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Comparing stable isotope values from vibrissae of newborn Antarctic fur seal pups (Arctocephalus gazella) to those of their mothers to better understand adult female foraging ecology and migration patterns

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Comparing stable isotope values from vibrissae of newborn Antarctic fur seal pups (*Arctocephalus gazella*) to those of their mothers to better understand adult female foraging ecology and migration patterns

A Thesis submitted in partial satisfaction of the requirements for the degree Master of Science

in

Biology

by

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The Thesis of David James Dannecker is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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ABSTRACT OF THE THESIS

Comparing stable isotope values from vibrissae of newborn Antarctic fur seal pups (*Arctocephalus gazella*) to those of their mothers to better understand adult female foraging ecology and migration patterns

by

David James Dannecker

Master of Science in Biology

University of California, San Diego, 2016

Professor Carolyn Kurle, Chair

Overexploitation of Southern Ocean fish stocks has caused significant trophic restructuring in Antarctic marine ecosystems, forcing Antarctic fur seals (*Arctocephalus gazella*) to forage on alternative prey. As a species that has faced near extinction in the recent past, it is important to have accurate data to monitor the effects ecosystem changes may have had on their diet, health, and behavior. Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope analysis is a proven technique for reconstructing foraging ecology and migration patterns in

marine mammals. Investigating whether the stable isotope values from Antarctic fur seal pup vibrissae grown in utero can provide valid data on their mothers' foraging ecology is useful as newborns are much more accessible research subjects than adults. Using samples collected at Cape Shirreff, Livingston Island in the South Shetland Islands during November to December 2012, I compared stable isotope values from vibrissae of 1- or 2-day-old pups with those from their mothers' vibrissae. I determined whisker growth rates for all individual seals in the study, using known total gestational whisker growth for pups, and known annual growth for mothers. I used spatial data from some mothers to determine if isotopic data corresponded to known migration routes. No distinct patterns emerged for the $\delta^{13}C$ values, whereas the $\delta^{15}N$ data suggest a correlation with the Antarctic Polar Front. Pup and mother isotope data show the appearance of a correlation when normalized for individual growth rates, however further research with revised sample collection parameters would be ideal to conclusively establish this relationship.

INTRODUCTION

Despite its many international environmental protections and safeguards (Fox 2014), Antarctica, like any other region of the world, is susceptible to a variety of human impacts, including pollution, fishing pressures, and the influence of global climate change (Turner et al 2005). Given these various and increasing ecosystem stressors, it is vital to examine and monitor their effects on Antarctic wildlife for better management and conservation of polar species and habitats.

One important human impact on Antarctic marine ecosystems is current and historical fishing in the Southern Ocean. From the 1960s to the 1980s many different Southern Ocean fish stocks were severely overexploited, reducing populations to less than 20 percent of their previous levels (Ainley & Blight 2009). These periods of overfishing drastically changed the food web structure in the Southern Ocean. Large predators, including the Antarctic fur seal (*Arctochepalus gazella*), that relied on annual supplies of young demersal fish turned to other prey options, such as krill, and they had to travel greater distances to find them (Ainley & Blight 2009). Collecting data on species that live in extreme habitats is inherently challenging, yet regular monitoring is important for understanding and managing the Antarctic marine ecosystem. Antarctica's remote location and harsh climate pose many logistical challenges for researchers to overcome. Ecological monitoring in the field is limited by

weather and the expense of maintaining long-term research sites. Additionally, many Antarctic species spend only a portion of the year in the Antarctic, migrating hundreds or thousands of kilometers away throughout the rest of the year. One promising and proven technique for collecting valuable ecological data from animals that are difficult to access is stable carbon ($^{13}C/^{12}C$, $\delta^{13}C$) and nitrogen ($^{15}N/^{14}N$, $\delta^{15}N$) isotope analysis of their tissues (Newsome 2010). These analyses are useful for tracking an organism's foraging habits in time and space and $\delta^{13}C$ values can inform us about particular taxa or geographical variations in diet sources, whereas $\delta^{15}N$ values can convey an animal's trophic position (Ben-David & Flaherty 2012).

The analysis of the stable isotope ratios in whole tissue samples collected from animals has the potential to reveal dietary and habitat use data over several time periods, depending upon the tissue sampled and the isotopic turnover rate of that tissue (Kurle 2009). The isotope values from tissues with high protein turnover rates such as liver or plasma yield foraging data on the order of one to two weeks previous and up to the time of tissue collection (Kurle 2009), whereas analysis of tissues with slower turnover rates, such as muscle, allow for examination of longer temporal patterns in foraging behavior (Kurle 2009). Stable isotope values can also be measured along a continuum from tissues with continuous growth such as the sequential, annual growth layers laid down in bones (Turner-Tomaszewicz 2015) or along the length of whiskers, or vibrissae (Scherer 2015), in order to obtain foraging

ecology data from multiple time periods in an animal's life. This technique is particularly useful for obtaining data on open ocean feeding or migration patterns from cryptic, migratory species such as marine mammals that are only accessible to researchers for a short period of time over the course of a year (Burns 1998, Cherel 2009, Lowther & Goldsworthy 2011, Beltran 2015).

Accessing pinnipeds that come ashore for only brief periods is still problematic as adults can be aggressive and require sedation or other specialized techniques for safe sampling (M Goebel, personal observation, CM Kurle, personal observation). Newborn pinnipeds are much more accessible research subjects than adults as their small size, relatively non-aggressive manner, and lack of territoriality make them easier to approach and handle. Stable isotope values from samples collected from pups in the initial days following parturition reflect their gestational growth, which in turn reflects their mother’s dietary choices during her non-breeding season migration (Rea et al 2015). Establishing that stable isotope data from tissues from newborn otariids can serve as valid proxies for isotope values from their mothers allows for alternative sampling and safer collection of data to better understand adult female migration and foraging patterns.

I compared stable isotope values from vibrissae collected from newborn Antarctic fur seals on South Georgia Island with those from their mother's vibrissae to determine the degree to which isotope values acquired during pregnancy are similar between mother and pup. To provide geographic

context for the stable isotope data, I also examined satellite collected geospatial data from a subset of the mother seals. I sought to determine a) the growth rates of vibrissae for Antarctic fur seal female adults and developing pups, b) foraging and migration patterns of adult female seals as reflected in the isotope data from their vibrissae, and c) how well the isotope values from pup vibrissae grown in utero reflect those of their mothers and thereby provide valuable insights into adult female diet and movement patterns during their non-breeding season in the southern hemisphere.

METHODS

The Antarctic fur seal is an otariid pinniped found in the Southern Ocean and the southernmost portions of the Atlantic, Pacific, and Indian Oceans. They are listed as a species of Least Concern by the IUCN (Hofmeyr 2014), however they are still recovering from a human-induced population bottleneck event that occurred in the 1800s and have only reached a stable population size in recent decades (Wynen 2000). In addition, the fur seal population recovery is tenuous in the face of climate change and overexploitation of Southern Ocean food resources (Boyd 1993). The majority of Antarctic fur seals breed on South Georgia Island, with a small number also breeding elsewhere, including in the South Shetland Islands (Polito & Goebel 2010) (Figure 1). The breeding season occurs during the Antarctic summer, from November to January. Female fur seals first give birth between the ages of 3 and 6 years then reach the age of prime breeding viability between 7 and 11 years (Lunn 1994). Pups are born within a few days of the females' arrival to the breeding site. The mother will care for her offspring on the island, mate, then set out to sea for a months-long migration at the end of the breeding season to find more productive feeding grounds and avoid the polar conditions of Antarctic winter. Implantation of the fetus occurs shortly after the March Equinox in response to photoperiod stimulation (York & Scheffer 1997) and

this event is relatively consistent within the species (Boyd 1991). Pup gestation takes place entirely during the migration period.

To obtain location data, adult females were captured at their breeding site near the Cape Shirreff research station on Livingston Island in the South Shetland Islands (Figure 2) during the 2011/12 breeding season and fitted with Lotek Nano-Lat 2900-series archival geolocation tags. These tags recorded latitude and longitude during the winter months for each individual. Reported spatial data from these devices tends to be accurate for longitude, but can be less so for latitude (Hinke et al 2015). For the purposes of contextual analyses, this study focuses exclusively on reported longitudes. Of the 17 mother seals included in this study, 11 were fitted with geolocation tags the prior year. Of those 11 individuals, eight yielded sufficient amounts of contemporaneous spatial data for contextualizing the stable isotope data.

For stable isotope analysis, one vibrissa was collected from each Antarctic fur seal pup and mother (n = 32 pups; n = 17 mothers; n = 17 complete mother-pup pairs) at Cape Shirreff (Figure 2) during November, December, and January of 2012/3. All vibrissae samples were clipped at the cheek, from the center of the cheek pad, and were collected from the newborn seal pups no more than two days after their birth. Vibrissae sampled from pups were prepared for stable isotope analysis in the Kurle lab at the University of California, San Diego. Vibrissae sampled from mothers were

prepared in the Antarctic Ecosystem Research Division at Southwest Fisheries Science Center.

All potential lipids and debris were removed by cleaning the whiskers with petroleum ether. Each vibrissa was first sealed individually into a glass test tube, with an aerated lid, that contained enough petroleum ether to cover the whisker. The tubes were placed into a rack, partially submerged in a sonicator containing 60°C water, then sonicated for 5 minutes. After the first round of sonication, whiskers were removed from the petroleum ether and rinsed thoroughly in a separate tube containing ultra-pure DI water. Each sample was then placed in a tube of ultra-pure water and submerged for another 60°C sonication bath lasting an additional 5 minutes. After the second sonication, each whisker was removed and dried. Each clean whisker was then weighed and the full length measured.

To construct a temporal sequence of stable isotope values, each vibrissa was divided into millimeter-length segments using a 1-mm biopsy punch. The biopsy punch retained each mm segment in its chamber after being separated from the whisker, which significantly reduced the chance of losing the tiny segments. Pup whiskers are thin, so not every 1 mm segment weighed enough for the 0.0005 to 0.001 g required for stable isotope analysis. Therefore, several adjacent segments were often combined into one sample to obtain the necessary mass (Rea et al 2015). Starting from the proximal end of the whisker, each segment was cut and weighed until they comprised a

sufficient quantity to form a sample. Each sample was then sealed into a tin capsule. Sample partitioning continued along each whisker until there was insufficient mass remaining to form a workable sample for stable isotope analysis. Each pup vibrissa provided between 2 and 12 usable samples, comprised of between 1 and 6mm of length (between 1 and 6 adjacent segments), and a total of 206 samples were produced from the 32 whiskers.

Vibrissae from the mothers were prepared with the same cleaning techniques as the pup vibrissae. Instead of a biopsy bunch, the Antarctic Ecosystem Research Division cut the larger, adult vibrissae into segments using a nail clipper. Whiskers were cut in an enclosed space to prevent the loss of samples. As with the pup whiskers, adult whiskers often required the combination of adjacent segments in order to provide sufficient mass for a stable isotope sample (Rea et al 2015). All samples were analyzed for $\delta^{13}C$ and $\delta^{15}N$ values at the UC Davis Stable Isotope Facility.

In order to directly compare the stable isotope data from mothers and pups, I determined whisker growth rates based on known temporal benchmarks. For the adults, vibrissae were clipped in November, December, and January of 2011/2, and again in 2012/3 to measure annual growth. This length was divided by 52 to estimate an average weekly growth rate for each individual. For the pups, sampled vibrissae were assumed to represent growth from when whiskers began growing in utero. The fetal development of otariids is not well studied, but based on previous observations of aborted fetuses of

other pinniped species (Scheffer 1962, Hewer 1968, Stewart 1989), I estimated that whiskers are likely to begin development within two months of implantation. For the purposes of calculating individual growth rates, and given the species' consistency of implantation dates, I marked the estimated start of whisker development as June 1 of the study year. The end date was the time the whisker was clipped just after the birth of the pup. Using these two dates, I estimated an average weekly growth rate for each pup. Whisker growth rate speeds up after birth (Rea et al 2015), but since the whiskers in this study were clipped within one or two days of birth, this accelerated growth would not have an effect on the rates in this study.

Using the individual whisker growth rate for each animal, I constructed a weekly time series of stable isotope values. Since many of the samples included differing lengths of whisker (because adjacent segments often needed to be combined to analyze an adequate amount of material) this step was needed to compare data points among individuals. Moving along the whisker from the proximal to the distal end, the individual's average weekly length of growth was used to select $\bar{\delta}^{13}C$ and $\bar{\delta}^{15}N$ values to represent each point in time. If a particular sample contained more than a week's growth, as was often the case, the value of that sample would be repeated in the time series in order to fairly represent the temporal significance of the sample.

The last factor needed to calibrate the mother and pup data, for the best comparison of the isotope data from each, was an estimate of the amount

of vibrissal tissue left behind when a whisker is clipped. The mothers' and pups' whiskers were all clipped, and not pulled, so a significant amount of vibrissal length was left behind in the cheek. Due to differing growth rates among individuals and age groups, this tissue could represent different growth times, so must be accounted for when comparing time series of isotope data from clipped whiskers. When a seal's whisker is plucked there is a visible distinction between subcutaneous root and supracutaneous tissue, so the amount of tissue that was positioned under the skin can be measured. Using samples of plucked whiskers from adult female (n = 11) and juvenile (n = 4) Antarctic fur seals collected from the same site in December 2011 and January/February 2013, I determined an average length of subcutaneous vibrissa. Coupling this length with an estimated growth rate for each cohort, I calculated the approximate amount of time represented by the tissue left behind when a sample was clipped. Following this, I applied appropriate temporal offsets to the data so that the pup and mother time series could be compared to one another directly.

Using the geospatial data and knowledge of common adult female migration routes (Polito & Goebel 2010), I hypothesized three broad geographic regions that could be occupied by female fur seals during the non-breeding season (Figure 3). Regions 1, 2, and 3 were organized along a longitudinal gradient and Region 1 was defined as below 70°W, which is in the Atlantic Ocean, east of Patagonia and which encompasses the Argentinian

coastal shelf, South Georgia Island and the South Shetland Islands. Region 2 was defined as between 70°W and 110°W, in the Pacific Ocean, and which includes the Chilean coastal shelf and the Polar front. Region 3 was defined as 110°W and above, which is in the Pacific Ocean past the boundary of the Polar front, further into the open ocean. Each geospatial data point representing a particular female was assigned to Region 1, 2, or 3, depending upon their location. Most individuals in this study stayed within one region; for individuals that visited multiple regions, their data were divided between the visited regions based on longitude. Stable carbon and nitrogen isotope values from the adult female vibrissae were assigned to each geospatial data point, using whichever $\delta^{13}C$ and $\delta^{15}N$ values were temporally closest to the date the geographic location of the female was recorded. These stable isotope data formed the foundation of group classification for the three longitudinal regions.

Discriminant analysis was used to determine the degree to which stable isotope values from the seals predicted their inclusion in each of these three non-breeding season foraging regions. Discriminant functions were based on the carbon and nitrogen isotope ratios for each individual. The isotope values from the three regions were also compared using ANOVA and Tukey's Honestly Significant Difference tests. I used the same regional classification matrix for discriminant analyses of the $\delta^{13}C$ and $\delta^{15}N$ data from the pups of these geotagged mothers, since their migration routes were known. We also compared the $\delta^{13}C$ and $\delta^{15}N$ values between mothers and pups using t-tests.

All statistical analyses were performed with Systat (Version 13) and significance was tested at the 0.05 level.

As all vibrissae were clipped, the isotope data from the most recent time periods that would have overlapped with the pups' vibrissae growth in utero was missing. Nonetheless, I compared isotope values between mother-pup pairs by graphically matching their time series to the best of my ability. To compare isotope values between mother-pup pairs, the time series for their vibrissae were matched, then the values graphed.

RESULTS

All stable isotope data for all individuals and each segment sample are listed in Appendix A, Tables 3 and 4. Individual pup vibrissae growth rates during gestation (Table 1) ranged from 1.5 to 2.7mm/week with a mean (±SD) of 2.2 ± 0.4 mm/week. This was adjusted to a range of 1.7 to 2.8mm/week and a mean of 2.4 ± 0.3 mm/week after accounting for subcutaneous growth (See Methods). Mean male pup whisker growth rates were faster (2.3 ± 0.3 mm/week, 2.5 ± 0.3 mm/week with subcutaneous growth) than females (2.1 ± 0.4 mm/week, 2.2 ± 0.4 mm/week with subcutaneous growth), but the difference was not significant (two-tailed T-test, $t = 1.9671$, $df = 29$, $p = 0.06$). Individual vibrissae growth rates for mothers were less variable, ranging from 0.4 to 0.6 mm/week (mean = 0.5 ± 0.1 mm/week). Subcutaneous growth did not affect calculated growth rates from the mothers' whiskers because these were based on a fixed annual length measured directly.

