Can the carbon and nitrogen isotope values of offspring be used as a proxy for their mother’s diet? Using foetal physiology to interpret bulk tissue and amino acid $\delta^{15}$N values

Nico Lübcker$^{1,*}$, John P. Whiteman$^{2,3}$, Seth D. Newsome$^{3}$, Robert P. Millar$^{4,5}$, P. J. Nico de Bruyn$^{1}$

$^1$Department of Zoology and Entomology, Mammal Research Institute, University of Pretoria, Private Bag X20, Hatfield, Pretoria, 0028, South Africa
$^2$Department of Biological Sciences, Old Dominion University, 5115 Hampton Boulevard, Norfolk, VA, 23529, USA
$^3$Department of Biology, University of New Mexico, Albuquerque, NM, 87131, USA
$^4$Centre for Neuroendocrinology and Department of Immunology, Faculty of Health Sciences, University of Pretoria, Pretoria, 0001, South Africa
$^5$Department of Integrative Biomedical Sciences, Neurosciences Institute and Institute of Infectious Diseases and Molecular Medicine, University of Cape Town, Anzio Road, Observatory 7925, South Africa

*Corresponding author: Department of Zoology and Entomology, Mammal Research Institute, University of Pretoria, Private Bag X20, Hatfield, Pretoria, 0028, South Africa. Email: nlubcker@zoology.up.ac.za

The measurement of bulk tissue nitrogen ($\delta^{15}$N) and carbon isotope values ($\delta^{13}$C) chronologically along biologically inert tissues sampled from offspring can provide a longitudinal record of their mothers’ foraging habits. This study tested the important assumption that mother–offspring stable isotope values are positively and linearly correlated. In addition, any change in the mother–offspring bulk tissues and individual amino acids that occurred during gestation was investigated. Whiskers sampled from southern elephant seal pups (Mirounga leonina) and temporally overlapping whiskers from their mothers were analyzed. This included $n = 1895$ chronologically subsampled whisker segments for bulk tissue $\delta^{15}$N and $\delta^{13}$C in total and $n = 20$ whisker segments for amino acid $\delta^{15}$N values, sampled from recently weaned pups ($n = 17$), juvenile southern elephant seals (SES) < 2 years old ($n = 23$) and adult female SES ($n = 17$), which included nine mother–offspring pairs. In contrast to previous studies, the mother–offspring pairs were not in isotopic equilibrium or linearly correlated during gestation: the $\Delta^{15}$N and $\Delta^{13}$C mother–offspring offsets increased by 0.8 and 1.2‰, respectively, during gestation. The foetal bulk $\delta^{15}$N values were $1.7 \pm 0.5$‰ ($0.9–2.7$‰) higher than mothers’ $\delta^{15}$N values before birth, while the foetal $\delta^{13}$C increased by $\sim 1.7$‰ during gestation and were $1.0 \pm 0.5$‰ ($0.0–1.9$‰) higher than their mothers’ $\delta^{13}$C at the end of pregnancy. The mother–offspring serine and glycine $\Delta^{15}$N differed by $\sim 4.3$‰, while the foetal alanine $\delta^{15}$N values were $1.4$‰ lower than that of their mothers during the third trimester of pregnancy. The observed mother–offspring $\delta^{15}$N differences are likely explained by shuttling of glutamate–glutamine and glycine–serine amongst skeletal muscle, liver, placenta and foetal tissue. Foetal development relies primarily on remobilized endogenous maternal proteinaceous sources. Researchers should consider foetal physiology when using offspring bulk tissue isotope values as biomarkers for the mother’s isotopic composition as part of monitoring programmes.

