POPULATION STRUCTURE AND FORAGING ECOLOGY OF SOUTHERN RIGHT WHALES (*EUBALAENA AUSTRALIS*): INSIGHTS FROM ISOTOPIC AND GENETIC ANALYSES

by

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ABSTRACT

Ocean warming will undoubtedly affect the migratory patterns of many marine species, but specific changes can be predicted only where behavioral mechanisms guiding migration are understood. Southern right whales (*Eubalaena australis)* show maternally inherited site fidelity to near-shore winter nursery grounds, but exactly where they feed in summer remains mysterious. Southern right whales are recovering from exploitation during the past two centuries, but their population numbers remain low at approximately 20% of pre exploitation estimates. However, their reproduction is influenced by reduction of food abundance linked to increased sea surface temperatures following El Niño events.

Here, I present the work conducted for my doctoral dissertation, which is aimed at increasing our understanding of the population structure, the foraging ecology and the migratory strategies of southern right whales.

In this dissertation I show that genetic and isotopic signatures, analyzed together, indicate that maternal lineages are structured over an isotopic range, and that the isotope ratios from adult females are more similar than expected among individuals sharing the same mitochondrial haplotype. This pattern suggests a strong maternally directed site fidelity to summer feeding grounds. Such fidelity would be expected to limit the exploration of new feeding opportunities and might explain why this population shows increased rates of reproductive failure in years following sea surface temperature anomalies in the western South Atlantic. By comparing isotope ratios from skin samples

with published and unpublished values of potential prey and other predators I have also discovered that southern right whales appear to have at least three different foraging strategies probably associated with different migratory patterns.

Additionally, I found significant genetic differentiation between live and dead animals, with an overrepresentation of dead calves within one of the two major phylogenetic clades known for this species. This overrepresentation is likely the outcome of a recent influx of migrants from populations where this clade is more abundant.

Finally, I present a study aimed at comparing mother and calf isotope ratios. I discovered interannual variability in the magnitude of nitrogen and carbon fractionation, which was interpreted as potential differences in the nutritional stress of the mother-calf pairs across years.

To my family in Argentina, for all their emotional support during these long years.

To Sydney, for all her love, friendship and help.

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CHAPTER 1

INTRODUCTION

Background

Many animal species are capable of some kind of movement across different geographical and temporal scales (Dingle and Drake 2007). For example, many shrimp species undergo daily vertical migrations in the water column, many birds and mammals have seasonal roundtrips between breeding and feeding grounds, and some insect species experience multigenerational migrations over long distances (Dingle and Drake 2007). As they move, these animals are subject to predictable changes across their environment and they adjust their physiology and behavior accordingly to maximize their fitness (Ramenofsky and Wingfield 2007). However, these animals are also subject to unpredictable changes such as climate variability, presence of invasive species and human impact occurring at different locations within their range. How these animals cope with such unpredictable changes is an important ecological and evolutionary question; Does a populations' migratory strategy change in accordance with environmental circumstances or do these changes result from a change at the evolutionary level?

Southern right whales (*Eubalaena australis*) migrate over long distances, travelling across a wide range of marine ecosystems (IWC 2001). Right whales migrate from cold, productive waters where they feed on large quantities of Antarctic krill (*Euphausia superba*) to coastal nursery grounds where little food is available. The evolutionary

constraints that such a predictable "feast and famine" migratory pattern enforces on these whales are unknown; but undoubtedly characteristics such as growth rate, foraging and lactating strategies, parental care, and social behavior have been affected (Clapham 2000).

Southern right whales have also suffered remarkably unpredictable changes that have affected their survival. The most notorious is the whaling industry, which reduced their populations to a small percentage (<20%) of prewhaling numbers (IWC 2001). Today, after the end of whaling activities, right whale populations are recovering at surprisingly high rates (about 7% for the entire southern hemisphere (IWC 2001). In addition, it appears that right whales reproductive output responds to fluctuations in krill abundance linked to El Niño-Southern Oscillation (Leaper *et al.* 2006). Yet, how is it possible that a population at a fraction of its historic levels is suffering from food shortage? Is this a density-dependent factor due to a decrease of the carrying capacity of the Southern Ocean ecosystem (Atkinson *et al.* 2004), or do the foraging strategies and population structure of the whales play an important role? Understanding these questions would be extremely useful in predicting the effect of climate change on these and other marine migrants (Kintisch 2006).

Summary of Research

Here, I present the work conducted for my doctoral dissertation, which is aimed at increasing our understanding of the population structure and foraging ecology of southern right whales. By combining isotopic and genetic analyses on skin samples, I found that maternal lineages are structured over an isotopic range, and that the isotope ratios from adult females are more similar than expected among individuals sharing the same

mitochondrial haplotype. This pattern indicates a strong maternally directed site fidelity to summer feeding grounds. Such fidelity would be expected to limit the exploration of new feeding opportunities and might explain why this population shows increased rates of reproductive failure in years following sea surface temperature anomalies off South Georgia. By comparing isotope ratios from skin samples with published and unpublished values of potential prey and other predators we have discovered that southern right whales appear to have at least three different foraging strategies probably associated with different migratory patterns.

Additionally, we found significant genetic differentiation between live and dead animals, with an overrepresentation of dead calves within one (clade A) of the two major phylogenetic clades known for this species. This overrepresentation is likely the outcome of a relatively recent influx of migrants from populations where clade A is more abundant. Furthermore, when comparing our samples with samples collected from stranded whales in the 1980s, we were able to detect an apparent shift in mortality between clades in recent years; a pattern likely produced by migration or environmental variability on a decadal scale. Finally, in a study aimed at comparing mother and calf isotope ratios we have discovered interannual variability in the magnitude of nitrogen and carbon fractionation, which we interpreted as differences in the nutritional stress of the mother-calf pairs across years.

The novel combination of stable isotopes and genetics that we employed, in which genetic structure was tested over a landscape of isotope ratios instead of a natural landscape, seems certain to stimulate similar interdisciplinary studies. Our investigations of southern right whale population structure and foraging strategies, as well as future

work on this and other species will provide an opportunity to better understand the unpredictable changes occurring in marine ecosystems which will allow a more accurate projection of the survival of many species.

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CHAPTER 2

ISOTOPIC AND GENETIC EVIDENCE FOR CULTURALLY INHERITED SITE FIDELITY TO FEEDING GROUNDS IN SOUTHERN RIGHT WHALES

Abstract

Ocean warming will undoubtedly affect the migratory patterns of many marine species, but specific changes can be predicted only where behavioral mechanisms guiding migration are understood. Southern right whales show maternally inherited site fidelity to near-shore winter nursery grounds, but exactly where they feed in summer (collectively and individually) remains mysterious. They consume huge quantities of copepods and krill, and their reproductive rates respond to fluctuations in krill abundance linked to El Niño Southern Oscillation (ENSO). Here we show that genetic and isotopic signatures, analyzed together, indicate maternally directed site fidelity to diverse summer feeding grounds for female right whales calving at Península Valdés, Argentina. Isotopic values from 131 skin samples span a broad range (-23.1 to -17.2‰ $\delta^{13}C$, 6.0 to 13.8‰ $\delta^{15}N$) and are more similar than randomly expected among individuals sharing the same mitochondrial haplotype. This pattern indicates that calves learn summer feeding locations from their mothers, and that the time scale of culturally inherited site fidelity to feeding grounds is at least several generations. Such conservatism would be expected to limit the exploration of new feeding opportunities, and might explain why this population

shows increased rates of reproductive failure in years following sea surface temperature anomalies off South Georgia, the richest known feeding ground for baleen whales in the South Atlantic.

Introduction

Might an animal population fail to use all of its available food resources for many generations because cultural traditions direct its foraging to a subset of the suitable locations? Southern right whales (*Eubalaena australis*) had six known feeding grounds in the South Atlantic, based on the locations of catches recorded by 19th and 20th century whalers (IWC 2001). Today, the only known feeding ground used in the western South Atlantic is South Georgia (Moore *et al.* 1999; IWC 2001), despite the species' sustained recovery from near extinction in the early 20th century to a population that probably exceeds 19,000 in 2008 [by extrapolation from population size and growth rate estimates for all Southern Ocean breeding grounds in 1990 (IWC 2001)]. Right whales make long annual migrations between mid-latitude coastal winter nursery grounds, and mostly high-latitude offshore summer feeding grounds (IWC 2001). If calves learn these routes from their mothers and then follow them faithfully for life, matrilines will continue to use the same feeding grounds for many generations, despite the availability of better foraging opportunities elsewhere. Here we combine genetic and stable-isotopic analyses of the population calving at Península Valdés, Argentina, to show that such cultural conservatism may help to explain why southern right whales, though recovering numerically, have appeared slow to return to many parts of their historic range throughout the Southern Hemisphere.

In baleen whales, site fidelity is thought to be maternally transmitted with calves learning the location of nursery and feeding grounds during their first annual migration (Hoelzel 1998). Over many generations, maternally directed site fidelity can result in genetic differentiation among seasonal subpopulations (Hoelzel 1998). In the most intensely studied species, humpback whales [*Megaptera novaeangliae*, (Palsboll *et al.* 1997; Baker *et al.* 1998*a,b*)] and North Atlantic right whales [*E. glacialis*, (Schaeff *et al.* 1993; Malik *et al.* 1999)], genetic differentiation of mitochondrial DNA (mtDNA) markers has been found among feeding grounds and among nursery grounds (the latter only in humpback whales in the North Pacific and Southern Hemisphere), consistent with female directed fidelity. Southern right whales show site fidelity to nursery grounds off the coasts of South America, South Africa, Australia and New Zealand (IWC 2001). Patenaude *et al.* (2007) detected mtDNA differentiation among these four nursery grounds and between feeding grounds off South Georgia and off south western Australia; however, within ocean basins, mtDNA haplotypes collected from the feeding grounds were shared with both nursery grounds (e.g.,, haplotypes from South Georgia were shared with Península Valdés and South Africa). Thus, Patenaude *et al.* (2007) confirmed southern right whale site fidelity to nursery grounds and suggested that whales from different breeding populations within an ocean basin mix on common feeding grounds. However, the difficulty of obtaining samples representing the whales' entire feeding range has prevented a thorough intra oceanic feeding ground comparison. Despite genetic evidence linking southern right whale nursery grounds to common feeding grounds, the population genetic structure (if any) on the feeding grounds remains unknown.

Leaper *et al.* (2006) recently showed that the reproductive success of southern right whales breeding at Península Valdés, Argentina is affected by sea surface temperature (SST) anomalies off South Georgia. High-SST anomalies at South Georgia have been correlated with periods of low krill abundance (Trathan *et al.* 2003). Although southern right whale populations are recovering well from their former exploitation (IWC 2001), reproductive failures resulting from food stress are cause for concern (Leaper *et al.* 2006). The correlation among breeding failures, SST anomalies and low krill abundance also suggests that a large proportion of whales that use the Península Valdés nursery ground may feed near South Georgia, which is only one of six major historic feeding grounds for right whales in the South Atlantic (IWC 2001). Furthermore, whaling records show that southern right whales killed south of 50°S had stomachs filled with krill, north of 40°S filled with copepods and between these latitudes stomachs were filled with a mix of krill and copepods (Tormosov *et al.* 1998). The exact number and location of current feeding grounds, and the proportion of whales associated with each have not been documented (IWC 2001). Understanding a species' migratory connections and genetic structure is critical to understanding the impact that fluctuations in food availability may have on it. If a species shows site fidelity to feeding areas, then the effects of changes in food abundance in one feeding ground may not spread throughout the entire breeding population, but be focused instead on particular genetic lineages.

Intrinsic markers such as stable isotopes and genetic variability have been used with varying degrees of success to study migratory biology (Webster *et al.* 2002; Rubenstein and Hobson 2004). Stable carbon and nitrogen isotope ratios in animal tissues are good indicators of food sources and have been used to study animal movements in a

broad range of species including butterflies, birds, fish and mammals (Hobson 1999; Rubenstein and Hobson 2004). The existence of predictable patterns of isotope ratios across landscapes provides the basis for the use of stable isotopes as tracers of migration (Hobson 1999; Rubenstein and Hobson 2004; West *et al.* 2006). For example, in marine ecosystems, carbon isotope ratio ($^{13}C/^{12}C$) declines with increasing latitude, and coastal waters have higher ratios than pelagic waters (Rau *et al.* 1982; Hobson 1999; Kelly 2000; Rubenstein and Hobson 2004). Population-specific genetic markers have been widely used to study animal movements, particularly bird migration (Bensch and Hasselquist 1999; Wennerberg 2001). However, few studies have combined genetic and isotopic markers to study animal migration (Clegg *et al.* 2003; Kelly *et al.* 2005). In one such study, Clegg *et al.* (2003) used a combination of hydrogen isotope ratios and genetic markers to study the migratory patterns and connectivity of Wilson's warbler (*Wilsonia pusilla*). These authors found a north-south structure of hydrogen isotope values and an east-west structure of genetic markers in breeding areas, which allow them to confirm a previously suspected migratory pattern and population structure across North America (Clegg *et al.* 2003).

Here we describe the population genetic structure of southern right whales on their feeding grounds by using a novel combination of genetic and stable-isotopic analyses of skin samples collected from live whales at Península Valdés, Argentina. Each skin sample provides the maternal lineage of the whale and information on its feeding location several months before sampling. We used stable carbon and nitrogen isotopes and mtDNA haplotypes to show that individuals from a given maternal lineage tend to

have similar isotopic values, apparently as a consequence of using the same culturally inherited feeding ground.

Materials and Methods

Fieldwork and sample collection

Skin samples were collected by biopsy darting adult female southern right whales on their nursery ground off Península Valdés (42° 30' S, 64° 10' W), Argentina. Sample collection took place over 4 consecutive years (2003 – 2006) at the time of peak whale abundance [September and October, (Payne 1986)]. Whales sampled in 2003 and 2006 correspond to the same calving cohort (Payne 1986). Adult females were recognized by the presence of an accompanying calf. To avoid including resampled whales, individuals were photographed for later identification based on callosity patterns (Payne *et al.* 1983). Each skin sample was divided into two subsamples in the field. One subsample was dried in preparation for stable carbon and nitrogen isotope analysis and the other was preserved in saturated NaCl with 20% DMSO for genetic analysis (Amos and Hoelzel 1991). Samples were frozen on return to the laboratory at the University of Utah.

Stable carbon and nitrogen isotope analysis

Dried samples were ground to a fine powder and lipid extracted following Todd *et al.* (1997). Approximately 1mg of material per sample was analyzed in a Carlo Erba 1108 elemental analyzer coupled to a Thermo Finnigan Delta S Isotope Ratio Mass Spectrometer at the Stable Isotope Ratio Facility for Environmental Research (SIRFER) at the University of Utah. Isotope ratios are expressed as $\delta^{13}C$ or $\delta^{15}N$ (‰) = $[(R_{sample}/R_{standard}) - 1] \times 100$, where R is the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ for $\delta^{13}C$ or $\delta^{15}N$,

respectively. Standards were referenced to Pee Dee Belemnite (PDB) for carbon and to atmospheric air for nitrogen. The reproducibility of these measurements was 0.2‰ for both carbon and nitrogen after repeated analyses of an internal laboratory standard (yeast).

Genetic analysis

DNA was extracted using standard protocols for cetacean skin described in Amos and Hoelzel (1991). Six hundred thirty base pairs of the mitochondrial control region were amplified by polymerase chain reaction (PCR) using primers AB6617 and H00034 (Malik *et al.* 1999). The purified PCR product was then directly sequenced in both directions either at the DNA Sequencing Core Facility at the University of Utah Health Science Center or at the High-Throughput Genomics Unit, University of Washington. Sequences were assembled using Sequencher 4.5 software (Gene Codes Corp.). Haplotype (h) and nucleotide (π) diversity (Nei 1987) were estimated using Arlequin 2.0 (Schneider *et al.* 2000). The degree of differentiation among years was estimated by Analysis of Molecular Variance (AMOVA; Excoffier *et al.* 1992) as implemented in Arlequin 2.0.

Statistical analysis

The $\delta^{13}C$ and $\delta^{15}N$ distributions were significantly non-normal (Shapiro-Wilk W test: $N = 131$; $p < 0.001$; Figure 2.1). Nonparametric statistics (Kruskal-Wallis analysis of variance by ranks and Dunn's Multiple Comparison test) were used to test for differences of isotopic values among years and among haplotypes (Dunn 1964; Sokal and Rohlf

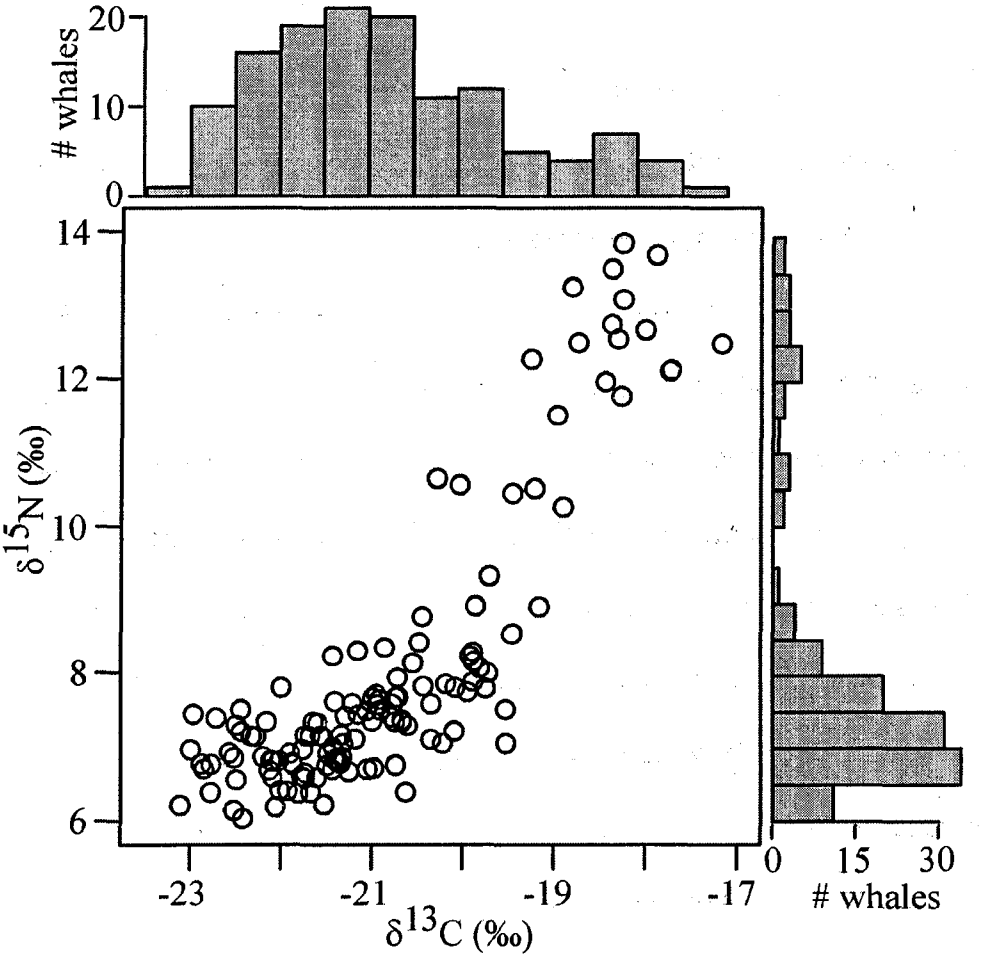

Figure 2.1: Scatter plot and histograms of stable carbon and nitrogen isotope values for 131 adult female southern right whales sampled at Península Valdés, Argentina from 2003 to 2006. $\delta^{13}C$ and $\delta^{15}N$ values are not normally distributed (Shapiro-Wilk W test; $p < 0.001$).

1981). α was set at 5% for all tests, which were conducted in R (R Development Core Team 2005) and JMP (SAS Institute Inc. 2005).

Under the null hypothesis of no site fidelity, it is expected that the isotopic variation within matrilines will be as great as isotopic variation between matrilines. Ideally this hypothesis would be tested using ANOVA with haplotypes as factors; however, the lack of normality and small sample size prevent this. Consequently, we decided to use a randomization test based on an F-distribution (Manly 1997) to see whether the isotopic variation within matrilines is as great as isotopic variation between matrilines. As in a traditional one-factor ANOVA, we estimated the F-ratio as the ratio of the between-group mean square to the within-group mean square, but in our test we compare this F-ratio with a randomly generated distribution of F-ratios instead of the conventional F-distribution (Manly 1997). To conduct the randomization test, we scrambled the isotope values and randomly assigned them to the haplotypes, maintaining the original haplotype frequencies; for each of these randomized datasets we estimated the F-ratio. We conducted 10,000 randomizations and generated an F-distribution to which the observed F-ratio was compared. The significance level for the randomization test was the proportion of randomly generated F-ratios that were greater than or equal to the observed F-ratio (Manly 1997).

If site fidelity is maternally directed, we predicted that the isotope difference between whales from the same matriline will be on average smaller than the distance between matrilines. We used the unsigned magnitudes of the isotope differences between whales as a metric referred to as pair-wise difference. We calculated the pair-wise difference for each possible combination and then separated the values into two groups: a

group where both members of the pair had the same haplotype (difference within haplotype, ΔWH) and a group were members had different haplotypes (difference between haplotype, ΔBH). We predicted that the mean pair-wise difference within haplotypes (ΔWH) would be smaller than that between haplotypes (ΔBH). Because each sample appears in many pair-wise comparisons, the assumption of independence for a two-way comparison (e.g., *t*-test of ΔWH versus ΔBH) is violated (Sokal and Rohlf, 1981). Consequently, we used a randomization test similar to the one described above. We randomly assigned the isotope values to the haplotypes and then calculated the mean pair-wise differences within (ΔWH) and between haplotypes (ΔBH), and their arithmetic difference (D = ΔWH - ΔBH). In this case, we repeated the randomization 1000 times, creating a distribution of randomly generated D, to assess the probability of obtaining the observed D. The significance level for this randomization test was the proportion of randomly generated values of D that were smaller than or equal to the observed D value from the original dataset (Manly 1997).

Results

Stable carbon and nitrogen isotopes

Skin samples collected in September and October from 131 adult female southern right whales from 2003 to 2006 show a wide range of stable carbon and nitrogen isotope values (Figure 2.1, Table 2.1), with $\delta^{13}C$ ranging from -23.1 to -17.2‰ (mean = -20.8‰, SD = 1.3‰) and $\delta^{15}N$ ranging from 6.0 to 13.8‰ (mean = 8.0‰, SD = 1.9‰). $\delta^{13}C$ values differed among years (Kruskal-Wallis $\chi 2 = 13.4$; $p = 0.004$), with values in 2006 (median = -20.2‰) being significantly higher than those in 2004 and 2005 (median

Table 2.1: Mean (SD), range and median $\delta^{13}C$ and $\delta^{15}N$ of southern right whale skin samples by year of collection and for all years combined. Non significant differences (Dunn's Multiple Comparisons test; $p < 0.05$) between years are indicated by the same letter next to the medians. *N* is sample size.

Year	Statistic	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
2003	Mean ± SD	-20.9 ± 1.0	7.7 ± 1.5
(*N* = 12)	Range	-22.3 to -19.2	6.7 to 12.3
	Median	-21.1 [a, b]	7.2 [a, b]
2004	Mean ± SD	-21.1 ± 1.3	8.0 ± 1.8
(*N* = 39)	Range	-23.1 to -17.9	6.1 to 13.7
	Median	-21.4 [a]	7.3 [a, b]
2005	Mean ± SD	-21.1 ± 1.3	7.8 ± 1.9
(*N* = 49)	Range	-23.0 to -18.3	6.0 to 13.5
	Median	-21.4 [a]	7.1 [a]
2006	Mean ± SD	-20.1 ± 1.3	8.7 ± 2.2
(*N* = 31)	Range	-22.0 to -17.2	6.4 to 13.8
	Median	-20.2 [b]	7.8 [b]
All years	Mean ± SD	-20.8 ± 1.3	8.0 ± 1.9
(*N* = 131)	Range	-23.1 to -17.2	6.0 to 13.8
	Median	-21.1	7.3

= -21.4 and -21.4‰ respectively; Dunn's Multiple Comparison tests; $p < 0.05$ for both comparisons). $\delta^{15}N$ values also differed among years (Kruskal-Wallis $\chi 2 = 8.2$; $p = 0.041$), with values in 2006 (median = 7.8‰) being higher than those in 2005 (median = 7.1‰; Dunn's Multiple Comparison test; $p < 0.05$).

