ross Sea lineage, RS); the other lineage (Antarctic lineage, A) was present across the continent, including the Antarctic Peninsula. Comparison of our 52 haplotypes with data archived in GenBank (Fig. 6.2) demonstrated that all haplotypes assayed from our study rookeries belonged to the A lineage as would be expected.

We detected an overall lack of genetic structure among regional breeding rookeries of Adélie penguins based on both marker types (Table 6.3), as well as our Bayesian clustering analysis using STRUCTURE. Therefore, our data confirm a panmictic population, which was also reported by Clucas et al. (2014) based on analyses of mtDNA only of Adélie penguins also of this region, as well as a circumpolar study of Adélie penguin nuclear markers (Roeder et al. 2001). Of note, the genetic survey we report here is representative of breeding rookeries not sampled by Clucas et al. (2014), therefore, our combined results offer a more complete understanding of Adélie penguin population genetic structure of the region, which generally suggests considerable standing genetic variation not accounted for by breeding location. However, one important distinction between our work and that reported by Clucas et al. (2014) is that our data were obtained from individuals specifically associated with breeding rookeries as samples were collected from reproductive adults or their chicks. Clucas et al. (2014) collected genetic data primarily from the calamus of shed feathers found at rookeries as late as the month of May, well after the summer breeding season of penguins that ends in February. Adélie penguins are known to typically molt on the pack ice after having migrated from breeding colonies (Ainley 2002). In regions where sea ice is less available, which is particularly true for the western AP, Adélie penguins will molt on land after leaving breeding rookeries to fatten as the molt process is energetically expensive. In these cases, Adélie penguins do not necessarily seek out molt sites at their breeding rookery (Ainley 2002). Because of the movements of penguins between breeding and molting, it is possible the shed feathers collected by Clucas et al. (2014) do not represent individuals who actually reproduced at their sampling locations, but more likely

individuals who molted there. Therefore, although results accord well from the only two regional western AP/Scotia Sea studies of Adélie penguin genetic diversity and spatial genetic structure, it is unclear to what degree results based on DNA obtained from shed feathers reflect breeding individuals, unlike our study. We suggest future research on the population genetics of breeding *Pygoscelis* penguins focus on collecting DNA samples from regions where the reproductive status of individuals is specifically known.

Significant population differentiation was detected at two of the three pairwise comparisons with Charcot Island based on mtDNA analyses only (Table 6.3). As pairwise population comparisons based on nuclear markers were all non-significant (Table 6.3), we interpret these results as being suggestive of sex-biased dispersal strategies occurring at Charcot Island only. The effective population size of nuclear markers is four-times that of mtDNA markers (Allendorf et al. 2013), therefore, it would be expected that more population structure be observed based on mtDNA markers in comparison with nuclear markers due to the inherent differences in coalescence times (Zink and Barrowclough 2008). When we compared pairwise estimates of rookery genetic differentiation between nuclear and mtDNA markers to the expected levels of nuclear structure given that observed in mtDNA to establish an equivalence between the two approaches, we found that all pairwise comparisons not including Charcot Island were distributed near equivalence (Fig. 6.3). However, all pairwise comparisons including Charcot Island fell notably outside the expected equivalence values of population differentiation between nuclear and mtDNA markers (Fig. 6.3). Many studies have interpreted such results as being reflective of sex-biased dispersal where females show philopatry to breeding areas, while males are more dispersive (see Zink and