**Key words:** Amino acid–specific stable isotopes, intrauterine, marine mammals, mother–offspring pairs, nutrition, whiskers

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Introduction

The interaction between the nutritional ecology and reproduction of organisms regulates their population dynamics (e.g. Gardner and Grafen, 2009; Bergman et al., 2019). Female fecundity often controls population growth rates (Pistorius et al., 1999; Gardner and Grafen, 2009; Birkhofer et al., 2017), and female fitness is highly correlated with diet composition (McMahon et al., 2000; Donnelly et al., 2003). However, traditional methods for dietary analyses, such as stomach lavage and scat analysis, only provide a ‘snapshot’ of the most recently ingested prey (Tollit et al., 2007, 2015; McNee et al., 2016; Roslin and Majaneva, 2016) and are often confounded by varied retention of diagnostic prey remains and an inability to account for spatial and temporal dietary variations. Sampling approaches that can provide longitudinal dietary data are required and are especially insightful for species with complex life histories that include extensive movement between foraging and breeding grounds (Young et al., 2015; Nielsen et al., 2018). However, such approaches must consider that the capture and handling of free-ranging large animals for dietary investigations are notoriously challenging.

Indirect biochemical analyses of non-lethally sampled tissues are increasingly utilized for longitudinal, retrospective studies of animal ecology and physiology (Newsome et al., 2010; Pauli et al., 2010; Trumble et al., 2018). Specifically, the carbon ($^{13}$C) and nitrogen ($^{15}$N) stable isotope values of animal tissues are particularly useful for reconstructing diet composition as well as inferring broad-scale patterns in habitat use and movement (Newsome et al., 2010; Ohkouchi et al., 2017; Pagani-Núñez et al., 2017). $^{13}$C and $^{15}$N values of a consumers’ tissue(s) reflect its diet but are offset in a predictable fashion due to physiologically mediated processes associated with nutrient assimilation and excretion (DeNiro and Epstein, 1976). For example, isotopically heavier $^{15}$N is preferentially retained in the whole-body nitrogen pool because deamination of amino acids tends to remove $^{14}$N that is excreted via urine and faeces (DeNiro and Epstein, 1976; Post, 2002). Such diet-to-tissue isotopic offsets ($\Delta^{15}$N$_{diet-tissue}$ or $\Delta^{15}$N$_{diet-tissue}$) are commonly called trophic discrimination factors (TDFs) and are required for the use of mixing models to quantify dietary inputs (Parnell et al., 2013).

In comparison to adults, offspring can often be handled more readily and safely (Jenkins et al., 2001) and as such are regularly targeted for isotopic analysis (Pagani-Núñez et al., 2017). It is safe to assume that the tissues of offspring sampled shortly after parturition were synthesized in utero (intrauterine synthesized tissues; Lowther et al., 2013). Paired mother–offspring stable isotope values of both income and capital breeders are often assumed to be either in isotopic equilibrium or positively correlated (e.g. Jenkins et al., 2001; Table 1 and S1). As such, the isotopic composition of tissue sampled from neonates or nursing offspring are widely used to infer the diet and foraging habitats of their mothers (e.g. Table 1). Shortly after birth, the isotopic values of offspring tissues begin to equilibrate with maternal milk, resulting in increased $^{15}$N values ($\sim +0.3$ to $+3.0\%$; Fogel, 1989; Newsome et al., 2006; De Luca et al., 2012). Patterns in $^{13}$C values between nursing offspring and their mothers are more variable and can be either positive or negative depending on the lipid content of maternal milk (Table 1 and S1). Although species-specific mother–offspring isotopic discrimination can occur during both gestation and lactation (Table 1), few studies have considered if or how such discrimination changes as gestation and lactation progress (but see Stricker et al., 2015; Habran et al., 2019). Moreover, studies validating this approach relied on a single, cross-sectional sampling approach focused on offspring that were wholly dependent on maternal milk for their nutrition (Dalerum et al., 2007; Drago et al., 2010; De Luca et al., 2012; but see Hindell et al., 2012; Table S1).