MtDNA sequence data

Sequence analysis of a 630 base pair region of the mitochondrial control region revealed 49 polymorphic sites defining 31 unique sequences or haplotypes (Table 2.2). The haplotypes were not equally represented in the sample; six haplotypes account for 53% of the sample while ten occurred only once (singletons, Table 2.2). Levels of haplotype and nucleotide diversity were similar across all four years (overall haplotype diversity $h = 0.94$ and nucleotide diversity $\pi = 1.6\%$, Table 2.2). A modest but almost significant differentiation was detected by AMOVA among years at the haplotype level (overall Fst = 0.01; $p = 0.06$), and a similarly slight differentiation was significant at the nucleotide level (overall Φst = 0.01; $p = 0.02$).

Isotopic values of individual haplotypes

Isotopic values are not independent of haplotypes (Figure 2.2). A statistical analysis of the distribution of haplotypes over the isotopic ranges revealed significant differences among haplotypes for $\delta^{13}C$ (Kruskal-Wallis $\chi 2 = 41.1$; $p = 0.004$) and $\delta^{15}N$ (Kruskal-Wallis $\chi 2 = 34.3$; $p = 0.024$). The randomization test based on an F-distribution also showed significant differences in $\delta^{13}C$ (F = 3.02, p = 0.0004,) and $\delta^{15}N$ (F = 3.64, p = 0.0002) values among haplotypes. Figure 2.2*a* shows the distribution of haplotypes

Table 2.2: Frequency of mtDNA haplotypes and diversity indices by year of collection and for all years combined. Singletons are haplotypes observed in only one whale. Haplotype (h) and nucleotide diversity (π) indices were indistinguishable across years. N is sample size.

Haplotype	2003	2004	2005	2006	All years
M	3	3	9	2	17
F	3	2	4	3	12
K		5	6	1	12
E	1	4	3	3	11
J	1	1	7		9
B		1	2	5	8
I		6		1	7
A	1	2	1	2	6
Q		2	1	3	6
O	1	1	1	1	4
W	1		1	2	4
P		2	2		4
H		1	2	1	4
D		1	1	1	3
C	1	1			2
N		2			2
L		1		1	2
X			2		2
Y			2		2
Z			1	1	2
BB			1	1	2
Singletons		4	3	3	10
h	0.91	0.95	0.93	0.95	0.94
(SD)	(0.06)	(0.02)	(0.02)	(0.02)	(0.01)
π (%)	1.6	1.7	1.6	1.4	1.6
(SD)	(0.97)	(0.88)	(0.84)	(0.72)	(0.82)
N	12	39	49	31	131

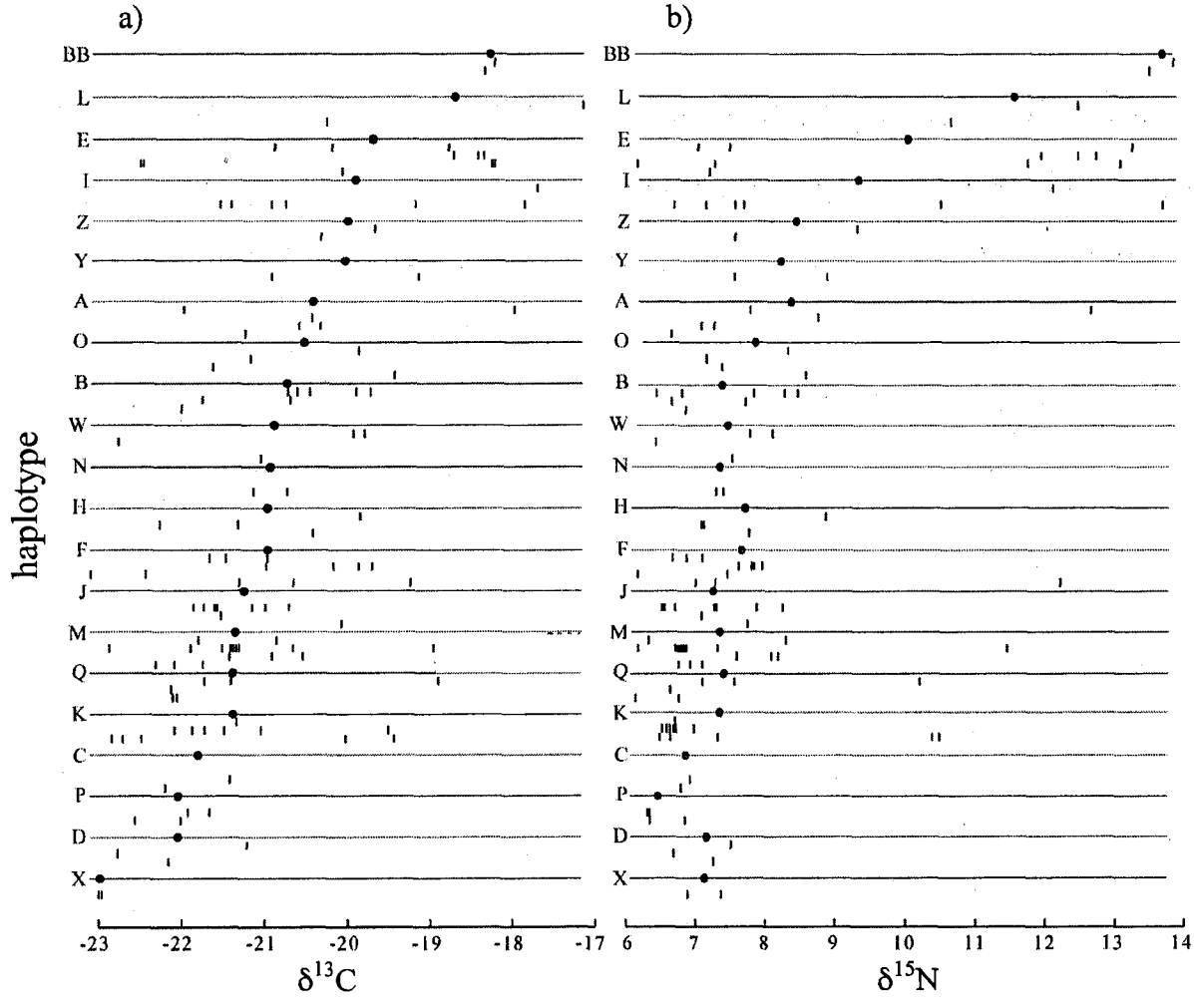

Figure 2.2: $\delta^{13}C$ (*a*) and $\delta^{15}N$ (*b*) values of individual haplotypes. For each haplotype, the filled circle represents the mean isotopic value and the vertical lines (below haplotype means) represent the raw values. Raw isotopic values are organized vertically by year, with 2003 at the bottom and 2006 at the top. Haplotypes are ordered from low (bottom of figure) to high (top) mean $\delta^{13}C$ values. Only haplotypes sampled more than once are presented.

along the $\delta^{13}C$ range. Some haplotypes tend to be associated with low $\delta^{13}C$ values (e.g., haplotype X); others show average values (e.g., haplotypes N and B); and others are concentrated at high values (e.g., haplotype BB). Some haplotypes are distributed throughout the isotopic range (e.g., haplotypes K, F and E), and some show variability between years (e.g., haplotype F) and variability within years (e.g., haplotype E in 2004). Figure 2.2*b* illustrates the distribution of haplotypes along the $\delta^{15}N$ range and shows a pattern that is comparable to that of $\delta^{13}C$. Again, some haplotypes appear only at low or high values of the distribution (e.g., haplotypes P and BB) while others appear throughout the distribution (e.g., haplotype M and K). For some of the haplotypes with larger ranges in $\delta^{15}N$ there is between year (e.g., haplotype E) and within year variation (e.g., haplotype F in 2003).

Overall, isotopic values were more similar among samples with identical haplotypes than among samples with different haplotypes (Table 2.3). For $\delta^{13}C$, the mean pair-wise difference in isotope values within haplotypes (ΔWH) was smaller than the mean pair-wise difference between haplotypes (ΔBH) in all comparisons except 2004 (Table 2.3). The difference between these two means (*D*) was significant when all samples were combined (all years) and also in 2005 and 2006 (Table 2.3). For $\delta^{15}N$, ΔWH was smaller than ΔBH in 2005, 2006 and all years combined, and *D* was significant in 2005 and all years combined (Table 2.3).

Discussion

Southern right whale mitochondrial haplotype diversity is structured with respect to stable isotope values. Three independent statistical tests show that the isotope values

Table 2.3: Mean pair-wise difference in isotopic values within haplotypes (ΔWH) and between haplotypes (ΔBH) for $\delta^{13}C$ and $\delta^{15}N$, for all samples combined (total) and for each sampling year. Singletons have been removed in each analysis. W<B? indicates whether (Y, N) ΔWH is smaller than ΔBH. *p* is the significance level of the randomization test (** $p < 0.01$, * $p < 0.05$) and indicates the proportion of randomly generated values of the difference (*D*) between ΔWH and ΔBH smaller than or equal to the observed *D*. *N* is sample size.

		$\delta^{13}C$				$\delta^{15}N$			
Year	*N*	ΔWH (‰)	ΔBH (‰)	W<B?	*p*	ΔWH (‰)	ΔBH (‰)	W<B?	*p*
Total	121	1.19	1.47	Y	0.002**	1.45	1.80	Y	0.001**
2003	6	0.88	1.65	Y	0.10	1.85	1.93	Y	0.19
2004	32	1.66	1.59	N	0.81	2.51	1.865	N	0.987
2005	43	0.85	1.43	Y	0.002**	0.82	1.71	Y	0.005**
2006	25	0.91	1.42	Y	0.023*	1.28	1.80	Y	0.16

are influenced by the haplotypes. The non-normal distribution of both isotope ratios (carbon and nitrogen) prevented us from using parametric tests with higher statistical power (Sokal and Rohlf 1981). However, the results from our three alternative and independent tests indicate a significant influence of the maternal lineages over the isotope ratios, a pattern most simply explained by maternally directed site fidelity to feeding areas. Individual haplotypes show isotopic values that are more similar than randomly expected, indicating that whales from the same matriline tend to consume isotopically similar food. Some haplotypes show larger ranges and more variation within and between years than others. Haplotypes with broad ranges may represent distinct but closely related matrilines that use different feeding grounds, and that could be distinguished by longer mtDNA sequences (work is currently underway to test this hypothesis). Nitrogen isotope values show larger variation than carbon, possibly because physiological processes and trophic position have a greater effect on $\delta^{15}N$ than on $\delta^{13}C$ (Hobson and Clark 1992*b*).

It is well accepted that isotope values in consumer's tissues reflect those of their food (Hobson 1999; Kelly 2000; Rubenstein and Hobson 2004). It is also well established that $\delta^{13}C$ (and to some extent $\delta^{15}N$) declines with latitude in marine plankton (Rau *et al.* 1982; Hobson 1999; Kelly 2000; Schmidt *et al.* 2003). Therefore, we consider that the large isotopic range detected in this study is indicative of a large feeding range. The lower isotopic values of skin samples seem to correspond with isotope values measured in krill and copepods around South Georgia and in waters of the Polar Front (Wada *et al.* 1987; Schmidt *et al.* 2003), while the higher isotope values seem to correspond with ratios measured in euphausiids and copepods collected on the Patagonian shelf (Rowntree *et al.* 2001) and collected offshore of Uruguay (Valenzuela LO *unpublished data*).

However, due to a lack of good isotopic sampling of zooplankton across the South Atlantic it is, currently, impossible to identify exact feeding locations for southern right whales using only the isotopic values from their skin. Work is underway to increase the isotopic coverage of zooplankton samples from the South Atlantic, as well as use other tracers such as sulphur isotopes and trace metals in whale skin, baleen plates and prey items identify more accurately the feeding locations.

The isotopic values measured in our study fall within the range previously detected along the length of baleen plates of southern right whales that stranded in South Africa [$\delta^{13}C$ range = -26.5 to -16.0‰, $\delta^{15}N$ range = 4.0 to 12.5‰, (Best and Schell 1996)] and in Argentina [$\delta^{13}C$ range = -26.1 to -15.7‰, (Rowntree *et al.* 2008)]. Both studies found that the isotopic values of the baleen plates oscillate in what appear to be annual cycles and suggested that these cycles correspond to the whales' annual migrations between isotopically different areas (Best and Schell 1996; Rowntree *et al.* 2008). Furthermore, Rowntree *et al.* (2008) found interindividual variations in the annual isotope cycles of five different whales and hypothesized that these variations reflect individually distinctive foraging patterns, associated with using different feeding grounds. For example, one of the baleen plates had higher mean and range $\delta^{13}C$ values (mean = -18.4‰; range = -19.7 to –15.7‰) than the other plates, three had lower values (mean –22.7‰; range = -26.1 to –19.6‰) and one baleen plate had intermediate values (mean = -20.9‰; range = -23.9 to –16.2‰).

Baleen, like hair, is a metabolically inert tissue after formation and provides a continuous record of dietary input (Schell *et al.* 1989; Rubenstein and Hobson 2004). However, skin is a metabolically active tissue and replaces its isotopes over time;

consequently, it integrates food consumed over a window of time (Rubenstein and Hobson 2004). Different tissues have different isotope turnover rates; some turn over within hours or days (e.g., blood plasma and liver) and provide information on more recent diets, while others take several weeks (e.g., muscles) or years (e.g., bone collagen) and provide a longer-term record of dietary integration (Rubenstein and Hobson 2004). The residence time of carbon and nitrogen isotopes in cetacean skin is unknown, and therefore, the temporal information recorded in the skin samples is also unknown, but it has been proposed to be between one month (Todd 1997) to several months (Ruiz and Cooley *et al.* 2004). Consequently, the isotope ratios measured on the skin samples probably represent the isotopic signal from the feeding grounds attenuated by isotope ratios incorporated from more recent feeding events along the migratory routes from the feeding grounds to Península Valdés. Regardless of this potential confounding factor, the isotope ratios of skin samples do represent individual preferences in the use of feeding grounds and migratory patterns.

The isotopic composition of an animal's tissues, particularly nitrogen ratios, can be influenced by its age, nutritional condition and reproductive status (Hobson and Clark 1992*a,b*; Roth and Hobson 2000; Fuller *et al.* 2004). In our study, all whales sampled were nursing females that had been under similar physiological stresses for at least a year, including 12 months of gestation, migration to the nursery ground, and lactation while fasting (Payne 1986; Cooke *et al.* 2001). To the degree that these stresses affect the isotopic values, the effect should be similar for all of the sampled whales. Thus, the differences we observed among whales would appear to be caused mainly by differences in the isotopic composition of their foods and not by their metabolism.

The interannual differences in the general distribution of $\delta^{13}C$ and $\delta^{15}N$ detected in this study are most likely a response to variations in the isotopic composition at the base of the food web produced by changes in ocean circulation or by modification of local biogeochemical processes (Peterson and Fry 1987; Druffel and Griffin 1999; Brix *et al.* 2004). An alternative explanation is that in 2006 the whales migrated to isotopically distinct feeding regions, but if this were true, the isotopic values of the samples from 2006 should not show any particular pattern. However, in 2006 the isotopic values of individual haplotypes were higher (see Figure 2.2). For example, in 9 of the 15 haplotypes sampled in 2006, the samples from 2006 had the highest $\delta^{13}C$ values, while 7 of the 15 haplotypes had the highest $\delta^{15}N$ values. The lack of genetic differentiation among years at the haplotype level indicates that the 2006 whales were not a genetically distinct subset of whales.

Our results show a pattern of fine-scale, long-term genetic substructuring within a breeding population that is most simply explained by maternally directed fidelity to different feeding sites. North Atlantic right whales (*Eubalaena glacialis*) and humpback whales (*Megaptera novaeangliae*) show similar site fidelity to feeding grounds, but mix on common breeding grounds (Schaeff *et al.* 1993; Palsbøll *et al.* 1995; Larsen *et al.* 1996; Malik *et al.* 1999). In contrast, Patenaude *et al.* (2007) suggest that southern right whales from different breeding populations within an ocean basin mix on common feeding grounds. For whale species that feed in the Southern Ocean, where there are no land barriers to circumpolar migrations, Hoelzel (1998) suggests that animals from different breeding grounds form mixed genetic assemblages on widely dispersed feeding areas. We suggest that the assemblages detected by Patenaude *et al.* (2007) represent

mixed subsets of maternal lineages with site fidelity to specific feeding grounds rather than an unstructured mix of animals migrating randomly from different nursery grounds. Therefore, southern right whale population structure may mirror that of humpback whales in the North Pacific, where the whales are genetically segregated on both the nursery and feeding grounds (Baker *et al.* 1998*b*).

The correlation of sea surface temperature (SST) anomalies off South Georgia and reduced calf output at Península Valdés (Leaper *et al.* 2006) suggests a strong migratory connection between these two areas, but our isotopic data imply that the whales feed in many different locations distributed over a large geographic range. We see two possible explanations for this apparent disagreement: 1) the SST anomalies detected at South Georgia might affect many other feeding locations, and therefore stress most of the whales that visit Península Valdés; or 2) the SST anomalies might affect only the whales that visit South Georgia, but so strongly that calf production is reduced detectably for the population as a whole. These alternatives could be distinguished by analyzing the individual reproductive histories of females using Península Valdés to see whether particular matrilines fail to reproduce in years following SST anomalies and low krill abundance near South Georgia.

Strong site fidelity may restrict animals to a set of culturally inherited areas and migratory patterns that represent only a portion of their potential range (Matthiopoulos *et al.* 2005; Clapham *et al.* 2008). The long-term mitochondrial haplotype substructuring presented here suggests that the timescale of culturally inherited site fidelity to feeding areas is long (at least several generations). The finding by Leaper *et al.* (2006) raises concerns about the future of southern right whales if krill fisheries and climate change

have a significant impact on krill abundance (Atkinson *et al.* 2004). If whales strictly follow the foraging strategies and migratory patterns learned from their mothers, can they be flexible enough to change their strategies and switch to other prey types with different spatial and temporal distributions? Some mitochondrial lineages show relatively large stable isotope ranges, suggesting that a few members of those lineages experimented with different locations (or prey types) in the relatively recent past. If we can better understand the causes and consequences of this limited plasticity, then we may be better able to predict the responses of southern right whales and other marine migrants to global climate change.

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CHAPTER 3

DIFFERENTIAL MORTALITY AMONG SOUTHERN RIGHT WHALE MATERNAL LINEAGES AT PENÍNSULA VALDÉS, ARGENTINA

Abstract

Strong site fidelity to breeding or feeding grounds may result in animals being less flexible to use different areas when their normal habitat becomes less suitable. Southern right whale populations are recovering from near extinction and as their numbers have increased they have begun to reutilize former habitats. A thorough assessment of the whale's intra- and interpopulation genetic heterogeneity will help understand and predict how dispersal influences the survival and reproduction of right whales. We assessed the genetic substructuring of southern right whales within Península Valdés, Argentina using sequence data from a 630 bp region of the mitochondrial genome. We tested for genetic differentiation between gulfs, age-sex classes, reproductive status, calving cohorts, and living versus dead whales. We found no genetic differentiation among different groups of living whales within Península Valdés, but did detect significant genetic differentiation between live and dead animals with one of the two major phylogenetic clades (A) over-represented among dead calves. We hypothesize that the overrepresentation results from a relatively recent migration from populations where clade A is more abundant. Newly arrived mothers may not be familiar

with the local coastal geography and consequently lose proportionally more calves than local whales. This hypothesis is supported by an overall increase in the proportion of whales within clade A, from 10% in past decades to 51% in our study. Moreover, because the majority of samples collected in the past were from stranded whales it appears that there has been a shift in mortality between clades in recent years.

Introduction

Genes and genotypes are often not randomly distributed between and within populations (Futuyma, 1986; Hartl and Clark, 1989). Factors such as habitat selection and site fidelity can produce genetic structure even in highly mobile species such as migratory birds and marine mammals (Hoelzel, 1998; Webster and Marra, 2005). Genetic structure of mitochondrial DNA (mtDNA) among populations as a consequence of maternally directed site fidelity is a recognized characteristic of many species of baleen whales (Hoelzel, 1998). However, the extent of genetic structure within populations and its consequences for survival and reproduction has not been fully explored (Rosenbaum *et al.,* 2002). Southern right whales (*Eubalaena australis*) are found in all the oceans of the southern hemisphere where they migrate between winter coastal nursery grounds and off shore, mostly unknown, summer feeding grounds (IWC, 2001). Nursery grounds are used primarily by adult females for raising their calves during their first three months of life (Payne, 1986; Best 1994). Long-term photo identification studies have shown that females return to the same nursery ground in their calving years, and that there is little exchange of animals between nursery grounds, despite the lack of obvious geographic barriers (Best *et al.,* 1993; IWC 2001). MtDNA analyses have confirmed that all major nursery grounds are genetically differentiated and that the differentiation is likely

maintained by maternally directed site fidelity (Portway 1998; Baker *et al.,* 1999; Patenaude *et al.,* 2007). A recent discovery of genetic heterogeneity on the nursery ground at Península Valdés appears to be driven by population structure on the feeding grounds (Valenzuela *et al.,* 2008). However, a thorough assessment of intrapopulation heterogeneity and its consequences for survival and reproduction of southern right whales is still lacking.

The overall genetic differentiation among southern right whale nursery areas has been well documented (Portway 1998; Baker *et al.,* 1999; Patenaude *et al.,* 2007). Portway (1998) and Baker *et al.* (1999) found population differentiation within the South Atlantic and within the Indo-Pacific ocean basins respectively, and Patenaude *et al.* (2007) reported genetic differentiation between these two ocean basins. Together, these studies document significant genetic differentiation at the haplotype and nucleotide levels among the four known calving grounds: Península Valdés and South Africa in the South Atlantic basin, and south western Australia and New Zealand in the Indo-Pacific basin. The limited gene flow reported in all of these studies is thought to result from maternal fidelity to nursery grounds (Schaeff *et al.,* 1993; Hoelzel, 1998). Within ocean basins, no genetic differentiation has been found between nursery grounds and feeding grounds (Portway 1998; Baker *et al.,* 1999; Patenaude *et al.,* 2007). This could be explained by mixing of lineages from neighboring nursery grounds on common feeding grounds (Patenaude *et al.,* 2007). At the level of ocean basins, differentiation is higher, with only one mtDNA haplotype shared by both basins (Patenaude *et al.,* 2007).

More sampling and new types of analyses are revealing population genetic structure on the feeding grounds and the existence of within-population substructure on

the nursery grounds. Recently, Valenzuela *et al.* (2008) combined genetic and stable isotope analyses of skin samples to show that mtDNA haplotypes of adult females from Península Valdés are not randomly distributed along an isotopic range. Furthermore, whales from the same matriline showed isotopic values more similar to each other than expected by chance (Valenzuela *et al.,* 2008). In addition, the mean isotope value for each haplotype was structured along a gradient of carbon and nitrogen isotopes, indicating feeding along a latitudinal gradient (Valenzuela *et al.,* 2008). Because isotope ratios in animal tissues are known to reflect the animal's diet and migratory origins (Hobson, 1999), the authors interpret this result as evidence of genetic structure on the feeding grounds. This is the first indication of genetic heterogeneity within a nursery-area subpopulation of southern right whales. The North Atlantic right whale (*Eubalaena glacialis*) has a similar pattern: a subgroup of females shows female directed site fidelity to the Bay of Fundy feeding ground, while a second group uses unknown feeding areas (Schaeff *et al.,* 1993; Malik *et al.,* 1999). The existence of a similar pattern in other southern right whale winter populations is unknown, as is the occurrence of any other internal substructuring.

The consequences of such substructuring within a breeding population are unknown; however, a likely result is that the effects of changes in food abundance within a particular feeding ground might not be spread across an entire breeding population, but instead focused on particular genetic lineages. On the nursery ground this could be reflected in changes in the haplotype frequencies of calving mothers in particular years (calving cohorts) or in the haplotype frequencies of stranded whales.

Although, little is known about other substructuring within southern right whale populations, there is evidence of internal substructuring on the breeding grounds of the ecologically similar grey whale (*Eschrichtius robustus*). Grey whales, in their calving lagoons along the Pacific coast of Baja California show significant genetic differentiation between calving females and mating females, as well as low, but not significant, differentiation between calving females using two different lagoons (Goerlitz *et al.,* 2003). This pattern is thought to result from a fine scale site fidelity to natal lagoons, especially during calving years (Goerlitz *et al.,* 2003). Similarly, on the shores of Península Valdés, southern right whales congregate primarily in two gulfs (Golfo San José, GSJ and Golfo Nuevo, GN), with similar numbers of mother-calf pairs in each gulf (Rowntree *et al.,* 2001). In the 1970s and early 1980s, different proportions of age-sex categories were founding different areas of Península Valdés (Payne, 1986). Adult males and single females predominated in GN, all age-sex classes were found in GSJ but with a higher proportion of juveniles, while the largest proportion of mother-calf pairs was found off the outer coast. With time, the distribution and proportion of animals in each area have changed. Females with calves abandoned the location that had the highest concentration of mother-calf pairs (the outer coast; Rowntree *et al.,* 2001). Although the observed change in distribution seems to work against the establishment of genetic structure due to site fidelity within subareas of the nursery ground, some other processes that produce and maintain genetic heterogeneity within an otherwise homogenous population might be taking place (e.g., kinship based social structuring).