Barrowclough 2008 for review and references therein for examples). Interestingly, female-biased philopatry and male-mediated dispersal is generally more typical of mammalian than avian systems (Greenwood 1980, Dobson 1982, Clarke et al. 1997). These issues raise the question as to why sex-biased philopatry/dispersal would be more evident at Charcot Island as we did not detect such patterns of population structure among other rookery comparisons along the western AP. Charcot Island is the most southern rookery of Adélie penguins in this region of the Antarctic. The marine environment here likely represents more suitable ecological conditions for the species similar to those found elsewhere around the continent that are characterized by more predictable, persistent sea ice throughout the year (Ainley 2002), unlike more northern regions along the western AP (Ducklow et al. 2013, Schofield et al. 2013). We speculate that female-biased philopatry and male-mediated dispersal may be a more characteristic element of breeding rookeries located at higher southern latitudes with more suitable ecological conditions. No other study to date has concurrently examined both marker types to lend insight on whether sex-biased philopatry/dispersal is a general feature of the Adélie penguin system that may be decoupled where environmental conditions are less stable such as the northwestern AP where the species exists at its northern range limit (Fraser et al. 1992, Williams 1995, Polito et al. 2011).

Estimates of gene flow based on a two population coalescent model (MIGRATE) were asymmetrical from the species regional core rookery at Avian Island to its northern range rookery at Anvers Island, however this result was not corroborated by our frequency-based analysis (BayesAss) that reflects up to the last 10 generations (Table 6.3). Gene flow estimates for Adélie penguins have not been reported previously. Our

result of asymmetrical gene flow may reflect the fact that the Anvers Island breeding rookery is located at the species northern range margin along the western AP, and therefore has historically always operated as a population sink.

Regarding our studies relevance to predicting future responses of Adélie penguins to ocean-climate warming, we conclude that Adélie penguins of the western AP hold a reasonable capacity to adapt to new environmental conditions given our observations of high genetic diversity. However, due to the fact the species is known to have considerable dispersal ability based on annual migratory patterns (e.g., Ballard et al. 2010) and a lack of genetic structuring as our study and others have reported, it is more likely that Adélie penguins would simply disperse to regions where environmental conditions are more suitable. Also within this context, the likelihood that Adélie penguins would respond to new environmental conditions via adaptation is made much more difficult by their long generation times. However, demographic structuring without genetic structuring is certainly a possible scenario for our study system. Therefore, changes in numbers of adults at breeding rookeries should continue to be studied from the perspective of environmental impacts on survival (e.g., Chapman et al. 2011, Fraser et al. 2013).

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Table 6.1. Microsatellite loci used to assess population genetic structure of Adélie penguins breeding west of the Antarctic Peninsula. Primer sequence (universal tail in parentheses), allele size range in base pairs, number of alleles, literature source for primers and GenBank accession numbers are reported.

Locus	Primer sequence (5'–3')[a]	Allele size range (bp)	A	Literature source	GenBank accession no.
AM12F	F: (M13F) AAAAACCCAACACAACAAAC	159-171[b]	8	Roeder et al., 2001	AF131242
AM12R	R: CCCAAGAAGAGATTTGTGAG				
AM13F	F: (M13R) TTTTCCCATCTCTCTCCTG	131-156	19	Roeder et al., 2001	AF131243
AM13R	R: CAGTTTTCAACAATCCTTCC				
Ech008-1F	F: (M13F) AAAGGGCACAAATACAGC[c]	157-161	3	Ahmed et al., 2009	FM878339
Ech008-1R	R: CTCTCCCATTTTGTAGGG [c]				
Ech060-1F	F: (SP6) CTAAGTTGCCTGCTAAGC[c]	131-141	6	Ahmed et al., 2009	FM878391
Ech060-1R	R: CATCTTAATGTCCCTAGC[c]				
Ech065-1F	F: (M13F) TCTCTGACATGTATGGGG[c]	177-193[b]	13	Ahmed et al., 2009	FM878396
Ech065-1R	R: CACAAAGGAACACTGGGC[c]				
Ech091-1F	F: (M13R) CATAAAAGCTGGCCTGTC[c]	176-206	9	Ahmed et al., 2009	FM878422
Ech091-1R	R: AGAACTACTGTTCCAAGG[c]				
Emm1-1F	F: (M13F) ACACCCCATTAAGGATGCC[c]	109-131	9	Billing et al., 2007	DQ837729
Emm1-1R	R: TGGGTGAAGTGCCTTGAGC[c]				
Emm4-1F	F: (SP6) GGCCTAACAAACTACAGC[c]	138-166[b]	17	Billing et al., 2007	DQ837732
Emm4-1R	R: AAACATTTCCCATCCTGC[c]				