Foetal nutritional demands may change as gestation progresses (Lindsay et al., 2015), and therefore, the assumption that mother–offspring isotopic offsets remain constant throughout pregnancy is unlikely. However, when using continuously growing, metabolically inert keratinous tissues such as whiskers or baleen to chronologically analyze the trophic ecology of mammals, it is assumed that TDFs remain constant during the period (months to years) of tissue synthesis (Lowther and Goldsworthy, 2011; Stricker et al., 2015). To our knowledge, the only measurements available for mother–offspring whisker isotopic offsets are for whiskers of income-breeding pinnipeds, including Australian (Neophoca cinerea; Lowther and Goldsworthy, 2011) and Stellar (Eumetopias jubatus; Stricker et al., 2015) sea lions. For sea lion pup whiskers grown during gestation, studies show negligible differences in $\Delta^{13}$C between mother and pup, but a slight and significant increase in foetus $\Delta^{15}$N values ($\sim 0.8\%$) relative to their mothers (Lowther and Goldsworthy, 2011; Stricker et al., 2015). For sea lion pup whiskers grown while nursing, mean mother–offspring $\Delta^{15}$N increased to $\sim 1.6\%$ (Stricker et al., 2015). The $\Delta^{15}$N and $\Delta^{13}$C values of blood sampled from phocid offspring, such as southern elephant seals (SES; Mirounga leonina) and northern elephant seals (M. angustirostris), have similarly been used to reconstruct the maternal trophic ecology (Ducatez et al., 2008; Habran et al., 2010, 2019). Until now, the possibility that mother–offspring isotopic offsets might change during the 7–9-month gestation period has not been investigated. Coupling isotope analysis of bulk keratin tissues and their constituent amino acids (Whiteman et al., 2019) could provide information on the maternal resource pool that supports foetal development as gestation progresses.

This study evaluates the assumption that bulk tissue $\Delta^{13}$C and $\Delta^{15}$N values measured along the length of phocid pup whiskers grown in utero predictably reflect the isotopic composition of their mothers (e.g. Lerner et al., 2018). Variation in $\Delta^{13}$C and $\Delta^{15}$N values of subsampled whiskers collected from recently weaned SES pups ($\sim 23$-day-old) and juvenile SES (<2-year-old) were used to isolate the por-
tions of the whisker grown during gestation, which was compared with similar segments of adult female whiskers to provide isotopic signatures between unpaired mother–offspring samples. Paired mother–offspring whisker samples were used to describe individual and temporal differences in the mother–offspring bulk tissue isotopic discrimination as well as patterns in the δ13C and δ15N values of individual amino acids in the whiskers. Our final objective was to assess changes in longitudinal amino acid isotopic signatures in the endogenous resource pool that supports foetal development. Few studies have analyzed concurrently synthesized tissues from both the mother and offspring (Borrell et al., 2016), and our study represents the first combined bulk tissue and amino acid isotope approach to investigate the resource pool contributing to foetal development, explore foetal amino acid metabolism and provide mechanistic explanations between mother and offspring isotopic offsets in SES.

Materials and methods

Study area and whisker collection

Whiskers from recently weaned SES pups (n = 17), juveniles (n = 23) and adult female SES (>3-year-old; n = 17) were cut as close to the skin as possible. All samples were collected from individually identified SESs (flipper tags) on Marion Island (46.7731° S, 37.8525° E) in the Southern Ocean (Pistorius et al., 2011). Sampling procedures and chemical immobilization techniques used on SES are detailed in Lübcker et al., (2017) and Bester (1988).

Pup whisker growth rates

Segments of SES pup whiskers that reflected growth in utero were identified. Foetal whiskers of SES are known to be ~10 mm long at 60-day post-conception when blastocyst implantation occurs at the end of the female pelage molt (Ling, 1966). A ~12-mm whisker segment remained after the initial whisker were removed from pups <2 days after weaning during the breeding seasons of October 2009–2014. This embedded whisker segment was grown during the ~21–23-day SES lactation period (Fig. S1; Lübcker et al., 2016). Regrown ex utero whisker growth sampled from SES juveniles (n = 17) was used to identify the lactation period. These whisker ‘regrowths’ were 68.7 ± 13.8 mm long and were collected during the annual molt in January 2013 and 2014 (Lübcker et al., 2016). Isotope values of whisker regrowths and (previously unsampled) fully grown whiskers collected from juvenile SES (<2 years old; n = 10; 102.0 ± 26.8 mm long) collected after these individual SES spent a year at sea (Fig. S2) were used to confirm that no portion of the whiskers grown ex utero was included when assessing the in utero mother–offspring whisker isotopic offsets.