Here we assess the genetic substructuring of southern right whales on Península Valdés nursery ground by using sequence data from a 630 bp region of the mitochondrial

genome; we compare gulfs, age-sex classes, reproductive status and calving cohorts, and living versus dead whales. We also reanalyze the population structure of southern right whales by combining our samples with those previously reported from this and other populations (Portway, 1998; Baker *et al.,* 1999; Malik *et al.,* 2000; Patenaude *et al.,* 2007). We detected an unexpected genetic differentiation between live and dead whales, characterized by an overrepresentation of dead calves within a major phylogenetic clade (clade A presented by Baker *et al.,* 1999). We explore the hypothesis that the high mortality of clade A and its overall increase in frequency in comparison to previous studies is the result of recent dispersal events from populations where clade A is known to be more abundant. We also discuss the hypothesis that the changes in the proportion of stranded whales within each clade at different time points is the result of some as yet unidentified environmental variability affecting whale survival at a decadal scale.

Materials and Methods

Sample collection

Skin samples were obtained by biopsy darting live animals off Península Valdés (42° 30' S, 64° 00' W; Figure 3.1), Argentina. Sample collection was concentrated in the northern gulf, Golfo San Jose (GSJ) though some skin samples were collected in the southern gulf, Golfo Nuevo (GN). In GSJ biopsy collection was carried out over four consecutive years (2003 – 2006) at the time of peak whale abundance (September and October; Payne, 1986). In GN sampling took place in 2005 and 2006, from August to

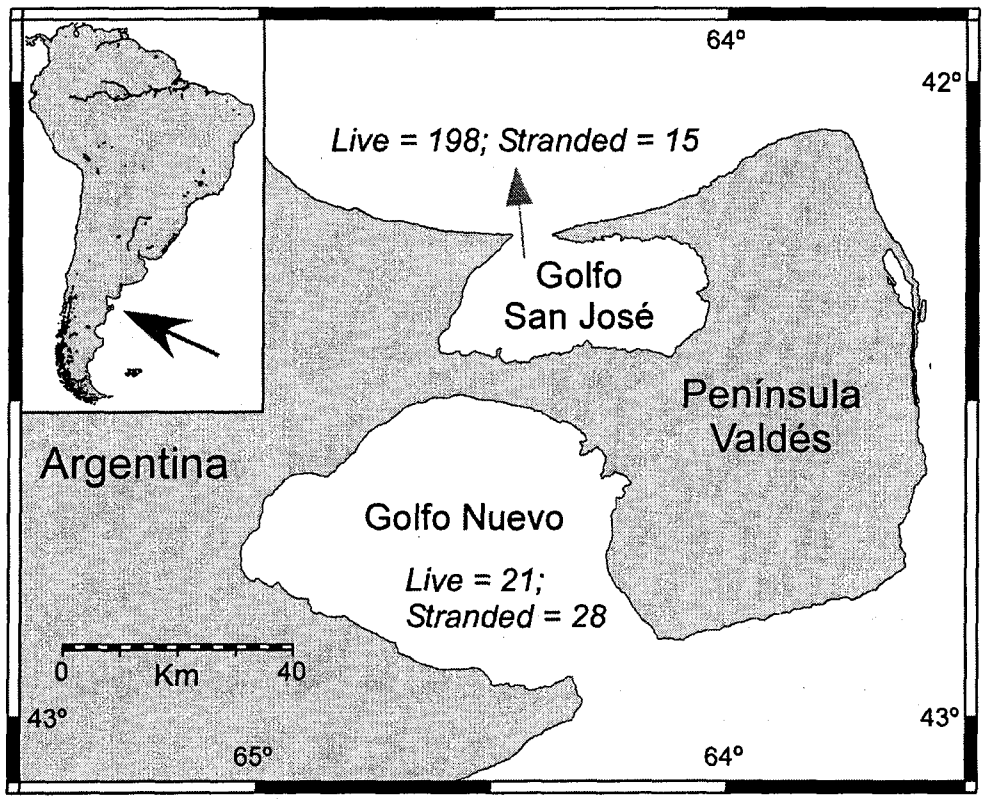

Figure 3.1: Map of Península Valdés, Argentina, showing the two gulfs where whales concentrate and the number of samples collected in each gulf. Inset shows the position of Península Valdés on the South America coast.

October. To avoid including resampled whales, individuals were photographed for later identification based on callosity patterns and other natural marks (Payne *et al.,* 1983). Samples were preserved in saturated NaCl with 20% DMSO for long-term storage (Amos and Hoelzel, 1991). Age classes (adults and juveniles) were identified primarily based on body size; adult females were recognized by the close proximity of a calf over an extended period of time. Gender of juveniles and single adults was determined whenever possible by observation of the genital area; otherwise, gender was identified by PCR amplification and electrophoresis of *Zfx* and *Zfy* introns following Shaw *et al.* (2003). Table 3.1 shows the number of samples used broken down by area, year and age-sex class.

Skin samples from whales that died and stranded at Península Valdés from 2003 to 2006 were provided by the Programa de Monitoreo Sanitario Ballena Franca Austral (PMSBFA), which is active from June through December (Uhart *et al.,* 2008). At each stranding, body measurements and skin tissue were collected; all samples were preserved in 70% EtOH and the stranded animals were tagged to avoid re-sampling. Whales were classified by age class (calves, juveniles and adults) based on their length and sex class based on observations of external or internal reproductive anatomy. Most of the stranded animals were calves (84%, n = 36) and their mtDNA was used to infer maternal haplotypes (Table 3.1).

MtDNA sequences

DNA was extracted using a standard phenol-chloroform procedure for cetacean skin as described in Amos and Hoelzel (1991). A 630 base pairs region of the mitochondrial

Table 3.1: Number of individuals included in our analyses. Adult-Females (AF), Adult-Males (AM), Juvenile-Females (JF) and Juvenile-Males (JM).

Number of samples			2003	2004	2005	2006	Total
Alive	Golfo San José	AF	18	47	47	34	146
		AM	2		1	2	5
		JF	9	5	5	4	23
		JM	9	4	6	5	24
		Total	38	56	59	45	198
	Golfo Nuevo	AF			7	6	13
		AM				2	2
		JF			1	1	2
		JM			2	2	4
		Total			10	11	21
Stranded	Golfo San José	AF	2	2	5	3	12
		JF			1	1	2
		JM				1	1
		Total	2	2	6	5	15
	Golfo Nuevo	AF	6	3	15	4	28

genome was amplified by polymerase chain reaction (PCR) using primers AB6617 and H00034 (Malik *et al.,* 1999). This sequence starts at nucleotide 40 of the t-Thr, and spans the complete t-Pro and the first 530 bp of the control region. The purified PCR product was sequenced in both directions either at the DNA Sequencing Core Facility at the University of Utah Health Science Center or at the High-Throughput Genomics Unit, University of Washington. Sequences were assembled using Sequencher 4.5 software (Gene Codes Corp.), and deposited at GenBank with accession numbers EU290462-EU290592. Sequence alignment was conducted using Clustal X 1.8 software (Thompson, 1997). Haplotypes were named following the format used in previous publications (Patenaude *et al.,* 2007), using a three-letter abbreviation of the author followed by an alphabetical code corresponding to individual haplotype identity.

Phylogenetic relationships

A phylogeny of the mtDNA haplotypes was reconstructed using neighbor-joining and maximum likelihood methods in the program PAUP* (Swofford, 2003). The tree was rooted with a homologous sequence extracted from the complete mitochondrial genome of a North Pacific right whale (*E. japonica*); GenBank accession number AP006474 (Sasaki *et al.,* 2005).

Genetic diversity and within-population differentiation.

Haplotype (h) and nucleotide (π) diversity (Nei, 1987) were estimated using Arlequin 2.0 (Schneider *et al.,* 2000). The degree of differentiation among groups (e.g., among areas, years, age and gender classes) was estimated by analysis of molecular

variance (AMOVA; Excoffier *et al.,* 1992) as implemented in Arlequin 2.0, using both haplotype frequencies (*Fst*; Wright, 1951) and molecular distances (Φst; Excoffier *et al.,* 1992).

Southern hemisphere population differentiation/structure

The population structure of southern right whales was reanalyzed by incorporating the 262 samples reported in this study to the 146 samples reported by Patenaude *et al.* (2007). The populations used were Argentina (AR, n = 282), South Africa (SA, n = 41), South Georgia feeding ground (SGF, n = 8), New Zealand (NZ, n = 42), southwestern Australia (SWA, n = 20) and southwestern Australia feeding ground (SWAF, n = 5). The haplotype (h) and nucleotide (π) diversity, and the degree of differentiation were calculated as described above.

We reconstructed the haplotypes reported by Patenaude *et al.* (2007) using the 37 variable sites from their paper (page 150, table 2), and as templates, the haplotypes reported by Malik *et al.* (2000; GenBank accession numbers AF395044-AF395053). The resulting haplotypes are 275 bp long, and the first nucleotide corresponds to base number 119 in the 630 bp sequences. In the case of sequences synonymous with previous studies, haplotypes kept the original names used by Patenaude *et al.* (2007).

Results

Sequence analysis and mtDNA phylogeny

Sequence analysis of a 630 base pair region of the mitochondrial genome from 262 whales revealed 35 unique haplotypes (Table 3.2). These haplotypes are defined by

Table 3.2: Polymorphic sites in 630 bp region of mtDNA of 262 southern right whales. Similarities are noted by "·", and differences are noted by proper nucleotide changes. The grey area represents the variable sites found in the same region studied by Patenaude *et al.* (2007); position 119 corresponds to position 1 in Patenaude *et al.* (2007). "N" represents the haplotype frequency.

Haplotype	N	12	13	16	48	50	100	104
valhapM	30	C	A	C	T	T	-	-
valhapJ	24	.	.	.	.	.	.	.
valhapF	23	.	.	.	.	C	.	.
valhapK	19	.	.	.	.	.		.
valhapD	16	.	.	.	.	C	.	.
valhapA	14	.	.	.	.	C	.	.
valhapE	14	.	.	.	.	C	.	.
valhapB	13	.	.	.	.	C	.	.
valhapL	10	.	.	.	.	.		.
valhapH	9	.	.	.	.	C	.	.
valhapQ	9	.	.	.	.	C	.	.
valhapZ	9	.	.	.	.	C	.	.
valhapI	8	.	.	.	.	.	.	.
valhapO	8	.	.	.	.	.	A	.
valhapW	7	.	.	.	.	.	.	A
valhapC	6	.	.	.	.	C	.	.
valhapP	5	.	.	.	.	.	.	.
valhapT	5	.	.	.	.	C	.	.
valhapX	5	.	.	.	.	C	.	.
valhapAA	4	.	.	.	.	C	.	.
valhapDD	4	.	.	.	C	.	.	.
valhapY	3	.	.	.	.	.	A	.
valhapBB	2	.	.	.	.	C	.	.
valhapEE	2	.	.	.	.	.	.	.
valhapN	2	.	.	.	.	.	.	.
valhapR	2	.	.	.	.	.	.	A
valhapCC	1	.	.	.	.	.	.	.

Haplotype	176	177	178	182	183	185	186	194	195	202	203	210	211	229	232	242	262	305	327	343	345	350	357	362	363	364	373	383	384	385	389	391
valhapM	A	T	C	T	A	T	C	G	T	T	A	G	G	T	C	T	C	C	C	T	G	A	G	C	T	C	A	A	T	T	T	C
valhapJ	.	.	.	.	.	C	T	A	C	C	G	.	.	.	T	.	.	.	.	.	A	.	.	.	.	.	.	.	C	.	.	T
valhapF	.	.	.	.	.	.	.	.	.	.	G	.	.	.	T	C	.	T	T	.	A	G	A	.	.	.	.	.	C	C	.	T
valhapK	.	.	.	.	.	.	T	.	C	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	C	.	.	T
valhapD	.	.	.	.	.	C	.	.	.	.	.	.	.	.	T	C	T	T	.	.	A	G	A	.	.	.	.	.	C	C	.	T
valhapA	.	.	.	.	.	.	.	.	.	.	G	.	.	C	T	C	.	T	.	.	A	G	A	.	.	.	.	.	C	C	.	T
valhapE	.	.	.	.	.	C	.	.	.	.	G	.	.	.	T	C	.	T	T	.	A	G	A	.	.	.	.	.	C	C	.	T
valhapB	.	.	.	.	.	.	.	.	.	.	G	.	.	.	T	C	.	T	.	.	A	G	A	.	.	.	.	.	C	C	.	T
valhapL	.	.	.	.	.	.	T	.	C	C	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	C	.	.	T
valhapH	.	.	.	.	.	.	T	.	.	.	G	.	.	.	T	C	.	T	T	.	A	G	A	.	.	.	.	.	C	C	.	T
valhapQ	.	.	T	.	.	.	.	.	.	.	G	.	.	.	T	C	.	T	.	.	A	G	A	.	.	.	.	.	C	C	.	T
valhapZ	.	.	T	.	.	C	.	.	.	.	.	.	.	.	T	C	.	T	.	.	A	G	A	.	.	.	.	.	C	C	.	T
valhapI	.	C	.	.	.	.	.	A	C	C	G	.	.	.	T	.	.	T	.	.	.	.	.	.	.	.	.	.	C	.	.	T
valhapO	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	T
valhapW	.	.	.	.	.	.	T	.	C	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	G	C	.	C	T
valhapC	.	.	.	.	.	C	.	.	.	.	.	.	.	.	T	C	.	T	.	.	A	G	A	.	.	.	.	.	C	C	.	T
valhapP	.	.	.	.	.	C	T	.	C	.	G	.	.	.	T	.	.	T	.	.	.	A	T	C	.	.	.	.	C	.	.	T
valhapT	.	.	.	.	.	C	T	.	.	.	G	.	.	.	T	C	.	T	T	.	A	G	A	.	.	.	.	.	C	C	.	T
valhapX	.	.	.	.	.	.	.	.	.	.	G	.	.	.	T	C	.	T	T	.	A	G	A	.	.	.	.	.	C	C	.	T
valhapAA	.	.	T	.	.	C	.	.	.	.	.	.	.	.	T	C	.	T	.	.	A	G	A	.	.	.	.	.	C	C	.	T
valhapDD	.	.	T	.	.	C	T	.	C	C	.	A	.	.	T	.	.	.	.	.	.	.	.	.	.	T	G	.	C	C	.	.
valhapY	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	C	T
valhapBB	.	.	.	.	.	.	.	.	.	.	G	.	.	.	T	C	.	T	T	.	A	G	A	.	.	.	.	.	C	.	.	T
valhapEE	.	.	.	.	.	C	T	.	.	C	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.
valhapN	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
valhapR	.	.	.	.	.	.	T	.	C	.	.	.	.	.	T	.	T	.	.	.	.	.	.	T	.	.	.	G	C	.	.	T
valhapCC	G	.	T	C	G	C	T	.	.	C	.	.	A	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	C	C	.	T

Haplotype	412	420	422	425	453	486	487	490	504	511	513	551	552	602	617	622	Synonyms[1]
valhapM	A	G	T	G	C	C	A	C	T	A	C	A	A	A	A	C	MalHapF
valhapJ	.	.	.	C	.	T	.	.	.	G	.	.	.	.	.	.	MalHapJ
valhapF	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	PorHap4
valhapK	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	PorHap16
valhapD	.	.	.	.	.	.	G	.	.	.	.	.	G	.	.	.	PorHap7
valhapA	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	PorHap3
valhapE	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	MalHapB
valhapB	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	MalHapD
valhapL	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	MalHapH
valhapH	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	BakHapA
valhapQ	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	MalHapE
valhapZ	.	.	.	.	.	.	.	T	.	.	.	.	G	.	.	.	PorHap11
valhapI	.	.	C	C	.	T	.	.	.	G	.	G	.	.	.	.	PorHap12
valhapO	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	
valhapW	.	.	.	.	.	.	.	T	.	.	.	G	G	.	.	.	PorHap25
valhapC	.	.	.	.	.	.	G	T	.	.	.	.	G	.	.	.	PorHap5
valhapP	.	.	.	C	.	T	.	.	.	G	T	.	G	.	.	.	
valhapT	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	PorHap6
valhapX	.	A	.	.	.	.	.	.	.	.	.	.	G	.	.	.	PorHap4
valhapAA	G	.	.	.	.	.	.	T	.	.	.	.	G	.	.	.	PorHap11
valhapDD	.	.	.	.	.	.	.	.	C	G	T	.	G	.	.	.	
valhapY	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	
valhapBB	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	
valhapEE	.	.	.	.	.	.	.	T	.	.	.	.	G	G	.	.	
valhapN	.	.	.	.	.	.	.	.	.	.	.	G	G	.	.	.	PatHap27
valhapR	.	.	.	.	T	.	.	T	.	.	.	G	G	.	.	.	
valhapCC	.	.	.	.	T	.	.	.	.	.	.	.	G	.	C	.	MalHapI

Table 3.2: Continued

Haplotype	N	1 1 2 3 6 8 0 0 4	1 1 1 1 1 1 1 1 1 2 2 2 2 2 2 2 2 3 3 3 3 3 3 3 3 3 3 3 3 3 3 7 7 7 8 8 8 8 9 9 0 0 1 1 2 3 4 6 0 2 4 4 5 5 6 6 6 7 8 8 8 8 9 6 7 8 2 3 5 6 4 5 2 3 0 1 9 2 2 2 5 7 3 5 0 7 2 3 4 3 3 4 5 9 1	4 4 4 4 4 4 4 5 5 5 5 5 6 6 6 1 2 2 2 5 8 8 9 0 1 1 5 5 0 1 2 2 0 2 5 3 6 7 0 4 1 3 1 2 2 7 2	Synonyms[1]
valhapFF	1	. . . . . A	. . . . . T . . . . . T . T . . . . . T . . . G C . . T	. . . . T . . T . . . G G . . .	
valhapG	1	. . . . C . .	. . . . . C T . . G . . . T C . T T . A G A . . . . C C . T	. . . . .	PorHap6
valhapGG	1	. . . . .	G . T C G C T . . C . . A . T . . . . . C C . T	. . . . T . . . . . G . . .	MalHapI
valhapHH	1	. . . . .	. . . . . C T . . . . . T . . . C . . . . T . . C C . T	. . . . .	PorHap17
valhapII	1	G	. . . . .	. . . . .	MalHapF
valhapS	1	. . G . C . .	. . . . . G . . . T C . T T . A G A . . . . C C . T	. . . . . G . . .	PorHap4
valhapU	1	. . . . .	. . . . T . C C . . . . T . . . . . T . . . C . . T	. . . . . G . . A	MalHapH
valhapV	1	. C	. . . . .	. . . . .	MalHapF

[1] Previously published haplotypes are from Portway (1998), Baker *et al.* (1999), Malik *et al.* (2000) and Patenaude *et al.* (2006)

55 polymorphic sites; from those, 47 are transitions, 6 are transversions and 2 are insertions (Table 3.2). The insertions represent an extra adenine within a small region of four consecutive adenines (from position 100 to 103). Because it is impossible to determine the exact position of the insertions, we arbitrarily placed them at position 100 for the clade with haplotypes O and Y, and at position 104 for the clade with haplotypes R, FF and W (Figure 3.2). The 35 haplotypes are not equally represented in the sample; for example, nine haplotypes account for 62% of the sample, while another nine occur only once (singletons) and account for just 3% of the sample (Table 3.2). The overall haplotype (h) and nucleotide (π) diversity were 0.95 ± 0.004 and 1.63 ± 0.82% respectively.

Phylogenetic reconstruction of these 35 haplotypes revealed two main clades that correspond to the previously known "A" and "W" clades (Baker *et al.,* 1999; Patenaude *et al.,* 2007; Figure 3.2). Clade A and W are supported by 72% and 63% bootstrap values respectively when out rooted with a North Pacific right whale sequence. Clade A contains 15 haplotypes and 51% (n = 134) of the total sample, while clade W has 20 haplotypes and 49% (n= 128) of the total sample (Figure 3.2). Haplotype diversity was similar between the two clades (clade A $h = 0.91 \pm 0.01$, clade W $h = 0.88 \pm 0.01$) while nucleotide diversity was three times higher in clade W ($\pi = 1.45 \pm 0.74\%$) than in clade A ($\pi = 0.48 \pm 0.28\%$).

Within-population differentiation

No genetic differentiation between the two gulfs of the peninsula was detected. When live whales were used to test for genetic differentiation between Golfo Nuevo (n =

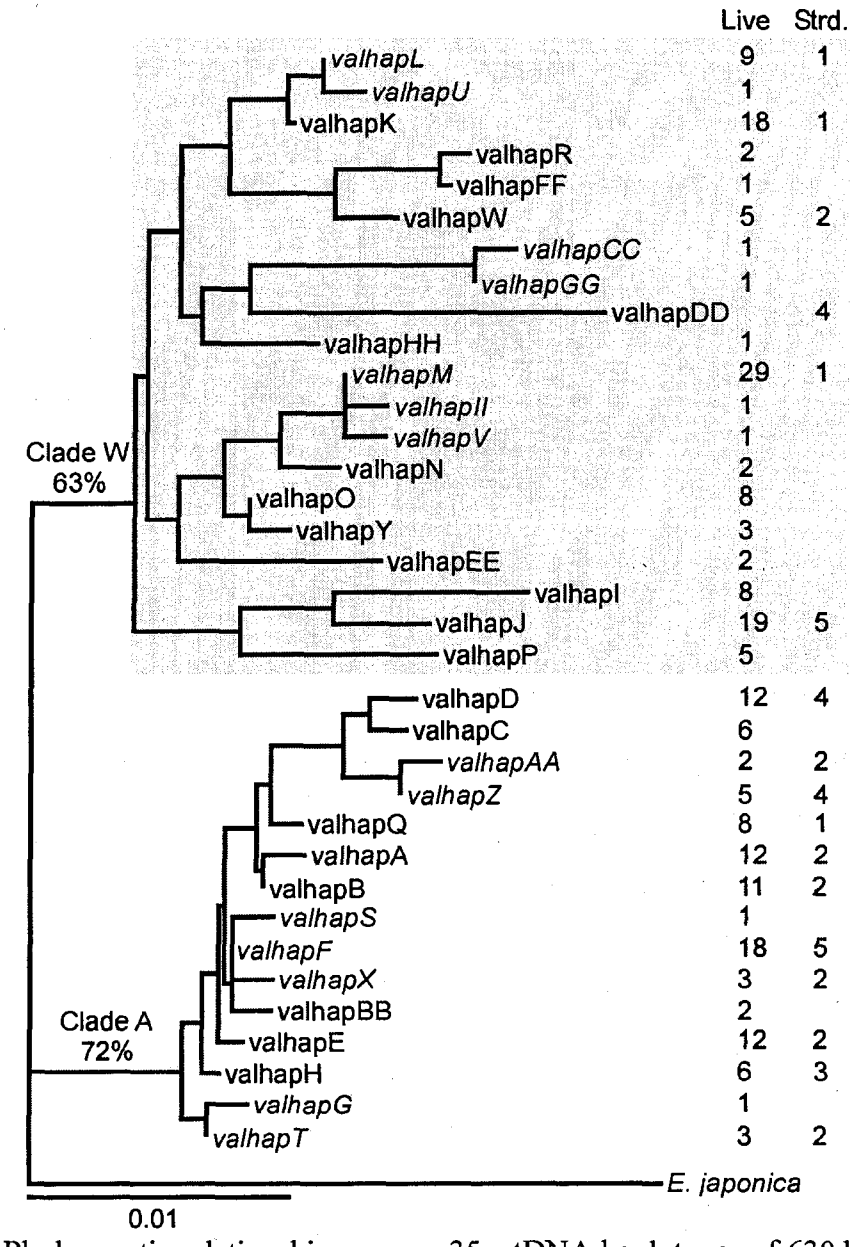

Figure 3.2: Phylogenetic relationships among 35 mtDNA haplotypes of 630 bp length from southern right whales off Península Valdés, Argentina. The tree is rooted with a homologous sequence of a North Pacific right whale (Sasaki *et al.,* 2005). Bootstrap percentages after 10,000 replications are indicated next to clades A and W. Haplotypes in i*talic* collapse to the nearest common ancestor (node) when using sequences of 275 bp long. Frequencies of live and stranded (Strd.) animals are adjacent to each haplotype. Note that stranded whales are over-represented in clade A.

21) and Golfo San José (n = 198), *Fst* was low and almost significant (*Fst* = 0.015, *P*-value = 0.051), while Φ_{ST} was also low and not significant (Φ_{ST} = 0.001, *P*-value > 0.1). No genetic differentiation was detected among age-sex groups of live whales at the haplotype or nucleotide level (overall *Fst* = 0.002, overall *Φst* = -0.008, *P*-value > 0.1 for both), or between calving females and single females (*Fst* = 0.001, *Φst* = 0.007, *P*-value > 0.1 for both). No significant differentiation in haplotype and nucleotide frequencies among years was detected when only live animals where compared (*Fst* = -0.001, *Φst* = 0.001, *P*-value >0.1 for both); or when only stranded animals where compared (*Fst* = -0.008, *Φst* = -0.006, *P*-value >0.1 for both).

