Genetic population structure of Patagonian right whales and assessment of foraging strategies by stable isotope analysis.

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ABSTRACT
Here we review the population genetic structure of southern right whales that calve at Península Valdés, Argentina. Furthermore, we focus most of this paper on presenting an assessment of the foraging strategies and potential feeding grounds used by right whales in the South Atlantic Ocean through stable isotope data from skin and baleen plates. We found a significant genetic heterogeneity among years for stranded individuals but not for living individuals at Península Valdés. We confirmed the pattern of genetic differentiation previously described between the subpopulations breeding off South America, South Africa, Australia and New Zealand. By comparing isotope ratios from skin samples with published and unpublished values of potential prey we have discovered that southern right whales appear to have at least three different foraging strategies probably associated with different migratory patterns. These different foraging strategies are supported by data derived from baleen plates.

INTRODUCTION
Ocean warming is changing the ocean ecosystems and affecting all trophic levels from phyplankton to large marine predators. A recent study predicts that a regional warming of 1ºC 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. In cases like this, ecological flexibility at the individual level might play an important role in the survival of the species. The whales' ability to use different feeding areas or consume different prey types will be a key factor in their survival. A recent study shows evidence of southern right whale site fidelity to unknown feeding grounds (Valenzuela *et al.,* 2009); 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. Understanding the whales' responses to global change events is dependent on understanding the whales' current population genetic structure, habitat use and foraging ecology.

Southern right whale population genetic structure. Southern right whales (*Eubalaena australis*) are found in all the oceans of the Southern Hemisphere and during the calving and nursing 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). 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 probably maintained by maternally directed site fidelity (Portway, 1998; Baker *et al.,* 1999; Patenaude *et al.,* 2007; Valenzuela *et al.,* 2010b).

Southern right whale feeding grounds. Southern right whales migrate seasonally between nursing grounds and feeding grounds. During the feeding season 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 nineteenth and twentieth century's whalers (Figure 1; IWC 2001). However, the extent to which these historic, or any 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 50ºS and between 30ºE and 10ºW; Mate and Best 2008). Other than these regional sights little is known of the whales current feeding distribution in the South Atlantic Ocean.

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 ^{13}C (Peterson and Fry 1987; Fry 2006). A second characteristic of isotopes that makes them useful to track animal movements is that they are incorporated directly from diet into animal tissues with varying degrees of fractionation.

Objectives. We first review the genetic data presented by Valenzuela *et al.* (2010b) to the International Whaling Commission Scientific Committee in Agadir, Morocco. We describe an assessment of the genetic substructuring of southern right whales on the Península Valdés nursery ground, using sequence data from a 630 bp region of the mitochondrial genome; we compare gulfs, age-sex classes, reproductive states and calving cohorts, and living versus dead whales. We also reanalyze the large-scale structure of southern right whales by combining our samples with those previously reported for other populations (Portway, 1998; Baker *et al.,* 1999; Malik *et al.,* 2000; Patenaude *et al.,* 2007).

Second, 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 of Península Valdés uses the southern hemisphere oceans and its resources, 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 (i.e. different age and sex classes). To assess the trophic position and location of feeding areas used by right whales we matched the stable isotope values from skin samples with published and unpublished isotope values of euphausiids and copepods. We used linear mixing models to estimate the proportional contribution of different feeding sources to the whale's diet. We also compared new and published (Rowntree *et al.,* 2008) isotopic data from baleen plates with the information obtained from skin samples to assess the whales' foraging strategies and migration.

MATERIALS AND METHODS

Sample collection

Live whales. Skin samples were obtained by biopsy darting live whales off Península Valdés (42º 30' S, 64º 00' W), Argentina. Sample collection was carried out over four consecutive years (2003 – 2006) at the time of peak whale abundance (September and October; Payne, 1986). To avoid resampling of whales, individuals were photographed for identification based on callosity patterns and other natural marks (Payne *et al.,* 1983). Each skin sample was divided into two subsamples in the field. One subsample was dried in preparation for stable isotope analysis and the other was preserved in saturated NaCl with 20% DMSO for genetic analysis and 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 Z*fx* and Z*fy* introns following Shaw *et al. (*2003).

*Dead whales. S*kin samples from dead animals were provided by the Programa de Monitoreo Sanitario Ballena Franca Austral (PMSBFA), which is active from June through December (Uhart *et al.,* 2008, 2009). Skin samples from the periods 2003-2006 and 2007-2009 were used. The first set (2003-2006) was sampled at the same time that biopsy samples were collected. At each stranding, body measurements and skin tissue were collected; all samples were preserved in 70% EtOH and the dead animals were tagged to avoid resampling.

Genetic analysis
The methods have been described previously (Valenzuela *et al.,* 2009; Valenzuela *et al.,* 2010b). DNA was extracted using a standard procedure for cetacean skin as described in Amos and Hoelzel (1991). A 630 base pairs region of the mitochondrial genome was amplified by PCR using primers AB6617 and H00034 (Malik *et al.,* 1999), and later sent for sequencing. The resulted sequences were deposited at GenBank with accession numbers EU290462-EU290592 and GQ389687-GQ389690. 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.

A phylogeny of the mtDNA haplotypes was reconstructed using neighbour-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). Haplotype (*h*) and nucleotide (π) diversity (Nei, 1987) were estimated using Arlequin 2.0 (Schneider *et al.,* 2000). The degree of differentiation among groups 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).