RM3F	F: (SP6) AATCAGGCTCCAAGGTCA	235-247[b]	8	Roeder et al., 2001	AF289546
RM3-1R	R: ATGCAAGTGACACAAAGGC[c]				
RM6-1F	F: (M13R) ACTGTCCTTTAGGCATGC[c]	170-182	6	Roeder et al., 2001	AF289547
RM6R	R: CTGTTTACATCCGATGCA				

Abbreviations: bp = basepair, A = number of alleles, no. = number.

[a]Universal tail primer sequences; M13F (CACGACGTTGTAAAACGAC), M13R (GGATAACAATTTCACACAGG), and SP6 (GATTTAGGTGACACTATAG).

[b]Signifies the presence of 1 base pair repeat.

[c]Microsatellite redesigned for this study.

Table 6.2. Estimates of genetic diversity of Adélie penguins breeding west of the Antarctic Peninsula. Number of alleles per breeding population, private alleles, average allelic richness, inbreeding coefficient, observed and expected heterozygosities, average relatedness and its variance were calculated using 10 microsatellite loci. Number of haplotypes, haplotype and nucleotide diversity, Fu's F_S and Tajima's D values estimated from mitochondrial DNA (mtDNA) control region loci. Bold values are significant at α = 0.05.

	Microsatellite loci									Mitochondrial DNA loci					
Study population	*n*	A	PA	AR	F_{is}	H_O	H_E	*rxy*	*rxy* variance	*n*	*K*	*h*	π	Fu's F_s	Tajima's *D*
Anvers Island	100	90	16	5.52	0.032	0.61	0.63	-0.011	0.041	17	16	0.99	0.020	**-7.91**	**-1.36**
Prospect Point	15	49	1	4.90	0.067	0.56	0.60	-0.064	0.051	11	11	1.00	0.021	**-4.70**	**-1.56**
Avian Island	46	71	5	5.27	-0.029	0.63	0.61	-0.022	0.039	16	16	1.00	0.018	**-10.31**	**-1.39**
Charcot Island	25	56	2	4.93	-0.004	0.61	0.61	-0.040	0.051	10	10	1.00	0.016	**-4.89**	**-1.53**

Abbreviations: *n* = sample size, A = number of alleles per breeding population, PA = private alleles, AR = allelic richness, F_{is} = inbreeding coefficient, H_O and H_E = observed and expected heterozygosities, *rxy* = average relatedness, *h* = haplotype diversity, π = nucleotide diversity, *K* = number of haplotypes.

Table 6.3. Pairwise and overall estimates of population differentiation among Adélie penguins breeding west of the Antarctic Peninsula based on θ_{ST} and R_{st} calculated from 10 microsatellite loci. Mitochondrial control region haplotypes were used to calculate Φ_{ST} values. Results of chi-square analysis of the distribution of alleles and haplotypes also are given. Bold values are significant at α = 0.05, Bonferroni corrections applied for microsatellite data.

Study population	Microsatellite loci			Mitochondrial DNA loci	
	θ_{ST}	χ^2	R_{st}	Φ_{ST}	χ^2
Anvers Island					
-Prospect Point	-0.005	11.327	-0.013	-0.018	∞[a]
-Avian Island	0.004	29.792	-0.003	0.011	∞[a]
-Charcot Island	-0.003	12.950	0.011	0.039	∞[a]
Prospect Point					
-Avian Island	-0.005	15.803	-0.019	-0.025	∞[a]
-Charcot Island	-0.006	19.215	-0.006	**0.056**	∞[a]
Avian Island					
-Charcot Island	0.002	23.947	0.001	**0.066**	∞[a]
Overall	0.00	17.791	-0.003	0.017	∞[a]

[a]α < 0.000001, df = 2.