Low but significant differentiation was found between live (n = 219) and stranded (n = 43) whales. The *Fst* statistic, based on haplotype frequencies, shows that 1% of the variance was accounted for by these two groups (*P*-value < 0.05). At the nucleotide level, the *Φst* statistic shows that 4% of the molecular variance is explained by the separation between samples from live and stranded whales (*P*-value < 0.005). The frequencies of certain haplotypes were remarkably different between the two groups (Figure 3.2); for example, valhapM, the most common haplotype (13.2%, n = 29) in live whales was found only once (a juvenile female) in the sample from stranded animals, while valhapDD was discovered four times (9.3%) in the stranded whales (including an adult female) and never in the live animals. Haplotype and nucleotide diversity were similar between live and stranded groups (live whales, $h = 0.94 \pm 0.005$, $\pi = 1.6\% \pm 0.8$; stranded whales, $h = 0.95 \pm 0.01$, $\pi = 1.6\% \pm 0.8$).

Sequences from stranded whales were over-represented in clade A (67.4%, n = 29) than in clade W (32.6%, n = 14; Figure 3.2). In fact, the proportion of stranded

whales in each clade was significantly different from expected based on the proportion of live whales in each clade ($X^2 = 5.47$, P-value =0.02). Most haplotypes (11 out of 17) discovered in the stranded whales belong to clade A (Figure 3.2). This pattern is primarily driven by calves; of the seven stranded adults and juveniles, three belong to clade W (haplotypes J, M and DD), and four to clade A (haplotypes D, E, X and Z).

Southern hemisphere population structure

Sequence alignment of the 35 haplotypes discovered in this study with 37 previously published haplotypes (Patenaude *et al.*, 2007) revealed 45 haplotypes of length 275 bp for the whole southern hemisphere. This represents 8 newly discovered haplotypes for the entire species, seven of which belong to clade W. With these additions, the overall haplotype diversity (h) for the southern right whales is 0.955 (±0.003) and the overall nucleotide (π) diversity is 2.80% (±1.45%). The Península Valdés sample is now composed of 33 haplotypes, of which fourteen are new for this population. Haplotype and nucleotide diversity in the Península Valdés whales were high and similar to the values for the whole southern hemisphere ($h = 0.94 \pm 0.005$, $\pi = 2.83\% \pm 1.47$).

Significant differentiation at the haplotype and nucleotide level was found among the 6 subpopulations analyzed (overall $Fst = 0.166$, P-value <0.001; overall $\Phi st = 0.158$, P-value < 0.001). Argentina shared haplotypes with all populations (Table 3.3); however, no single haplotype was present in all the populations and only two haplotypes were shared by the South Atlantic and Indo-Pacific ocean basins (BakHapA and BakHapE). Pairwise comparisons at the haplotype level (Fst) revealed differences between Argentina and all other populations (P-value < 0.01; Table 3.3) except the South Georgia feeding

Table 3.3: Pair wise genetic comparisons between Península Valdés, Argentina, and five seasonal subpopulations. For each group, the total number of haplotypes followed by the sample size is shown between parentheses.

Argentina (33/282)	*Fst*	*Φst*	Shared haplotypes
South Africa (21/41)	0.034***	0.056***	15
South Georgia F. (8/8)	0.007	-0.016	6
New Zealand (4/42)	0.192***	0.208***	1
Australia (5/20)	0.130***	0.113***	2
SWAustralia F (3/5)	0.125**	0.078	1

*** *P*-value <0.001, ** *P*-value <0.01

ground (*Fst* = 0.007, *P*-value > 0.05; Table 3.3). In comparisons at the nucleotide level (*Φst*), the differentiation was statistically significant when compared to other nursery areas (*P*-value < 0.001; Table 3.3) but not to either feeding ground (*Φst*= -0.016 for SG, *Φst* = 0.078 for SWA F; *P*-value > 0.05 for both comparisons); although the sample sizes for these two areas are too small for statistical comparisons (Table 3.3).

Discussion

Genetic diversity and phylogeny

Although southern right whales in the South Atlantic were hunted for a long period of time (as recently as the 1960s), levels of genetic diversity do not seem to have been greatly affected. The haplotype diversity currently detected at Península Valdés is relatively high and similar to levels previously reported for this, and other, recovering southern right whale populations (Portway, 1998; Baker *et al.,* 1999; Malik *et al.,* 2000; Patenaude *et al.,* 2007). The nucleotide diversity appears lower than previously reported for this population, but this is due to an artifact produced by the use of a longer sequence. When nucleotide diversity was recalculated using shorter sequences we found values similar to those previously reported (Portway, 1998; Patenaude *et al.,* 2007). As pointed out by Portway (1998), commercial whaling appears not to have reduced genetic diversity in the Península Valdés population to an extent that has affected its recovery.

The large number of samples and longer mtDNA sequences used in this study has revealed 35 haplotypes in the whales from Península Valdés, as many haplotypes as were previously known for the entire southern hemisphere (37 haplotypes presented by Patenaude *et al.,* 2007). The phylogenetic reconstruction of these 35 haplotypes reveals two well-supported clades that correspond to the previously reported A and W clades

(Baker *et al.,* 1999; Patenaude *et al.,* 2007). The low-diversity clade A may represent a clade that historically had a smaller population size or suffered more depletion than clade W. We found that the Argentinean right whale population is divided equally between the two clades. In contrast, Patenaude *et al.* (2007) found 90% (n = 18) of the samples from Península Valdés concentrated within clade W and only 10% (n = 2) within clade A. The large increase in the number of haplotypes within clade A is most simply explained by the larger sample size in our study. An alternative explanation for the equal frequency of both clades in our sample involves the influx of immigrants from areas that are rich in clade A (South Africa and New Zealand; Patenaude *et al.,* 2007), indicating contemporary gene flow between, or complete mixing of, formerly isolated populations (Portway, 1998; Patenaude *et al.,* 2007).

Within-population heterogeneity

The most unexpected finding in this study was the non-random distribution of mitochondrial lineages among stranded whales. Although the degree of genetic differentiation between live and stranded whales was small, it is similar in magnitude (as reflected by *Fst* and Φst) to the genetic differentiation between Argentina and South Africa. More surprising was the differential mortality of clade A over clade W. Two, not mutually exclusive, hypotheses could explain this finding: 1) an increase in calf mortality (36 out of the 43 strandings were calves) among recent migrants from areas with high clade A abundance, and 2) differential susceptibility to environmental variability affecting food abundance on feeding grounds used predominantly by whales from clade A.

Increased mortality among whales from clade A could occur if migration from areas rich in clade A, such as South Africa (65% of samples belong to clade A) or New Zealand (90%; Patenaude *et al.,* 2007), had increased in recent years. It is plausible that newly arrived females with no previous experience of the Península Valdés area might be prone to lose calves. Lack of experience with the local coastal geomorphology and tides, and lack of "social structure" that might benefit mothers with calves have been proposed as important factors in the reproductive success of southern right whales (Elwen and Best, 2004). Furthermore, migrants might suffer higher nutritional stress due to longer migratory paths. Although no direct evidence of recent migration or dispersal from South Africa to Argentina exists, southern right whales are capable of long distance movements (Best *et al.,* 1993). A few known animals from both populations have been photographed in a common area in the middle of the South Atlantic Ocean that is approximately 4,424 Km from Península Valdés and 2,769 Km from South Africa (Best *et al.,* 1993). Additionally, a few (n = 3) adult females from the Península Valdés population have been photographed with calves in a nursery area off southern Brazil, 2,051 Km to the north of the Argentinean nursery area (Best *et al.,* 1993). Further comparisons of photo-identification catalogues among seasonal subpopulations would be needed to detect recent migrants and validate this hypothesis.

The second hypothesis that could explain the differential mortality between clades is that whales from clade A are more susceptible to environmental variability affecting their food supply. This hypothesis implies that the whales forage in different locations according to the mitochondrial DNA phylogeny revealed in our study. Changes in krill abundance in the western South Atlantic have been correlated to sea surface temperature

anomalies and changes in the amount of sea ice around Antarctica (Trathan *et al.*, 2006; Murphy *et al.*, 2007). These anomalies affect the reproductive output of marine predators, including the southern right whale (Leaper *et al.*, 2006; Trathan *et al.*, 2006). If the whales are suffering of food shortages, adult females may not be able to provide the calves with enough energy, with the result of an increase in calf mortality. Although there is evidence of substructuring of maternal lineages on feeding areas (Valenzuela *et al.*, 2008), there is no indication, based on isotope ratios, that haplotypes from clade A and clade W use different feeding grounds (LOV *personal observation*). Future research should examine with more detail the population structure of southern right whales throughout their entire migratory range by, taking skin samples and photographs on the feeding grounds or, using indirect methods to infer migratory origins (such as stable isotopes or trace metals).

Is differential mortality a recent phenomenon? An examination of past records of live and dead whales may help answer this question. In their comprehensive analysis of southern right whale population structure, Patenaude *et al.* (2007) reported an overrepresentation of clade W among samples from Argentina, 12 haplotypes from 18 samples clustered within clade W while only 1 haplotype from 2 samples belonged to clade A (Table 3.4). It is important to note that 19 of those 20 samples were collected from *stranded* whales between 1994 and 1996 (Portway, 1998). In a different set of samples collected in 1988 and 1989, 10 haplotypes from 16 *live* whales were equally divided between the two clades (Table 3.2; Malik *et al.*, 2000). Although the frequency of each of these 10 haplotypes and the number of samples within each clade has not been reported, it is possible to estimate a range of haplotype frequencies for each clade. Either

Table 3.4: Proportion of samples and haplotypes (Haps.) within clades A and W from this and previous studies. Data presented by Patenaude *et al.* (2007) comes originally from: [1] Portway (1998) and [2] Malik *et al.* (2000).

	This study				Patenaude et al. (2007)		
Collected in	2003-06				1994-96 [1]		1988-89 [2]
Collected from	Living		Stranded		19 stranded, 1 living		16 living
	Samples	Haps.	Samples	Haps.	Samples	Haps.	Haps.
Clade A	46.6 %	44 %	67.4 %	65 %	10 %	8 %	50 %
Clade W	23.4 %	56 %	32.6 %	35 %	90 %	92 %	50 %
Total number	219	34	43	17	20	13	10

clade could contain a minimum of 31% (n = 5) and a maximum of 69% (n = 11) of the samples, with the other clade having the complementary proportion.

If the 16 samples collected from live whales in 1988 and 1989 are also concentrated within clade W as were those collected from dead whales in the mid 1990s, our results showing a similar proportion of samples and haplotypes in both clades would indicate an increase of clade A at Península Valdés in recent years (2003-2006). This increase could result from our larger sample size or from migration from other populations (as discussed earlier). However, if the samples collected from live whales (in 1988 and 1989) are not concentrated in clade W (if they are randomly distributed or concentrated in clade A), it would suggests that clade A was as abundant in the 1980s as it is today, indicating a biased mortality of clade W during the 1990s. If the latter pattern is correct, it would provide evidence of a "switch" in differential mortality in recent years, which might be explained by environmental variability affecting different feeding areas on a decadal scale. A better understanding of past genetic heterogeneity may come from reanalyzing archived skin samples, bones (Rastogi *et al.,* 2004) or baleen plates (Kimura *et al.,* 1997; Rosenbaum *et al.,* 1997).

Other than the differential mortality between the two clades, no evidence of genetic heterogeneity was found within the Península Valdés. The weak, yet not significant, differentiation in haplotype frequencies detected between Golfo Nuevo and Golfo San José is probably the result of the small number of samples collected from Golfo Nuevo. The lack of heterogeneity between gulfs is supported by observations of the same whales using different gulfs in different years as well as some whales moving between gulfs within the same season (Rowntree *et al.,* 2001). We did not find

differentiation either among years or between calving and single females, but this would be expected only if primiparous females gave birth at the same time as their mothers and no "migration" between cohorts existed. Although Patenaude *et al.* (2006) found genetic differentiation between two years in whales sampled off New Zealand, a later study of the same population showed no differentiation (Carroll, 2006).

Southern hemisphere population structure

Additional sampling substantially increased the genetic differentiation between Argentina and South Africa at the haplotype level, but decreased the genetic differentiation between Argentina and both nursery areas in the Indo-Pacific basin (Australia and New Zealand) at the haplotype and nucleotide levels. No genetic differentiation was found between Argentina and both feeding grounds (South Georgia and southwestern Australia); however the small sample sizes from these feeding grounds prevent further statistical analyses and conclusions. Although the use of a large number of samples is likely responsible for the changes in genetic differentiation among populations, the lower differentiation detected between Argentina and the Indo-Pacific basin could result from a recent increase in gene flow between populations. Further sampling on different feeding grounds would increase our understanding of the population structure of southern right whales across the southern hemisphere.

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CHAPTER 4

ASSESSMENT OF SOUTHERN RIGHT WHALE FORAGING ECOLOGY USING STABLE ISOTOPE ANALYSES OF SKIN SAMPLES

Introduction

Ocean warming will undoubtedly change the ocean ecosystems and affect all trophic levels from phyplankton to large marine predators (McClintock *et al.* 2008). A recent study indicates that a regional warming of 1°C could lead to a 95% reduction in the abundance of krill over the next 100 years across the south western South Atlantic (Murphy *et al.* 2007). Southern right whales consume large quantities of zooplankton (Reilly *et al.* 2004), and their reproductive output responds to fluctuations in krill abundance linked to El Niño-Southern Oscillation (ENSO; Leaper *et al.* 2006). Adapting to such changes will be extremely difficult for these whales because it will happen during an individual's lifetime (life spans for right whales is thought to be a minimum of 65 years; Hamilton *et al.* 1998). Furthermore, southern right whales reproduce slowly, with mature females giving birth to one calf every 3 years (Payne 1986). In cases like this, ecological flexibility at the individual level might play an important role in the survival of the species. Their ability to use different feeding areas or consume different prey types will be a key factor in their survival. A recent study shows indirect evidence of southern right whale site fidelity to unknown feeding grounds (Valenzuela *et al.* 2008); strong site

fidelity may hinder the whales' flexibility to use other feeding grounds. However, our understanding of southern right whale foraging behavior and distribution is sparse and relies heavily on information collected by whaling ships in 1800s and 1900s (IWC 2001), recent observations of feeding behavior near shore (Hamner *et al.* 1988; Moore *et al.* 1999), and line transect surveys of the Southern Ocean in summer (Reilly *et al.* 2004). Understanding the whales' responses to short-term responses to ENSO events is dependent on understanding the whales' current habitat use and foraging ecology.

Southern right whale feeding distribution

Southern right whales migrate seasonally between nursing grounds and feeding grounds. During the nursery season (August to November) they congregate along the coastal waters of Argentina, Brazil, South Africa, Australia and New Zealand, where they have been intensely studied (IWC 2001). During the rest of the year they travel off shore to unknown feeding areas where research opportunities are scarce. Six historic feeding grounds are recognized in the South Atlantic based on catch locations of the 19th and 20th century's whalers (Figure 4.1; IWC 2001). However, the extent to which historic, and other feeding grounds, are used today is unknown. Southern right whales have been sighted in the waters near the Antarctic Peninsula and South Georgia (Hamner *et al.* 1988; Moore *et al.* 1999; Reilly *et al.* 2004); three whales photo-identified on their nursery ground at Península Valdés have been resighted off South Georgia (Best *et al.* 1993). A recent study using satellite tags showed that South African whales apparently use three different feeding grounds, only one of which matches a historic feeding ground (south of 50°S and between 30°E and 10°W; Mate and Best 2008). Other than these

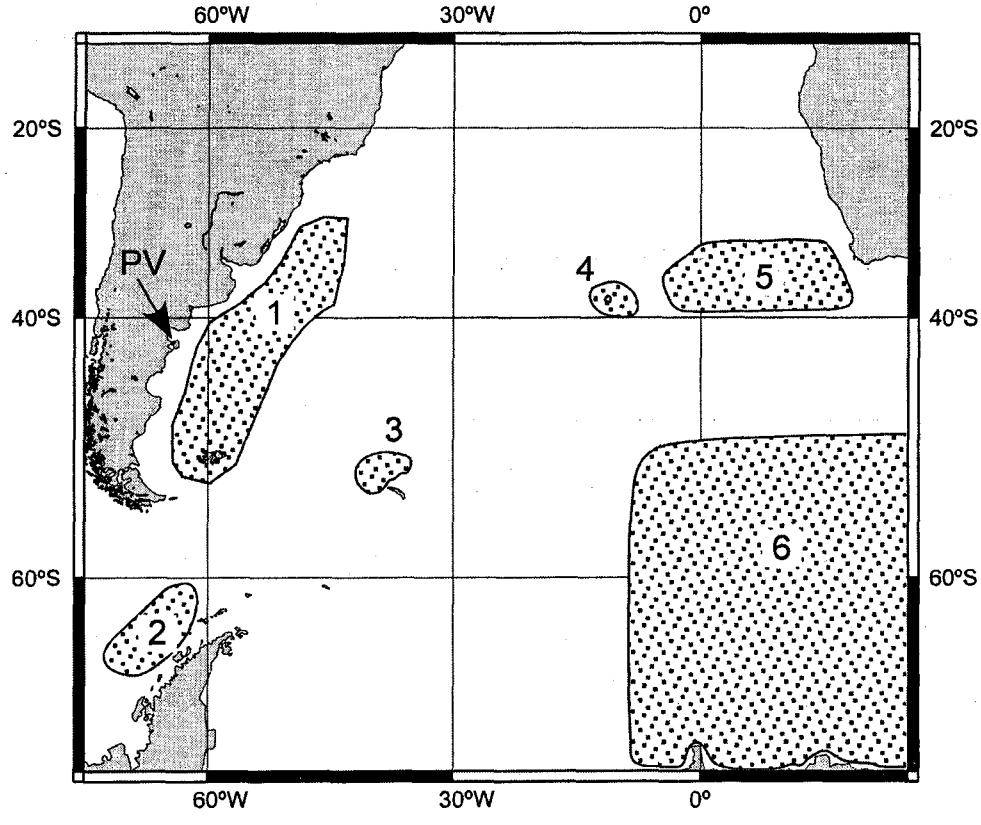

Figure 4.1: Map of the South Atlantic and Atlantic sector of the Southern Ocean indicating the six historic feeding grounds recognized by the International Whaling Commission (IWC 2001). 1) offshore South America, 2) Antarctic Península, 3) South Georgia, 4) Tristan da Cunha, 5) Cape Town-Tristan da Cunha, a band (30°S-40°S) of catches between Gough Island and South Africa, and 6) a diffuse area of catches south of 50°S and between 30°E and 10°W. The location of Península Valdés (PV), Argentina is also marked in the map.

regional sights little is known of the whales current feeding distribution in the South Atlantic Ocean.

Southern right whale trophic position

Southern right whales feed on krill (primarily *Euphausia superba*) and copepods. The stomach of right whales killed in the South Atlantic during an illegal hunt by Soviet whalers in the 1960s showed that whales taken south of 50°S had stomachs full of unidentified euphausiids, those north of 40°S were full of Calanoid copepods, and between 40° and 50°S the stomachs contained a mixture of both groups of crustaceans (Tormosov *et al.* 1998). Hamner *et al.* (1988) observed right whales feeding on Antarctic krill near the Antarctic Peninsula and South Georgia. This pattern seems to be the result of prey distribution, as swarms of Antarctic krill are not found north of 50°S (Atkinson *et al.* 2004; Nicol 2006).

Stable isotopes as indicators of foraging locations

Regional variations in stable isotopes have been used to identify the feeding locations and migratory patterns of many species of birds and mammals, including whales (Schell *et al.* 1989; Hobson 1999; Rubenstein and Hobson 2004). This is possible because stable isotopes ratios have predictable patterns of change across landscapes (Hobson 1999, Rubenstein and Hobson 2004; West *et al.* 2006). In marine ecosystems for example, carbon isotope ratios ($^{13}C/^{12}C$) decrease with increasing latitude, and more shallow waters are more enriched compared to pelagic waters in both carbon and sulphur ($^{34}S/^{32}S$) ratios (Peterson and Fry 1987; Fry 2006). The carbon isotope patterns are in part

the result of temperature differences, CO_2 concentrations and differences in plankton metabolism (Peterson and Fry 1987; Fry 2006), while the differences in sulphur reflect differences in the anoxic conditions of the waters (Peterson and Fry 1987; Fry 2006). A second characteristic of isotopes that makes them useful to track animal movements is that isotopes are incorporated directly from diet into animal tissues with varying degrees of fractionation. Some isotopes (e.g., carbon) reflect the diet without much change, while others (e.g., nitrogen) show considerable enrichment and are affected by variables such as nutritional stress and food quality (Deniro and Epstein 1981; Roth and Hobson 2000; McCutchan *et al.* 2003; Fuller *et al.* 2004). When incorporated into body tissues, nitrogen isotope ratios ($^{15}N/^{14}N$) increase an average of 3‰ per trophic level over the whole animal body (Deniro and Epstein 1978, 1981; Peterson and Fry 1987; Kelly 2000). The stable isotope values recorded in an animal's tissues represent an integration of the food consumed anywhere from the last few hours or weeks (blood plasma or muscle) up to the animal's entire life (bone collagen), depending on the isotope turnover rate of the tissue that is analyzed (Rubenstein and Hobson 2004).

Isotope as tracers of whale migration

Schell *et al.* (1989) documented annual cycles of $\delta^{13}C$ values in the baleen of bowhead whales and showed that these values matched those of the whales' prey at locations along their migratory route between the Bering, Chukchi and Beaufort Seas. The baleen of southern right whales contains a continuous 6 to 7 year record of their foraging paths (Best and Schell, 1996; Rowntree *et al.* 2008). Best and Schell (1996) found that the baleen from South Africa right whales show annual cycles that appear to

correspond to regular north-south movements across the subtropical convergence. Rowntree *et al.* (2008) compared the carbon isotope ratios in baleen plates from different individuals and found inter- and intra individual variations in annual foraging cycles and hypothesized that these variations reflect individually distinctive foraging behaviors.

Measurements of stable isotope ratios from skin biopsies have been successfully used to study the foraging ecology of free-ranging cetaceans (Todd *et al.* 1997; Hooker *et al.* 2001; Ruiz-Cooley *et al.* 2004). The main constraints when using skin samples are the unknown isotopic fractionation and unknown isotope turnover rate; neither of these parameters has been experimentally determined for cetacean skin. Isotope fractionation between diet and cetacean skin has been assumed to be 3‰ for nitrogen and 1‰ for carbon. Turnover rate has been proposed to be between one (Todd *et al.* 1997) and several months (Ruiz-Cooley *et al.* 2004). However, skin is the only tissue that can be relatively easily collected from a large number of live baleen whales with a minimally invasive collection procedure, and produce enough material to be used for several different types of analyses.

In this paper we used stable isotope analyses to assess the feeding ecology of southern right whales. In an attempt to understand how the southern right whale population at Península Valdés uses the southern hemisphere oceans and its resources (Rowntree *et al.* 2008; Valenzuela *et al.* 2008), we have focused on identifying the number and location of current feeding grounds and the differences in isotopic profiles between different segments of the population (different age and sex classes). Skin samples collected from live whales off Península Valdés, Argentina, were analyzed for stable carbon, nitrogen and sulphur isotopes. To assess the trophic position and location

of feeding areas used by right whales we matched isotope values from skin samples with published and unpublished isotope values of euphausiids, copepods and marine predators (fish, seabirds, pinnipeds and odontocetes) from different areas across the south western South Atlantic and the Atlantic sector of the Southern Ocean. We used linear mixing models to estimate the proportion of contribution from different feeding sources to the whale's diet.

Our analyses indicate that southern right whales have at least three distinct prey sources. Each prey source may represent a single feeding ground or a combination of feeding grounds with different contributions to the diet. The latter option likely represents a foraging strategy associated with different proportions of time spent in different feeding grounds according to a whale's migratory path. The four feeding grounds that seem to contribute more to the diet of right whales are Uruguay, the Patagonian shelf, South Georgia and the waters from the Polar Front. We found differences between age and sex classes, but these differences may be due to differences in physiology and anatomical characteristics of the feeding system (baleen plates).

Materials and Methods

Sample collection and analyses

Skin samples (n = 196) were obtained by biopsy darting live whales off Península Valdés, Argentina (42° 30' S, 64° 00' W) over four consecutive years (2003 – 2006) at the time of peak whale abundance [September and October, Payne 1986]. To avoid including resampled whales, individuals were photographed for later identification based on callosity patterns and other natural marks (Payne *et al.* 1983). Age classes (adults and juveniles) were identified primarily based on body size; adult females were recognized by

the presence of a calf. Gender of juveniles and single adults, was determined whenever possible by observations of the genital area; otherwise, gender was later identified by PCR amplification and electrophoresis of *Zfx* and *Zfy* introns following Shaw *et al.* (2003).