The population structure of southern right whales across the Southern Ocean was reanalyzed using the following populations: Argentina (AR, n = 282), South Africa (SA, n = 41), South Georgia feeding ground (SGF, n = 8), New Zealand (NZ, n = 42), south-western Australia (SWA, n = 20) and south-western Australia feeding ground (SWAF, n = 5). Data obtained from Patenaude *et al. (*2007).

Stable Isotope analysis
Skin samples were dried, ground to a fine powder and lipid extracted using Soxhlet extraction following Todd *et al. (*1997). Stable isotope analyses were conducted 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 $\delta^{13}C$ and $\delta^{15}N$ values. Baleen plates were analyzed following Rowntree *et al.* (2008).

Food web stable isotope ratios. Stable isotope values of copepods and euphausiids were primarily obtained from published literature (Appendix Table A). We focused our effort on studies reporting samples collected in the western South Atlantic Ocean and in the Atlantic sector of the Southern Ocean (from 20ºE to 70ºW), 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 Patagonia, Argentina were analysed for carbon and nitrogen isotope ratios (Appendix Table A). 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. Non-parametric statistics were used due to the lack of normality in both isotope distributions. For all tests, statistical significance was set at 5%.

IsoSource modelling. Standard linear mixing models were used to estimate the proportion of different food sources contributing to the whales' diet (Phillips 2001). The model was implemented using the software *IsoSource* (Phillips and Gregg, 2003) and requires the mean isotope values of the mixture of interest (a predator's tissue) and the sources (its potential prey). For a description of the actual *IsoSource* model see the Appendix. We used the isotope values measured in skin samples (mixture) and selected isotopic values from potential prey samples (sources) from

Appendix Table A to conduct an *IsoSource* modelling. To compensate for trophic level enrichment, we corrected the isotope ratios of skin samples by subtracting 3‰ for nitrogen and 1‰ for carbon.

RESULTS

Genetic analysis

Sequence analysis and mtDNA phylogeny. Sequence analysis of a 630 base pair region of the mitochondrial genome from 374 whales revealed 37 unique haplotypes (Table 1). The overall haplotype (h) and nucleotide (π) diversity were 0.95 ± 0.01 and 1.63 ± 0.82% respectively. Phylogenetic reconstruction of the 37 haplotypes revealed two main clades that correspond to the previously known "A" and "W" clades (Figure 2; Baker *et al.,* 1999; Patenaude *et al.,* 2007). Clade A contains 16 haplotypes and 54% (n = 201) of the total sample, while clade W has 21 haplotypes and 51% (n= 189) of the total sample (Table 1, Figure 2). The haplotype diversity was similar between the two clades (clade A h = 0.91 ± 0.01, clade W h = 0.88 ± 0.01) while nucleotide diversity was ~3 times higher in clade W (π = 1.45 ± 0.74%) than in clade A (π = 0.48 ± 0.28%).

Within-population differentiation. The stranded whales show significant genetic differentiation among years (*Fst* = 0.008, P = 0.03), and this contrasts with the live whales, which show no differentiation among years (*Fst* = -0.001, $P > 0.1$). 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, $P > 0.1$ for both), or between calving females and single females (*Fst* = 0.001, Φst = 0.007, $P > 0.1$). Significant differentiation was found between live (n = 219) and stranded whales sampled in the same period (2003-2006, n = 43, Table 1). Stranded whales were more abundant in clade A (Table 1). The *Fst* statistic, based on haplotype frequencies, shows that 1% of the variance was accounted for by these two groups ($P < 0.05$). 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 ($P < 0.005$). However, when all samples from stranded whales were compared with live whales no differentiation was found (*Fst* = 0.001, $P > 0.1$).

Southern hemisphere population structure. Sequence alignment of the 37 haplotypes discovered in this study with the 37 haplotypes previously published by Patenaude *et al.* (2007) revealed 45 unique haplotypes (of length 275 bp) for the whole Southern Hemisphere. The overall haplotype diversity (h) for southern right whales is 0.955 (±0.003) and the overall nucleotide (π) diversity is 2.80% (±1.45%). Haplotype and nucleotide diversities in the Península Valdés whales are high and similar to values for the whole Southern Hemisphere (h = 0.94 ± 0.005, π = 2.83% ± 1.47).

Significant differentiation at the haplotype and nucleotide level was found among the six subpopulations analyzed (overall *Fst* = 0.166, $P < 0.001$; overall Φst = 0.158, $P < 0.001$). Argentina shared haplotypes with all populations (Table 2); 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 except the South Georgia feeding ground (Table 2). In comparisons at the nucleotide level (Φst), the differentiation was statistically significant when compared to other nursery areas but not to either feeding ground, although the sample sizes for these two areas are too small for reliable statistical comparisons (Table 2).

Stable Isotopes analysis

Stable isotope ratios from skin samples. Southern right whale skin samples had an overall mean $\delta^{13}C$ value of -20.8‰ ± 1.4‰ (range: -23.9 to -17.2‰; n = 196) and a mean $\delta^{15}N$ value of 8.4‰ ± 2.1 (range: 6.0 to 15.0‰; n = 196). Carbon and nitrogen distributions are not normally distributed and are positively skewed (Shapiro-Wilk normality tests $P < 0.001$; Figure 3). Carbon and nitrogen distributions appear to

be multimodal (Figure 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 7.5‰ (range 6.0 to 9.9‰, n = 159), a '*Mid*' group with a mean $\delta^{15}N$ of 10.5‰ (range 10.3 to 10.7‰) and a '*High*' group with a mean $\delta^{15}N$ of 12.8‰ (range 11.5 to 15.0‰). When samples are separated by age-sex classes, the gaps become wider and the multimodal pattern more apparent (Figure 3). 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).