Identifying segments of adult female whiskers reflecting gestation

Isolating the adult SES female whisker segments reflecting gestation, and subsequently aligning these segments with the foetal (intrauterine) whisker growth, requires detailed information about the adult female whisker growth rate and history (e.g. McHuron et al., 2019). The segments of the adult female whiskers grown on land while fasting can be identified based on the chronology of both bulk tissue and amino acid δ15N values (McHuron et al., 2019; Lübcker et al., 2020). The fasting-enriched δ15N values of adult female whiskers start declining at the onset of the post-molt foraging period, which were assumed to overlap with the delayed blastocyst implantation that occurs during the molt, although the timing of blastocyst implantation can be variable (Ling, 2013; McHuron et al., 2019; Lübcker et al., 2020). The whisker segments of the same n = 17 breeding adult females were used to confirm that no portion of the whiskers grown ex utero was included when assessing the in utero mother–offspring whisker isotopic offsets.

Table 1: Differences between the mother–offspring bulk tissue δ15N and δ13C values measured in offspring tissue (whiskers, whole blood, serum/plasma, red blood cells, skin biopsy, hair; studies of fossilized material are excluded) of 31 mammal species in 25 studies to infer the foraging habitats of their mothers during gestation and lactation.

| Breeding strategy | Period | Δ15N mother-offspring (%) | Δ13C mother-offspring (%) |
|-------------------|--------|--------------------------|---------------------------|
| Income breeder    | Intrauterine/late gestation | +0.8 ± 1.5 | +0.4 ± 1.1 |
| | Lactation | −0.8 ± 3.0 | −2.9 ± 1.2 |
| Capital breeder   | Intrauterine/late gestation | +0.5 ± 2.0 | +0.1 ± 1.1 |
| | Lactation | +0.3 ± 2.6 | −1.2 ± 1.9 |

Applications

Trophic ecology reconstructions

Foraging ecotypes

Traced breast feeding or weaning

Inter-colony/site variation in maternal foraging habits

References

Lübcker et al., 2020; S. Porras-Peters et al., 2008; D. Ducatez et al., 2008; S. Stricker et al., 2015; A. Hindell et al., 2012; B. Borrell et al., 2016; J. Jenkins et al., 2001; J. Habran et al., 2010; D. Dalermo et al., 2007; D. De Luca et al., 2012; J. Habran et al., 2019; F. Fogel et al., 1997; M. Polischnik et al., 2001; C. Cherel et al., 2015; A. Hobson et al., 2000; E. Elorriaga-Verplancken et al., 2016; S. Sare et al., 2005; K. Krakkenko et al., 2015; T. Wolf et al., 2008; A. Auriolos et al., 2006; B. Baylis et al., 2016; D. Drago et al., 2010; A. Lerner et al., 2018; K. Lowther et al., 2013; S. Scherer et al., 2015. See Table S1 for additional details of studies listed herein.

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that reflected gestation were contrasted to isotope values of unpaired, in utero grown pup whiskers.

The amino acid $\delta^{13}N$ values measured in whiskers sampled from five recently weaned SES pups were compared to temporally matched amino acid $\delta^{15}N$ values measured in the whiskers of their mothers (Lübcker et al., 2020). From these five SES mothers, we analyzed two segments per whisker that were grown during the first/second trimester of pregnancy (middle of whisker: T1/2-mother) and the third trimester (base of whisker: T3-mother; $n=10$ samples; Fig. S1). The maternal amino acid $\delta^{15}N$ values measured during T1/2 and T3 were compared to the temporally overlapping in utero grown whiskers sampled from their offspring ($n=5$ pairs; detailed below).