I compared plucked whiskers (those with subcutaneous vibrissal growth still intact) collected in previous years with those that had been cut for use in my study. I found that a mean of 5.9 ± 1.9 mm of subcutaneous vibrissal tissue is left behind when a juvenile whisker is clipped, and a mean of 6.2 ± 1.0 mm of subcutaneous vibrissa is left behind when an adult female whisker is clipped. Based on the cohort-specific growth rates estimated above, this

subcutaneous tissue was estimated to represent approximately 12 weeks of growth for adults and three weeks of growth for pups.

Because the mothers' whiskers were cut, the stable isotope values from the subcutaneous portion that would have coincided in time with the pups' whisker growth in utero were missing. Therefore, the overlap between the mother-pup time series of stable isotope values collected from whiskers was small or absent and the number of paired data points was insufficient for statistical analysis. This lack of data reflecting a direct overlap between the mother and pup time series also prevented the calculation of a correction factor (similar to a trophic enrichment or stable isotope discrimination factor) to adjust the pups' $\delta^{13}C$ and $\delta^{15}N$ values to account for potential differences in isotope ratios between mothers and pups that likely arose during the pups' in utero development. However, we did observe that overall mean (±SD) pup $\delta^{13}C$ were significantly higher and $\delta^{15}N$ values were significantly higher (-20.3±1.2 and 11.5±0.9, respectively) than those from the adult females (-20.5±1.3 and 9.8±0.9, respectively) ($\delta^{13}C$ t-test: $t = 1.9888$, $df = 940$, $p = 0.047$; $\delta^{15}N$ t-test: $t = 21.8237$, $df = 922$, $p < 0.0001$).

Visual comparisons of the graphed time series of $\delta^{13}C$ and $\delta^{15}N$ values from vibrissae from mother-pup pairs show the pup time series often begin in close vicinity to where the mother time series leave off (Figures 4, 5, and 8-22 in Appendix B), even when there is no overlap present. Pairs 6, 9, 10, 16, and 17 show particularly closely matched time series (Figures 4 and 5, and Figures

15, 16, and 22 in Appendix B). This appearance of correlation is present in both the $\delta^{13}C$ and $\delta^{15}N$ values to varying degrees, though they are generally more closely matched for $\delta^{13}C$ values. To better visualize the overall patterns in stable isotope values over the course of a year, I combined all $\delta^{13}C$ and $\delta^{15}N$ values from all sampled mothers into two figures (Figure 6a and b).

Discriminant analysis classifying mother seals into three regions based upon their stable isotope data showed weak categorical sorting. The jackknifed classification matrix from the discriminant analysis using the classification factors derived from the $\delta^{13}C$ and $\delta^{15}N$ values from the adult female whiskers assigned females to the correct regions only 52% of the time, with 44% correct for Region 1, 40% for Region 2, and 62% for Region 3. Despite this, the separations were statistically significant (Pillai's trace, $F = 7.10$, $df = 4$, $p < 0.01$), but the canonical scores plot showed significant overlap between regions (Figure 7a). The results of the ANOVA showed that the $\delta^{13}C$ values were not significantly different between regions ($F_{1,81} = 1.017$, $p = 0.316$), whereas the $\delta^{15}N$ values were significantly different (See Table 2) ($F_{2,80} = 6.123$, $p = 0.003$), with the mean $\delta^{15}N$ values from animals known to be in Regions 1 and 2 significantly higher (10.3±1.5‰ and 9.9±0.8‰, respectively) than those in Region 3 (9.1±1.0‰) (Tukey's, $p = 0.006$ and $p = 0.047$, respectively).

The jackknifed classification matrix from the discriminant analysis using the classification factors derived from the $\delta^{13}C$ and $\delta^{15}N$ values from the pup

whiskers assigned females to the correct regions only 46% of the time, with 83% correct for Region 1, 52% correct for Region 2, and 31% correct for Region 3, however the separations were statistically significant (Pillai's trace, $F = 5.75$, $df = 4$, $p < 0.01$) despite significant overlap among regions (Figure 7b). There were no differences in the mean $\delta^{13}C$ or $\delta^{15}N$ values from pup whiskers among regions (ANOVAs, $F_{1,67} = 0.10$, $p = 0.75$ and $F_{1,67} = 3.424$, $p = 0.07$, respectively).

DISCUSSION

The average whisker growth rates I found for mothers and pups are close to previously reported growth rates in similar species of otariid. The average of 0.5 mm/week (~0.07 mm/day) of whisker growth for adult females is slightly slower than the average whisker growth of 0.10-0.17 mm/day reported for adult female Steller sea lions (*Eumetopias jubatus)* (Hirons et al 2001). The average growth rates reported for Steller sea lions in utero of 6.5-8.1 mm/month (~0.22-~0.27 mm/day) (Rea et al. 2015) were slightly slower than the average in utero growth rate of 2.4 mm/week (~0.34 mm/day) I found for Antarctic fur seal pups. In addition, adult female Steller whisker growth rates of 2.0-9.0 mm/month (~0.07 to ~0.3 mm/day) were equal to or greater than the 0.5 mm/week (~0.07 mm/day) I observed for adult female Antarctic fur seals

To our knowledge, this is the first study to determine whisker growth rates for Antarctic fur seals. In addition, recent studies have specifically pointed to the uncertainty associated with gestational whisker growth for Steller sea lions (Stricker 2015). Given how vital growth rates are for guiding interpretations of isotopic analyses of whiskers, the greater the body of growth rate data available, the more viable this technique will be. The estimates of in utero whisker growth rates will be useful for future efforts to use newborn pup

whiskers as sources of foraging data for their mothers and for calibrating early life growth rates for a more complete analysis of adult whiskers.

The stable isotope time series from pups do appear to closely match those from their mothers. Many of the time series I analyzed display patterns resembling correlation in the transition from mother stable isotope data to pup stable isotope data (see Figures 4, 5 and Appendix B Figures 13, 16, 17). Further study is required to support this more conclusively as limitations in sample size and collection methodology prevented direct statistical comparison. One primary limiting factor in this study was the method of clipping whiskers at time of sampling instead of plucking them whole from the seals' cheeks. Leaving behind the most recently grown vibrissae segments severely limited the option to compare the mother and pup isotopic time series in parallel. Since vibrissae grow so much faster in the womb than in adulthood, the entire sampled length of a pup vibrissa can often represent the same span of time that is left behind in the cheek by a slower-growing adult vibrissa. In order to provide more conclusive results when comparing pup-mother pairs, I advise future studies pluck sampled whiskers instead of clipping them so as not to leave valuable data behind. If it can be shown that mother and pup data are indeed statistically related, clipped pup whiskers do appear to fill in the temporal gap left by clipped mother whiskers. It is possible that pup vibrissae sampled with the less invasive clipping method may still be used alongside

their mothers' clipped whiskers to yield a more comprehensive isotopic representation of the late migration season.

While this study was not able to conclusively answer the question of whether a gestating pup's whisker can serve as a direct proxy to the mother's whisker, it does lend some tentative support to the methodological concept, as well as explores some of the limitations inherent to this data collection approach. Other studies have previously utilized stable isotope data from pups in conjunction with data from their mothers in various species (Lowther & Goldsworthy 2010, Cherel 2015, Rea et al 2015, Scherer et al 2015, Stricker 2015), though the hypothesis of pups being valid proxies for mothers has yet to be thoroughly tested.

There are many factors that could complicate the relationship between stable isotope values of a mother and her offspring developing in utero. For example, differential fractionation of carbon and nitrogen stable isotopes could cloud the trophic signal during the development and growth of the fetus (Pinnegar & Polunin 1999). Alternatively, the resources devoted to growing a pup in utero may not always reflect a mother's most recently consumed prey. During times of nutritional stress, consumers can use tissue reserves rather than recently ingested food to contribute to tissue growth (Hobson & Clark 1992). Nutritional stress is possible, if not likely, under the high-energy demand of the later months of gestation (Perez & Mooney 1986) which is the time period best represented isotopically in the pups' vibrissal growth. Finally,

the very thin size of the distal end of pup vibrissae places a limitation on how far back in time a pup whisker's stable isotope data can capture. The thinner the whisker tissue, the more segments are required for an analyzable stable isotope sample, meaning that toward the distal end of the whisker, so much length is required for a single sample that the data are not informative about fine-scale foraging activity. Vibrissae development begins in June, yet the end of July was the farthest back in time any pup whisker in this study could reach, and most whiskers only yielded six to nine weeks of data from September to November.

Examination of all of the mothers' $\delta^{13}C$ and $\delta^{15}N$ data together yielded evidence of predictable patterns. Overall there was a general pattern in $\delta^{13}C$ values over the course of the year (Figure 6a): higher $\delta^{13}C$ values at the breeding site at Cape Shirreff, followed by lower $\delta^{13}C$ values during migration, then a return to higher $\delta^{13}C$ values as the mother returns to the breeding site. This pattern corresponds well with the expected isotopic influence of the Polar Front. The Antarctic Polar Front, which encircles Antarctica, is the area with the highest recorded $\delta^{13}C$ values in this region of the Southern Ocean, with declining gradients to both the north and south (Charles & Fairbanks 1990). The South Shetland Islands, where the breeding site is located, are right on the edge of the Polar Front. The cyclical nature of an increase in the $\delta^{13}C$ values from the vibrissae in mothers as they approach then forage near the

breeding site and their subsequent decrease as they travel north is consistent with the underlying stable isotope patterns observed in these waters.

The $\delta^{15}N$ data from the mothers' vibrissae taken together show no strong annual trend, which is also consistent with expectations. Adult female Antarctic fur seals have been previously observed to occupy consistent trophic niches throughout the year (Cherel et al 2007). The niche may increase during migration to utilize a wider array of prey options, but in general, female fur seals appear to forage at an isotopically consistent level from summer to winter (Cherel et al 2007). They may still change prey or forage at higher or lower trophic positions, but to determine this with certainty would require compound specific stable isotope analysis of amino acids (CSIA-AA) from the whiskers to untangle the influence of the underlying nitrogen dynamics in the different oceanic areas from that of true prey switching (Bradley et al. 2015).

However, some females did display variation in their vibrissal $\delta^{15}N$ values between the breeding and non-breeding seasons (Figure 4, Appendix Figure 16). It is known that adult male Antarctic fur seals switch their primary prey between fish during the non-breeding season and krill during the breeding season when they are in Antarctic waters (Cherel et al 2007). It is possible that variation in $\delta^{15}N$ values in adult females is also representative of individual and seasonal prey switching patterns from fish to krill (Polito & Goebel 2010). Isotopic evidence that krill form a substantial portion of breeding females' diet in the Antarctic would be valuable information for

understanding how females are responding to the pressures of variable prey availability and help inform management of the burgeoning Antarctic krill fishery (Nicol et al 2011).

I observed significantly higher mean $\delta^{15}N$ values from vibrissae from females that encompassed time periods when they were known to be foraging in Regions 1 and 2 over those from Region 3, indicating that these regions do exhibit isotopic differences. This could be driven by differences in female foraging choices or prey availability or by the underlying nitrogen dynamics driving overall $\delta^{15}N$ values in each region. As mentioned above, without further investigation using CSIA-AA of fur seal vibrissae or comparing the stable isotope values from prey in each region, it is difficult to be sure exactly what is causing these differences.

The $\delta^{13}C$ values were not different among regions, which could be explained by a number of factors. The $\delta^{13}C$ values' ratios are a reflection of certain processes that drive oceanic $\delta^{13}C$ values and that can create geographic patterns. The primary spatial patterns in $\delta^{13}C$ values observed in the Antarctic are an inshore-offshore gradient and a latitudinal gradient (Cherel & Hobson 2007). Based on the geospatial distribution of the mother fur seals' stable isotope data, neither pattern should have much of an influence. There was very little latitudinal spread in the mothers' positions as indicated by the tag data (Figure 3) and almost all of the mothers were situated well away from the Polar Front for the duration of the geospatial monitoring, so the latitudinal

gradient in $\delta^{13}C$ values would not appear to be much of a factor. Additionally, given the limitations of the Lotek geolocation tags' latitudinal accuracy, I was unable to utilize the reported latitudinal data to define categorical regions with the same degree of reliability available for the longitudinal data. In addition, all individuals migrating during the time the geospatial data were recorded were a sizeable distance from any coastline, removing the potential for an inshore/offshore influence on the $\delta^{13}C$ values. A more robust future study with more individuals and a more thorough contextual understanding of the Southern Ocean isoscape could potentially better detect $\delta^{13}C$ patterns.

Even though the $\delta^{15}N$ values from the mothers exhibited some regional structure and there was a general pattern for the $\delta^{13}C$ values between breeding and non-breeding season months, predicting the mother's migration region based on pup data alone was not possible. The discriminant analyses attempting to classify the foraging regions of the mothers based on the stable isotope data from the pups were weak. The number of pups whose mothers' locations were known was very low (N=8) and, as the separations among regions were statistically significant, perhaps an increase in sample size could strengthen the assignments of females to different regions. However, as there were no differences in the $\delta^{13}C$ values from mothers or pups among the regions, they may not be isotopically disparate enough to truly segregate individuals using these types of analyses.

I observed that the mean $\delta^{13}C$ values from pup and mother whiskers were very similar (though statistically distinct by ~0.2‰), whereas the mean $\delta^{15}N$ values were higher in pups by ~1.7‰. It appears that the $\delta^{13}C$ from the pups closely mirror that of their mothers, which is expected since studies of elephant seals have shown that there is generally little isotopic fractionation between mothers and their gestating offspring in utero (Aurioles et al. 2006). In particular, otariid offspring $\delta^{13}C$ values tend to start close to the mothers' $\delta^{13}C$ values, and decline during nursing due to ^{13}C-depleted milk (Lowther & Goldsworthy 2010). The difference in the $\delta^{15}N$ values between pups and mothers is consistent with data from Steller sea lion pups that demonstrated elevated $\delta^{15}N$ values of 0.8‰ and 1.7‰ during gestation and nursing, respectively (Stricker et al 2015). The patterns I observed between the isotope values from mothers and pups are consistent with other studies underscoring the promise of using stable isotope values from pup whiskers to uncover foraging and migration patterns of their mothers.

There are definite strengths to using stable isotope data from pup vibrissae for studying the migration period. Determining a specific time scale can be difficult with adult whiskers, which contain multiple years. Despite the annual patterns typically apparent in stable isotope data from vibrissae (Cherel et al 2007), determining signals of particular events or time frames can be inexact (Kernaléguen et al 2012). When analyzing stable isotope values from pup vibrissae, the total time frame captured is shorter but much better defined

as the start of vibrissae development in utero is the earliest possible date shown in the pup's data. All of the data contained in the pup's vibrissae must reflect the most recent migration period as the entirety of the tissue was grown during gestation, so determining the extent of the time series, while still inexact, may be significantly easier than with adult vibrissae. In addition, capturing and sampling a whisker from a pup is significantly easier than doing so from an adult female.

CONCLUSION

My findings show that there is much promise in the concept of using newborn Antarctic fur seal pup whiskers to delve into the ecological patterns of their mothers' migration. However, any investigation attempting to utilize this technique should have a plan to obtain accurate growth rates. To accomplish the goal of direct comparison of pup and mother stable isotope data, plucking whiskers would be a superior sample collection method than clipping. I observed that the pup stable isotope data followed predictable patterns in relation to the mothers' stable isotope data. I found that the mothers' data displayed ecologically consistent $\delta^{13}C$ and $\delta^{15}N$ patterns, matching expectations with regard to the Antarctic Polar Front as well as seasonal fluctuations. Mothers exhibited higher $\delta^{13}C$ values when they were near the breeding site. They also exhibited lower $\delta^{15}N$ values if they migrated past 110°W in the South Pacific than if they stayed east of that boundary. Fluctuations in the time series of the $\delta^{15}N$ values from some of the mothers suggested that some individuals might vary their seasonal prey selection. However, in general, adult females appear to occupy fairly consistent trophic niches throughout the year. Going forward, investigations that utilize proper sample collection methods, the compilation of a larger dataset of whisker growth rates, the collection of more geographic data from migrating seals, and a better understanding of the Southern Ocean isotopic environment should

allow for a better understanding and application of the promising concept of using newborn pup vibrissae to study mother fur seal migration and foraging ecology.