Skin samples were dried, ground to a fine powder and lipid extracted using Soxhlet extraction following Todd *et al.* (1997). Approximately 1mg of material per sample was analyzed for carbon and nitrogen isotopes using a Carlo Erba 1108 elemental analyzer coupled to a Thermo Finnigan Delta S Isotope Ratio Mass Spectrometer at the Stable Isotope Ratio Facility for Environmental Research (SIRFER) at the University of Utah. Sulphur ($\delta^{34}S$) analysis was conducted at the Colorado Plateau Stable Isotope Laboratory (CPSIL), using a Costech ECS4010 elemental analyzer interfaced to a Thermo-Electron Delta Plus Advantage IRMS. The isotope ratios are expressed as $\delta^{13}C$, $\delta^{15}N$ or $\delta^{34}S$ (‰) = $[(R_{sample}/R_{standard}) - 1] \times 10^3$, where R is the $^{13}C/^{12}C$, $^{15}N/^{14}N$ or $^{34}S/^{32}S$ for $\delta^{13}C$, $\delta^{15}N$ or $\delta^{34}S$, respectively. Standards were referenced to Pee Dee Belemnite for carbon, Atmospheric air for nitrogen and Canyon Diablo Triolite for sulphur isotopes. The reproducibility of these measurements is 0.2‰ for $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ after repeated analyses of internal laboratory standards.

Food web stable isotope ratios

Stable isotope values of copepods, euphausiids and marine predators (fish, cephalopods, seabirds, pinnipeds and odontocetes) were primarily obtained from the published literature (Table 4.1). We focused our effort on studies reporting samples

Table 4.1: Previously published and unpublished stable carbon and nitrogen isotope values of zooplankton and predators sampled in the South Atlantic and Atlantic sector of the Southern Ocean.

Taxa	$\delta^{13}C$ Min	$\delta^{13}C$ Mean	$\delta^{13}C$ SD	$\delta^{13}C$ Max	$\delta^{15}N$ Min	$\delta^{15}N$ Mean	$\delta^{15}N$ SD	$\delta^{15}N$ Max	n	Study site	Year Season	Source
Copepoda												
Calanoides acutus		-31.50				2.70			4	African quadrant (LQW)	1999 Fa	1 i
Calanus propinquus		-28.60				4.00			4	African quadrant (LQW)	1999 Fa	1 i
Metridia gerlachei		-30.60				4.20			3	African quadrant (LQW)	1999 Fa	1 i
Rhincalanus gigas		-29.40				4.30			2	African quadrant (LQW)	1999 Fa	1 i
C. propinquus		-30.00				3.70			4	American quadrant (LQW)	1999 Fa	1 i
Ctenocalanus		-30.80				3.00			2	American quadrant (LQW)	1999 Fa	1 i
M. gerlachei		-29.70				4.80			4	American quadrant (LQW)	1999 Fa	1 i
C. acutus		-30.60				3.40			4	Lazarev Sea (LQW)	1999 Fa	1 i
C. propinquus		-29.40				4.60			19	Lazarev Sea (LQW)	1999 Fa	1 i
Ctenocalanus		-29.20				4.40			4	Lazarev Sea (LQW)	1999 Fa	1 i
M. gerlachei		-29.40				5.00			16	Lazarev Sea (LQW)	1999 Fa	1 i
C. acutus		-25.40				8.20			13	Marguerite Bay (MB)	1999 Fa	1 i
Euchaeta		-24.30				9.90			8	Marguerite Bay (MB)	1999 Fa	1 i
M. gerlachei		-27.00				9.60			9	Marguerite Bay (MB)	1999 Fa	1 i
Calanoid		-27.00#				-1.80#				Marion Island	1999 Fa	2 e
Calanoid		-25.00#				-1.20#				Marion Island	1999 Fa	2 e
Acartia lilljeborgi		-22.00	0.30						3	Northeast Brazil (Br)	1995 Su	1 d
Copepods	-19.80			-17.20						Northeast Brazil (Br)	1995 Su	1 d
Copepods		-23.00				8.40				Patagonian Shelf (PS)	1998	1 f
Copepods		-19.10				8.80				Patagonian Shelf (PS)	1998	1 f
Copepods		-20.20				14.60				Patagonian Shelf (PS)	1998	1 f
Copepods		-20.50				10.70				Patagonian Shelf (PS)	1998	1 f
C. propinquus		-29.40				2.00			9	Polar Front (PF)	1999 Fa	1 i
C. simillimus		-24.60				3.20			23	Polar Front (PF)	1999 Fa	1 i
Heterorhabdus		-25.10				6.10			6	Polar Front (PF)	1999 Fa	1 i

Table 4.1: Continued

Taxa	Min	$\delta^{13}C$ Mean	SD	Max	Min	$\delta^{15}N$ Mean	SD	Max	n	Study site	Year Season	Source
M. gerlachei		-28.30				3.60			5	Polar Front (PF)	1999 Fa	1 i
M. lucens		-27.50				3.20			8	Polar Front (PF)	1999 Fa	1 i
Pleuromanna robusta		-24.50				4.50			6	Polar Front (PF)	1999 Fa	1 i
Rhincalanus gigas		-23.40				2.00			16	Polar Front (PF)	1999 Fa	1 i
Calanus		-19.58				9.45			5	Uruguay (Ur)	2005 Su	1 p
Copepods		-22.90				4.45			14	Uruguay (Ur)	2004 Sp	1 p
Copepods		-23.49	0.18			3.27	0.18			Uruguay (Ur)	2004 Sp	1 p
Copepods		-21.92				5.49			8	Uruguay (Ur)	2004 Sp	1 p
Copepods		-22.34				4.98			6	Uruguay (Ur)	2004 Sp	1 p
Copepods		-19.69				9.63			25	Uruguay (Ur)	2005 Su	1 p
Copepods		-19.76				9.25			17	Uruguay (Ur)	2005 Su	1 p
Neocalanus		-21.52				7.80			18	Uruguay (Ur)	2005 Su	1 p
Paracalanus		-22.59				4.80			7	Uruguay (Ur)	2004 Sp	1 p
Pleuromamma		-20.97				6.79			18	Uruguay (Ur)	2005 Su	1 p
Calanoides acutus		-30.20				0.90			2	Weddell Gyre (LQW)	1999 Fa	1 i
C. propinquus		-28.70				0.90			8	Weddell Gyre (LQW)	1999 Fa	1 i
Ctenocalanus		-30.30				-0.10			2	Weddell Gyre (LQW)	1999 Fa	1 i
M. gerlachei		-30.10				2.20			8	Weddell Gyre (LQW)	1999 Fa	1 i
C. acutus		-31.10				4.00				Weddell Sea (LQW)	1986 Su	1 b
Calanus propinquus		-31.00				4.50				Weddell Sea (LQW)	1986 Su	1 b
M. geriachei		-30.40				3.00				Weddell Sea (LQW)	1986 Su	1 b
Rhincalanus gigas		-33.30				2.90				Weddell Sea (LQW)	1986 Su	1 b
Euphausiids												
Euphausiids		-27.00	2.00			5.10	1.30		3	American quadrant (LQW)	1999 Fa	1 a
Thysanoessa		-29.40				2.30			7	American quadrant (LQW)	1999 Fa	1 i
Euphausia frigida		-28.00				3.40			6	Lazarev Sea (LQW)	1999 Fa	1 i
E. superba		-31.30				3.60			20	Lazarev Sea (LQW)	1999 Fa	1 i
E. superba		-31.20				2.10			23	Lazarev Sea (LQW)	1999 Fa	1 i
E. superba		-27.50				2.10			16	Lazarev Sea (LQW)	1999 Fa	1 i

Table 4.1: Continued

Taxa	Min	$\delta^{13}C$ Mean	SD	Max	Min	$\delta^{15}N$ Mean	SD	Max	n	Study site	Year Season	Source
Thysanoessa		-29.10				3.20			15	Lazarev Sea (LQW)	1999 Fa	1 i
E. superba		-28.20							6	Marguerite Bay (MB)	1999 Fa	1 i
E. superba		-26.10				5.70			4	Marguerite Bay (MB)	1999 Fa	1 i
E. superba		-24.70				6.10			31	Marguerite Bay (MB)	1999 Fa	1 i
E. vallentini		-25.80#				4.00#				Marion Island	1999 Fa	2 e
E. vallentini		-20.50#				3.00#				Marion Island	1999 Fa	2 e
E. superba		-29.80	0.60			3.60	0.20		12	Palmer station (AP)	1999 Fa	7 f
E. superba		-25.70				3.70			4	South Georgia (SG)	1996 Su	1 i
E. superba		-27.80				3.60			4	South Georgia (SG)	1996 Su	1 i
E. superba		-23.90				4.30			4	South Georgia (SG)	1996 Su	1 i
E. superba		-27.90				2.40			4	South Georgia (SG)	1996 Su	1 i
E. superba		-24.50				4.20			4	South Georgia (SG)	1996 Su	1 i
E. superba		-26.80				3.50			4	South Georgia (SG)	1996 Su	1 i
Euphausiids		-18.10				10.50				Patagonian Shelf (PS)	1998	1 f
Euphausiids		-18.00				12.30				Patagonian Shelf (PS)	1998	1 f
Euphausiids & mysids		-17.80				12.30				Península Valdés (PS)	2000 Sp	1 p
Euphausiids & mysids		-18.40				12.60				Península Valdés (PS)	2000 Sp	1 p
Euphausiids & mysids		-19.20				12.30				Península Valdés (PS)	2000 Sp	1 p
Euphausiids & mysids		-18.20				12.40				Península Valdés (PS)	2000 Sp	1 p
Euphausiids & mysids		-18.50				12.30				Península Valdés (PS)	2000 Sp	1 p
E. frigida		-22.50				2.50			31	Polar Front (PF)	1999	1 i
E. frigida		-24.20				4.20			15	Polar Front (PF)	1999	1 i
E. frigida		-23.10				3.60			4	Polar Front (PF)	1999	1 i
E. triacantha		-22.20				3.20			16	Polar Front (PF)	1999	1 i
Thysanoessa spp		-21.80				2.60			8	Polar Front (PF)	1999	1 i
Thysanoessa spp		-22.20				5.30			3	Polar Front (PF)	1999	1 i
Thysanoessa spp		-22.60				2.90			103	Polar Front (PF)	1999	1 i
E. superba		-27.20	0.50			3.80	0.20		6	South Shetland Is. (SG)	2000 Fa	1 k
E. superba		-25.10	0.90			4.20	0.40		9	South Shetland Is. (SG)	2000 Fa	1 k

Table 4.1: Continued

Taxa	$\delta^{13}C$ Min	$\delta^{13}C$ Mean	$\delta^{13}C$ SD	$\delta^{13}C$ Max	$\delta^{15}N$ Min	$\delta^{15}N$ Mean	$\delta^{15}N$ SD	$\delta^{15}N$ Max	n	Study site	Year Season	Source
E. superba		-28.30	0.70			2.90	0.40		9	South Shetland Is. (SG)	2000 Fa	1 k
Euphausiids		-21.91				4.74				Uruguay (Ur)	2005 Sp	1 p
Euphausiids		-21.41				4.57			5	Uruguay (Ur)	2004 Sp	1 p
Euphausiids		-19.93				7.66			1	Uruguay (Ur)	2004 Sp	1 p
Euphausiids		-23.04				3.42			6	Uruguay (Ur)	2004 Sp	1 p
Euphausiids		-20.91				7.08			5	Uruguay (Ur)	2004 Sp	1 p
Euphausiids		-20.90				6.60			9	Uruguay (Ur)	2005 Su	1 p
Euphausiids		-21.76				5.25			3	Uruguay (Ur)	2004 Sp	1 p
E. frigida		-28.70				4.80			8	Weddell Gyre (LQW)	1999 Fa	1 i
Thysanoessa		-29.20				0.00			24	Weddell Gyre (LQW)	1999 Fa	1 i
E. superba	-31.50			-25.50	1.50			4.80		Weddell Sea (LQW)	1986 Fa	1 b
Fish												
Gobionotothen marionensis	-18.80	-18.30	0.70	-17.80	9.80	10.40	0.90	11.10		Marion Island	2003 Fa	2 l
G. marionensis	-21.20	-20.60	0.60	-20.00	7.80	8.50	0.60	9.10		Marion Island	2003 Fa	2 l
Lepidonotothen larseni	-22.40	-22.10	0.40	-21.70	6.50	7.20	0.80	8.60	5	Marion Island	2003 Fa	2 l
L. larseni		-21.20#				8.00#				Marion Island	1999 Fa	2 e
Chaenocephalus aceratus		-24.90	0.10			11.00	0.60		4	Palmer station (AP)	1997 Fa	2 f
G. gibberifrons		-24.90	0.90			10.10	0.70		3	Palmer station (AP)	1997 Fa	2 f
Harpagifer antarcticus		-20.70	0.50			11.80	0.20		5	Palmer station (AP)	1997 Fa	2 f
Notothenia coriiceps		-20.40	0.50			12.00	0.30		3	Palmer station (AP)	1997 Fa	2 f
Acanthistius brasilianus	-16.00	-15.50	0.30	-14.60	20.20	20.40	0.10	20.50	4	Patagonian Shelf (PS)	1999-01	7 j
Callorhynchus callorhynchus		-15.00	0.00			17.70	0.00		1	Patagonian Shelf (PS)	1999-01	7 j
Eleginops maclovinus	-16.90	-15.40	0.70	-13.40	16.90	18.20	0.50	19.00	4	Patagonian Shelf (PS)	1999-01	7 j
Engraulis anchoita	-19.00	-17.70	0.10	-16.90	14.80	16.40	0.10	17.30	18	Patagonian Shelf (PS)	1999-01	7 j
Genypterus blacodes	-16.70	-15.40	0.70	-13.70	17.20	18.00	0.20	18.60	5	Patagonian Shelf (PS)	1999-01	7 j
Macruronus magellanicus	-17.50	-17.40	0.10	-17.30	15.80	16.20	0.20	16.50	3	Patagonian Shelf (PS)	1999-01	7 j
Merluccius hubbsi	-17.50	-16.50	0.40	-14.70	15.70	17.50	0.40	19.60	9	Patagonian Shelf (PS)	1999-01	7 j

Table 4.1: Continued

Taxa	$\delta^{13}C$ Min	$\delta^{13}C$ Mean	$\delta^{13}C$ SD	$\delta^{13}C$ Max	$\delta^{15}N$ Min	$\delta^{15}N$ Mean	$\delta^{15}N$ SD	$\delta^{15}N$ Max	n	Study site	Year Season	Source
Odonthestes incise	-17.40	-16.30	0.30	-14.40	13.50	16.50	0.40	18.00	10	Patagonian Shelf (PS)	1999-01	7 j
O. smitty	-16.10	-15.20	0.20	-13.70	16.60	18.10	0.20	18.90	13	Patagonian Shelf (PS)	1999-01	7 j
Pseudopercis semifasciata	-15.20	-14.70	0.40	-13.50	18.90	19.50	0.20	19.80	4	Patagonian Shelf (PS)	1999-01	7 j
Raneya fluminensis	-16.40	-15.20	0.40	-13.50	18.20	18.70	0.10	19.20	7	Patagonian Shelf (PS)	1999-01	7 j
Riveiroclinus eigenmani	-17.70	-16.70	0.20	-15.80	17.10	17.90	0.20	18.70	8	Patagonian Shelf (PS)	1999-01	7 j
Salilota australis	-15.90	-15.6	0.20	-15.40	16.50	16.60	0.10	16.70	3	Patagonian Shelf (PS)	1999-01	7 j
Seriolella porosa		-17.80	0.00			18.32	0.00		1	Patagonian Shelf (PS)	1999-01	7 j
Trematomus bernacchir		-23.40				10.40			1	Elephant Island (AP)	1982 Su	1 a
Bathylagus antarcticus	-27.90			-26.70	9.10			9.40	2	Weddell Sea (LQW)	1986 Su	2 c
Electrona antarctica	-31.50			-29.40	8.20			8.50	3	Weddell Sea (LQW)	1986 Su	2 c
Gymnoscopelus braueri	-30.00			-27.50	8.00			8.50	3	Weddell Sea (LQW)	1986 Su	2 c
Notolepis coasti		-27.40				8.50			1	Weddell Sea (LQW)	1986 Su	2 c
Cephalopoda												
Kondakoria longimama		-25.40	0.80			6.90	0.40		3	American quadrant (LQW)	1983 Su	1 a
Loligo and *Illex*	-21.00	-17.00	0.50	-15.10	12.30	16.30	0.50	18.30	17	Patagonian Shelf (PS)	1999-01	7 j
Octopus	-15.50	-12.50	1.00	-11.30	17.50	17.80	0.40	18.90	4	Patagonian Shelf (PS)	1999-01	7 j
Aves												
Pagodroma nivea		-23.90	0.70			15.20	1.60		9	Dronning Maud Land (DL)	1999-92	6 n
Macronectes giganteus		-21.50#				13.30#				South Georgia (SG)	1998 Sp	3 m
M. giganteus		-23.30#				12.00#				South Georgia (SG)	1998 Sp	3 m
Catharacta antarctica	-19.20	-16.80	0.50	-15.70	18.20	19.00	0.00	20.40	89	Northern Patagonia (PS)	1999-01	3 j
Larus atlanticus	-15.70	-12.60	0.40	-11.30	14.70	16.50	0.30	19.40	11	Northern Patagonia (PS)	1999-01	3 j
L. dominicanus	-20.70	-17.50	0.10	-13.60	13.20	18.10	0.10	20.30	229	Northern Patagonia (PS)	1999-01	3 j
Leucophaeus scoresbii	-17.10	-15.30	0.10	-14.20	17.40	20.50	0.10	22.20	65	Northern Patagonia (PS)	1999-01	3 j
M. giganteus	-18.00	-17.00	0.10	-16.30	18.10	19.40	0.10	21.00	50	Northern Patagonia (PS)	1999-01	3 j
Phalacrocorax bougainvillii	-16.50	-16.50	0.00	-16.50	19.00	19.10	0.10	19.20	2	Northern Patagonia (PS)	1999-01	3 j
P. atriceps albiventer	-17.80	-16.20	0.00	-14.60	15.10	19.40	0.10	20.90	236	Northern Patagonia (PS)	1999-01	3 j
P. a. atriceps	-16.70	-16.30	0.10	-15.90	19.40	20.00	0.10	20.60	15	Northern Patagonia (PS)	1999-01	3 j

Table 4.1: Continued

Taxa	$\delta^{13}C$ Min	$\delta^{13}C$ Mean	$\delta^{13}C$ SD	$\delta^{13}C$ Max	$\delta^{15}N$ Min	$\delta^{15}N$ Mean	$\delta^{15}N$ SD	$\delta^{15}N$ Max	n	Study site	Year Season	Source
P. Hybrid	-17.00	-16.60	0.10	-16.30	19.00	19.70	0.10	20.30	14	Northern Patagonia (PS)	1999-01	3 j
P. magellanicus	-17.90	-16.30	0.10	-14.80	17.60	19.30	0.10	21.50	48	Northern Patagonia (PS)	1999-01	3 j
P. olivaceus	-17.00	-16.10	0.10	-15.30	18.70	20.20	0.10	21.30	59	Northern Patagonia (PS)	1999-01	3 j
Spheniscus magellanicus	-18.60	-16.70	0.00	-14.80	14.10	18.80	0.00	19.90	414	Northern Patagonia (PS)	1999-01	3 j
Sterna hirundinacea	-17.40	-16.80	0.00	-16.40	17.10	18.30	0.10	19.00	35	Northern Patagonia (PS)	1999-01	3 j
Thalasseus maximus	-17.50	-16.80	0.10	-16.00	18.30	18.70	0.00	19.20	49	Northern Patagonia (PS)	1999-01	3 j
T. sandwichensis	-16.00	-16.80	0.00	-17.50	16.60	18.40	0.10	19.50	83	Northern Patagonia (PS)	1999-01	3 j
Thalassoica antarctica		-24.80	1.00			13.20	0.60		9	Dronning Maud Land (DL)	1999-92	6 n
Aptenodytes patagonicus		-23.20	0.90			9.70	0.40			Malvinas Is. (PS)	2001 Su	5 h
A. patagonicus		-22.50	0.40			9.70	0.30			Malvinas Is. (PS)	2001 Su	4 h
Pygoscelis adeliae		-23.70	0.30			12.50	1.60		6	Palmer station (AP)	1989	2 f
P. Antarctica		-27.00				6.90			1	Palmer station (AP)	1997	2 f
Fulmarus glacialoides	-28.60	#		-26.00		6.70			15	Weddell Sea (LQW)	1986 Su	2 c
M giganteus		-24.20#				8.70			1	Weddell Sea (LQW)	1986 Su	2 c
T. Antarctica	-29.00	#		-27.10		5.60			32	Weddell Sea (LQW)	1986 Su	2 c
Halobaena caerula	-28.00	#		-27.20		6.30			15	Weddell Sea (LQW)	1986 Su	2 c
P. adeliae	-26.90	#		-24.80		5.90			9	Weddell Sea (LQW)	1986 Su	2 c
Daption capense	-28.40	#		-26.10		6.50			12	Weddell Sea (LQW)	1986 Su	2 c
P. nivea	-28.50	#		-26.00		7.20			22	Weddell Sea (LQW)	1986 Su	2 c
Pacyphtila desolata	-26.80	#		-24.50		6.40			11	Weddell Sea (LQW)	1986 Su	2 c
Pterodroma brevirostris	-27.70	#		-24.50		7.80			19	Weddell Sea (LQW)	1986 Su	2 c
Oceanites oceanicus	-27.80	#		-22.00		8.30			17	Weddell Sea (LQW)	1986 Su	2 c
Sterna vitatta	-27.70	#		-26.90		5.40			4	Weddell Sea (LQW)	1986 Su	2 c
S. paradisaea	-28.80	#		-27.00		5.90			11	Weddell Sea (LQW)	1986 Su	2 c
Pinnipeds												
Lobodon carcinophaga	-26.50	#		-23.00	5.40			6.60	16	Weddell Sea (LQW)	1986 Su	2 c
Arctocephalus gazelle	-25.80	#		-24.30	7.20			8.80	7	Weddell Sea (LQW)	1986 Su	2 c
Hydrurga leptonyx	-24.50	#		-24.00	7.10			8.90	5	Weddell Sea (LQW)	1986 Su	2 c
Ommatophoca rossii		-22.90#			9.00			9.40	2	Weddell Sea (LQW)	1986 Su	2 c

Table 4.1: Continued

Taxa	Min	$\delta^{13}C$ Mean	SD	Max	Min	$\delta^{15}N$ Mean	SD	Max	n	Study site	Year Season	Source
Odentocetes												
Cephalorhynchus commersonii		-12.7	1.3	17.2	0.7				121	Southern Patagonia (PS)	1975-08*	6 o
Grampus griseus		-11.6	0.5	19.5	1.3				23	Southern Patagonia (PS)	1975-08*	6 o
G. griseus		-13.9	0.9	15.5	1.4				25	Southern Patagonia (PS)	1975-08*	6 o
Lagenorhynchus australis		-10.8	1.2	19.3	1.6				39	Southern Patagonia (PS)	1975-08*	6 o
L. cruciger		-17.2	1	10.2	0.8				5	Southern Patagonia (PS)	1975-08*	6 o
Lissodelphis peronii		-12.9	0.9	15.3	2.4				37	Southern Patagonia (PS)	1975-08*	6 o
Pseudorca crassidens		-12.8	0.4	13.1	0.7				27	Southern Patagonia (PS)	1975-08*	6 o
Phocoena dioptrica		-17.9	1.2	10.1	0.9				87	Southern Patagonia (PS)	1975-08*	6 o
P. spinipinnis		-13	1	17.9	0.7				7	Southern Patagonia (PS)	1975-08*	6 o

[#] values obtained from figures

Study site: between parentheses is the acronym of the region used in figure 4.5.

Year Season: 1975-08* refers to a long term study of cetaceans stranding on the coast of Tierra del Fuego, Argentina that continues to the present date.

Sources: Tissue analysed: 1 Whole body; 2 Muscle; 3 Whole blood; 4 Blood cells; 5 Plasma; 6 Bone collagen; 7 soft tissues. References: a Wada et al. 1987; b Rau et al. 1991; c Rau et al. 1992; d Schwamborn et al. 1999; e Kaehler et al. 2000; f Dunton 2001; g Rowntree et al. 2001; h Cherel et al. 2002; i Schmidt et al. 2003; j Forero et al. 2004; k Schmidt et al. 2004; l Bushula et al. 2005; m Forero et al. 2005; n Steele 2005; o Riccialdelli et al. 2008; p This study. Treatments: Lipids were removed in "h" and "1 e". Samples preserved in ethanol for "h" and "7 j".

collected in the western South Atlantic Ocean, and in the Atlantic sector of the Southern Ocean (from 20°E to 70°W), as well as on studies reporting both carbon and nitrogen isotope values. In some cases isotope values were derived from figures within published literature and as a result may contain a degree of error.