Whales from different age-sex classes show significant differences in $\delta^{13}C$ (Kruskal-Wallis $X^2 = 9.3$, $P < 0.05$) and $\delta^{15}N$ values (K-W $X^2 = 20.7$, $P < 0.001$). Adult females median $\delta^{15}N$ value (7.40‰; n = 143) is statistically lower than juvenile females (8.2‰; n = 24) and juvenile males (8.6‰; n = 20; $P < 0.05$ for both comparisons; Figure 3). Juvenile males median $\delta^{13}C$ value (-20.3‰) is statistically higher ($P < 0.05$) than both female classes (adults = -21.1‰, juveniles = -21.4‰; Figure 3). Adult males show a large isotopic range (Figure 3), 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 $\delta^{15}N$ values (K-W $X^2 = 12.4$, $P = 0.006$). Post hoc Dunn's multiple comparison tests indicate that the median carbon and nitrogen isotope values for the year 2006 ($\delta^{13}C$ = -20.1‰, $\delta^{15}N$ = 8.2‰, n = 50; figure 4) are statistically higher than for any other year ($\delta^{13}C$ = -21.3‰, $\delta^{15}N$ = 7.4‰ for the other three years combined; Figure 4). No correlation was found between isotope values and sampling date for any isotope when all the samples were pooled or analyzed by year ($P < 0.05$).

Trophic structure and feeding areas. 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 coast of Uruguay, the Patagonian shelf, the Polar Front and South Georgia (Figure 5). 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.

IsoSource Modeling. Eight sources were used in *IsoSource* (Figure 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 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 2001), 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 6). The modeling was conducted for the mean isotope value of each of the three subgroups of whales previously presented (*High*, *Mid* and *Low*). 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 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 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 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 *High* and *Mid* groups, with minimum contributions of 64% and 24% for each group respectively. Euphausiids 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%.

Comparison of skin samples with baleen plates. As reported in previous papers the stable carbon and nitrogen isotope ratios measured along the length of baleen plates from southern right whales show what appear to be yearly migratory cycles (Best and Schell, 1996; Rowntree *et al.,* 2008). Here we examined data from baleen plates obtained from stranded adults (six) and calves (fourteen). We do not analyze temporal trends as previous authors have done but rather we used bivariate plots to compare them with the information obtained from skin samples. Figure 8 is presented only to illustrate the typical cycles found in baleen plates.

Figure 9a shows three baleen plates with what appear to be migratory cycles restricted to specific isotopic zones that match the *High*, *Mid* and *Low* groups defined using skin samples. On the other hand, Figure 9b shows three baleen plates that appear to span all isotope areas throughout their migratory cycles. Figure 9c shows the isotope ratios of baleen plates from calves. The data derived from the calves' plates show a similar pattern to the skin samples: a large group of samples in the area of *Low* isotope values, and then two baleens with isotope values corresponding to the *Mid* and *High* values.

DISCUSSION

Genetic analysis

Genetic diversity and phylogeny. The haplotype diversity detected at Península Valdés resulted 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). 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.

We found that the Argentinean population was divided equally between the two clades (A and W). 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. It is important to note that 19 of those 20 samples presented by Patenaude *et al. (*2007) were collected from *stranded* whales between 1994 and 1996 (original data in Portway, 1998). An alternative explanation for the equal frequency of both clades in our sample involves the influx of migrants 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 mtDNA lineages among dead whales. The among-year differentiation of the stranded animals does not result from just one year being distinct from all the others; instead, most pairwise comparisons present positive values of *Fst*, and some of these are individually significant (data not shown). The observed difference between live and stranded whales for the period 2003-2006, as well as the differential proportion of clade A and W in the stranded sample are intriguing, but the small sample size of some years preclude a rigorous statistical analysis and interpretation of the data. Furthermore, when all stranded whales are included in the analysis we found no differentiation between stranded and live whales and no differences in the frequency of haplotypes and samples within clades. Unfortunately, there are no samples from live whales in the period 2007-2009 which could be compared directly to the samples of dead whales. Other than this heterogeneity among stranded calves across years, no evidence of genetic heterogeneity was found within the Península Valdés population.

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 south-western 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 genetic structure of southern right whales across the Southern Hemisphere.

Stable isotope analysis

Stable isotope of skin samples. The lack of normality in carbon and nitrogen isotope distributions suggests a non-homogenous food source for the overall population (Hobson and Schwarcz, 1986). Our stable isotope analysis of skin samples indicates that the Patagonian right whales appear to use at least three isotopically distinct feeding sources. Whether the supposed *Mid* group is "real", or just an artifact of sampling is yet to be determined. However, the fact that this intermediate group is also represented the data from baleen plates is encouraging. In the rest of the discussion we assume that the *Mid* group indeed represents a third feeding group.

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. The *Low* group may represent a single feeding source or a "continuum of feeding sources", as indicated by its lack of normality and the positive correlation between $\delta^{13}C$ and $\delta^{15}N$ values. 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 at least 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 *et al.,* 2004).