FIGURES

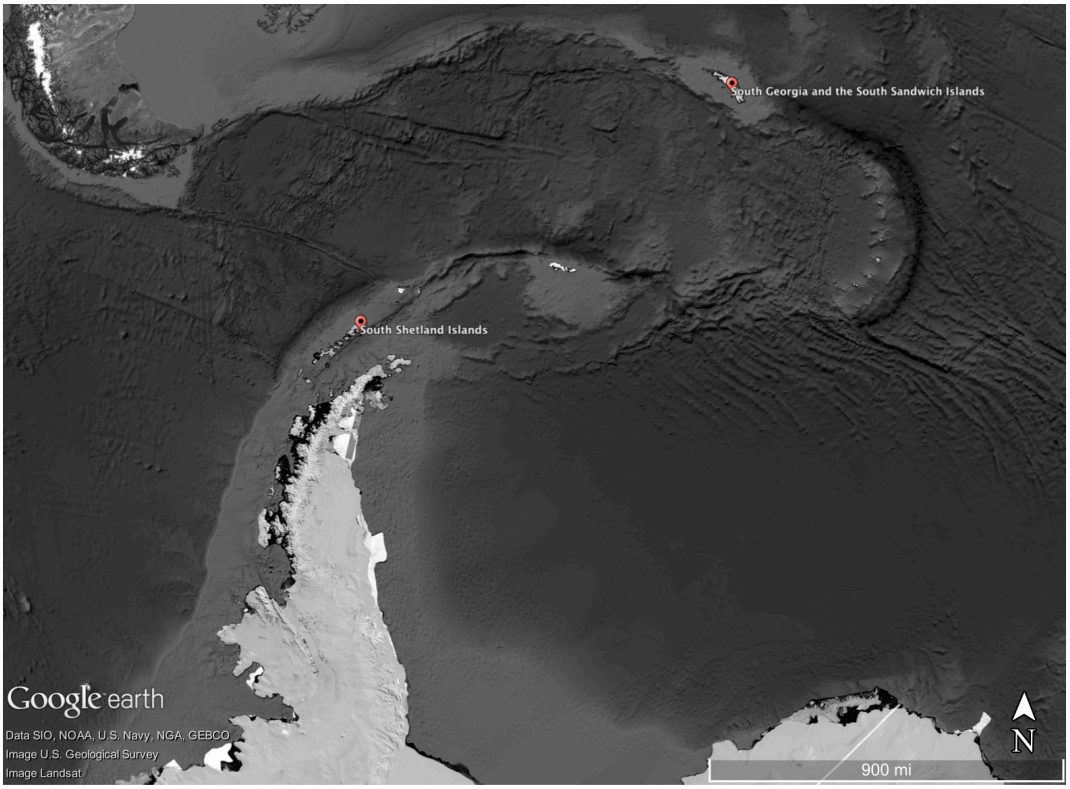

Figure 1: Map showing the locations of South Georgia Island and the South Shetland Islands relative to the Antarctic Peninsula and South America.

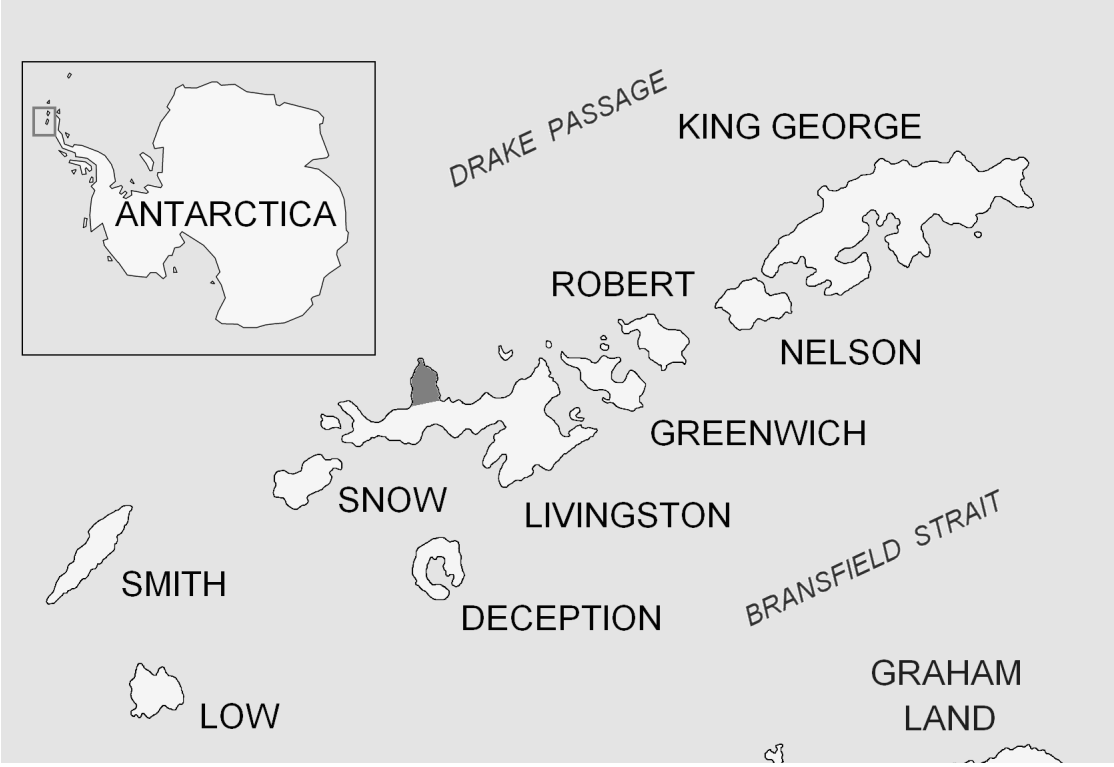

Figure 2: Map of the South Shetland Islands, with location relative to Antarctica shown in inset. Shaded area shows the position of the Cape Shirreff research station on the Ioannes Paulus II Peninsula on Livingston Island. Original map created by Wikimedia Commons user Apcbg.

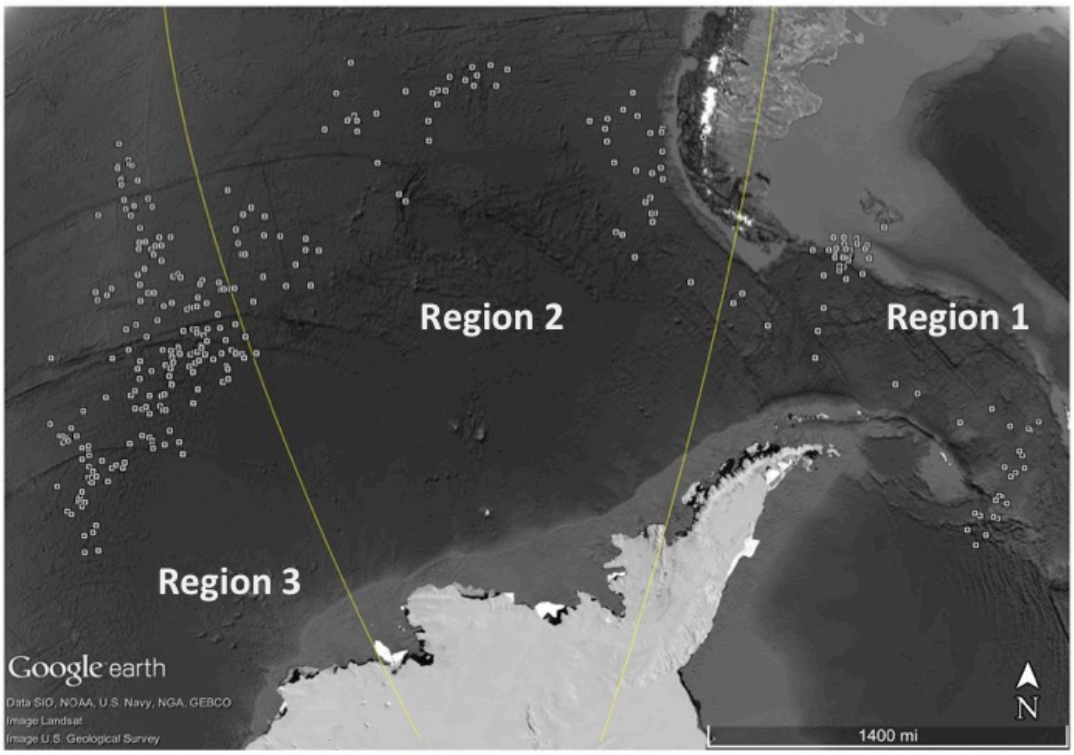

Figure 3: Migration data collected via satellite tags during the non-breeding season in 2012 for adult female Antarctic fur seals (n=12) whose whiskers and whose pups' whiskers were collected for use in this study. Each point represents the migration route of a separate individual. These data demonstrate three distinct regions occupied by fur seal females during the austral winter and the regional isotopic data are likely reflected in the $\delta^{13}C$ and $\delta^{15}N$ values from the pups' vibrissae that were grown in utero from that same year and that reflect the mothers' foraging regions.

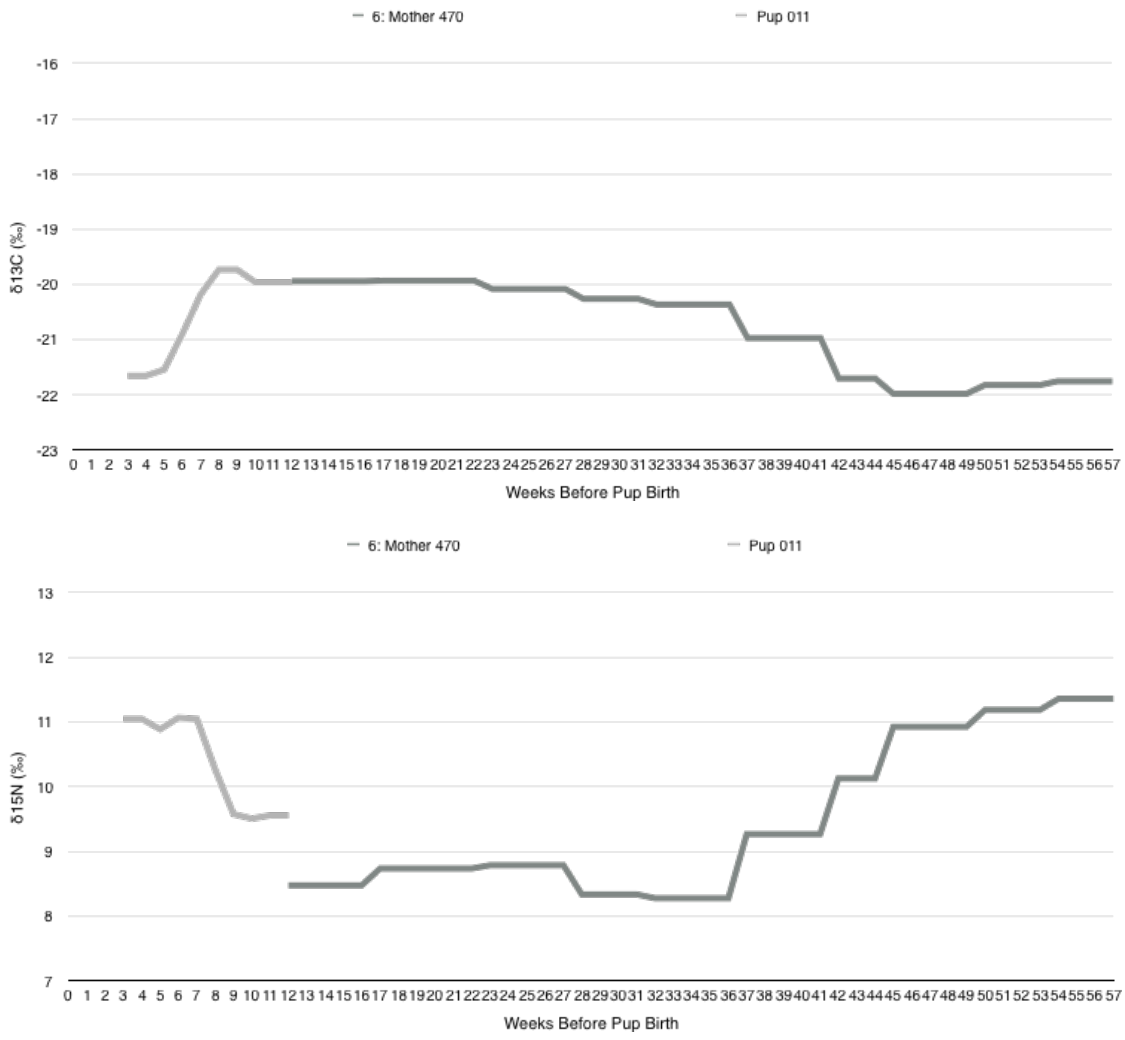

Figure 4: An example of a normalized time series of stable carbon and nitrogen isotope values for mother-pup pairs of Antarctic fur seals. These time series are from mother-pup pair 6. The time series do not overlap because both the mother and pup's whiskers were cut, leaving behind the length that would reflect the isotope values from the time period immediately prior to parturition (see Methods). Week zero on the x-axis marks the pup's birth.

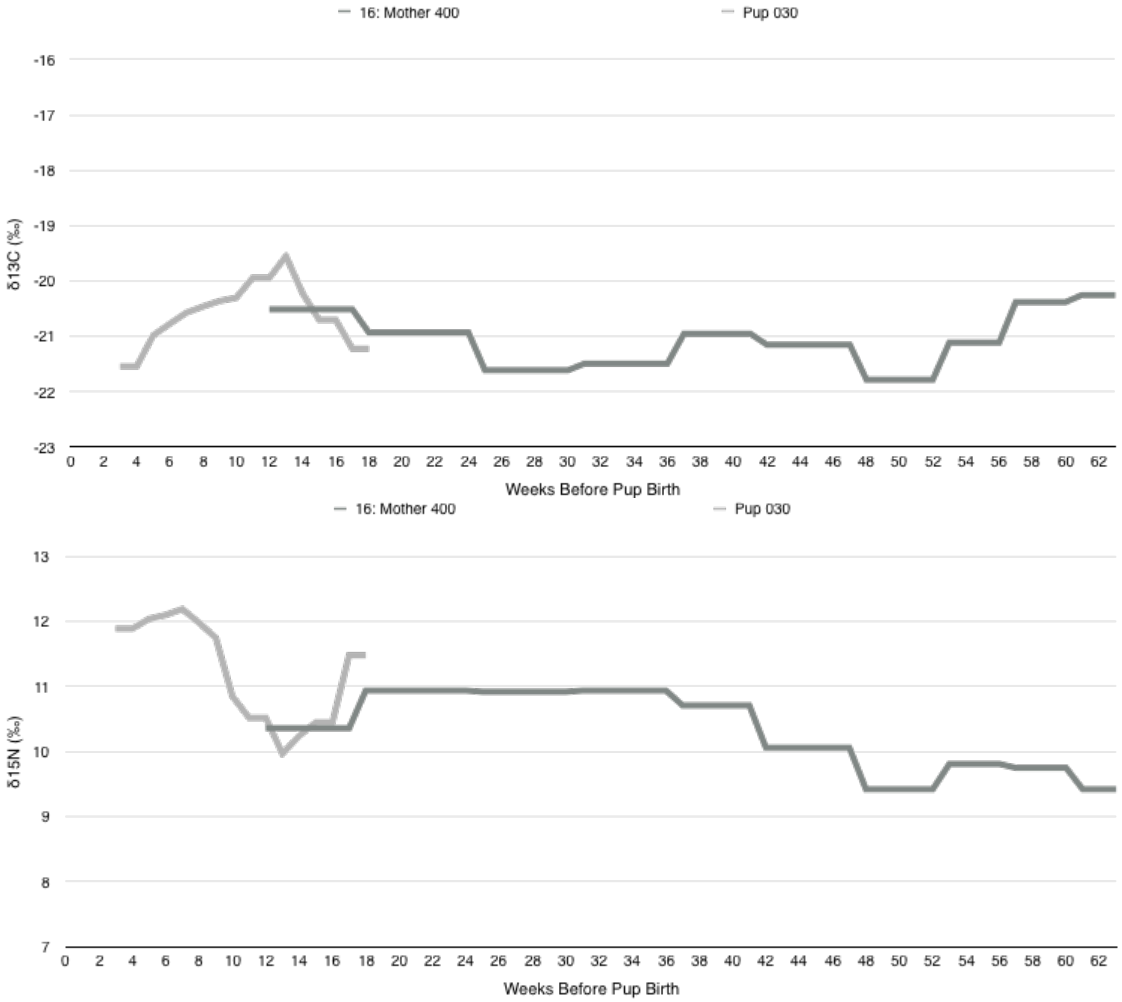

Figure 5: An example of a normalized time series of stable carbon and nitrogen isotope values for mother-pup pairs of Antarctic fur seals. These time series are from mother-pup pair 16. The time series do not overlap because both the mother and pup's whiskers were cut, leaving behind the length that would reflect the isotope values from the time period immediately prior to parturition (see Methods). Week zero on the x-axis marks the pup's birth. All other graphs of mother pup pairs are in the appendix.