Additionally, samples of euphausiids and copepods collected off Uruguay and northern Patagonia, Argentina were analyzed for carbon and nitrogen isotope ratios (Table 4.1). Stable isotope analyses were conducted at SIRFER using the same methodology as explained for skin samples; however, lipids were not extracted for these samples.

Statistical analyses

Differences in isotopic values among whales of different age-sex classes (adult, juveniles, males and females), and among whales sampled in different years were evaluated using Kruskal-Wallis tests (Sokal and Rohlf 1981). Post hoc Dunn's multiple comparison tests were used to detect which groups were different (Dunn 1964). Non-parametric Spearman's correlation (Sokal and Rohlf 1981) was used to test for correlations between isotope ratios of different elements, and between isotope ratios and sampling date. For all tests, statistical significance was set at 5%.

IsoSource modeling

Standard linear mixing models are used to estimate the proportion of different sources contributing to a mixed diet (Phillips 2001). For example, they have been used to estimate the proportion of each food source an animal's diet (Urton and Hobson 2005; Inger *et al.* 2006; Samelius *et al.* 2007). These models work well as long as the number of

sources does not exceed the number of isotopes by more than one (Phillips 2001). If there are n isotopes and more than $n + 1$ sources, the system is mathematically undetermined and no unique solution exists (Phillips 2001). However, in nature the number of sources is commonly big and requires alternative methods. Phillips and Gregg (2003) developed an iterative model that presents the distribution of all feasible combinations of sources that produce a mixture with an isotopic value equal to the observed mixture. The model is implemented using the software IsoSource (Phillips and Gregg 2003), and requires the mean isotope values of the mixture of interest (a predator's tissue for example) and the sources (its potential prey); either mixture or sources should be corrected for isotope fractionation if necessary.

The model has three basic steps. First, it creates each possible combination of source proportions by some small increment determined by the user (usually 1% to 2%). Second, it predicts the isotope values for the mixtures created by each source combination. Third, it stores in a database all possible source combinations that produce a mixture with an isotope value equal to or within a small user-defined tolerance (e.g., 0.1‰) of the observed isotope value of the original mixture. At the end of the iteration process, all the source combinations that represent feasible solutions can be accessed and descriptive statistics (mean, range and quartiles) are calculated. We used the isotopic values measured in skin samples (mixture) and selected isotopic values from potential prey samples (sources) from Table 4.1 to conduct an IsoSource modeling. To compensate for trophic level enrichment, we corrected the isotope ratios of skin samples by subtracting 3‰ for nitrogen and 1‰ for carbon.

Results

Stable isotope ratios from skin samples

Southern right whale skin samples have an overall mean $\delta^{13}C$ value of -20.78‰ ± 1.41‰ (range: -23.90 to -17.17‰; n = 196), a mean $\delta^{15}N$ value of 8.42‰ ± 2.11 (range: 6.03 to 15.01‰; n = 196) and a mean $\delta^{34}S$ value of 18.33‰ ± 0.68 (range: 17.57 to 20.37‰; n = 27). Carbon, nitrogen and sulphur distributions are not normal and are positively skewed (Shapiro-Wilk normality tests $p < 0.001$; Figures 4.2 and 4.3). Carbon and nitrogen distributions appear to be multimodal (Figure 4.2 and 4.3); in particular, nitrogen isotope ratios segregate into three main groups separated by gaps of approximately 0.8‰: a 'Low' group with a mean $\delta^{15}N$ of 7.49‰ (range 6.03 to 9.90‰, n = 159), a 'Mid' group with a mean $\delta^{15}N$ of 10.54‰ (range 10.26 to 10.71‰) and a 'High' group with a mean $\delta^{15}N$ of 12.85‰ (range 11.50 to 15.01‰). When samples are separated by age-sex classes, the gaps become wider and the multimodal pattern more apparent (Figure 4.2). Overall, there is a positive correlation between $\delta^{13}C$ and $\delta^{15}N$ values (Spearman's $\rho = 0.7$; $p < 0.001$; n = 196; Figure 4.2) but no correlation between $\delta^{34}S$ and either of the other two isotopes ($p > 0.1$; n = 27; Figure 4.3). Carbon and nitrogen isotope ratios are positively correlated in samples of the Low group (Spearman's $\rho = 0.45$, $p < 0.001$) but not among those in the Mid or High groups.

Whales from different age-sex classes show significant differences in $\delta^{13}C$ (Kruskal-Wallis $X^2 = 9.3$, $p < 0.05$) and $\delta^{15}N$ (K-W $X^2 = 20.7$, $p <= 0.001$) but not in $\delta^{34}S$ (K-W $X^2 = 3.0$, $p = 0.4$). Adult females median $\delta^{15}N$ value (7.40‰; n = 143) are statistically lower than juvenile females (8.25‰; n = 24) and juvenile males (8.62‰; n = 20; $p < 0.05$ for both comparisons; Figure 4.2). Juvenile males median $\delta^{13}C$ value

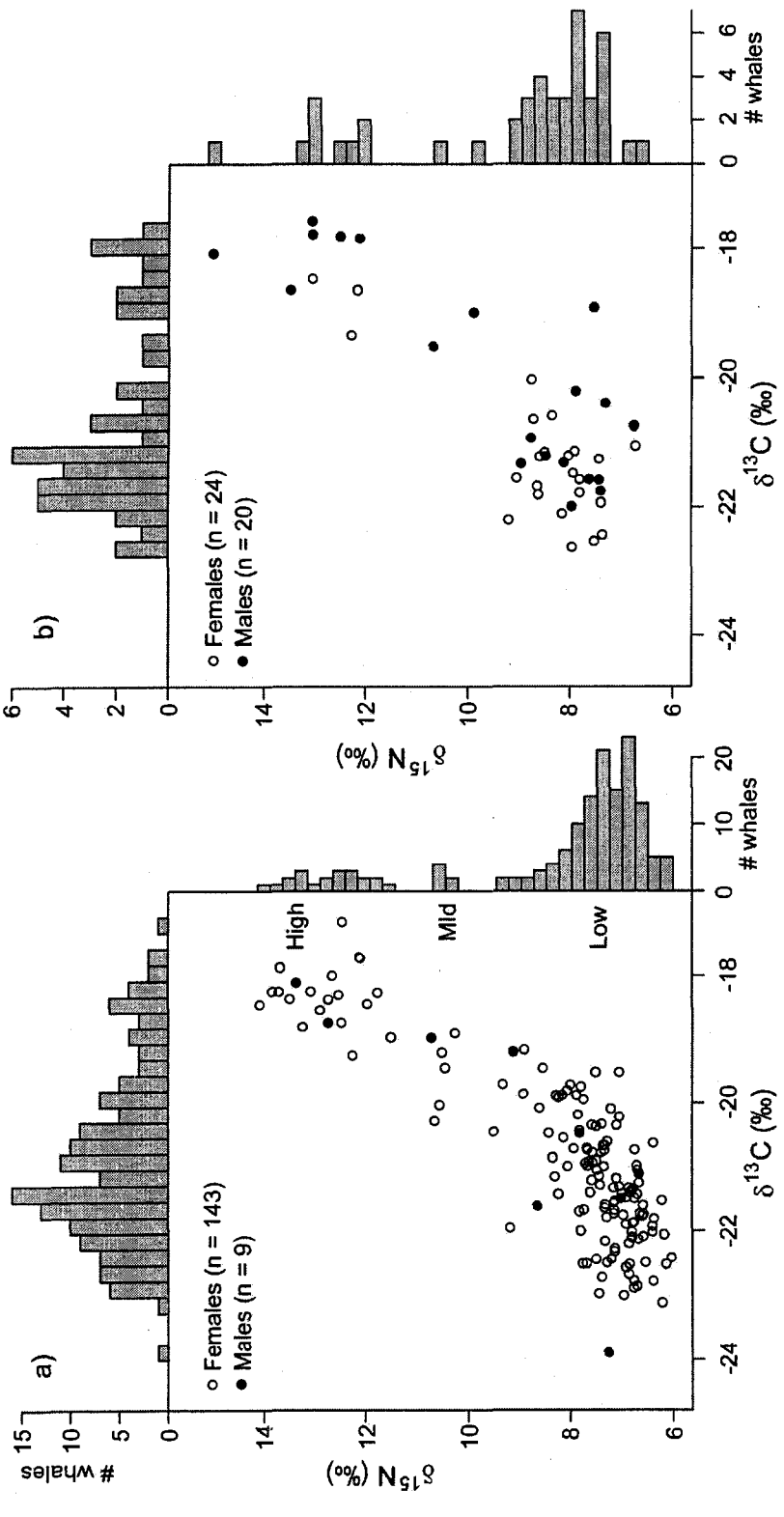

Figure 4.2: Scatter plots of $\delta^{15}N$ and $\delta^{13}C$ values from 196 southern right whale skin samples collected off Península Valdés: a) Adults, b) Juveniles. Frequency distributions of $\delta^{15}N$ and $\delta^{13}C$ are shown as marginal histograms. Isotope distributions are not normal (Shapiro-Wilk test $p < 0.001$) and appear to be multimodal. The names High, Mid and Low refer to the three isotopic groups defined by the $\delta^{15}N$ distribution.

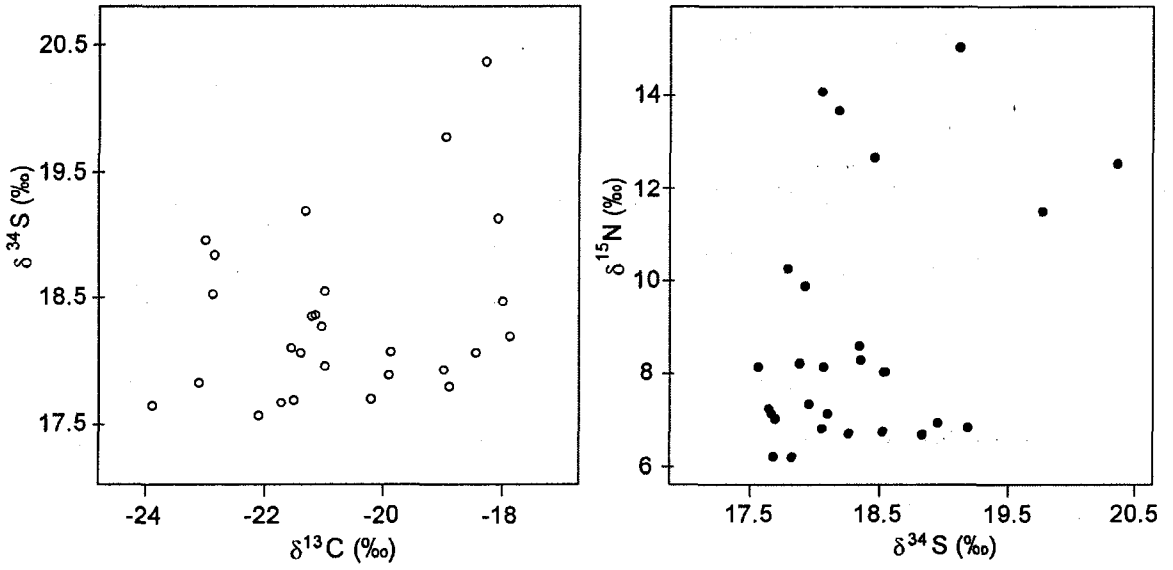

Figure 4.3: Sulphur ($\delta^{34}S$) values from 27 skin samples in relation to $\delta^{13}C$ (left) and $\delta^{15}N$ (right) values. No correlation between sulphur isotope vales and carbon or nitrogen values was found. Sulphur isotope distribution is not normal (Shapiro-Wilk test $p < 0.001$).

(-20.30‰) is statistically higher ($p < 0.05$) than both female classes (adults = -21.06‰, juveniles = -21.36‰; Figure 4.2). Adult males show a large isotopic range (Figure 4.2a), and are not statistically different from any other class for either isotope; however, this group has a small sample size (n = 9).

Skin samples collected in different years show significant differences in $\delta^{13}C$ (K-W $X^2 = 19.7$, $p < 0.001$) and $\delta^{15}N$ (K-W $X^2 = 12.4$, $p = 0.006$) but not in $\delta^{34}S$ (K-W $X^2 = 6.8$, $p = 0.08$). Post hoc Dunn's multiple comparison tests indicate that the median carbon and nitrogen isotope values for the year 2006 ($\delta^{13}C$ = -20.08‰, $\delta^{15}N$ = 8.25‰, n = 50; Figure 4.4) are statistically higher than for any other year ($\delta^{13}C$ = -21.31‰, $\delta^{15}N$ = 7.44‰ for the other 3 years combined; Figure 4.4). No correlation was found between isotope values and sampling date for any isotope when all the samples were pooled or analyzed by year.

Trophic structure

Overall, zooplankton and predators (excluding whales) from northern and coastal areas (Patagonian shelf and Uruguay) tend to have higher isotope ratios than the same taxa sampled in colder waters, such as the Southern Ocean (Table 4.1; Figure 4.5). Predators from the Patagonian shelf and the Southern Ocean are isotopically distinct (t-test $p < 0.001$) and there is a large gap in the isotopic distribution between these two regions (Figure 4.5b). The only animals from the Patagonian shelf that did not differ isotopically from the Southern Ocean were king penguins (*Aptenodytes patagonicus*) sampled on the Malvinas/Falkland Islands and two cold water odontocetes (*Lagenorhynchus cruciger* and *Phocoena dioptrica*) that stranded on the coast of

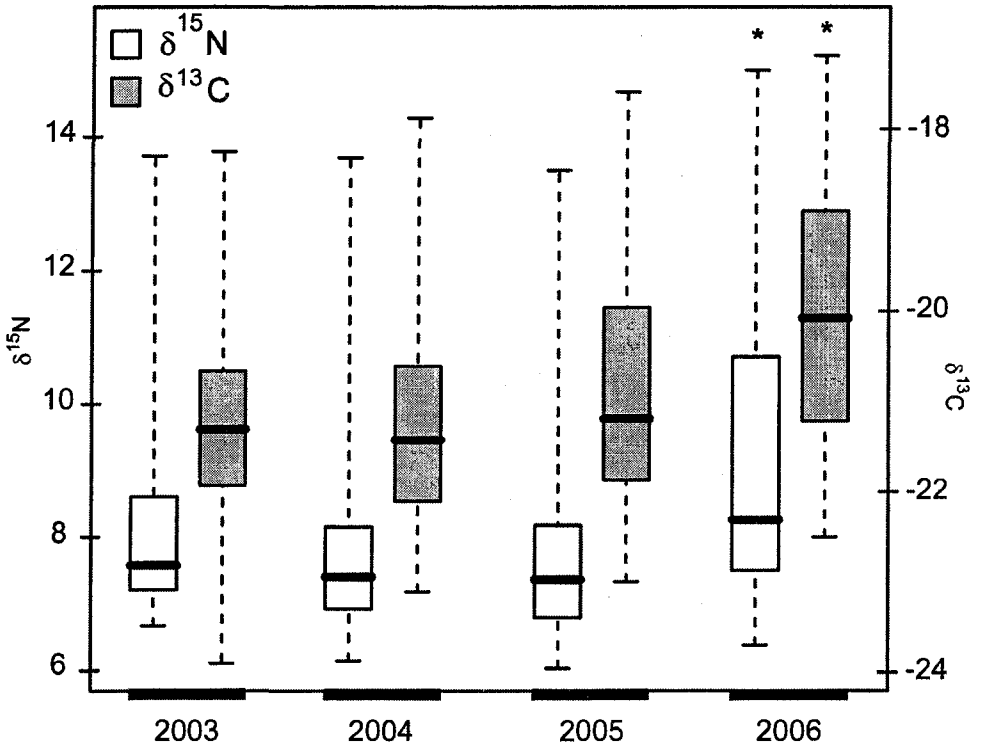

Figure 4.4: Boxplots of isotope values by year. Boxplots present the median, 25th-75th percentiles, minimum and maximum values. $\delta^{15}N$ values are presented in open boxes and $\delta^{13}C$ values in grey boxes. Year 2006 had higher stable isotope values than any other year.

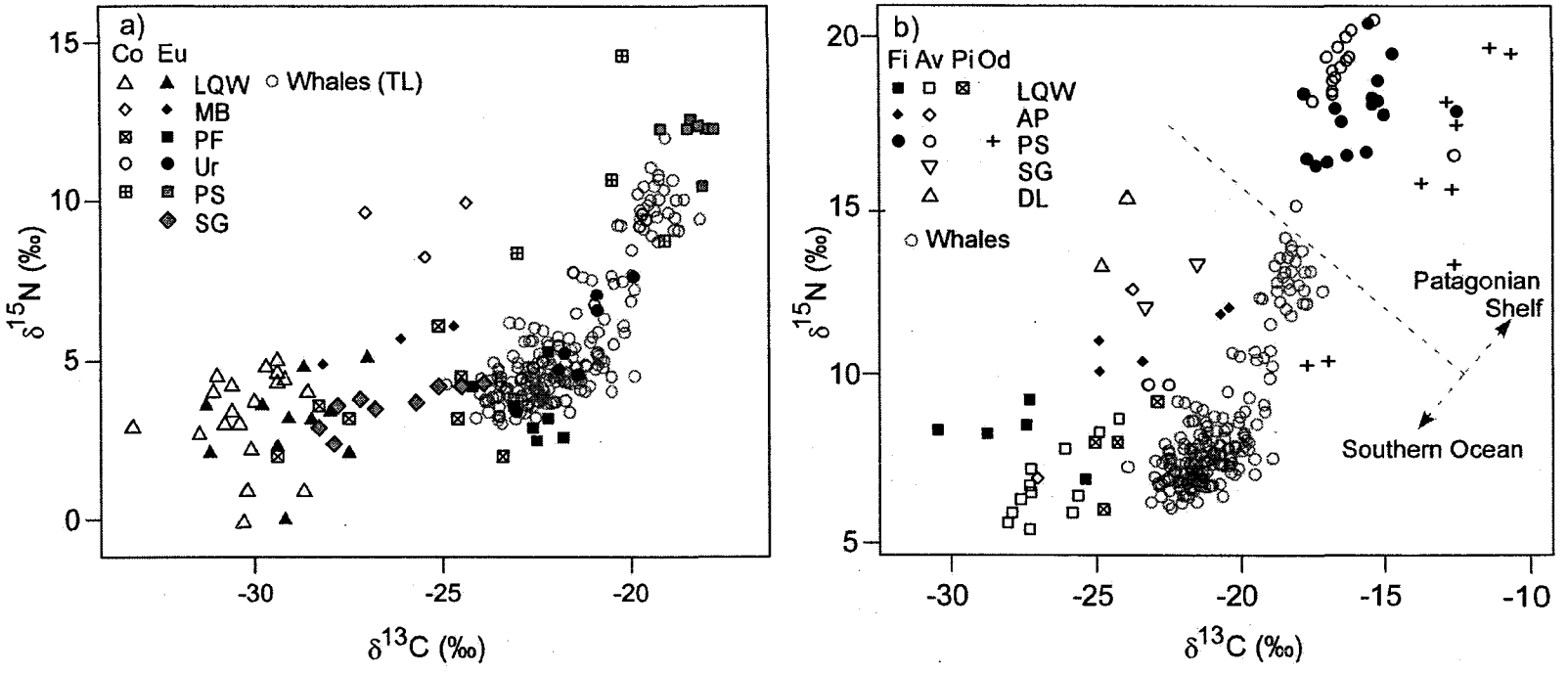

Figure 4.5: Stable carbon and nitrogen values of prey (a) and predators (b) from the South Atlantic and Southern Ocean. The $\delta^{13}C$ and $\delta^{15}N$ from skin samples are corrected for trophic level in pane 5a and not corrected in 5b. Copepods (Co), Euphausiids (Eu), Fish (Fi), Aves (Av), Pinnipeds (Pi) and Odontocetes (Od) values are from Table 4.1. Samples are from Uruguay (Ur), Patagonian shelf (PS), South Georgia (SG), Marguerite Bay (MB), Antarctic Peninsula (AP), Polar Front (PF), Draunning Maud Land (DL), and from a large area that includes the Lazarev sea, the American and African quadrants and the Weddell Sea (LQW). Note the isotope separation of samples taken from (PS) and those from higher latitudes (Southern Ocean), particularly from predators.

southern Patagonia (Table 4.1, Figure 4.5b). The Patagonian shelf and the Southern Ocean are also isotopically distinct for copepods and euphausiids (t-test $p < 0.001$; Figure 4.5a). However the isotope separation for prey species is not as large as the gap for predators. Zooplankton samples from Uruguay are intermediate and have a large isotopic range (Figure 4.5a). Copepods from Marguerite Bay, west of the Antarctic Peninsula, have the highest $\delta^{15}N$ values for zooplankton collected in higher latitudes; however, for carbon they are within the range of other samples from the Southern Ocean (Figure 4.5a).

When corrected for trophic enrichment (3‰ for $\delta^{15}N$ and 1‰ for $\delta^{13}C$), southern right whale isotope ratios overlap with a large range of isotope values of zooplankton collected from several locations, including the Uruguay, the Patagonian shelf, the Polar Front and South Georgia (Figure 4.5a). Different groups of right whales cluster with values from different regions. For example, whales with the lowest carbon and nitrogen isotope ratios (Low group) cluster with zooplankton from the Polar Front and South Georgia, and whales with the highest isotope values cluster with zooplankton from the Patagonian shelf. Skin samples show similar $\delta^{15}N$ values to other predators sampled in the Southern Ocean (Figure 4.5b). The $\delta^{13}C$ values do not overlap with any particular area, but rather range from the isotope values of Southern Ocean to the Patagonian shelf (Figure 4.5b). However, the isotope ratios of the predators in Figure 4.5 are derived from a variety of tissues and were subject to different treatments, therefore, no correction for trophic enrichment was attempted.

IsoSource Modeling

Eight sources were used in IsoSource (Figure 4.6): Euphausiids (Eu) from Uruguay (Ur), Patagonian shelf (PS), Marguerite Bay (MB), South Georgia (SG) and Polar Front (PF), as well as copepods (Co) from the Patagonian shelf and Marguerite Bay (Figure 4.6). These sources were chosen based on the need to draw a convex polygon that includes the mean isotope values of the skin samples (Phillips and Gregg), and a priori knowledge of modern right whale sightings off the Antarctic Peninsula, South Georgia and Uruguay (). Although copepods sampled off Uruguay and within the Polar front also met these criteria, we did not use them as sources because they present a particularly large isotopic range and other sources from similar areas showed narrower ranges and similar isotope means (Figure 4.6). The modeling was conducted for each of the three subgroups of whales previously presented (High, Mid and Low $\delta^{15}N$ groups). Results are presented for a 2% source increment and a tolerance of 1.5‰.