The range of stable isotope values from skin samples also falls within the range of $\delta^{13}C$ and $\delta^{15}N$ values measured along baleen plates collected from whales that stranded at Península Valdés (Rowntree *et al.,* 2008). Baleen plates show annual oscillations of $\delta^{13}C$ and $\delta^{15}N$ values, with three distinct regions characterised by a trough (with isotope values as low as -26‰ and 4‰ in $\delta^{13}C$ and $\delta^{15}N$ values, respectively), followed by an area of no dramatic isotopic change (a plateau of intermediate values), followed by a sharp peak in $\delta^{13}C$ and $\delta^{15}N$ values (see Rowntree *et al.,* 2008 for detailed explanation of the cycles). 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 $\delta^{13}C$ and $\delta^{15}N$ values of skin samples are mostly restricted to the range of isotope values between the plateaus and the peaks, and do not reach values as low as the troughs (Rowntree *et al.,* 2008). Assuming that the food-skin isotope fractionation is equal to the food-baleen plate fractionation, this previous observation suggests that the skin samples may represent a temporal integration of feeding activities over a period of time that do not correspond to the southernmost migration. However, according to the current literature our assumption of equal fractionations for both tissues is likely incorrect (Kelly, 2000).

Individual variations. Age segregation with 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 3). 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 whales 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. 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 have been proposed to increase the nitrogen isotope ratios in animal tissues (Kelly, 2000; Fuller *et al.,* 2004; Valenzuela *et al.,* 2010a); although, the magnitude of these effects is still controversial and a subject of intensive research.

The interannual differences in the general distribution of $\delta^{13}C$ and $\delta^{15}N$ values 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 and Fry, 1987; Druffel and 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 values. At this point we cannot discriminate between these two hypotheses.

Feeding areas and IsoSource modeling. A source of uncertainty in our *IsoSource* modeling is the use of prey sources with isotope values from very diverse origins (Appendix Table A). The large array of sites, years, seasons, tissues and methodological treatments that these sources cover are 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 6) reveals that the mean values for the subgroups of whales lie 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 (or any other with similar isotope ratios) 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 sources 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 or "not sampled" feeding area currently being used by southern right whales is probable. 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 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 elements.

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 north-south along the shelf break following seasonal blooms in productivity (Romero *et al.,* 2006). These three scenarios of migration are supported by the isotope data obtained from a small set of baleen plates (Figure 9a). The three individuals represented in Figure 9a seem to have migratory routes restricted to specific isotopic regions. On the other hand, the three individuals represented in Figure 9b do not seem to be restricted to a particular isotopic region, but rather span the full range. Although with such a small set of baleen plates is problematic to make inferences about the whole population, it seems that southern right whales show individual differences in foraging strategies and migratory patterns. These individual differences might be showing the whales flexibility to use different feeding areas and consume different prey types, an ability necessary to cope with changes affecting the ocean ecosystems.

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FIGURES

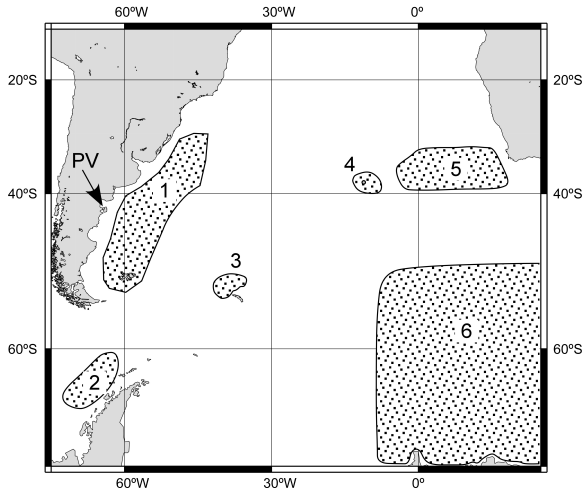

Figure 1: Map of the South Atlantic and Atlantic sector of the Southern Ocean indicating the 6 historic feeding grounds recognized by the International Whaling Commission (IWC 2001). 1) offshore South America, 2) Antarctic Península, 3) South Georgia, 4) Tristan da Cunha, 5) Cape Town-Tristan da Cunha,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

Figure 2: Phylogenetic relationships among 37 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.

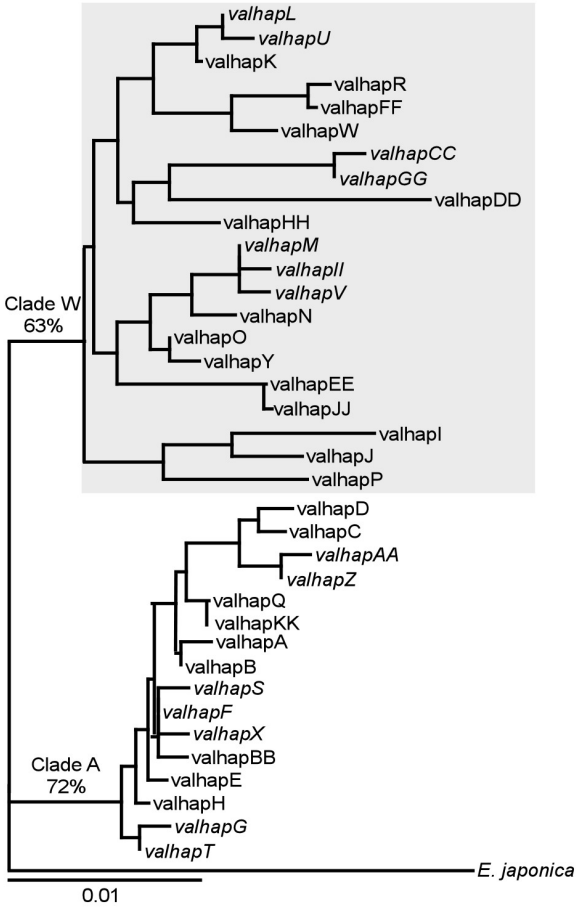

Table 1: Haplotype (630 bp) distribution by year of collection for live and stranded animals. Grey upper 21 rows correspond to clade W and white lower 16 rows to clade A. *Syn* corresponds to synonymic haplotype names based on using 275 bp sequences from Portway (1998), Baker *et al.* (1999), Malik *et al.* (2000) and Patenaude *et al.* (2007).