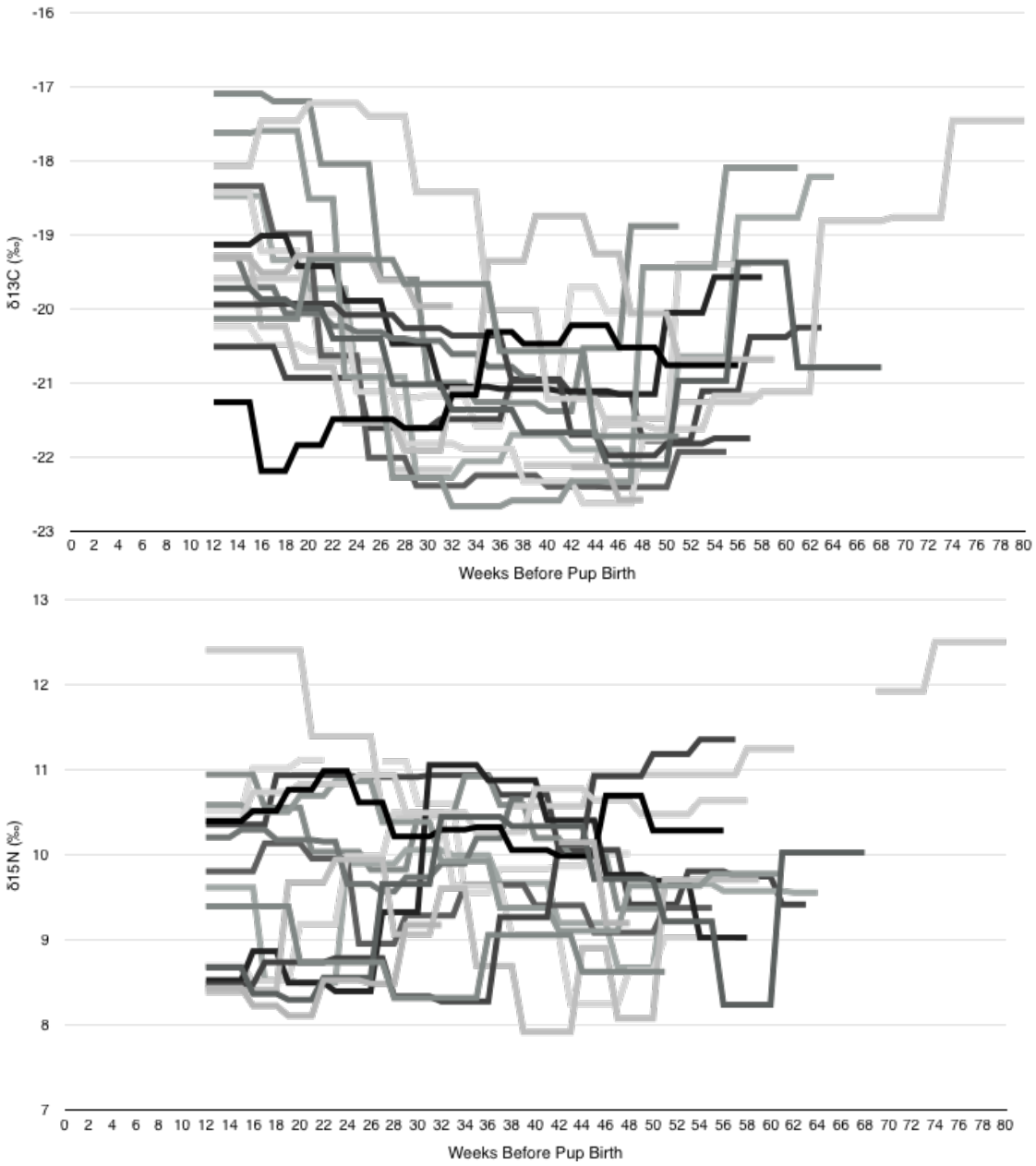

Figure 6a and 6b: Graphs showing all normalized time series of stable carbon (a) and nitrogen (b) isotope values for mother fur seals. These series display the overall seasonal patterns for mothers between the breeding season and non-breeding/migration season for approximately one annual period.

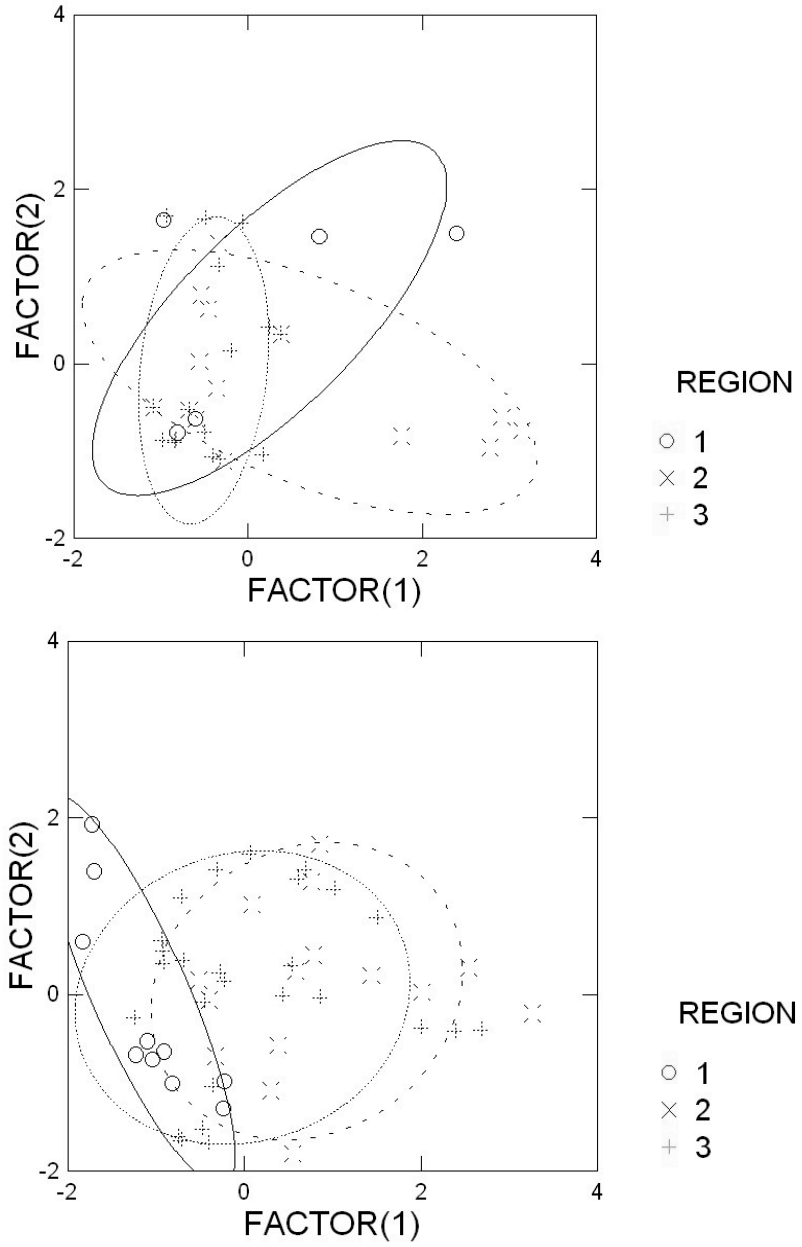

Figure 7: Position of adult female Antarctic fur seals and their separations into 3 foraging/migration regions during the non-breeding season in the southern hemisphere based upon the $\delta^{15}N$ and $\delta^{13}C$ values of whisker segments from a) adult females and b) their pups. Canonical discriminant functions standardized by within-variances comprise Factors 1 and 2. Factor 1 ranges from a) 0.789 for N to 0.832 for C and b) -0.985 for N to 0.476 for C; Factor 2 ranges from a) -0.607 for C to 0.663 for N and b) 0.275 for N to 0.905 for C. Ellipses represent 95% confidence regions around each cluster of points.

TABLES

Table 1: Mean vibrissae growth rates, ranges and standard deviation for various groups. All pup rates refer to growth in utero. Unadjusted growth rates refer only to directly measured tissue from clipped whiskers. Adjusted growth rates include an estimate for subcutaneous tissue left behind when whiskers were clipped. Mean male pup whisker growth rates were faster than females, but the difference was not significant (two-tailed T-test, t = 1.9671, df = 29, p = 0.06).

Group	Mean (mm/week)	±SD (mm/week)	Range (mm/week)	Sample Size
All pups	2.2	0.4	1.5 – 2.6	31
All pups adjusted	2.4	0.3	1.7 – 2.8	31
Male pups	2.3	0.3	1.7 – 2.6	16
Male pups adjusted	2.5	0.3	1.9 – 2.8	16
Female pups	2.1	0.4	1.5 – 2.6	15
Female pups adjusted	2.2	0.4	1.7 – 2.8	15
Female adult	0.5	0.1	0.4 – 0.6	17

Table 2: Table showing mean $\delta^{13}C$ and $\delta^{15}N$ values for mother and pup cohorts with known migration regions. Regions 1, 2, and 3 are defined in Figure 3.

Group	Region	Mean δ 13C (‰)	±SD	Mean δ 15N (‰)	±SD
Mothers	1	-20.3	0.7	10.3	1.5
Mothers	2	-19.3	1.6	9.9	0.8
Mothers	3	-20.4	0.9	9.1	1.0
Pups	1	-20.5	1.3	12.6	0.9
Pups	2	-19.9	1.2	11.1	0.8
Pups	3	-20.2	1.3	11.6	0.9

APPENDIX A

Table 3: Raw δ13C and δ15N data from all mother Antarctic fur seals in the study. Sample number refers to the individual segment from the whisker from the proximal end to the distal end. Pair ID indicates which pup from Table 4 matches with each mother here.

Mother ID	Pair ID	Sample	δ13C (‰)	δ15N (‰)
A03	1	1	-20.13	9.40
A03	1	2	-19.33	8.74
A03	1	3	-19.66	8.32
A03	1	4	-20.57	9.06
A03	1	5	-21.72	8.63
459	2	1	-19.31	8.44
459	2	2	-19.51	8.23
459	2	3	-19.28	8.11
459	2	4	-19.28	8.53
459	2	5	-19.61	8.49
459	2	6	-19.96	9.18
459	2	7	Missing	Missing
459	2	8	Missing	Missing
459	2	9	-22.14	10.15
459	2	10	-22.58	9.20
486	3	1	-19.72	8.68
486	3	2	-19.87	8.37
486	3	3	-19.99	8.30
486	3	4	-20.40	8.56
486	3	5	-21.02	9.66
486	3	6	-21.36	10.45
486	3	7	-21.67	10.34
486	3	8	-22.11	9.72
486	3	9	-20.97	9.22
486	3	10	-19.37	8.24
486	3	11	-20.79	10.03
A05	4	1	-21.26	10.40
A05	4	2	-22.19	10.52
A05	4	3	-21.84	10.77
A05	4	4	-21.49	10.99
A05	4	5	-21.49	10.62
A05	4	6	-21.61	10.22
A05	4	7	-21.16	10.30
A05	4	8	-20.31	10.33
A05	4	9	-20.47	10.06

A05	4	10	-20.22	9.99
A05	4	11	-20.52	10.70
A05	4	12	-20.76	10.29
479	5	1	-18.07	10.42
479	5	2	-17.46	10.74
479	5	3	-17.22	10.84
479	5	4	-17.39	10.94
479	5	5	-18.42	10.50
479	5	6	-20.01	10.27
479	5	7	-21.21	10.78
479	5	8	-21.56	10.65
479	5	9	-21.62	10.48
479	5	10	-21.17	10.64
470	6	1	-19.94	8.48
470	6	2	-19.93	8.74
470	6	3	-20.08	8.79
470	6	4	-20.26	8.34
470	6	5	-20.36	8.28
470	6	6	-20.97	9.27
470	6	7	-21.70	10.13
470	6	8	-21.98	10.93
470	6	9	-21.82	11.19
470	6	10	-21.75	11.36
359	7	1	-17.62	10.59
359	7	2	-17.59	10.51
359	7	3	-18.51	10.70
359	7	4	-20.92	10.87
359	7	5	-22.28	10.39
359	7	6	-22.67	9.93
359	7	7	-22.59	9.38
359	7	8	-22.33	9.11
359	7	9	-19.44	9.64
359	7	10	-18.09	9.78
381	8	1	-19.28	8.39
381	8	2	-20.24	8.42
381	8	3	-20.79	9.68
381	8	4	-21.54	9.95
381	8	5	-21.91	9.07
381	8	6	-21.08	9.61
381	8	7	-19.35	8.70
381	8	8	-18.74	7.92
381	8	9	-19.25	8.91
381	8	10	-20.06	8.08
381	8	11	-20.68	9.71

496	9	1	-19.30	10.21
496	9	2	-19.71	10.30
496	9	3	-20.06	10.18
496	9	4	-20.23	10.16
496	9	5	-20.31	9.66
496	9	6	-20.40	9.57
496	9	7	-20.43	9.74
496	9	8	-20.61	9.90
496	9	9	-20.78	10.20
496	9	10	-20.92	10.66
267	10	1	-19.13	8.53
267	10	2	-19.01	8.87
267	10	3	-19.42	8.50
267	10	4	-19.89	8.40
267	10	5	-20.47	9.33
267	10	6	-21.05	11.06
267	10	7	-21.08	10.88
267	10	8	-21.11	10.41
267	10	9	-21.15	9.77
267	10	10	-20.05	9.70
267	10	11	-19.57	9.03
A01	11	1	-18.43	8.70
A01	11	2	-19.22	8.52
A01	11	3	-20.06	9.19
A01	11	4	-21.12	9.99
A01	11	5	-21.82	10.50
A01	11	6	-21.89	9.64
A01	11	7	-22.33	9.06
A01	11	8	-22.63	8.25
A01	11	9	-21.76	8.65
A01	11	10	-19.40	9.03
452	12	1	-18.34	9.81
452	12	2	-18.98	10.14
452	12	3	-20.63	9.96
452	12	4	-22.01	8.96
452	12	5	-22.39	9.29
452	12	6	-22.25	9.65
452	12	7	-22.40	9.41
452	12	8	-22.41	9.09
452	12	9	-21.93	9.38
408	13	1	-18.47	9.62
408	13	2	-19.34	8.53
408	13	3	-19.73	8.50
408	13	4	-20.92	9.90

408	13	5	-22.28	10.06
408	13	6	-22.06	10.00
408	13	7	-21.70	9.67
408	13	8	-21.90	9.20
408	13	9	-22.15	8.68
408	13	10	-20.64	9.66
408	13	11	-18.76	9.58
408	13	12	-18.21	9.56
482	14	1	-19.59	12.41
482	14	2	-20.70	11.40
482	14	3	-22.17	10.46
482	14	4	Missing	Missing
482	14	5	-22.11	10.58
482	14	6	-21.48	10.95
482	14	7	-21.26	10.95
482	14	8	-21.12	11.25
482	14	9	-18.81	Missing
482	14	10	-18.76	11.93
482	14	11	-17.45	12.51
A09	15	1	-17.09	10.95
A09	15	2	-17.19	10.56
A09	15	3	-18.04	10.04
A09	15	4	-19.60	9.83
A09	15	5	-21.00	10.47
A09	15	6	-21.26	10.93
A09	15	7	-21.27	10.59
A09	15	8	-21.38	10.20
A09	15	9	-20.53	9.99
A09	15	10	-18.88	9.37
400	16	1	-20.51	10.36
400	16	2	-20.93	10.94
400	16	3	-21.61	10.92
400	16	4	-21.49	10.94
400	16	5	-20.95	10.71
400	16	6	-21.15	10.06
400	16	7	-21.78	9.42
400	16	8	-21.11	9.81
400	16	9	-20.38	9.75
400	16	10	-20.25	9.42
A06	17	1	-20.24	10.52
A06	17	2	-20.48	11.02
A06	17	3	-20.57	11.12
A06	17	4	Missing	Missing
A06	17	5	-21.20	11.10

A06	17	6	-21.17	10.61
A06	17	7	-21.58	9.56
A06	17	8	-21.02	9.84
A06	17	9	-19.71	9.87
A06	17	10	-20.04	10.02

Table 4: Raw δ13C and δ15N data from all Antarctic fur seal pups in the study. Sample number refers to the individual segment from the whisker from the proximal end to the distal end. Pair ID indicates which mother from Table 3 matches with each pup here.