Table 6.4. Two population model gene flow matrix for Adélie penguins breeding west of the Antarctic Peninsula calculated from 10 microsatellite loci using frequency- and coalescent-based methods. Parameter estimates for number of migrants per generation (BayesAss: m%, MIGRATE: Nem) are listed for the Anvers and Avian Island population pair only, and are reported as immigration to and emigration from Anvers Island. The 95% confidence intervals are given in parentheses.

Study population	BayesAss – m (%)			MIGRATE - Nem		
	Im	Em	Gene flow direction	Im	Em	Gene flow direction
Anvers Island						
-Avian Island	21.1 (0.0–43.1)	24.4 (6.6–42.2)	None	8.9 (7.8–10.2)	6.2 (5.4–7.1)	Dispersal into Anvers Island

Abbreviations: m = percent migrants per generation, Nem = number of migrants per generation, Im = immigration, Em = emigration.

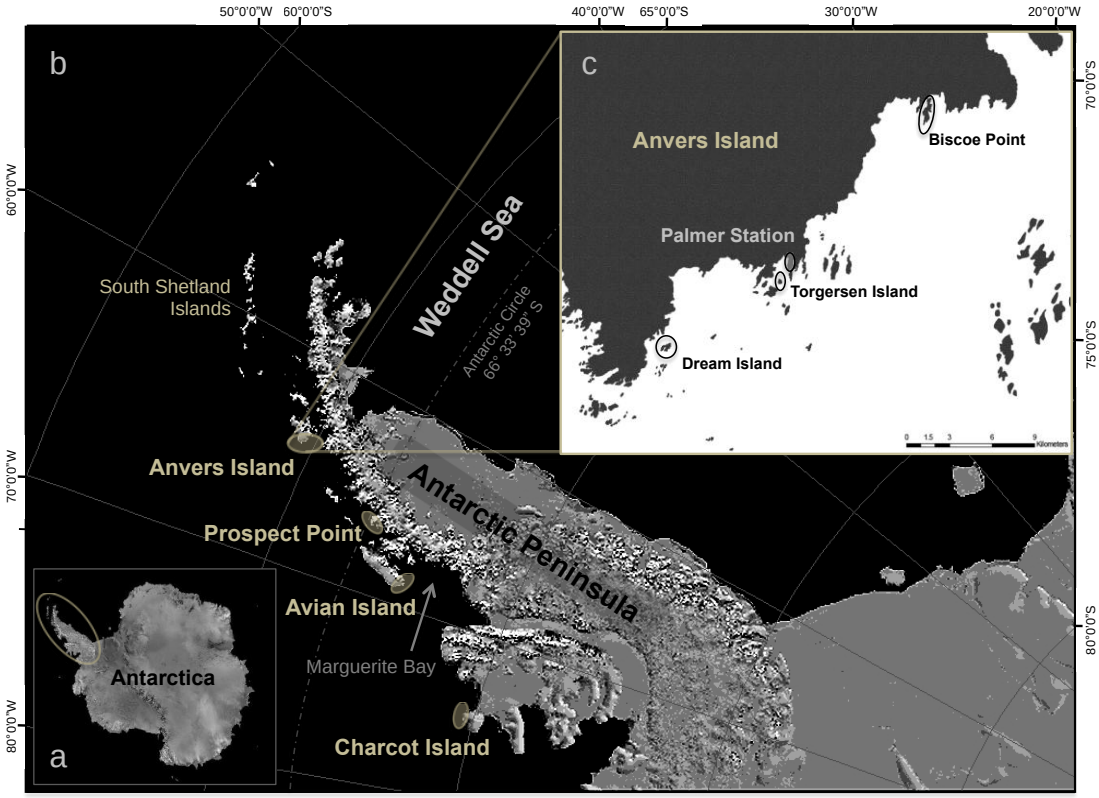