**Paired mother–offspring whisker sampling**

Whiskers collected from recently weaned SES pups ($n=5$) were subdivided into two segments per whisker to obtain the required sample mass ($\approx 8$ mg) needed for amino acid $\delta^{15}N$ and $\delta^{13}C$ analysis; this sampling strategy produced 10 total samples that were categorized as either from the tip (T1/2-foetus) or base (T3-foetus) of the whisker (Fig. S1). The distal T1/2-foetus segment was grown during the first and second trimesters of pregnancy while the basal T3-foetus segment reflected the third trimester of pregnancy (Table 2). The position along the length of the maternal whiskers where the overlapping foetal whisker growth would have started was then identified (Supplementary Material). Furthermore, to ensure that the bulk tissue isotope data are temporally comparable to the amino acid $\delta^{13}N$ data and that the maternal and foetal isotope data overlapped; the first-to-second trimester (T1/2-mother) and third-trimester (T3-mother) bulk tissue isotope data of adult females were pooled (Fig. S1). Similarly, the bulk tissue isotope data of each recently weaned SES pup that reflect the time periods represented by T1/2-foetus and T3-foetus were pooled to correspond to the time periods for which accompanying amino acid $\delta^{13}N$ data were available (Fig. 2). Grouping data into two time points (T1/2 and T3) reduced the risk that autocorrelation influenced the statistical comparisons and that any temporal mismatching of the mother–offspring isotope data could affect our conclusions. The difference in the mother–offspring stable isotope values was reported as the mean difference ($\Delta^{13}N$ or $\Delta^{15}C$) for each time point.

Bulk tissue $\delta^{15}N$ and $\delta^{13}C$ tissue values representing SES foetal growth were compared with their mother’s isotopes during the same time period ($n=4$ pairs). All paired mother–offspring samples, which included four pairs for bulk tissue analysis and five pairs for amino acid analysis, were collected during the 2015 breeding season. The logistical coordination and associated risk involved with the temporary marking of unweaned pups (De Bruyn et al., 2008) and subsequent sampling of mother–offspring pairs when the pups are weaned before the mothers return to sea, restricted our sample size of mother–offspring pairs (Stricker et al., 2015).

**Bulk tissue- and amino acid stable isotope analysis**

For bulk tissue isotope analysis, all whiskers were chronologically sectioned into $2.0 \pm 0.4$ mm sections, and surficial contaminants were removed by rinsing in a (2:1) chloroform:methanol solvent solution (Lübcker et al., 2017). A $\sim 0.5$-mg aliquot of each whisker segment was weighed into a tin capsule and measured $\delta^{13}C$ and $\delta^{15}N$ values using a Thermo Scientific Flash 1112 Series Elemental Analyzer (ThermoFinnigan, Bremen, Germany) coupled to a Thermo Scientific Delta V Plus isotope ratio mass spectrometer (EA-IRMS; Thermo Finnigan, Bremen, Germany) at the Stable Isotope Laboratory at the University of Pretoria (Pretoria, South Africa). The thinner distal portion of the whiskers required larger sections to obtain the required sample mass for $\delta^{13}C$ and $\delta^{15}N$ analysis. Isotopic measurements were corrected to the international reference standards, Vienna Pee Dee Belemnite (VPDB) for $\delta^{13}C$ and atmospheric air for $\delta^{15}N$. In each run, two internal reference materials (Merck gel and DL-alanine) were used to assess analytical precision. The results are expressed in parts per mil ($\%\delta$) relative to the international standard atmospheric N2. Sample precision (SD) was $\pm 0.3\%\delta$ for $\delta^{13}C$ and $\pm 0.2\%\delta$ for $\delta^{15}N$.