Pup ID	Pair ID	Sample	δ13C (‰)	δ15N (‰)
002	1	1	-20.91	11.64
002	1	2	-20.31	11.80
002	1	3	-19.78	10.34
004	2	1	-21.09	12.32
004	2	2	-20.30	12.81
004	2	3	-19.91	12.62
004	2	4	-19.77	12.60
004	2	5	-19.49	12.11
004	2	6	-19.12	11.80
004	2	7	-18.53	12.25
004	2	8	-19.78	10.34
005	3	1	-19.87	12.55
005	3	2	-19.08	12.63
005	3	3	-18.57	12.45
005	3	4	-18.22	12.24
005	3	5	-18.38	11.71
005	3	6	-18.38	11.35
005	3	7	-18.22	11.69
005	3	8	-18.17	11.36
005	3	9	-18.61	10.85
006	4	1	-20.90	12.49
006	4	2	-20.07	12.65
006	4	3	-20.04	12.49
006	4	4	-19.74	12.61
006	4	5	-19.93	12.33
006	4	6	-19.96	11.96
007	5	1	-21.94	11.20
007	5	2	-21.69	11.00
007	5	3	-21.42	11.03
007	5	4	-21.19	10.97
007	5	5	-21.40	10.58
007	5	6	-21.68	10.02

007	5	7	-19.72	10.90
011	6	1	-21.65	11.05
011	6	2	-21.54	10.89
011	6	3	-20.89	11.07
011	6	4	-20.18	11.05
011	6	5	-19.73	10.27
011	6	6	-19.73	9.58
011	6	7	-19.99	9.58
011	6	8	-19.96	9.51
011	6	9	-19.96	9.56
012	7	1	-21.91	10.46
012	7	2	-22.13	10.32
012	7	3	-20.51	11.39
012	7	4	-19.03	11.89
012	7	5	-18.25	11.89
012	7	6	-18.06	11.50
012	7	7	-18.06	11.49
013	8	1	-21.81	11.06
013	8	2	-21.85	10.49
013	8	3	-21.55	10.82
013	8	4	-20.44	11.34
013	8	5	-18.91	12.16
013	8	6	-17.82	12.98
013	8	7	-17.67	12.46
015	9	1	-22.39	11.53
015	9	2	-22.35	11.57
015	9	3	-22.33	11.28
015	9	4	-22.15	11.40
015	9	5	-21.53	11.51
015	9	6	-20.02	11.59
015	9	7	-19.58	11.37
018	10	1	-20.41	11.96
018	10	2	-20.05	11.88
018	10	3	-20.03	11.31
018	10	4	-19.91	10.98
018	10	5	-19.98	10.48
018	10	6	-19.94	9.97
018	10	7	-19.86	9.66
018	10	8	-19.74	9.44
019	11	1	-22.14	10.51
019	11	2	-21.37	10.99
019	11	3	-20.72	11.13
019	11	4	-19.65	11.31
019	11	5	-19.36	11.23

019	11	6	-19.42	10.64
019	11	7	-19.45	10.13
019	11	8	-18.94	9.84
019	11	9	-19.32	9.08
020	12	1	-22.10	10.32
020	12	2	-20.25	11.61
020	12	3	-18.83	11.99
023	13	1	-21.67	11.85
023	13	2	-21.34	11.41
023	13	3	-20.83	12.04
023	13	4	-20.11	12.23
023	13	5	-19.47	12.59
023	13	6	-18.89	12.47
023	13	7	-18.18	12.84
023	13	8	-17.63	13.22
023	13	9	-17.57	12.92
023	13	10	-17.62	12.90
027	14	1	-21.40	12.14
027	14	2	-21.25	12.08
027	14	3	-21.42	11.42
027	14	4	-21.80	11.31
027	14	5	-21.65	11.86
027	14	6	-21.40	12.31
027	14	7	-21.17	12.27
027	14	8	-20.96	12.72
027	14	9	-20.06	13.28
027	14	10	-19.06	13.50
027	14	11	-18.43	13.73
028	15	1	-21.11	11.62
028	15	2	-20.37	11.93
028	15	3	-20.14	12.11
028	15	4	-19.54	11.94
028	15	5	-18.90	11.99
028	15	6	-18.37	11.58
028	15	7	-17.82	11.68
030	16	1	-21.54	11.89
030	16	2	-20.98	12.04
030	16	3	-20.77	12.10
030	16	4	-20.57	12.19
030	16	5	-20.46	11.98
030	16	6	-20.36	11.75
030	16	7	-20.30	10.85
030	16	8	-19.94	10.52
030	16	9	-19.55	9.97

030	16	10	-20.22	10.24
030	16	11	-20.70	10.45
030	16	12	-21.22	11.48
NA	17	1	-21.54	11.63
NA	17	2	-20.75	12.21
NA	17	3	-20.72	12.22
NA	17	4	-20.63	12.17
NA	17	5	-20.51	11.96
NA	17	6	-20.42	12.00
NA	17	7	-20.33	11.02
NA	17	8	-20.28	10.71
NA	17	9	-20.02	10.26
NA	17	10	-20.10	10.10
NA	17	11	-20.48	10.30

APPENDIX B

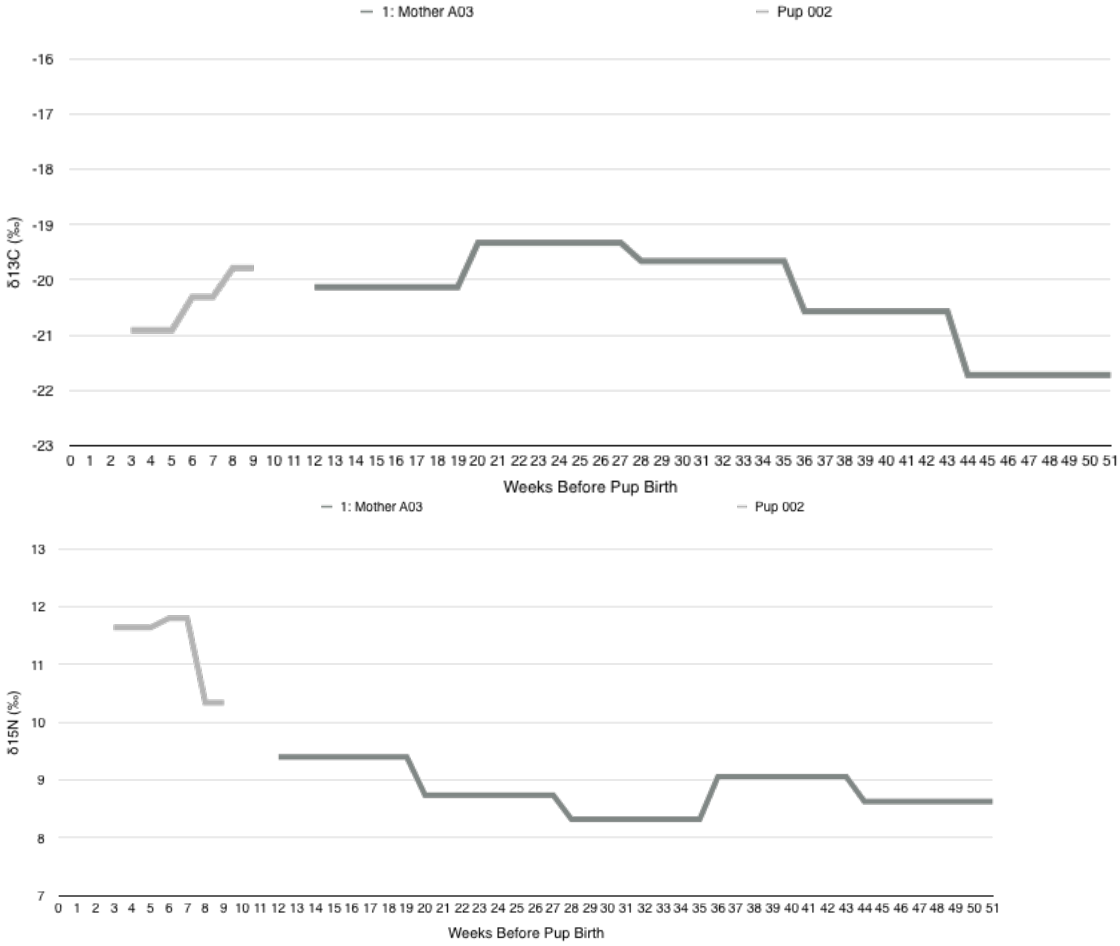

Figure 8: An example of a normalized time series of stable carbon and nitrogen isotope values for mother-pup pairs of Antarctic fur seals. These time series are from mother-pup pair 1. The time series do not overlap because both the mother and pup's whiskers were cut, leaving behind the length that would reflect the isotope values from the time period immediately prior to parturition (see Methods). Week zero on the x-axis marks the pup's birth.

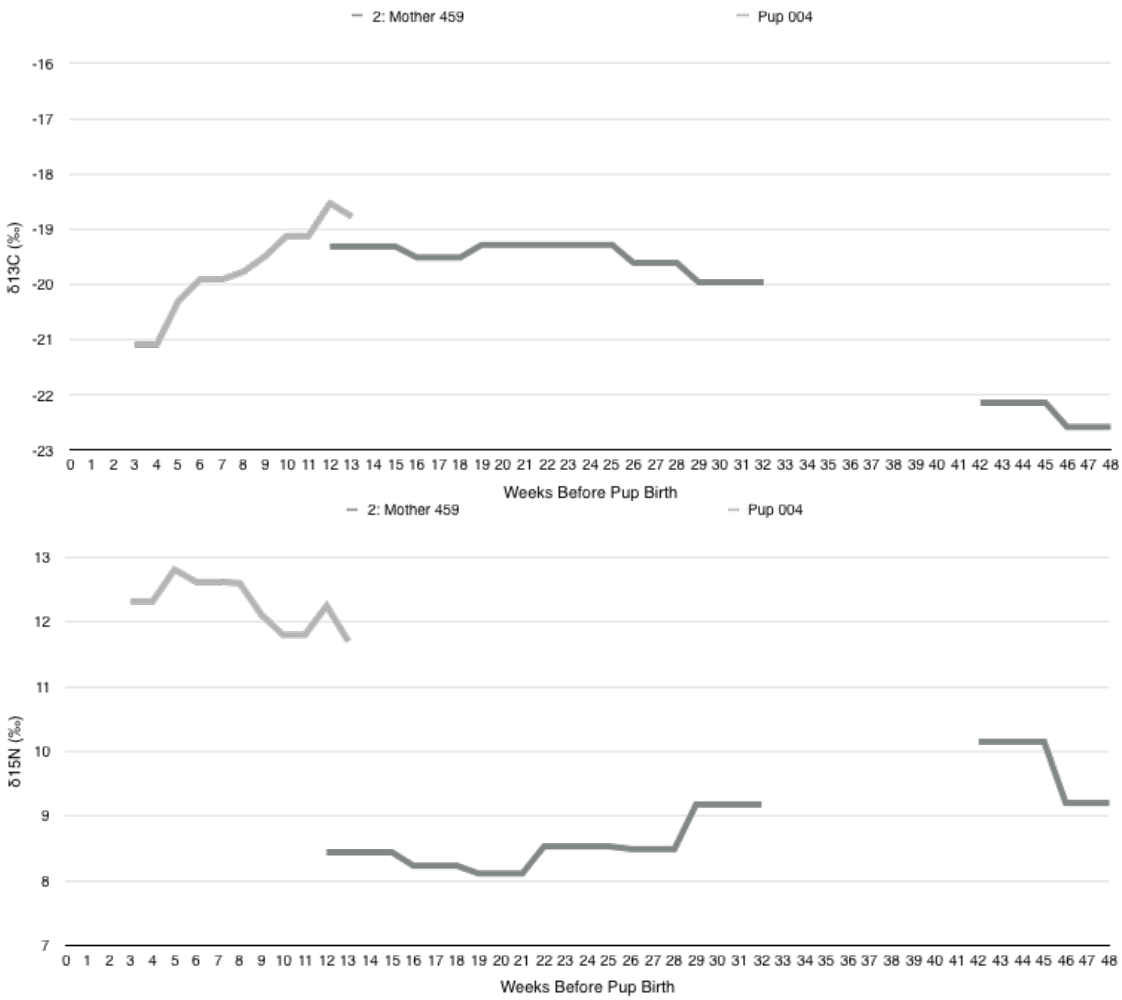

Figure 9: An example of a normalized time series of stable carbon and nitrogen isotope values for mother-pup pairs of Antarctic fur seals. These time series are from mother-pup pair 2. The time series do not overlap because both the mother and pup's whiskers were cut, leaving behind the length that would reflect the isotope values from the time period immediately prior to parturition (see Methods). Week zero on the x-axis marks the pup's birth.

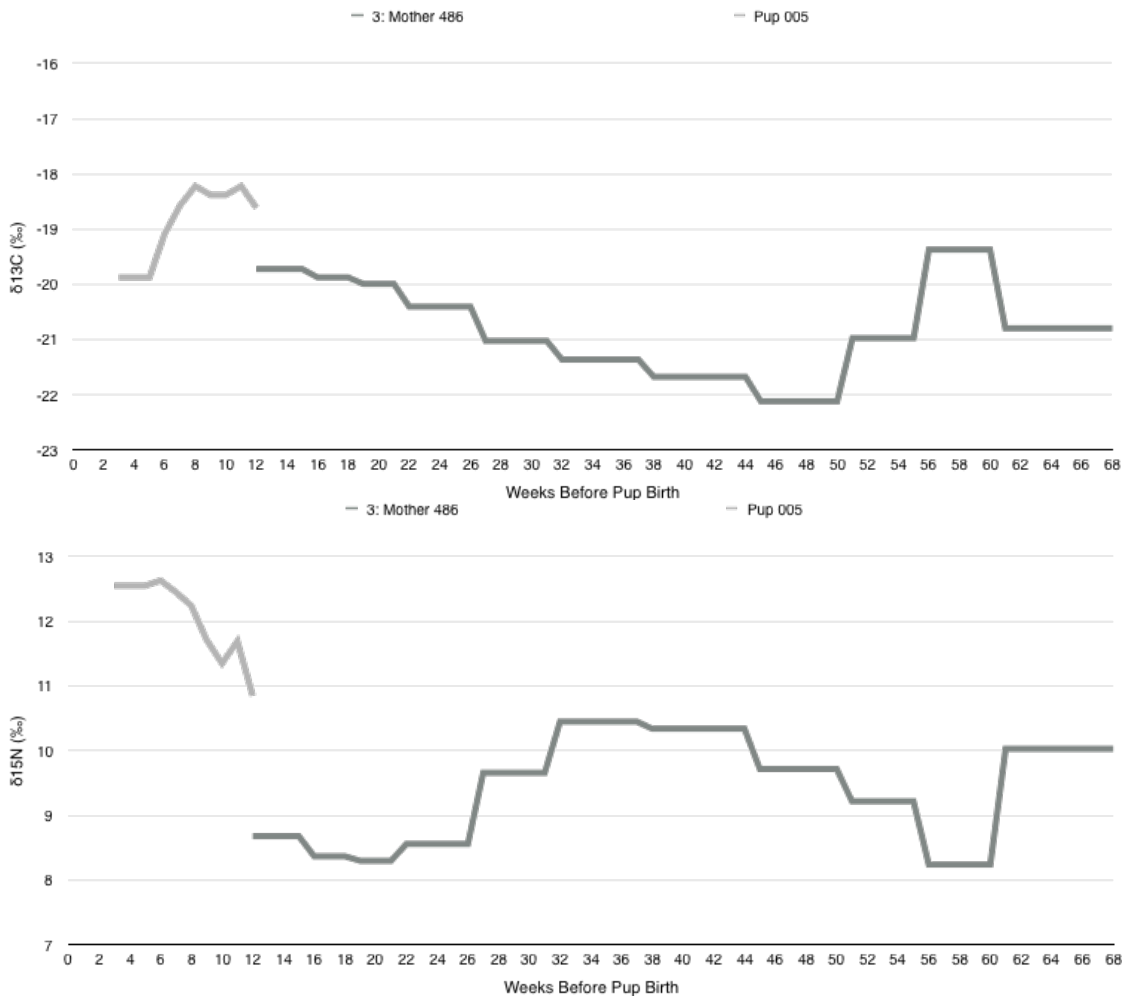

Figure 10: An example of a normalized time series of stable carbon and nitrogen isotope values for mother-pup pairs of Antarctic fur seals. These time series are from mother-pup pair 3. The time series do not overlap because both the mother and pup's whiskers were cut, leaving behind the length that would reflect the isotope values from the time period immediately prior to parturition (see Methods). Week zero on the x-axis marks the pup's birth.