Whales from the Low group, have the largest contribution from euphausiids from the Polar front (10^{th} – 90^{th} percentile: 42-70%; mean: 57%; Figure 4.7), followed by South Georgia (4-26%; 15%) and Uruguay (0-26%; 12%). Whales from the Mid group, have the largest contribution from euphausiids from Uruguay (18-66%; 45%; Figure 4.7), followed by euphausiids from the Patagonian shelf (28-42%; 35%) and the Polar Front (2-38%; 17%). The High group has the largest contribution from euphausiids from the Patagonian shelf (65-72%; 69%; Figure 4.7), followed by euphausiids from Uruguay (6-32%; 20%) and the Polar Front (0.6-20%; 10%). Euphausiids collected on the Patagonian shelf are always part of the combination of sources for the Mid and High whale groups, with minimum contributions of 64% and 24% for each group respectively. Euphausiids

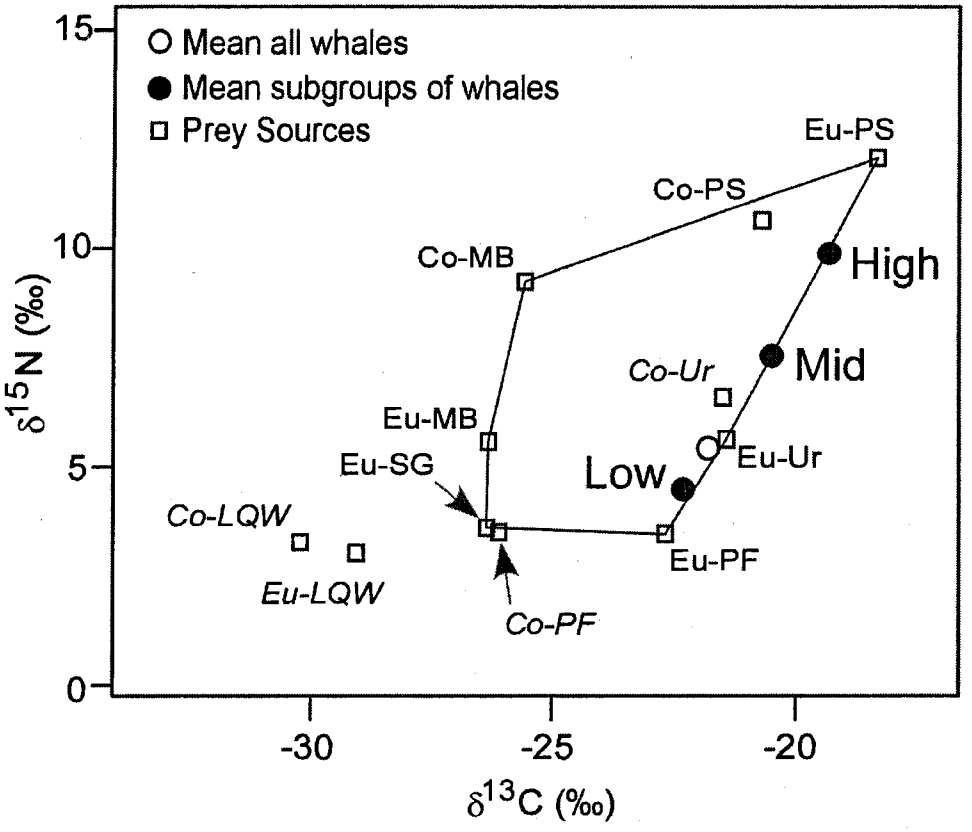

Figure 4.6: IsoSource polygon. Bivariate representation of the isotope sources (whales corrected for trophic level) and mixtures used for the IsoSource modeling. The mean isotope value of each prey source (squares) and subgroups of whales (circles) are presented. In *italics* are also presented a few prey groups not used for the IsoSource modeling. Co and Eu indicate copepods and euphausids respectively. Acronyms of locations are the same as Figure 4.5 and Table 4.1.

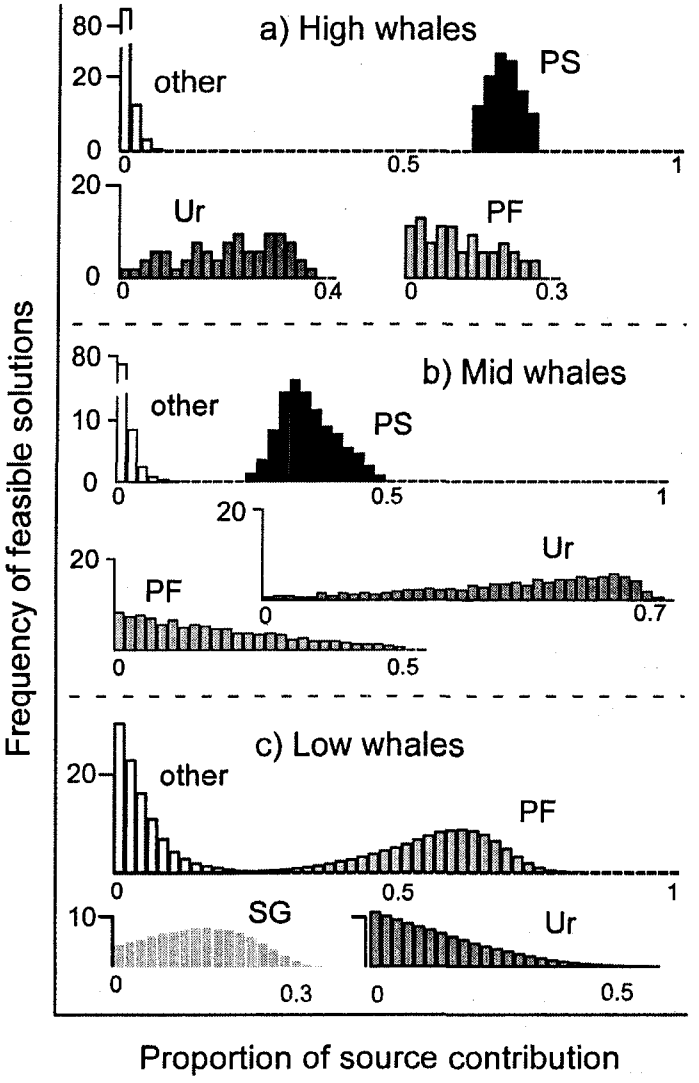

Figure 4.7: Results from IsoSource modeling. Distribution of proportions of prey source contribution to the diet of the High (a), Mid (b) and Low (c) subgroups of whales. For each subgroup, the three main sources are presented, all are euphausiids; the other four sources that do not contribute much are combined ("other").

from the Polar Front are always present in the combination of sources recorded as feasible solutions for the Low group, with a minimum contribution of 12%.

Discussion

Stable isotope of skin samples

Our stable isotope analyses of skin samples indicate that the Patagonian right whales appear to use at least three different feeding sources. The lack of normality in carbon and nitrogen isotope distributions suggests a non homogenous food source for the overall population (Hobson and Schwarcz 1986). The High and Mid groups appear to represent two distinct food sources, as suggested by the normal distribution of isotope values within each group and the lack of correlation between carbon and nitrogen isotopes. On the other hand, the Low group may represent a single feeding source or continuum of feeding sources, as indicated by its lack of normality and the positive correlation between $\delta^{13}C$ and $\delta^{15}N$. If all other physiological and ecological aspects are assumed to be equal among the sampled whales, the three isotopic groups (High, Mid and Low) may represent three different feeding grounds. Whether these feeding grounds correspond to the ultimate migratory origin of each whale or represent intermediate feeding locations is unknown due to a lack of understanding of the temporal information represented in the skin samples (Todd *et al.* 1997; Ruiz-Cooley 2004).

Sulphur isotope ratios were within the range expected for a pelagic ecosystem (Peterson and Fry 1987; Fry 2006). The lack of correlation with carbon and nitrogen isotopes suggests that the three putative feeding sources are from an area with a homogenous sulphur isotope signature. The two samples with the highest $\delta^{34}S$ values correspond to samples from the middle of the $\delta^{15}N$ distribution, which may indicate a

food source with a more benthic or coastal contribution (Peterson and Fry 1987; Fry 2006).

The range of stable carbon isotope values from skin samples also falls within the $\delta^{13}C$ range measured along five baleen plates collected from whales that stranded at Península Valdés (Rowntree *et al.* 2008). Baleen plates show annual oscillations of $\delta^{13}C$ values and three distinct regions characterized by a trough (with isotope values as low as -26‰ in $\delta^{13}C$), followed by an area of no isotopic change (a plateau of intermediate $\delta^{13}C$), followed by a peak in $\delta^{13}C$ values (as high as -15‰). The high and low isotope values found in the peaks and troughs are thought to represent the isotopic end members of the whales annual migratory range, while the plateaus may correspond to the fasting period when the whales are in the nursery area (Rowntree *et al.* 2008). Interestingly, the $\delta^{13}C$ values of skin samples are restricted to the range of isotope values between the plateaus and the peaks of four out of five baleen plates, and do not reach values as low as the troughs (Rowntree *et al.* 2008). This latter observation suggests that the skin samples may represent a temporal integration of feeding activities over a period of time that correspond to several months before migrating to Península Valdés, from the plateau to the peak (Rowntree *et al.* 2008).

Individual variations

Age segregation in regards to feeding grounds has not been reported in baleen whales. The significant isotope differences among age-sex classes could indicate some physiological differences, but could also indicate different foraging strategies. Juveniles had higher nitrogen values than adult females (Figure 4.2). Two factors may influence the

difference in isotope ratios between juveniles and adults. First, juveniles may not have fully developed baleen plates (Schell *et al.* 1989; Best and Schell 1996). Best and Schell (1996) speculate that the post weaning pause in body growth detected in southern right whales and in bowhead whales is the result of juvenile baleen plates being inadequate for effective filtering. An inadequate feeding system may prevent juveniles from capturing all possible sizes of prey (Mayo *et al.* 2001), thus the isotope ratios being incorporated may represent those from the largest zooplankton stages and species. Schmidt *et al.* (2003) found that the $\delta^{15}N$ values of Antarctic copepods increase significantly with body size, and that Antarctic krill juvenile stages sampled in the Lazarev Sea tend to have lower $\delta^{15}N$ than adults. Second, juvenile right whales grow rapidly (Whitehead and Payne 1981; Best and Schell 1996) and maintain higher activity levels in the nursery area than lactating females (Sironi 2004). Body growth as well as increased physical activities has been proposed to increase the nitrogen isotope ratios in animal tissues (Kelly 2000).

The interannual differences in the general distribution of $\delta^{13}C$ and $\delta^{15}N$ detected in this study could be explained as a response to changes in the isotopic composition of the whales' food sources or a response to changes in foraging strategy. Interannual and seasonal variations in the isotopic composition at the base of food webs produced by changes in ocean circulation or by modification of local biogeochemical processes may be responsible for the higher isotope ratios detected in 2006 (Peterson & Fry 1987; Druffel & Griffin 1999; Brix *et al.* 2004). However, it is also possible that prior to the 2006 nursing season, food was not abundant in areas with normally low isotope values, causing the whales to switch to prey with higher isotope. It has been observed that

changes in the abundance of Antarctic krill have affected the foraging strategies of marine predators in the South Georgia area (Trathan *et al.* 2006).

Trophic structure and IsoSource modeling

The isotopic patterns detected among the species and regions analyzed provide a whale-based view of the South Atlantic and Southern Ocean food webs. A broader representation of the trophic relationships within food webs from the regions mentioned can be found in the original papers listed in Table 4.1. The isotopic separation between food webs from the Patagonian shelf and Southern Ocean relates directly to the trophic ecology of southern and suggests the existence of two isotopically distinct food webs. Forero *et al.* (2005) has shown that southern giant petrels (*Macronectes giganteus*) from the Patagonian coast have higher $\delta^{13}C$ and $\delta^{15}N$ than the same species sampled on South Georgia by almost 5‰ in carbon and 6‰ in nitrogen (the original values in Table 4.1; Forero *et al.* 2005). The authors suggest that the difference between the two areas is primarily due to two factors. First, animals from the Patagonian shelf forage while in a more neritic (from the low tide to the continental shelf) on the environment with a longer food web and animals from South Georgia forage in a pelagic environment with a shorter food web (Forero *et al.* 2005). Many predators in the Southern Ocean ecosystem rely primarily on the herbivorous Antarctic krill, while the same or similar species of predators on the Patagonian shelf rely primarily on small fish and squids, which occupy a higher trophic level than krill (Forero *et al.* 2005). Therefore, it is expected that most predators from the Patagonian shelf would have higher stable isotope ratios than their counterparts from the Southern Ocean. However, copepods and euphausiids from the Patagonian shelf have isotope values higher than copepods and euphausiids from the

Patagonian shelf have isotope values higher than copepods and euphausiids from the Southern Ocean. Consequently, it appears that both areas have different isotope signatures across trophic levels, probably because their respective primary producers use different sources of carbon and nitrogen isotopes that relate to their locations (northern-neritic versus southern-pelagic waters; Peterson and Fry 1987; Fry 2006).

A source of uncertainty in our IsoSource modeling is the use of prey sources with isotope values from very diverse origins (Table 4.1). The large range of sites, years, seasons, tissues and methodological treatments that these sources (Table 4.1) cover is likely to introduce error to the analyses. Although the results should be taken as preliminary, the modeling exercise has been useful for pointing out potential feeding areas as well as some known and unknown problems. An examination of the IsoSource polygon (Figure 4.6) reveals that the mean values for the subgroups of whales lies near the periphery of the polygon connecting Uruguay, the Patagonian shelf, and the Polar Front sources. Such a configuration suggests that these three food sources are the main contributors to the isotope value of the whales (Phillips and Gregg 2003). Furthermore, the High and Mid groups are almost outside the polygon; in fact, half of the individual whale samples from these groups are outside its limits. A pattern like this suggests two main problems: first, the fractionation factor is not correct, and second, at least one more unknown source could be contributing to the whales' isotope values (Phillips and Gregg 2003). If we used a larger fractionation value for carbon, then all samples would fall within the limits of the polygon. However, there is no empirical evidence to suggest that 1‰ fractionation between diet and cetacean skin is incorrect. Furthermore, isotope values

measured from humpback and sperm whale skin and their prey suggest that 1‰ is an adequate fractionation factor (Todd *et al.* 1997; Ruiz-Cooley *et al.* 2004).

The possibility of an unknown feeding area currently being used by southern right whales is highly likely. The stable isotope values of prey that were used in our study include only four of the six historic feeding areas recognized by the International Whaling Commission (IWC 2001): the Antarctic Peninsula, South Georgia, the Polar Front (area 6 in Figure 4.1) and the coast of South America. However, the samples representing the latter are from a small number of locations off Uruguay and Patagonia, and may not accurately represent the isotopic range of the whales' historic feeding ground. We have no copepods or euphausiids samples from other areas where whales formerly concentrated during the feeding months, such as the waters of the subtropical convergence or the confluence of the Malvinas and Brazil currents.

Even with the sources of error previously mentioned, the results of the IsoSource modeling for the three different groups of whales provide interesting insights with respect to the food sources of the Patagonian right whales. Because it is physically impossible that a whale would have simultaneous contributions from prey species geographically separated by large distances (e.g., Uruguay to South Georgia), the information obtained from the IsoSource modeling has to be interpreted as a combination of dietary and migratory information.

Considering the whales migration, the Low group may represent a segment of the population that feeds primarily in higher latitudes (Polar Front and South Georgia) and while migrating towards Península Valdés consumes prey with a higher isotope signature. The whales from the Mid group may feed primarily in higher latitudes, on prey with an

isotopic signature similar to euphausiids from Uruguay, and then travel to feed on zooplankton on the Patagonian shelf as they migrate towards Península Valdés. The High group may represent whales that stay primarily on the Patagonian shelf, probably moving from north to south along the shelf break following seasonal blooms in productivity (Romero *et al.* 2006). These scenarios of habitat use and migration by the three groups are supported by the distribution of catch positions of right whales in the 1800s and 1900s (Townsend 1935; Tormosov *et al.* 1998; IWC 2001) and by recent sightings (IWC 2001). The continental shelf off the eastern coast of South America (from southern Brazil to the Malvinas/Falkland Islands) was a rich whaling ground, with a markedly seasonality in catch positions, The whales were caught in northern areas from October to January and in southern areas from February to May. South Georgia was also an important whaling ground and continues to be an important right whale feeding area where they are sighted year round.

The results from the IsoSource modeling suggest that southern right whale skin samples integrate isotope values over a large geographic range and possibly over a long period of time. The large geographic and temporal integration is likely to produce the isotope values that do not fully cluster within the Patagonian shelf or Southern Ocean ecosystems, but rather span both. Other highly mobile predators such as penguins, albatrosses, petrels and seals should show a similar pattern, but for the most part they cluster within one ecosystem or the other (Figure 4.5b), indicating that their movements are restricted within one ecosystem or the other.

Summary

Our findings show that different groups of whales use diverse food sources characterized by differential proportions of prey items from several regions. We detected isotopic differentiation between age classes, which could result from physiological (higher metabolism) or anatomical (not fully developed baleen) causes or from foraging in different areas on different prey. The detected interannual variability in mean carbon and nitrogen values result from feeding in different areas or from oceanographic changes affecting isotope sources. More data are needed to test these hypotheses. We detected a large separation of isotope ratios in zooplankton from the Patagonian shelf and the Southern Ocean that seems to explain a similar separation previously described among predators from both areas (Forero *et al.* 2005). We have shown that IsoSource modeling using isotope values from skin samples may be a useful technique to determining the proportion of prey sources in the diet of cetaceans. With better understanding of the skin turnover rate, IsoSource can be used to model the proportions of food consumed and time spent in a feeding ground and therefore infer migratory patterns for southern right whales or any other animal species with food sources with disjoint geographic distribution.

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CHAPTER 5

STABLE ISOTOPE DIFFERENCES BETWEEN MOTHERS AND THEIR CALVES IN SOUTHERN RIGHT WHALES

Abstract

Lactation is the most energetically expensive part of mammalian reproduction. As capital breeders, lactating southern right whales are completely dependent on their stored nutrients. The abundance of food prior to the nursing period and the whales' capacity to store nutrients have enormous effects on their reproductive output. Stable isotope ratios in an animal's tissues respond to diet quality, and nutritional stress. The proportion of exogenous (food) to endogenous (body tissues) nutrients as well as the relative use of different endogenous pools (blubber versus muscle) during the lactation period can be assessed using stable isotopes. We estimated the stable carbon and nitrogen isotope ratio difference between skin samples of southern right whale mothers and their calves. When samples from 3 consecutive years were pooled, the mean $\delta^{15}N$ value of calves was 0.51‰ higher than that of their mothers, but their $\delta^{13}C$ values were identical. However, the isotope differences between mothers and offspring varied by year. Mother-calf pairs showed no isotope differences in 2004, but calves showed significant enrichment in $\delta^{15}N$ (about 0.85‰) and $\delta^{13}C$ (about 0.63‰) in 2003 and 2005. We hypothesize that the interannual variability was a consequence of different levels of nutritional stress caused by environmental variability. A decline in food abundance prior to the 2003 and 2005

nursing seasons could result in relatively poorer physical condition of the mothers, who would then not be able to meet the high energetic demands of their rapidly growing offspring. In this case, the calves would be forced to utilize proteins as well as lipids to meet their own energy demands, and this increase their nitrogen and carbon isotope ratios. This hypothesis is supported by variation in rates of strandings over the same time period.

Introduction

Lactation is the most energetically expensive part of mammalian reproduction; it involves the export of significant quantities of maternal nutrients to supply offspring demands (Oftedal 1993, 2000). For most mammals, this period is accompanied by an increase in food consumption (Oftedal 1993). However, in capital breeders (those that fast during lactation, e.g., seals, bears and baleen whales) the large nutrient demand on mothers must be supplied from energy stores such as body fat, blubber and muscle tissue accumulated during previous feeding events (Oftedal 2000). Because capital breeders are unable to increase their energy intake during lactation, they have to deal with nutrient demands associated with milk production and nutrient conservation associated with a fasting metabolism (Sare *et al.* 2005; Dalerum *et al.* 2007). If a lactating female is unable to meet these demands, she must either reduce milk production (putting offspring survival at risk), or compromise her own health and future reproductive output (Oftedal 2000). Thus, the reproductive success of capital breeders is strongly influenced by food abundance prior to lactation, capacity to store nutrients and maternal ability to balance offspring demands and individual needs.

Southern right whales (*Eubalaena australis*) are capital breeders with an extreme and energetically demanding lifestyle. They migrate between low latitude coastal nursery grounds and higher latitude feeding grounds (IWC 2001). Calves are born in winter and females depend exclusively on internal reserves to nurse their young during their first 2 to 3 months of life (IWC 2001). During this period, when mothers are fasting, calves grow approximately 2.4 cm/day (Best and Rüther 1992). Their reproductive cycle reflects the demands of lactation. The normal reproductive cycle includes 1 year of gestation, 1 year of lactation, and 1 year of resting (Knowlton *et al.* 1994; Cooke *et al.* 2003). The resting year has been attributed to the need to recover from the long lactation and accumulate enough energy to begin the next cycle (Knowlton *et al.* 1994). Deviations from the mean 3-year calving interval have been attributed to calf loss (Knowlton *et al.* 1994; Cooke *et al.* 2003) and environmental variability affecting food (primarily krill) abundance during the resting year (Cooke 2003). Leaper et al (2006) found a reduction in calf output following years with high Sea Surface Temperatures (SST) on the South Georgia feeding ground. High-SST anomalies at South Georgia are correlated with periods of low krill abundance, which in turn are correlated with low reproductive output of local populations of Antarctic fur seals (*Arctocephalus gazella*) and gentoo penguins (*Pygoscelis papua*; Trathan *et al.* 2006).

Stable isotopes have been used to study transfer of maternal nutrients to offspring (Polischuk *et al.* 2001; Sare *et al.* 2005; Dalerum *et al.* 2007). For example, the onset and duration of the weaning period, when maternal nutrients are replaced by food, has been assessed using stable nitrogen and carbon isotopes in polar bear (*Ursus maritimus*) and meerkat (*Suricata suricatta*; Polischuk *et al.* 2001; Dalerum *et al.* 2007). Stable nitrogen

and carbon isotope ratios in animal tissues reflect those of their diet (Peterson and Fry 1987; Michener and Schell 1994). In general, the stable nitrogen isotope ratio ($^{15}N/^{14}N$) in the tissues of a consumer are enriched (positive fractionation) about 3 ‰ over the diet, while the stable carbon isotope ratio ($^{13}C/^{12}C$) change is relatively small (1‰; Deniro and Epstein 1978, 1981; Peterson and Fry 1987; Kelly 2000). In mammals, nitrogen fractionation occurs because there is preferential excretion of the lighter isotope, ^{14}N during the production of urea, thus leaving the animal's tissues enriched in the heavier isotope, ^{15}N (Kelly 2000). Nitrogen fractionation is affected mainly by the quality of dietary protein, nutritional stress, and the nitrogen balance of the consumer (Deniro and Epstein 1981; Roth and Hobson 2000; McCutchan *et al.* 2003; Fuller *et al.* 2004). The extent of nitrogen and carbon isotope fractionation is also affected by tissue type, with keratin-rich tissues (i.e., hair, feather, and baleen) generally showing higher values than other tissues (Kelly 2000). Lipid-rich tissues show lower carbon isotope ratios due to preferential use of the lighter ^{12}C during lipid synthesis (Tieszen and Buotton 1988). The fact that nitrogen and to some extent carbon isotopes can be affected by diet, has motivated studies to determine the proportions of exogenous (i.e., food) and endogenous (i.e., maternal reserves) nutrients that are allocated to the offspring (Polischuk *et al.* 2001; Sare *et al.* 2005). In capital breeders, where all transferred nutrients are endogenous, stable isotope analyses have been used to identify the differential use of various nutrient pools (e.g., muscle *versus* fat) used throughout the lactation period (Polischuk *et al.* 2001). For example, Polischuck *et al.* (2001) found that nitrogen fractionation between polar bear mothers and cubs was larger in mothers with lower body fat content. The difference among mother-cub families was attributed to relatively higher use of maternal

lean tissue (muscle proteins) as compared to fat as an energy source (Polischuk *et al.* 2001).

Isotope fractionation between mothers and offspring is not well understood. One attractive idea is that offspring are a trophic level higher than their mothers because the offspring "consume" their mothers (Hobson *et al.* 1997; Hobson *et al.* 2000; Newsome et al 2006; Knoff *et al.* 2008). Nitrogen enrichment from mother to offspring has been detected in all studies in which it has been directly measured (Table 5.1; Fogel *et al.* 1989; Hobson *et al.* 1997; Hobson *et al.* 2000; Jenkins *et al.* 2001; Polischuk *et al.* 2001; Sare *et al.* 2005; Dalerum *et al.* 2007). However, few studies found the expected trophic level fractionation of 3‰ for nitrogen and 1‰ for carbon (Table 5.1; Fogel *et al.* 1989; Hobson *et al.* 1997; Hobson *et al.* 2000). Furthermore, reported fractionation values show large variability both within and between studies and species.

The "trophic level" model oversimplifies the many processes acting during nutrient transfer from mother to offspring, and assumes implicitly that milk is isotopically similar to the mother's tissues. Jenkins *et al.* (2001) studied 11 different mammalian species and detected isotopic depletion in carbon and nitrogen from mother to milk, and enrichment from milk to offspring, producing a "balancing effect" with the net effect being a small difference in nitrogen (0.9‰) and no difference in carbon isotopes between mothers and offspring. Milk with high fat content is expected to have lower $\delta^{13}C$ because fats are depleted in ^{13}C relative to protein and carbohydrates (Oftedal 1993). On the other hand, little is known about the processes by which milk is ^{15}N-depleted.

Table 5.1: Average values of nitrogen and carbon isotopic differences directly measured from mothers and their offspring.

Species	Tissue	$\Delta\delta^{15}N$ (‰)	$\Delta\delta^{13}C$ (‰)	Reference
Humans	Fingernails	2.4	0.0	Fogel *et al.* 1989
Northern fur seal	Muscle	1.9	-0.7	Hobson *et al.* 1997
Black bears	Hair	2.5	0.7	Hobson *et al.* 2000
Eleven species [a]	Plasma	0.9	0.0	Jenkins *et al.* 2001
Polar bears	Plasma	1	-0.8	Polischuk *et al.* 2001
Red-backed voles	Hair	1.8	-1.5	Sare *et al.* 2005
Meerkat	Hair	1.0	NR	Dalerum *et al.* 2007

[a] Moose, caribou, black-tailed deer, coyotes, grizzly bears, domestic rabbits, rats, cows, sheep, pigs, and domestic cats. NR: not reported.