For amino acid $\delta^{13}N$ analysis, pup whisker segments with a mean ($\pm$ SD) sample mass of $8.2 \pm 2.0$ mg (minimum: 5.6 mg; $n=10$ samples) and adult female whisker segments with a mass of $9.9 \pm 2.9$ mg ($n=30$ samples) were hydrolyzed in 1 ml 6 N hydrochloric acid (HCl) for 20 h at 110°C in muffled glassware. The SES whisker segment amino acids were hydrolyzed using 2-isopropanol and N-TFAA (Fante et al., 1999). The analyses were performed using a 60 m DB-5 column (SGE Analytical Science) in a Thermo Scientific Trace 1310 gas chromatograph coupled to a Isolink II and Thermo Scientific Delta V Plus IRMS at the University of New Mexico Center for Stable Isotopes (Albuquerque, NM, USA). This method provided $\delta^{15}N$ measurement of 13 amino acids: alanine (Ala), isoleucine (Iso), leucine (Leu), valine (Val), proline (Pro), glycine (Gly), serine (Ser), phenylalanine (Phe), lysine (Lys), tyrosine (Tyr), threonine (Thr), glutamic acid (Glu) and aspartic acid (Asp). The within-run precision (SD) for amino acid $\delta^{15}N$ analysis for multiple injections of duplicate unknown samples averaged $0.3\%\delta$ and ranged from $0.3\%\delta$ for Lys and $0.4\%\delta$ for Ile. The precision of the stock internal stock standard consisting of pure amino acids (Sigma–Aldrich Co.) that bracketed each set of unknown samples averaged $0.6\%\delta$ and ranged from $0.4\%\delta$ for Thr and $0.8\%\delta$ for Tyr.

**Statistical analyses**

A piecewise linear regression model was applied to characterize isotopic variation along the length of the whisker that corresponds to specific life-history events, using the package **segmented** (Muggeo, 2008) in R (version 3.4.4). Breakpoints in the bulk tissue $\delta^{15}N$ and $\delta^{13}C$ values were estimated by visual inspection of the plotted data and the fitted Loess
smoothing polynomial regression. A least trimmed squares robust regression model (ltsReg package in R) was applied to describe the correlation of maternal and foetal bulk tissue isotope data over the whisker length, respectively. A similar approach was used to characterize the correlation of the mother–offspring isotopic differences for each pair (Table S4). Lastly, linear mixed-effect models (lme4 package in R 1.1–21; Bates et al. 2015) were used to assess the influence of maternal whisker bulk isotope values on the temporally paired offspring whisker values. In these models, the stable isotope value of offspring whisker (e.g. δ\(^{15}\)N\(_{\text{offspring}}\)) was predicted by the fixed effect of the corresponding value from the mother (e.g. δ\(^{15}\)N\(_{\text{mother}}\)) and the random effects of period (i.e. T1/2-foetus or T3-foetus) and pairs (pair 1–4). These mixed-effect models included data from 67 paired mother–offspring whisker segments (39 from T1/2-foetus, 28 from T3-foetus). The inclusion of pairs and period as random effects accounted for non-independence of repeated measures between the mother and offspring. Where applicable, residual and data normalities were assessed using a Shapiro–Wilk test. Visual inspection of residual plots then confirmed homoscedasticity and normality. The P-values of the full model were ascertained via restricted likelihood ratio tests and calculated based on Satterthwaite’s approximations (Luke, 2017) by comparison with reduced models that excluded the random effects (e.g. Grecian et al., 2015) using the lmerTest package in R (Kuznetsova et al., 2017; version 3.1–1). Model selection was based on Akaike’s information criterion (AIC) and Bayesian Information Criterion (BIC) values (Burnham and Anderson, 2002). The proportion variance explained by the final models is expressed as marginal and conditional R\(^2\) values (\(R^2_{\text{GLMM(m)}}\) and \(R^2_{\text{GLMM(c)}}\); Nakagawa and Schielzeth, 2013). The error (residual) term (\(\epsilon\)) in the models can be attributed to intra-individual variability in δ\(^{13}\)C and δ\(^{15}\)N (Hückstädt et al., 2012). For bulk tissue δ\(^{15}\)N, the mean and standard deviation (SD) are reported, while the medians and associated upper and lower 95% confidence intervals are reported for the amino acid δ\(^{15}\)N results. The mean difference between