Figure 6.1. The marine ecosystem west of the Antarctic Peninsula (a) extends from northern Alexander Island (approximately Charcot Island) to the South Shetland Islands (b). Fieldwork for the present study was conducted at major penguin rookeries within the Palmer Archipelago near Anvers Island and Palmer Station, a United States supported research base, as well as at Prospect Point, Avian and Charcot Islands (b, c). Northern penguin rookeries were located at Dream and Torgersen Islands, as well as Biscoe Point (c). Image generated from base maps provided by the National Snow and Ice Data Center's map server A-CAP (http://nsidc.org/agdc/acap/).

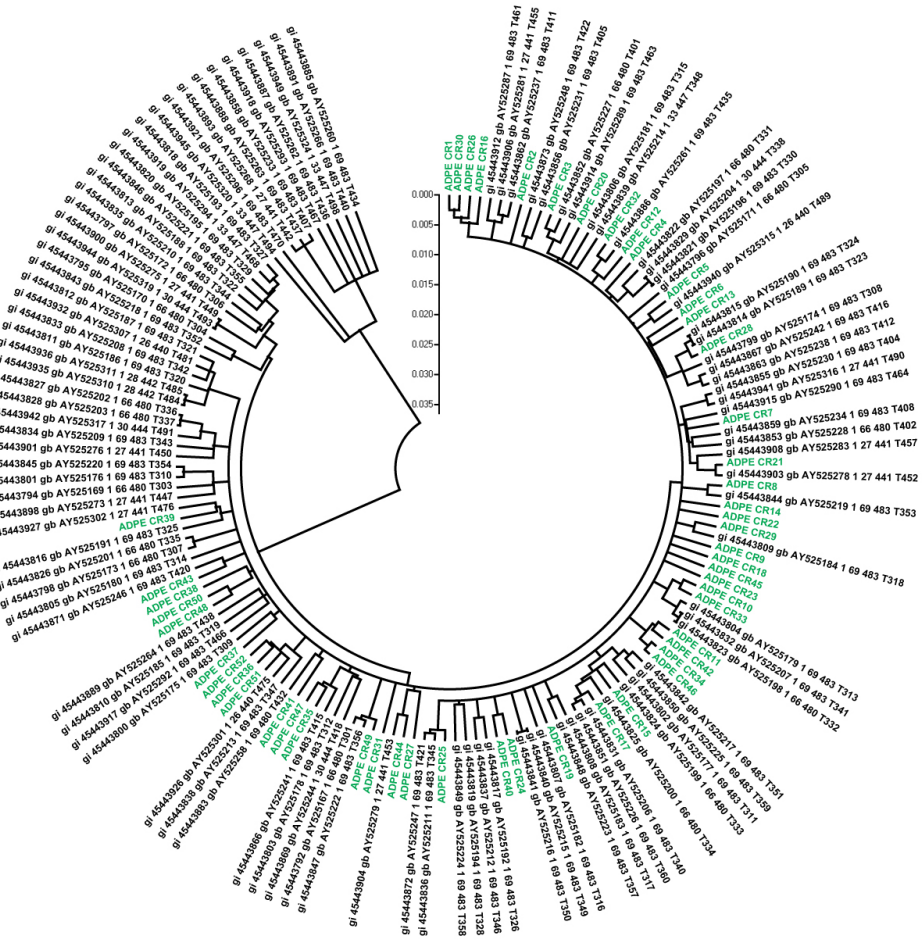

Figure 6.2. Minimum evolution (ME) phylogenetic tree showing relationships among 52 mitochondrial DNA control region haplotypes (ADPE CR01-52) assayed from 54 Adélie penguins from among four major breeding rookeries located west of the Antarctic Peninsula (see Fig. 1) relative to 105 haplotypes reported in Ritchie et al. (2004), retrieved from GenBank. All haplotypes assayed were found to belong to the Antarctic clade A following Ritchie et al. (2004).