In this paper, we report isotopic differences between lactating right whale mothers and their calves by examining stable nitrogen and carbon isotope ratios measured in skin samples collected during the first months of the nursing period. The isotope differences described here integrate several physiological processes affecting the isotope ratios of carbon and nitrogen in the mothers as well as in their calves. We expected a small isotope difference in nitrogen and no difference in carbon ratios, reflecting the balancing effect described by Jenkins *et al.* (2001). By examining stable isotope measurements over the course of 3 consecutive years we found interannual variability in isotopic differences between mothers and calves. We hypothesize that the interannual variability is a consequence of different average levels of nutritional stress caused by environmental variability. This hypothesis is supported by variation in rates of strandings during the same sequence of years.

Materials and Methods

Skin samples from each member of 42 mother-calf pairs were collected by biopsy darting whales on their nursery ground off Península Valdés, Argentina (42° 30' S, 64° 10' W). Samples were collected over 3 consecutive years (2003 – 2005) at the time of peak whale abundance (September and October, Payne 1986). The start date and duration of sampling differed each year: in 2003 sampling started on September 14 and lasted 21 days, in 2004 sampling started on September 21 and lasted 25 days, and in 2005 sampling started on September 8 and lasted 33 days. All sampled calves were estimated to be older than one week of age based on their size relative to that of their mother (Thomas and Taber 1984), skin characteristics (Reeb *et al.* 2005), and the behavioral patterns of mother-calf pairs (Thomas and Taber 1984). To avoid including resampled individuals,

adult females were photographed for later identification based on callosity patterns (Payne *et al.* 1983, Best 1990).

Skin samples were dried, ground to a fine powder and lipid extracted using Soxhlet extraction following Todd *et al.* (1997). Approximately 1mg of material per sample was analyzed for carbon and nitrogen isotopes using a Carlo Erba 1108 elemental analyzer coupled to a Thermo Finnigan Delta S Isotope Ratio Mass Spectrometer at the Stable Isotope Ratio Facility for Environmental Research (SIRFER) at the University of Utah. The isotope ratios are expressed as $\delta^{13}C$ or $\delta^{15}N$ (‰) = $[(R_{sample}/R_{standard}) - 1] \times 10^3$, where R is the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ for $\delta^{13}C$ or $\delta^{15}N$, respectively. Standards were referenced to Pee Dee Belemnite for carbon and atmospheric air for nitrogen. The reproducibility of these measurements is 0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$ after repeated analyses of an internal laboratory standard (yeast).

The $\delta^{13}C$ and $\delta^{15}N$ values of mothers and calves were not normally distributed (Shapiro-Wilk W test, $p < 0.01$, $n = 42$ for all four distributions). Non parametric Kruskal-Wallis analysis of variance and Spearman correlation were used to test for the influence of sampling year and sampling date on isotope values (Sokal and Rohlf 1981). We used the isotopic difference between calf and mother as an indicator of isotope fractionation, $\Delta\delta^{15}N = \delta^{15}N_{calf} - \delta^{15}N_{mother}$, and $\Delta\delta^{13}C = \delta^{13}C_{calf} - \delta^{13}C_{mother}$ for nitrogen and carbon differences respectively. The $\Delta\delta^{15}N$ and $\Delta\delta^{13}C$ values were normally distributed (Shapiro-Wilk W test, $p > 0.1$, $n = 42$ for both distributions). Parametric pair wise t-tests were used to test for isotopic differences between mothers and calves and Pearson correlation was used to test for the influence of sampling date and for correlations between nitrogen and carbon isotope differences (Sokal and Rohlf 1981). Statistical tests

were conducted in *R* (R Development Core Team 2005) and JMP (SAS Institute).

Results

Stable isotope ratios

Average stable nitrogen and carbon isotope ratios of calves varied among years (Kruskal-Wallis n = 42: $H_{nitrogen} = 14$, $p < 0.001$; $H_{carbon} = 9$, $p = 0.012$; Figure 5.1). Calves sampled in 2004 had significantly lower values of $\delta^{15}N$ (mean = 7.98 ± 2.10‰) and $\delta^{13}C$ (mean = -21.61 ± 1.65‰) than calves sampled in 2003 and 2005 (mean $\delta^{15}N_{2003} = 8.67 \pm 1.28$, mean $\delta^{13}C_{2003} = -20.50 \pm 1.36$‰, and mean $\delta^{15}N_{2005} = 8.75 \pm 1.92$‰, mean $\delta^{13}C_{2005} = -20.32 \pm 1.66$‰). Mothers sampled in different years showed no significant differences in nitrogen (mean $\delta^{15}N = 7.84 \pm 2.04$‰) or carbon (mean $\delta^{13}C = -21.20 \pm 1.33$‰) isotope values (Kruskal-Wallis n = 42, $H = 1$, $p > 0.1$ for both nitrogen and carbon; Figure 5.1). Isotope ratios were not correlated with sampling dates when all samples were analyzed together or separately by year (Spearman's $\rho < 0.27$ and $p > 0.5$ for all correlations).

Stable isotope differences

The nitrogen ($\Delta\delta^{15}N$) and carbon ($\Delta\delta^{13}C$) differences between mothers and their calves were weakly correlated ($r^2 = 0.24$, $p = 0.001$; Figure 5.2). However, when the isotope differences were analyzed by year, no correlations were found ($r^2 < 0.18$ and $p > 0.05$ for all years). Total mean $\Delta\delta^{15}N$ (0.51 ± 0.62‰) was significantly different from zero ($t = 5.4$, $p < 0.001$), while total mean $\Delta\delta^{13}C$ (0.21 ± 0.73‰) was not significantly different ($t = 1.9$, $p = 0.07$; Figure 5.3). For 2004, neither mean $\Delta\delta^{15}N$ (0.15 ± 0.45‰) nor mean $\Delta\delta^{13}C$ (-0.24 ± 0.64‰) were significantly different from zero ($p > 0.1$; Figure

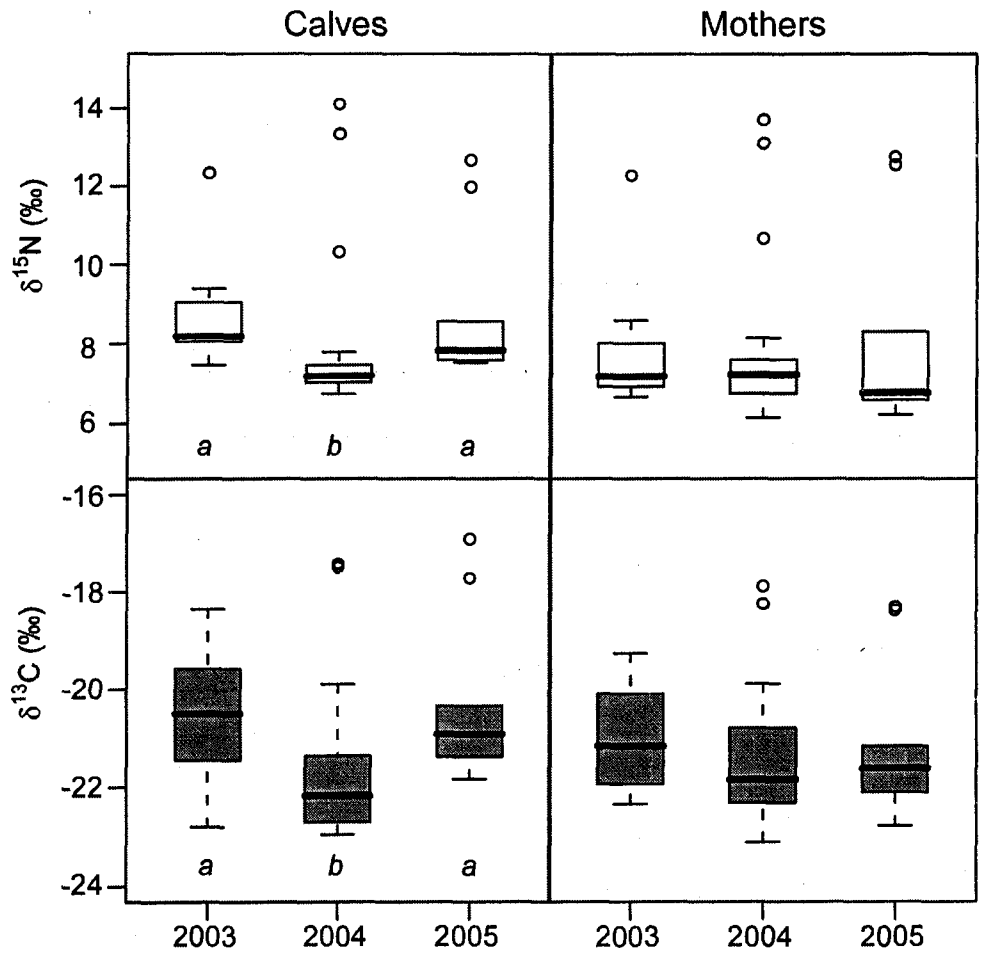

Figure 5.1: Boxplots of $\delta^{15}N$ (open boxes) and $\delta^{13}C$ (grey boxes) for calves (left plots) and mothers (right plots) by year of collection. Calves sampled in 2004 have isotope values statistically smaller than calves sampled in 2003 and 2005; non-significant differences between years are indicated by the same letter under the boxes. For the mothers, no significant differences in isotope values were detected among years.

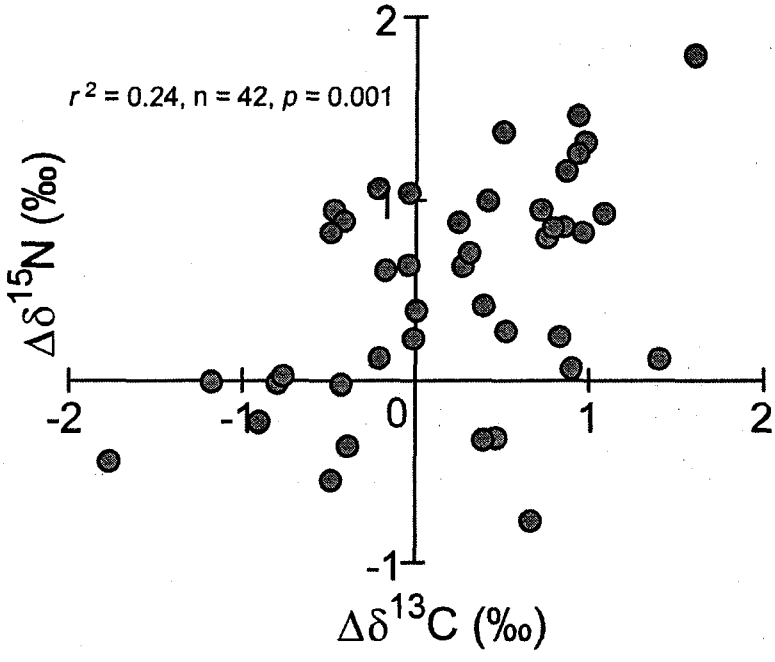

Figure 5.2: Scatter plot of $\Delta\delta^{15}N$ versus $\Delta\delta^{13}C$. Each point represents the isotope difference of a calf from its mother. Note that there is a weak but significant correlation; however, this correlation disappears when samples are analyzed by year.

5.3). However, for 2003 and 2005, the mean $\Delta\delta^{15}N$ and mean $\Delta\delta^{13}C$ were significantly different from zero ($\Delta\delta^{15}N_{2003} = 0.93 \pm 0.43‰$, $t = 7.4$, $p < 0.001$; $\Delta\delta^{13}C_{2003} = 0.46 \pm 0.66‰$, $t = 2.4$, $p = 0.03$; $\Delta\delta^{15}N_{2005} = 0.74 \pm 0.69‰$, $t = 3.4$, $p = 0.008$; $\Delta\delta^{13}C_{2005} = 0.81 \pm 0.31‰$, $t = 8.4$, $p < 0.001$; Figure 5.3). Total nitrogen and carbon isotope differences appear to be negatively correlated with sampling date ($\Delta\delta^{15}N$, $r^2 = 0.24$, $p = 0.001$; $\Delta\delta^{13}C$, $r^2 = 0.10$, $p = 0.04$). However, when the three years were considered separately, sampling date did not influence nitrogen ($r^2 < 0.15$ and $p > 0.1$ for all years) or carbon isotope differences ($r^2_{2003} < 0.1$ and $p > 0.1$ for all years).

Discussion

As predicted, we found a small isotope difference between the skin samples from mothers and calves in nitrogen (mean = 0.51‰) and no difference in carbon isotopes. Jenkins *et al.* (2001) also found small nitrogen differences and no carbon differences between mothers and their offspring across eleven mammalian species. However, the mean nitrogen difference in our study (0.51‰) is smaller than the interspecific mean (0.9‰) estimated by Jenkins *et al.* (2001). Two other studies (Polischuk *et al.* 2001; Dalerum *et al.* 2007) have reported small (1‰) nitrogen fractionation from mother to offspring, but the isotope fractionation of carbon was negative (-0.8‰ for Polar bears; Polischuk *et al.* 2001) or not reported (for meerkat; Dalerum *et al.* 2007). When the stable carbon and nitrogen differences between right whale mothers and calves were analyzed by year, two different scenarios emerged. For the 2004 samples, stable isotope ratios from mothers and calves were statistically indistinguishable from each other. In the other

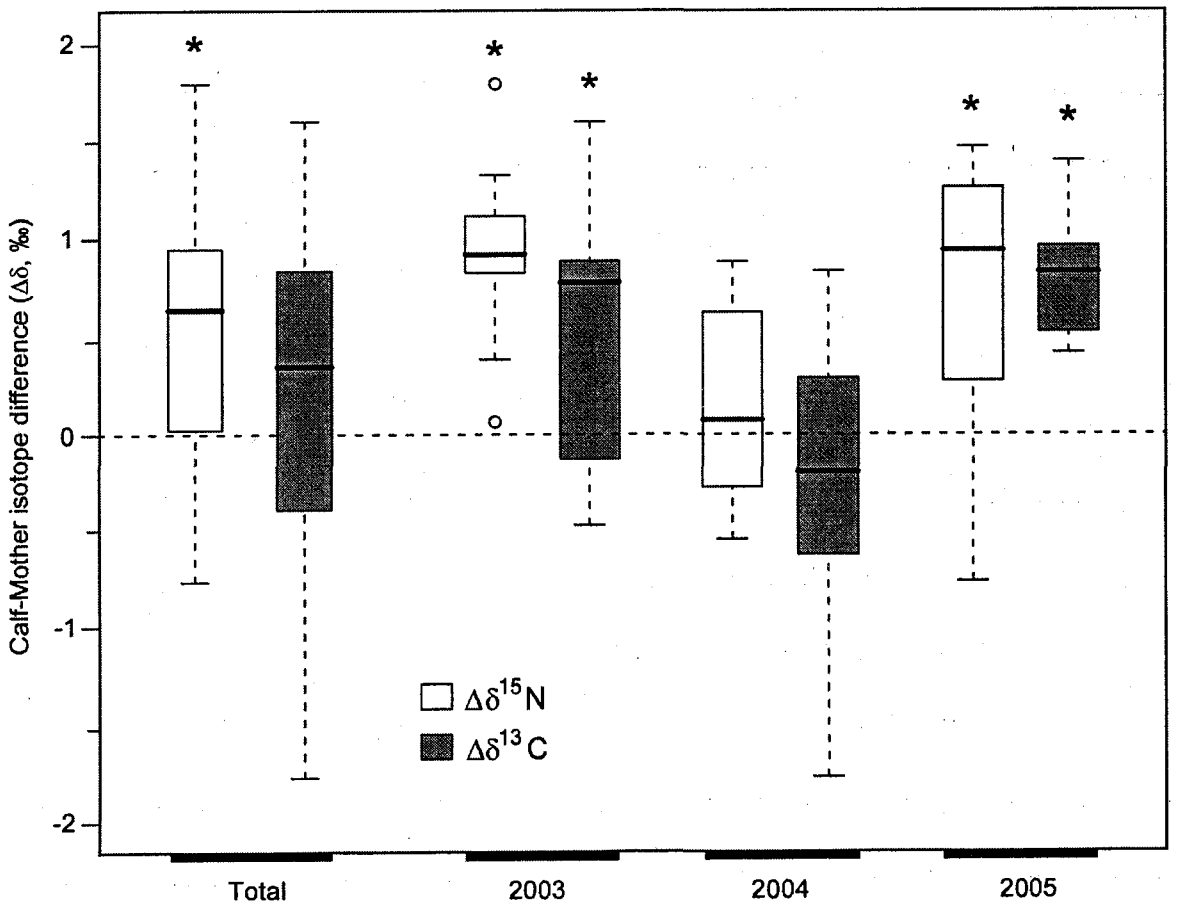

Figure 5.3: Boxplots of calf-mother isotope differences (Δδ) by year of sampling and total. Nitrogen differences are presented in open boxes and carbon differences in grey boxes. Isotope differences significantly different from zero (paired *t*-test) are indicated by *.

two years, 2003 and 2005, calves showed significant enrichment over their mothers in nitrogen (about 0.85‰) and carbon (about 0.63‰).

We are confident that the stable isotope ratios measured from the calves' skin reflects the lactation period rather than tissue formation *in utero*. The mean date of birth for southern right whales is estimated to be 24 August (Best 1994; Whitehead and Payne 1981). According to Reeb *et al.* (2005), southern right whale calves shed their fetal skin an average of 1 week after birth. Our earliest sample was taken on 8 September (in 2005), which is about 2 weeks after the estimated mean date of birth. Our visual observations of the skin appearance (rough in newborns vs. smooth in older calves; Reeb *et al.* 2005) and the behavior patterns of mother-calf pairs (fast swimming for mothers with newborns vs. resting for mothers with older calves; Thomas and Taber 1983) also indicated that we sampled calves that were at least 2 weeks old.

No isotopic differences in 2004

As capital breeders, southern right whales derive all the components of milk from internal reserves, with blubber as the main source of fat and lean body tissue as the main source of protein (Oftedal, 1993, 2000). The high fat content of their milk (30-40%) serves as the primary source of energy (Oftedal, 1993, 2000). High protein content is also needed to fulfill the requirements of their extremely rapid growth (Oftedal, 1993, 2000). The growth rate of right whale calves is estimated to be 2.4 cm/day during the first 3 months of life (Best and Rüther 1992). The large quantity of lipids consumed by calves probably accounts for the lack of fractionation in $\delta^{13}C$ observed in the samples collected in 2004, because lipids are ^{13}C-depleted. Data from 2004 are the first report of no $\delta^{15}N$

enrichment between a mother and her offspring. Jenkins *et al.* (2001) detected $\delta^{15}N$ depletion from mother to milk, but from mother to offspring they found that a positive fractionation. It is possible that southern right whale milk is so depleted in ^{15}N that a 'complete' balancing effect brings the calves back up to the same isotope values as their mothers. The process by which nitrogen in milk is depleted in comparison to the mother's tissues is unknown.

Another possible cause for the lack of nitrogen fractionation in 2004 is that the positive nitrogen balance of fast growth in the calves reduces the nitrogen isotope fractionation. During anabolic states such as pregnancy and neonatal growth, animals enter positive nitrogen balance, decreasing nitrogen excretion and potentially increasing urea recycling, and as a consequence, $\delta^{15}N$ fractionation is reduced (Fuller *et al.* 2004). For southern right whales, the first months of lactation are critical and any imbalance due to low energy reserves or high energetic demands could affect isotope ratios in both the mother and the calf.

Isotopic differences in 2003 and 2005

Alternative hypotheses could explain the large isotope differences between mothers and their calves observed in 2003 and 2005. The first hypothesis considers nutritional stress (primarily caused by low food abundance) and the second addresses the possibility of temporal decoupling of mother-calf isotope ratios measured from skin samples.

In 2003 and 2005 nutritional stress could have caused mother-calf pairs to use more energy reserves than in 2004. Calves could suffer nutritional stress if their mothers

do not provide enough milk or if the quality of the milk is somehow reduced; the calves would then be forced to utilize proteins as well as lipids to meet the energy demands of growing. Catabolism of body proteins during periods of nutritional stress increases $\delta^{15}N$ values (Hobson and Clark 1992; Hobson *et al.* 1993). The $\delta^{13}C$ values of the calves' skin may reflect the catabolism of both the lipid and protein pools. Nutritional stress may also cause mothers to use proteins to meet their own energy demands. This catabolism of proteins would lead to the production of isotopically enriched milk (high values of $\delta^{15}N$). As a result, calves would have higher isotope ratios than their mothers.

The lack of correlation between isotope differences and sampling date may seem inconsistent with our previous argument of nutritional stress. As the nursing season progresses the isotope fractionation of fasting animals would be expected to increase as a result of using other body tissues rather than blubber as energy reserves. The lack of seasonal effect is particularly clear in 2005, the year with the broadest sampling time (33 days). It is possible, however, that a month may not be enough time to detect such changes in the isotopic composition of the skin (Ruiz-Cooley *et al.* 2004).

An independent observation that supports our hypothesis of nutritional stress is an increase in the proportion of stranded whales detected in the same population for the years 2003 and 2005. The proportion of stranded to live calves (PSC) is estimated as the number of stranded calves recorded during the nursing season (Uhart *et al.* 2008), divided by the total number of live calves counted during annual aerial surveys (VJ Rowntree *pers. obs.*). PSC appears to be correlated with the mean isotope differences between live mothers and their calves; although not significantly different, PSC was lower in 2004 (0.15) than in 2003 (0.19) and 2005 (0.20). Furthermore, in 2003 and 2005, in addition to

calves, adults and juveniles were also reported among the stranded whales (3 in 2003 and 11 in 2005), indicating that nutritional stress may also affect other segments of the population. Years of elevated mortality (2003 and 2005) correspond with years of larger isotope differences; both increments can be explained by an increase in nutritional stress caused by reduced food intake during the previous feeding season.

Variability in reproductive success in southern right whales has been tied to changes in sea surface temperature (SST) that in turn is correlated with changes in krill abundance off South Georgia (Leaper *et al.* 2006). Fewer calves than expected are born following years of high SST and low krill abundance (Leaper *et al.* 2006). Analysis of calving intervals of known whales suggests that in these years females appear to extend their resting period but do not appear to suffer increased calf mortality or stress during nursing (Cooke *et al.* 2003). The Antarctic fur seal (*Arctocephalus gazella*) shows increased pup mortality in years of low krill abundance around South Georgia (Trathan *et al.* 2006) as well as variation in milk composition (Arnould and Boyd 1995). Despite being in poorer condition, female fur seals produce milk with higher lipid content in years with reduced prey abundance (Arnould and Boyd 1995). This paradoxical situation results from the mothers extending the length of their foraging trips. As capital breeders, southern right whales cannot compensate in the same way because there is no food in the nursery area. A nursing female's only option to meet her calf's demands is to adjust nutrient mobilization and milk production from her own body reserves (Oftedal 1993, 2000).

An alternative hypothesis to explain the variation among years in isotope differences between mothers and their offspring addresses the timing of tissue formation

and the possible decoupling of isotope ratios. Different tissues have different isotope turnover rates; some turn over within hours or days (e.g., blood plasma and liver) while others take several weeks (e.g., muscles) or years (e.g., bone collagen; Rubenstein and Hobson 2004). It is possible that whales in 2003 and 2005 may have fed on an isotopically depleted diet that was integrated during formation of their skin, but fed on an enriched diet when storing nutrients in their blubber and muscle reserves for later milk production. Alternatively, in 2004 the whales could have had isotopically similar diets during both time periods. Hobson *et al.* (2000) remark on a similar mother-offspring decoupling problem in black bear hair. The turnover rate of cetacean skin is unknown, although it has been suggested to be less than a month (Todd *et al.* 1997) to several months (Ruiz-Cooley *et al.* 2004). Because the turnover rate and thus the time for tissue formation are unknown, we cannot determine the influence of this type of decoupling on the interannual isotope differences we have observed between mother-calf pairs.

In summary, stable isotope differences between southern right whale mothers and their calves do not follow the usual pattern of trophic enrichment (3‰ enrichment in nitrogen and 1‰ in carbon). The substantial interannual variability presented here is probably associated with variability in the nutritional condition of mother-calf pairs. We suggest that nutritional stress, caused by food shortages, causes some mother-calf pairs to use both protein and lipid stores to meet the energetic demands of calf growth and maintenance. We do not have enough information to reject the decoupling hypothesis, but the association between a higher proportion of stranded animals and larger isotopic differences between mothers and their calves supports the nutritional stress hypothesis. Regardless of whether one or both of these hypotheses are true, the data presented here

probably reflect changes in the foraging ecology of southern right whales as a consequence of fluctuations in food abundance or distribution. When we better understand the processes affecting stable isotopes during milk production and consumption, and during offspring growth, it should become possible to study nutritional stress at the individual and population levels, in some detail, by measuring isotopic differences between mothers and their offspring.

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