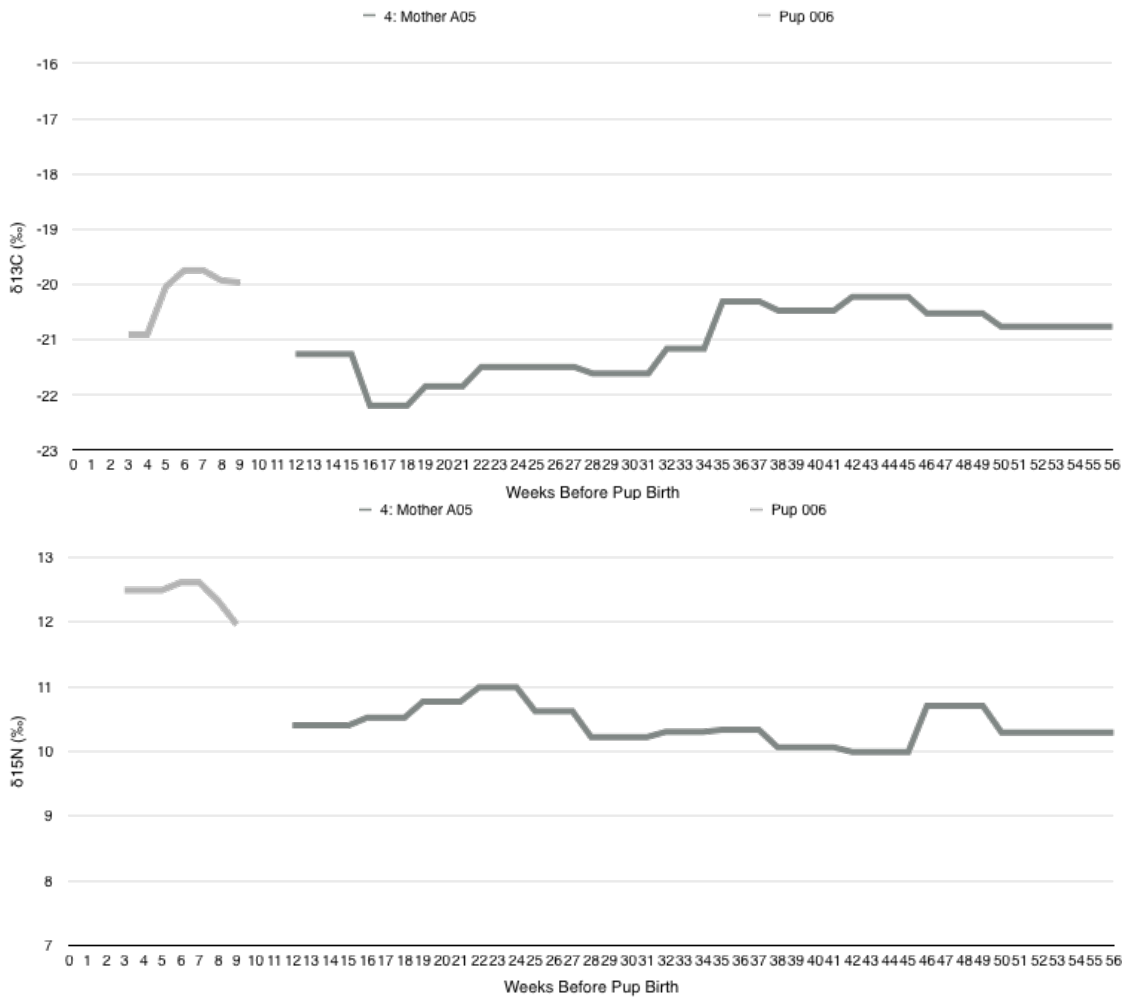

Figure 11: An example of a normalized time series of stable carbon and nitrogen isotope values for mother-pup pairs of Antarctic fur seals. These time series are from mother-pup pair 4. The time series do not overlap because both the mother and pup's whiskers were cut, leaving behind the length that would reflect the isotope values from the time period immediately prior to parturition (see Methods). Week zero on the x-axis marks the pup's birth.

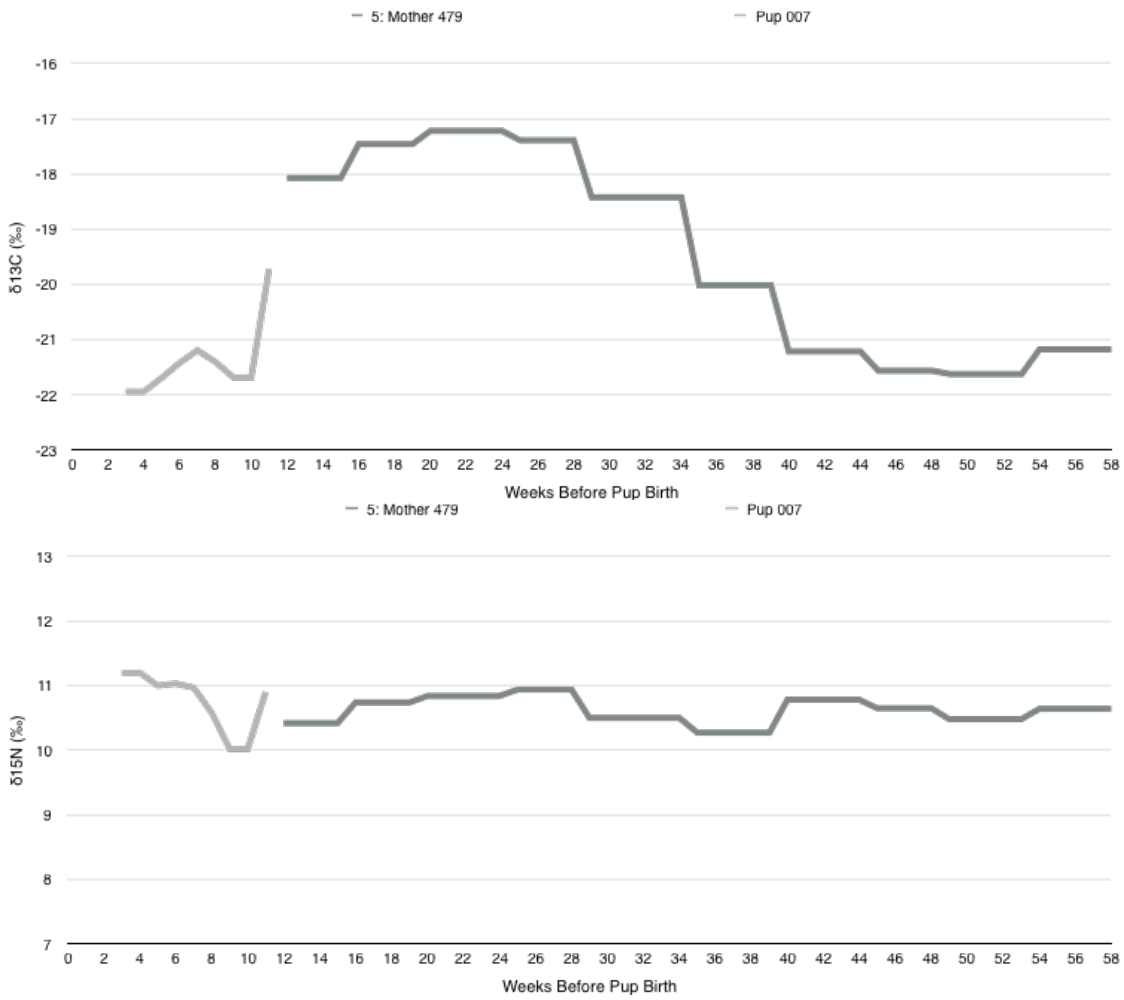

Figure 12: An example of a normalized time series of stable carbon and nitrogen isotope values for mother-pup pairs of Antarctic fur seals. These time series are from mother-pup pair 5. The time series do not overlap because both the mother and pup's whiskers were cut, leaving behind the length that would reflect the isotope values from the time period immediately prior to parturition (see Methods). Week zero on the x-axis marks the pup's birth.

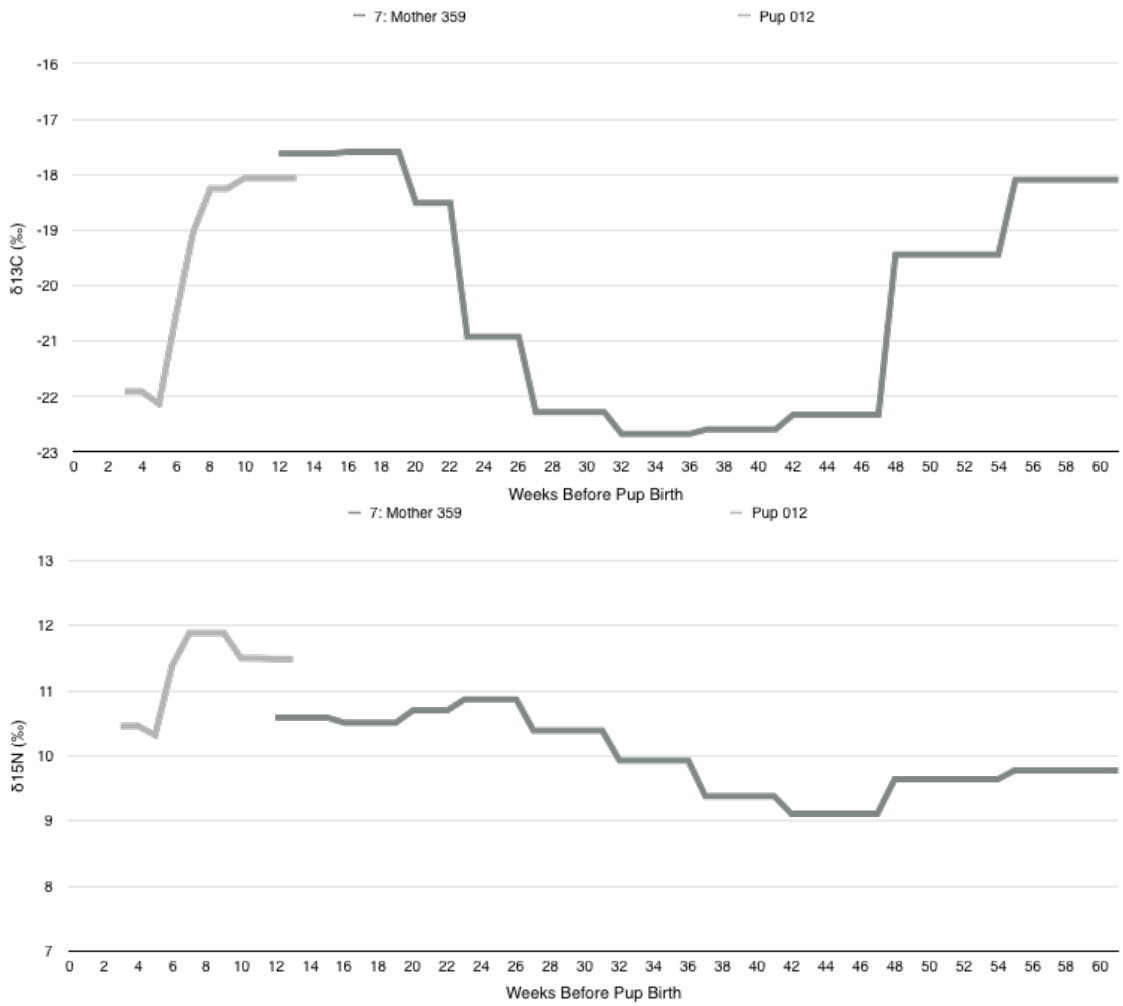

Figure 13: An example of a normalized time series of stable carbon and nitrogen isotope values for mother-pup pairs of Antarctic fur seals. These time series are from mother-pup pair 7. The time series do not overlap because both the mother and pup's whiskers were cut, leaving behind the length that would reflect the isotope values from the time period immediately prior to parturition (see Methods). Week zero on the x-axis marks the pup's birth.

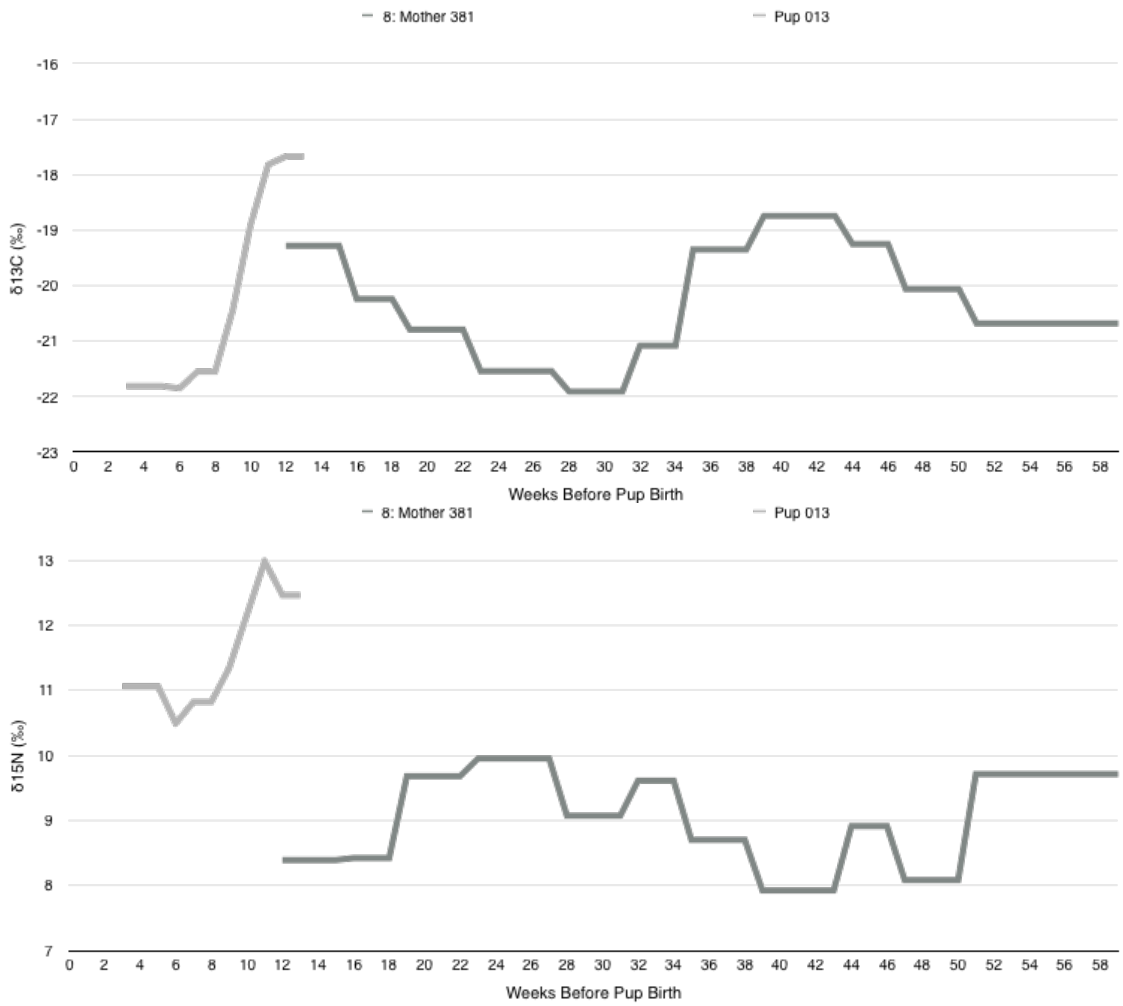

Figure 14: An example of a normalized time series of stable carbon and nitrogen isotope values for mother-pup pairs of Antarctic fur seals. These time series are from mother-pup pair 8. The time series do not overlap because both the mother and pup's whiskers were cut, leaving behind the length that would reflect the isotope values from the time period immediately prior to parturition (see Methods). Week zero on the x-axis marks the pup's birth.