		Live					Stranded							
Hap	***Syn***	**03**	**04**	**05**	**06**	**Total**	**03**	**04**	**05**	**06**	**07**	**08**	**09**	**Total**
M	*Mal.F*	7	7	10	5	29			1		4	9	1	15
J	*Mal.J*	4	2	9	4	19		1	3	1	3	4		12
K	*Por.16*	2	6	7	3	18			1		3	3	2	9
L	*Mal.H*	1	2	4	2	9				1	4	7		12
W	*Por.25*	2		1	2	5			2		1	3		6
O		2	3	1	2	8					1		1	2
I	*Por.12*		6	1	1	8					1			1
P			2	2	1	5						2		2
Y				3		3						1		1
EE				1	1	2								
N	*Pat.27*		2			2								
R		1	1			2								
GG	*Mal.I*				1	1							1	1
CC	*Mal.I*			1		1								
FF				1		1								
HH	*Por.17*				1	1								
II	*Mal.F*				1	1								
U	*Mal.H*		1			1								
V	*Mal.F*		1			1								
DD								1	3			1		5
JJ											2			2
F	*Por.4*	5	4	6	3	18	2		2	1	5	5	3	18
D	*Por.7*	2	4	2	4	12		1	2	1		3	2	9
B	*Mal.D*	1	2	2	6	11				2	2	4		8
A	*Por.3*	3	2	2	5	12			1	1	1	2		5
E	*Mal.B*	2	4	3	3	12		1	1		1	1		4
Q	*Mal.E*	1	2	1	4	8	1				2	3	2	8
Z	*Por.11*			2	3	5	1		3		3	2		9
H	*Bak.A*	1	1	3	1	6	2	1			2	1		6
C	*Por.5*	2	1	1	2	6							4	4
T	*Por.6*	1	1	1		3	1		1			2	1	5
X	*Por.4*			3		3	1			1	1	1		4
AA	*Por.11*	1		1		2			1	1	1		1	4
BB				1	1	2					1			1
G	*Por.6*		1			1						1		1
S	*Por.4*		1			1								
KK												1		1
Total		38	56	69	56	219	8	5	21	9	38	56	18	155

Table 2: Pairwise 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	***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
SW Australia F (3/5)	0.125**	0.078	1

*** $P < 0.001$, ** $P < 0.01$

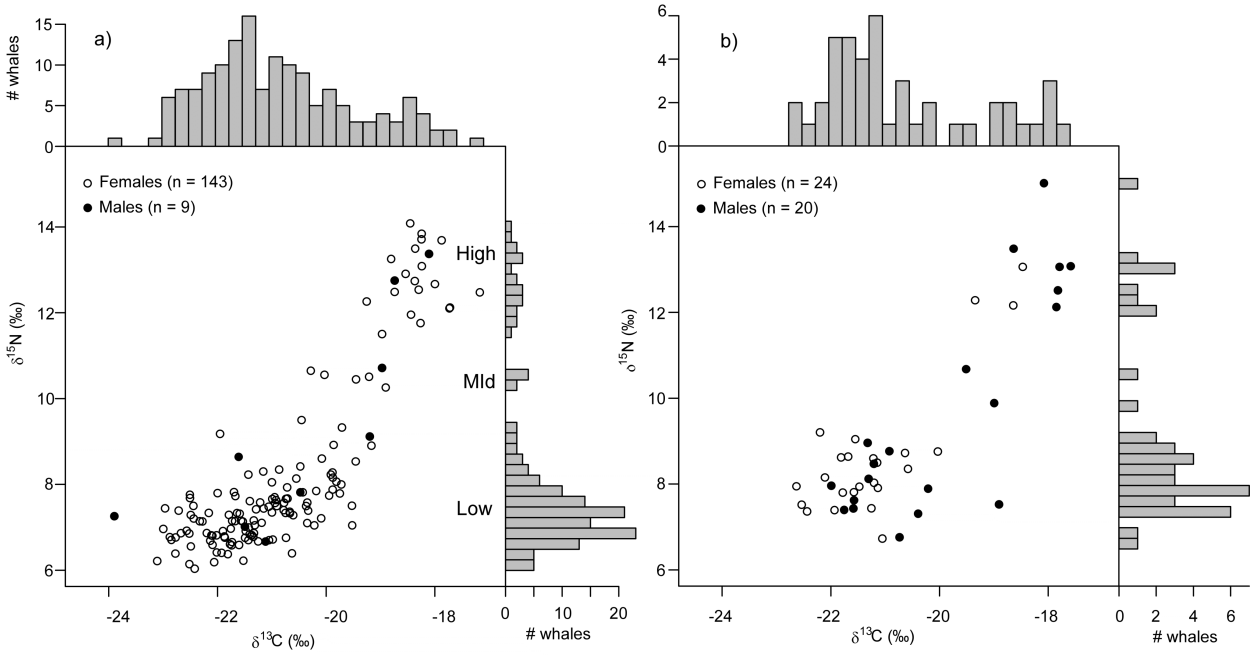

Figure 3: Scatter plots of $\delta^{15}N$ and $\delta^{13}C$ values from 196 southern right whale skin samples collected in Península Valdés: a) Adults, b) Juveniles. Frequency distributions of $\delta^{15}N$ and $\delta^{13}C$ values are shown as marginal histograms. Isotope distributions are not normal (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 distribution of $\delta^{15}N$ values.