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Chronology of corresponding bulk tissue δ\(^{15}\)N (a, b) and δ\(^{13}\)C values (c, d) measured along the length of intrauterine grown whiskers sampled from recently weaned pups (a, c), and (b, d) ex utero grown whisker regrowths of \(n = 17\) juvenile southern elephant seals (Mirounga leonina), fitted with a linear regression (black line) and a Loess smoother (SE in grey = 0.12‰, blue line). The growth starts from the onset of gestation (left) ending just before birth. The plot position (x axis) was set to zero based on the beginning of the post-weaning δ\(^{15}\)N depletion (b, d), with light grey (far left) reflecting the intrauterine grown segment of the whiskers, followed by lactation and the post-weaning fast deplicted in darker grey (right). The red vertical line indicates the start of the predicted independent foraging.}
\end{figure}
Table 2: Bulk tissue δ¹⁵N and δ¹³C values (Mean ± SD) reflecting different life-history events captured along the length of whiskers sampled for recently weaned pups, juvenile and breeding adult female southern elephant seals (Mirounga leonina), corresponding to the first-to-second trimester of gestation (T1/2) and the third trimester of pregnancy (T3).

| Age class        | Sample size (number of individuals) | Period      | Whisker/segment length (mm) | Number of segments (average/individual) | δ¹⁵N (%) | δ¹³C (%) |
|------------------|-------------------------------------|-------------|----------------------------|-----------------------------------------|----------|----------|
| Recently weaned  | 12                                  | Full intrauterine | 78.4 ± 8.3 | 252 (21.0 ± 6.0) | 10.2 ± 0.5 | −20.3 ± 0.7 |
|                  |                                     | T1/2-foetus  | 35.0 ± 4.2 | 110                      | 10.0 ± 0.5 | −20.6 ± 0.6 |
|                  |                                     | T3-foetus    | 13.4 ± 5.7 | 76                       | 10.5 ± 0.5 | −19.9 ± 0.6 |
| Juveniles        | 17                                  | Full regrowth | 68.7 ± 13.8 | 560 (32.9 ± 6.6) | -         | -         |
|                  |                                     | Intrauterine | 10.0 ± 8.2 | 77                       | 10.9 ± 0.5 | −19.3 ± 0.7 |
|                  |                                     | Lactation    | 6.6 ± 1.4  | 73                       | 11.3 ± 0.8 | −19.9 ± 0.6 |
|                  |                                     | Transition   | 10.3 ± 0.8 | 105                      | -         | -         |
|                  |                                     | Fasting      | 20.5 ± 1.1 | 186                      | 11.9 ± 0.7 | −20.2 ± 0.6 |
|                  |                                     | Foraging     | 13.4 ± 9.8 | 119                      | 8.7 ± 0.5  | −20.2 ± 0.4 |
| Adult females    | 17                                  | Full whisker | 116.4 ± 19.1 | 805 (47.4 ± 10.0) | -         | -         |
|                  |                                     | Fastinga    | 21.4 ± 12.1 | 136                     | -         | −19.4 ± 0.9 |
|                  |                                     | Fastingb    | 11.5 ± 1.3  | 108                     | 11.6 ± 0.8† | −19.2 ± 0.8 |
|                  |                                     | Transition   | 25.6 ± 1.3  | 231                     | 11.2 ± 0.8† | −19.6 ± 0.9 |
|                  |                                     | Gestation    | 40.1 ± 19.5 | 330                     | 10.0 ± 0.7† | −20.0 ± 1.1 |
|                  |                                     | T1/2-mother  | 35.1 ± 7.1  | 309                     | 10.1 ± 0.6 | −20.1 ± 1.0 |
|                  |                                     | T3-mother    | 10.5 ± 6.2  | 68                      | 9.6 ± 0.8  | −20.3 ± 1.2 |

*Excludes n = 16 plucked whisker segments. Mean (± SD) attributes of full whiskers are given in bold.
†Values correspond to piecewise regression of nitrogen isotope values, as reported in Lübcker et al., (2020).
- = Not applicable.