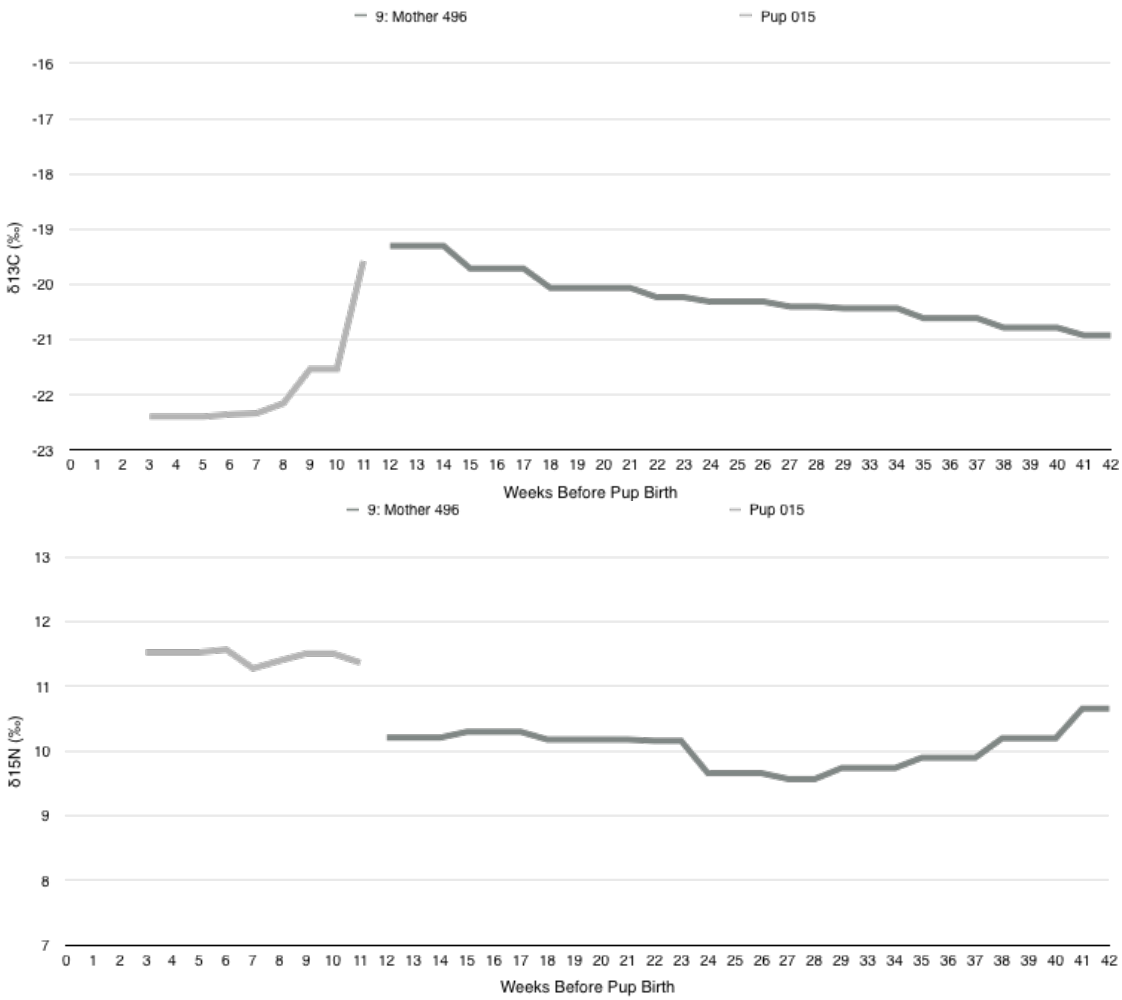

Figure 15: An example of a normalized time series of stable carbon and nitrogen isotope values for mother-pup pairs of Antarctic fur seals. These time series are from mother-pup pair 9. The time series do not overlap because both the mother and pup's whiskers were cut, leaving behind the length that would reflect the isotope values from the time period immediately prior to parturition (see Methods). Week zero on the x-axis marks the pup's birth.

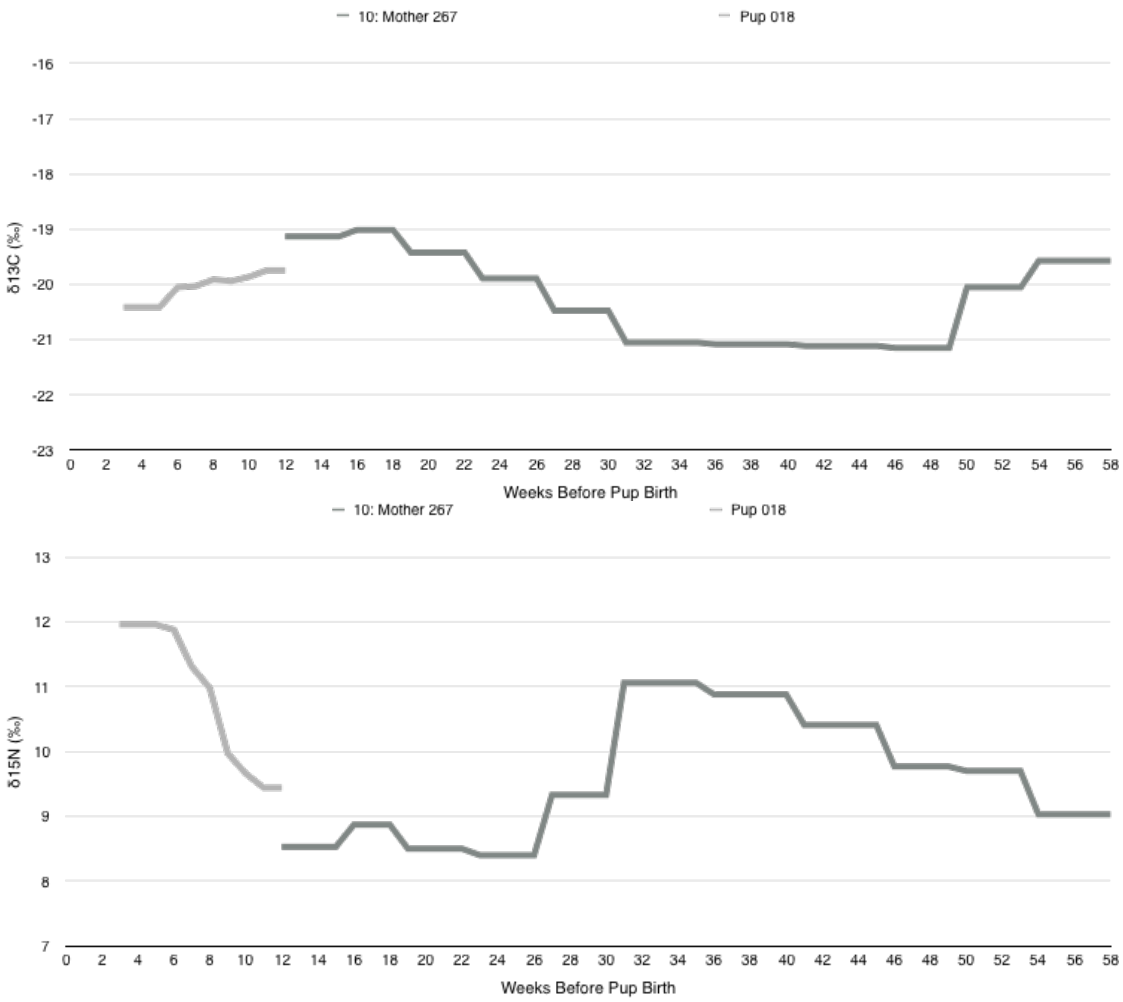

Figure 16: An example of a normalized time series of stable carbon and nitrogen isotope values for mother-pup pairs of Antarctic fur seals. These time series are from mother-pup pair 10. The time series do not overlap because both the mother and pup's whiskers were cut, leaving behind the length that would reflect the isotope values from the time period immediately prior to parturition (see Methods). Week zero on the x-axis marks the pup's birth.

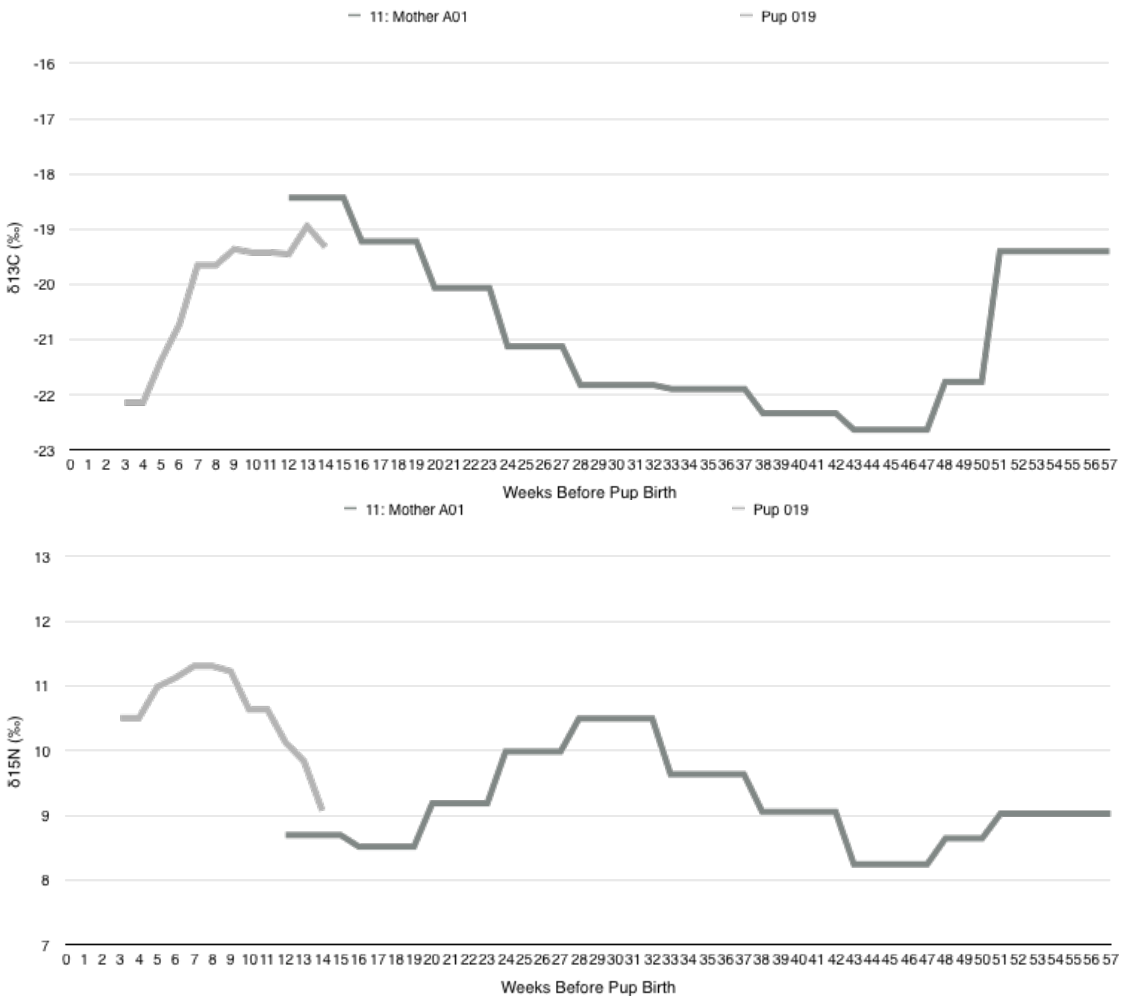

Figure 17: An example of a normalized time series of stable carbon and nitrogen isotope values for mother-pup pairs of Antarctic fur seals. These time series are from mother-pup pair 11. Week zero on the x-axis marks the pup's birth.

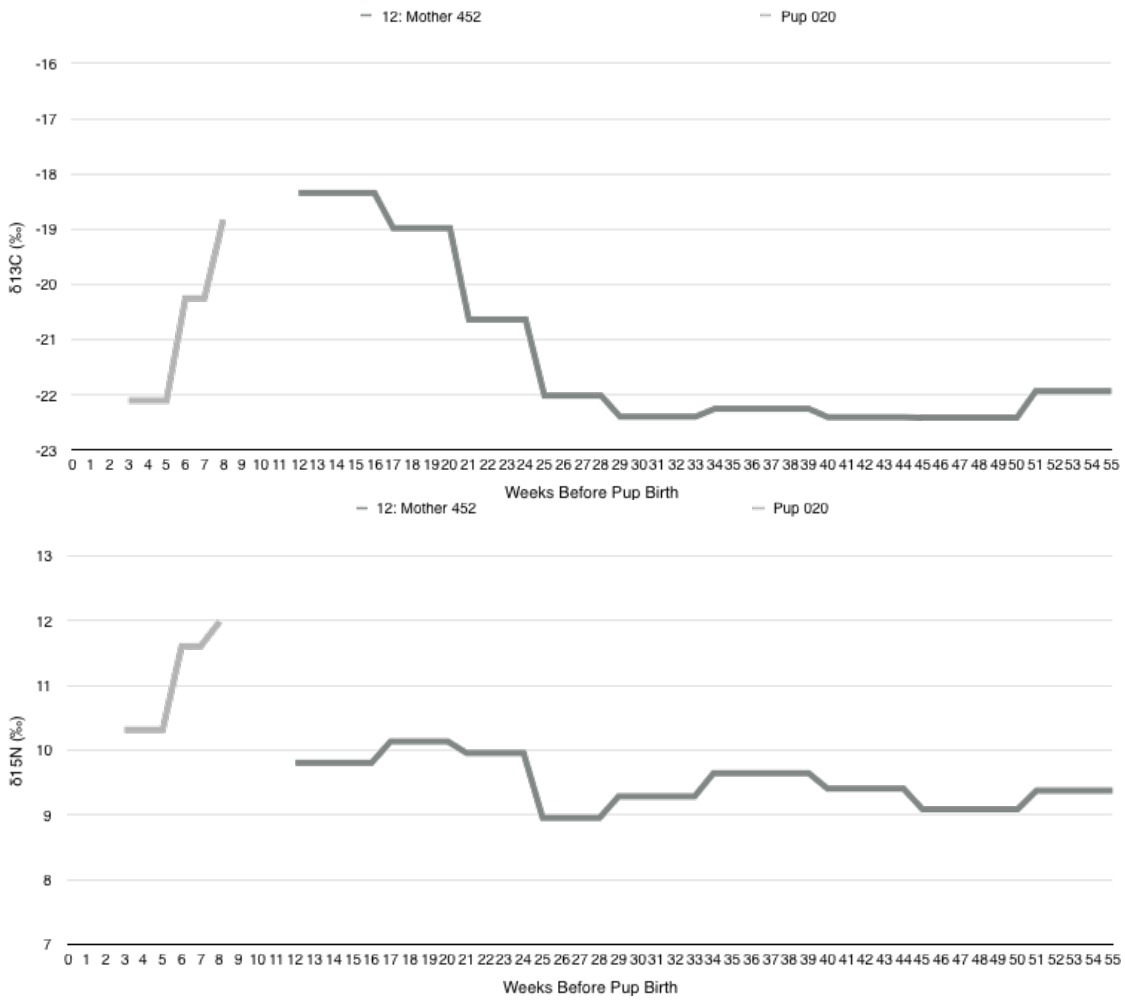

Figure 18: An example of a normalized time series of stable carbon and nitrogen isotope values for mother-pup pairs of Antarctic fur seals. These time series are from mother-pup pair 12. The time series do not overlap because both the mother and pup's whiskers were cut, leaving behind the length that would reflect the isotope values from the time period immediately prior to parturition (see Methods). Week zero on the x-axis marks the pup's birth.