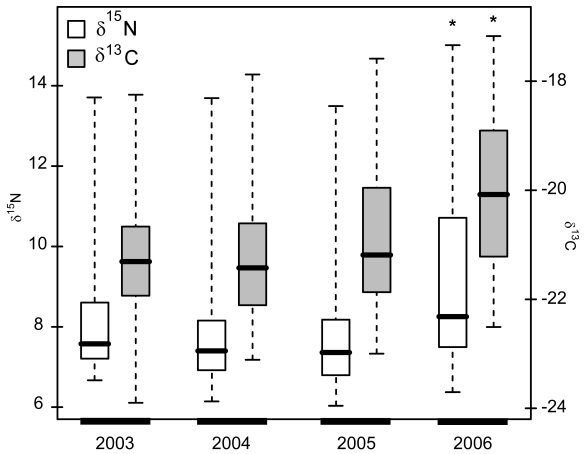

Figure 4: Boxplots of isotope values by year. Boxplots present the median, 25th-75th percentiles, minimum and maximum values. $\delta^{15}N$ values are presented in open boxes and $\delta^{13}C$ values in grey boxes. Year 2006 had higher stable isotope values than any other year.

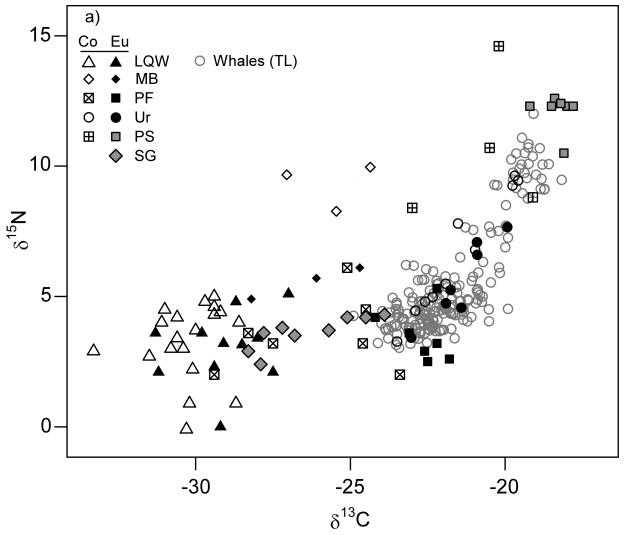

Figure 5: Stable carbon and nitrogen values of prey samples from the South Atlantic and Southern Ocean. The $\delta^{13}C$ and $\delta^{15}N$ values from skin samples are corrected for trophic level. Copepods (Co), Euphausids (Eu) values are from Table A in the appendix. Samples are from Uruguay (Ur), Patagonian shelf (PS), South Georgia (SG), Marguerite Bay (MB), Polar Front (PF), and from a large area that includes the Lazarev sea, the American and African quadrants and the Weddell Sea (LQW).

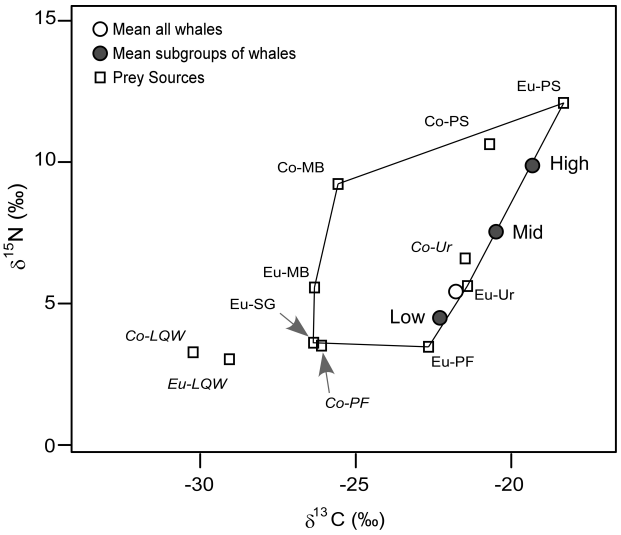

Figure 6: IsoSource polygon. Bivariate representation of the isotope sources (prey groups) and mixtures (whales corrected for trophic level) used for the IsoSource modelling. The mean isotope value of each prey source (squares) and subgroup of whales (circles) are presented. In *italics* are also presented a few prey groups not used for the IsoSource modelling. Co and Eu indicate copepods and euphausids respectively. Acronyms of locations are the same as Figure 4 and Table A in the appendix.