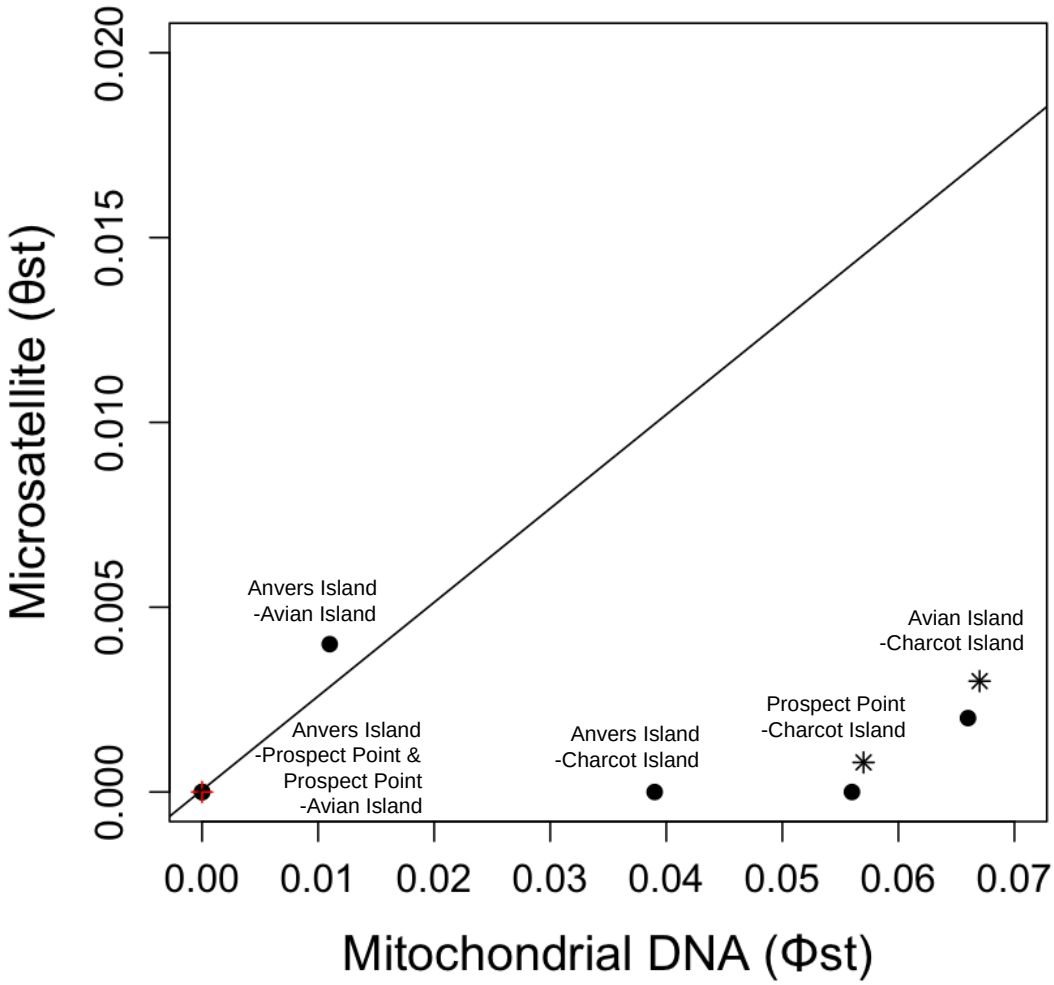

Figure 6.3. Comparison of pairwise estimates of rookery differentiation among Adélie penguins breeding west of the Antarctic Peninsula based on mitochondrial DNA Φ_{ST} and microsatellite θ_{ST} values. Regression line indicates the level of genetic structure expected at nuclear loci given the level of structure observed at mtDNA markers. Rookery pairwise comparisons (significant comparisons noted by *) located below the regression line are suggestive of female-biased philopatry or male-biased dispersal. Red cross indicates a second data point at that position.