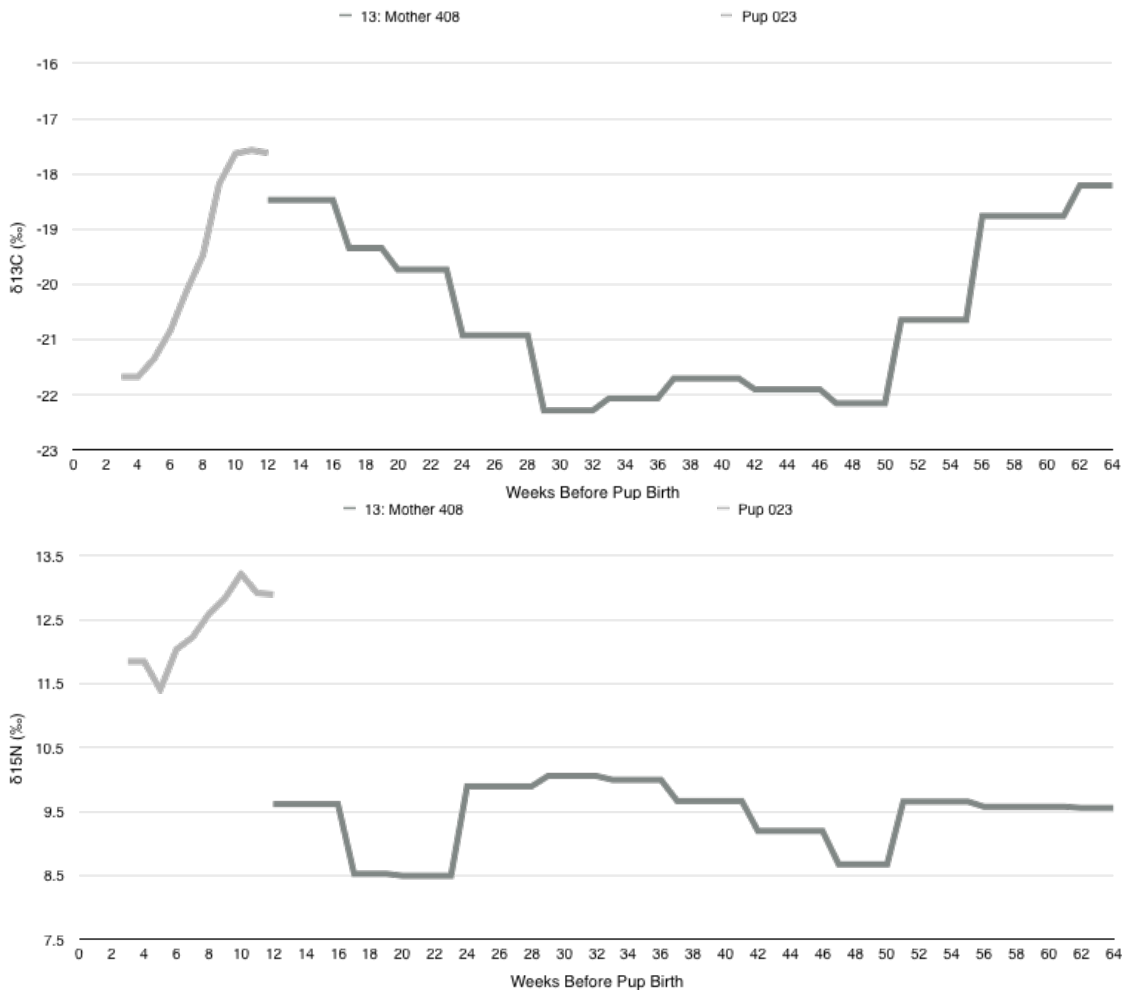

Figure 19: An example of a normalized time series of stable carbon and nitrogen isotope values for mother-pup pairs of Antarctic fur seals. These time series are from mother-pup pair 13. The time series do not overlap because both the mother and pup's whiskers were cut, leaving behind the length that would reflect the isotope values from the time period immediately prior to parturition (see Methods). Week zero on the x-axis marks the pup's birth.

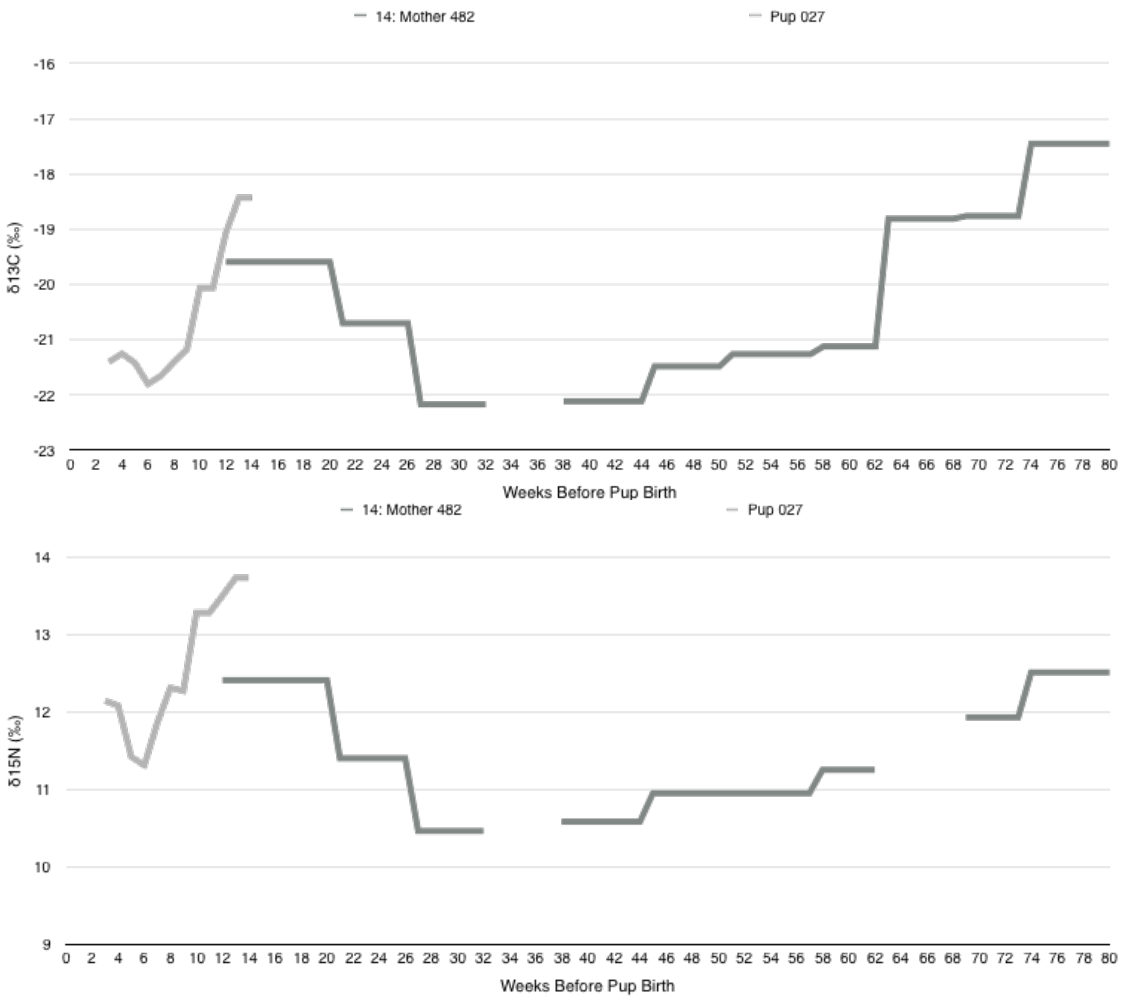

Figure 20: An example of a normalized time series of stable carbon and nitrogen isotope values for mother-pup pairs of Antarctic fur seals. These time series are from mother-pup pair 14. The time series do not overlap because both the mother and pup's whiskers were cut, leaving behind the length that would reflect the isotope values from the time period immediately prior to parturition (see Methods). Week zero on the x-axis marks the pup's birth.

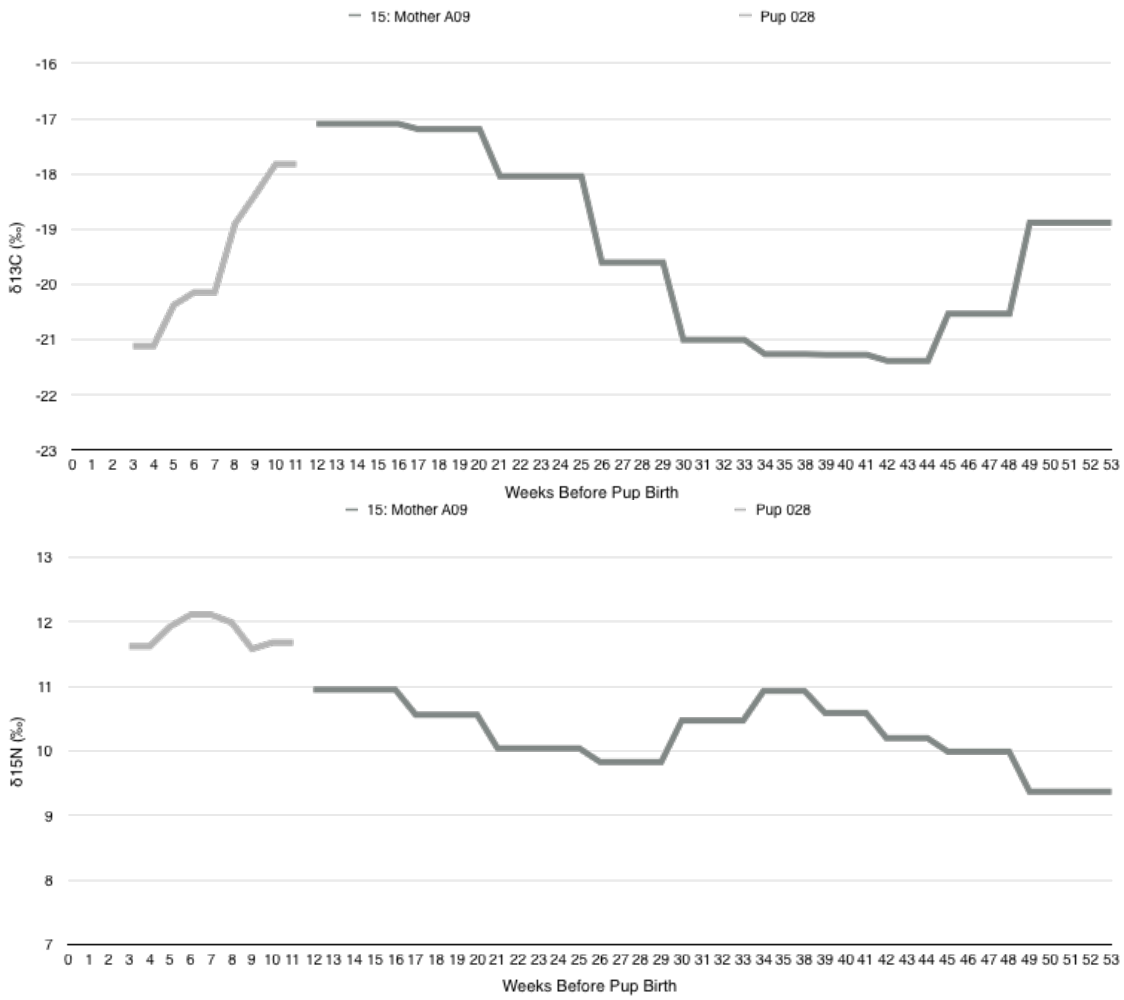

Figure 21: An example of a normalized time series of stable carbon and nitrogen isotope values for mother-pup pairs of Antarctic fur seals. These time series are from mother-pup pair 15. The time series do not overlap because both the mother and pup's whiskers were cut, leaving behind the length that would reflect the isotope values from the time period immediately prior to parturition (see Methods). Week zero on the x-axis marks the pup's birth.

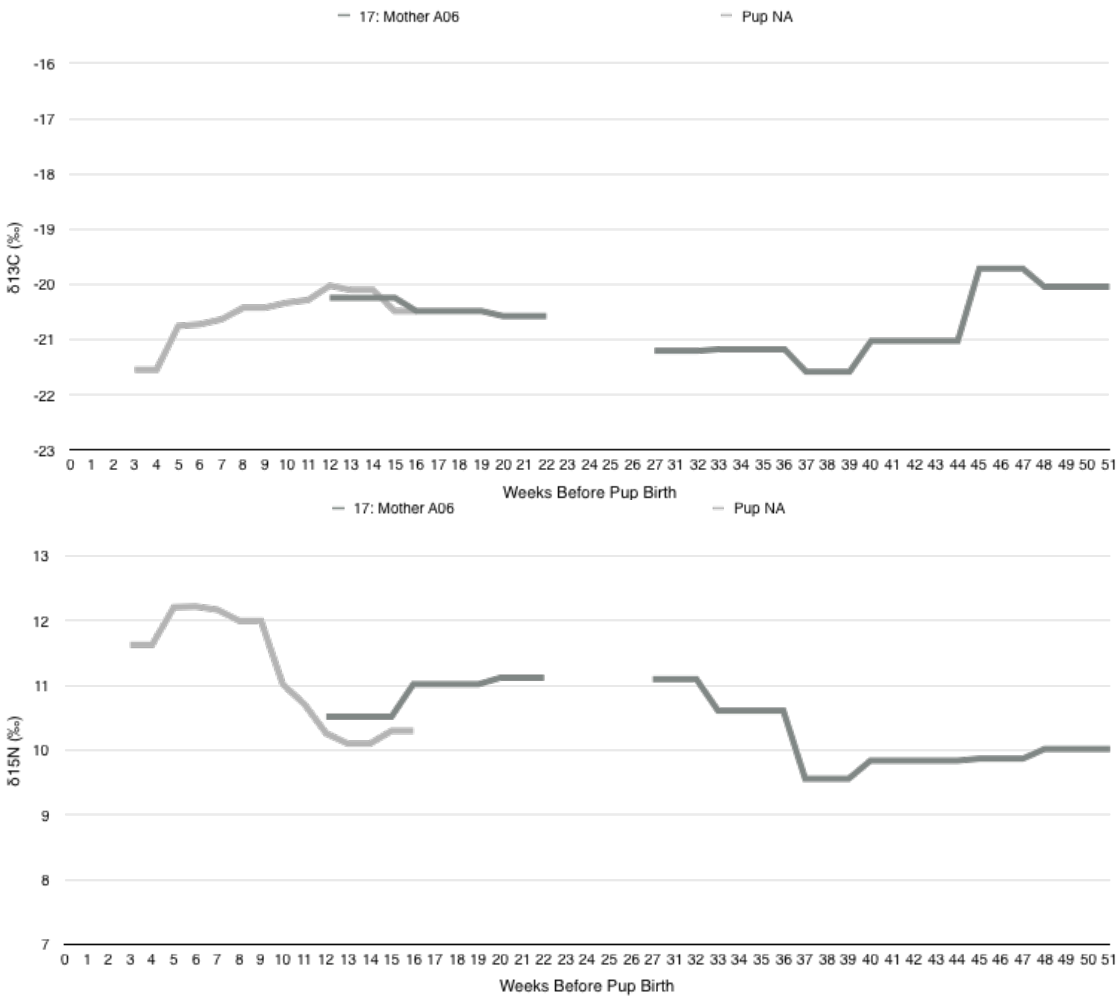

Figure 22: An example of a normalized time series of stable carbon and nitrogen isotope values for mother-pup pairs of Antarctic fur seals. These time series are from mother-pup pair 17. Week zero on the x-axis marks the pup's birth.

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