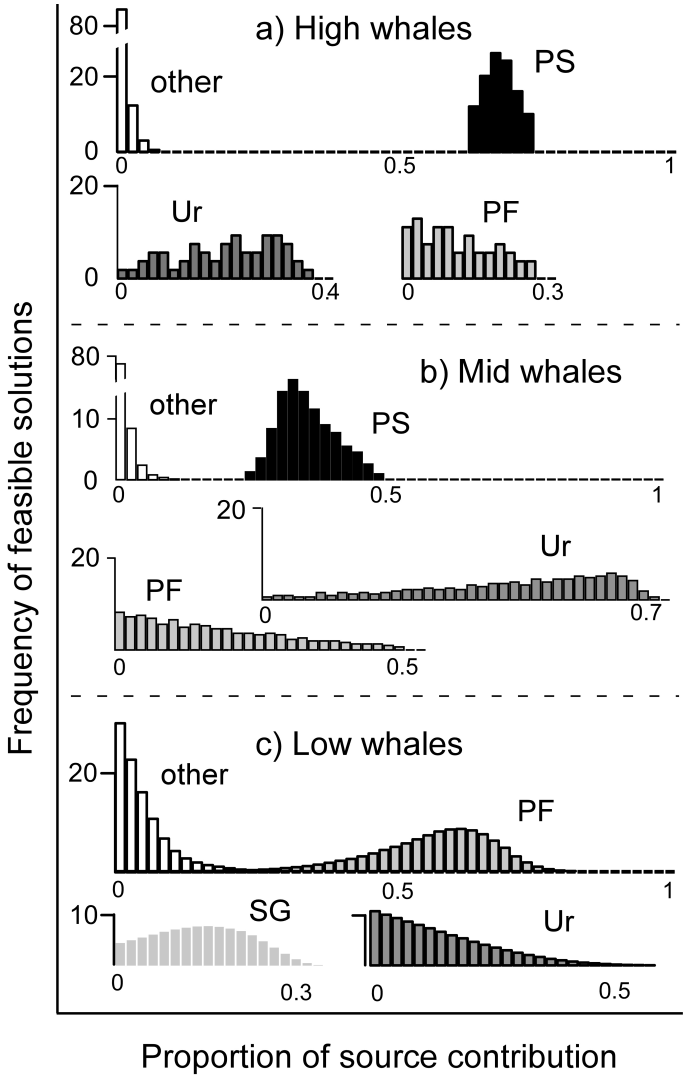

Figure 7: Results from *IsoSource* modelling. Distribution of proportions of prey source contribution to the diet of the *High* (a), *Mid* (b) and *Low* (c) subgroups of whales. For each subgroup, the three main sources are presented, all are euphausiids; the other four sources that do not contribute much are combined ("other").

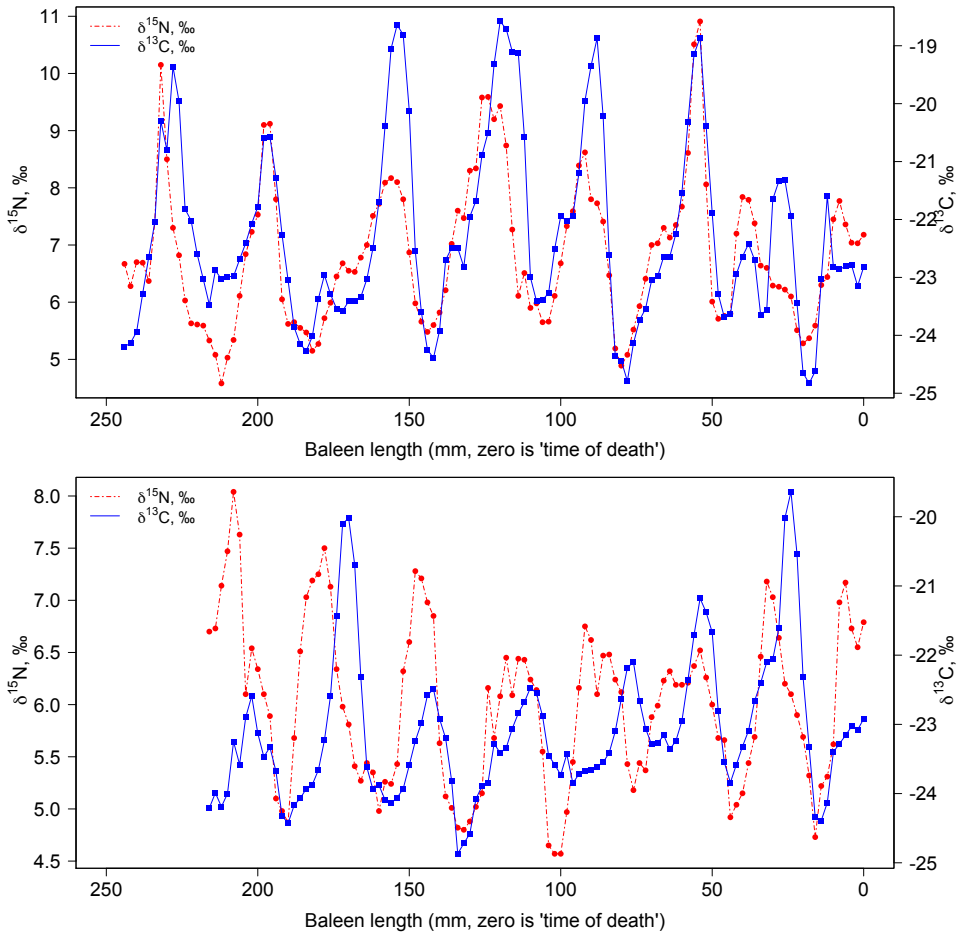

Figure 8: Examples of isotopic profiles of baleen plates from two different adult whales stranded at Península Valdés, Argentina. Samples were collected every 20mm. Carbon isotope data were previously presented by Rowntree *et al.,* (2008)

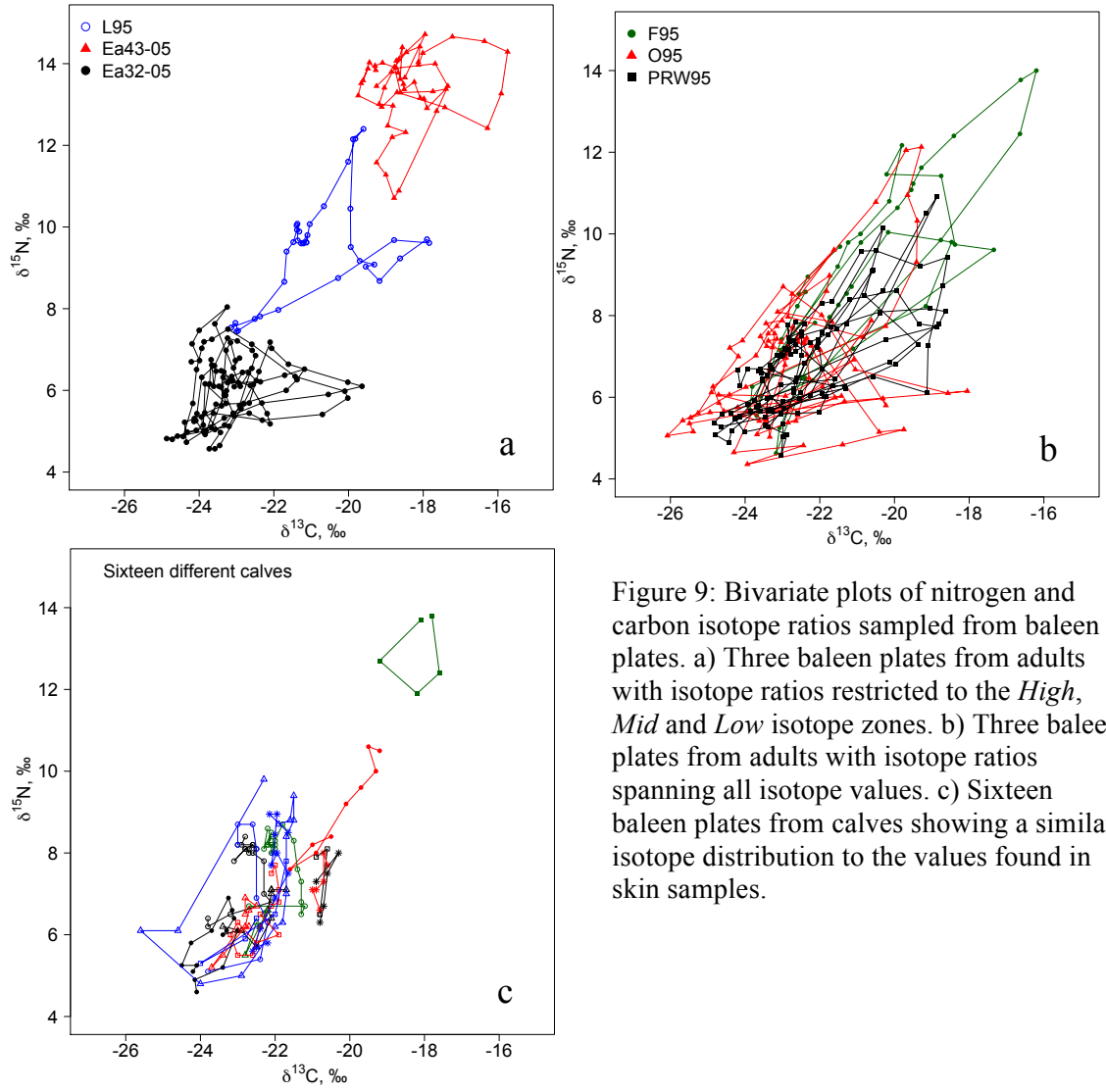

Figure 9: Bivariate plots of nitrogen and carbon isotope ratios sampled from baleen plates. a) Three baleen plates from adults with isotope ratios restricted to the *High*, *Mid* and *Low* isotope zones. b) Three baleen plates from adults with isotope ratios spanning all isotope values. c) Sixteen baleen plates from calves showing a similar isotope distribution to the values found in skin samples.

APPENDIX.

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

	$\delta^{13}C$				$\delta^{15}N$						Year	
Taxa	**Min**	**Mean**	**SD**	**Max**	**Min**	**Mean**	**SD**	**Max**	**n**	**Study site**	**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
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
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

continued		$\delta^{13}C$				$\delta^{15}N$					Year	
Taxa	**Min**	**Mean**	**SD**	**Max**	**Min**	**Mean**	**SD**	**Max**	**n**	**Study site**	**Season**	**Source**
E. superba		-29.80	0.60			3.60	0.20		12	Palmer station (AP)	1999 Fa	3 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
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

Notes:
[#] values obtained from figures
Study site: between parentheses is the acronym of the region used in figure 5.
Source:

Tissue analysed: 1 Whole body; 2 Muscle; 3 soft tissues.
References: a Wada *et al.,* 1987; b Rau *et al.*, 1991; d Schwamborn *et al.,* 1999; e Kaehler *et al.,* 2000; f Dunton, 2001; i Schmidt *et al.,* 2003; k Schmidt *et al.,* 2004; p This study.

Description of IsoSource modelling

***IsoSource*: stable isotope mixing model for partitioning an excess number of sources**
Developed by Dr. Don Phillips, Environmental Protection Agency.
IsoSource version 1.3 is a Microsoft Visual Basic™ software package that calculates ranges of source proportional contributions to a mixture based on stable isotope analyses when the number of sources is too large to permit a unique solution.

Standard linear mixing models were 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 1 to conduct an *IsoSource* modelling. To compensate for trophic level enrichment, we corrected the isotope ratios of skin samples by subtracting 3‰ for nitrogen and 1‰ for carbon.

Each of the individual solutions represents a combination of source proportions that satisfies isotopic mass balance in the mixing model. Descriptive statistics are provided simply as a way to characterize this entire distribution of feasible solutions. To avoid misrepresenting the results, users of this procedure should report the distribution of feasible solutions rather than focusing on a single value such as the mean.

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