Results

Juvenile SES whisker bulk tissue δ¹⁵N and δ¹³C values

Bulk tissue δ¹⁵N values measured along the length of whiskers sampled from both recently weaned pups (Fig. 1a) and juvenile SES (Fig. 1b) increased significantly (Kruskal–Wallis χ² = 351.75, df = 4, P < 0.001) from the onset of gestation to the end of the post-weaning fast (also see Fig. S2). The lactation-associated δ¹⁵N values (Fig. S1) of the ∼12-mm whisker segment left embedded in the muzzle of recently weaned pups had significantly higher δ¹⁵N values by ∼0.7‰ than pre-parturition δ¹⁵N values in the distal 12 mm of the resulting whisker regrowths (Table 2; Fig. 1b). It was confirmed that the entire whisker sampled from recently weaned pups only reflects whisker growth that occurred in utero and does not include post-parturition growth influenced by nursing. δ¹⁵N values increased by ∼1.0‰ during gestation as measured along the length of the whiskers sampled from n = 12 recently weaned SES pups (least-squared linear regression; y = 0.013 x + 9.6, SE = 0.4‰; df = 242; Adj. R² = 0.32; P < 0.001; Fig. 1a).

The corresponding δ¹³C values measured along the length of intrauterine grown whiskers sampled from recently weaned pups increased by ∼1.7‰ as gestation progressed (Fig. 1c; least-squared linear regression: SE = 0.5‰; df = 244; Adj. R² = 0.40; P < 0.001). δ¹³C values then declined by ∼0.6‰ during lactation relative to pre-parturition values (Kruskal–Wallis χ² = 102.86, df = 4, P < 0.001; Fig. 1d) and decreased by a further 0.3‰ during the post-weaning fast (piecewise linear regression: SE = 0.6‰; df = 554; Adj. R² = 0.29; P < 0.001; Table S2).
Figure 2: Temporally overlapping bulk tissue δ¹⁵N (a) and δ¹³C (b) values measured chronologically along the length of whiskers sampled from unpaired adult female (n = 17), and intrauterine grown whiskers sampled from n = 12 recently weaned southern elephant seal pups (Mirounga leonina). The plot position (x axis) was set to zero based on the onset of the post-molt fast-associated δ¹⁵N depletion, with grey reflecting the segments of the adult female whiskers predicted to have grown on land. The solid red vertical line indicates the position along the adult female whiskers where the foetal (intrauterine) whisker growth started with the standard deviation indicated by the vertical dashed lines. The whisker segments were divided into two periods corresponding to the first to second trimester of gestation (T1/2) and the third trimester of pregnancy (T3) for further analyses. The blue line with grey bars represents the fitted Loess smoother (span=0.3), while the piecewise linear regression model is indicated by black lines. Least trimmed squares robust regression models were used to illustrate the correlation of the foetal isotope values (red dashed lines).

Adult female whisker bulk tissue δ¹⁵N and δ¹³C values

The increase of the bulk whisker δ¹⁵N values of ~1.8‰ measured in the distal segments of adult female whiskers are likely associated with fasting (piecewise linear regression: SE = 0.7‰; df = 799; Adj. R² = 0.48; P < 0.001; Fig. 2a), and coupled with the subsequent precipitous decrease of 1.4‰ over the length of their whiskers associated with the post-molt foraging trip, enabled identification of the switch from endogenous to exogenous resource use (Table 2). After this initial steep decline, δ¹⁵N values continued to decrease by on average ~1.3‰ over the length of their whiskers and reflect at sea foraging during which females are pregnant (P < 0.001; δ¹⁵N = −0.012 × + 10.6).

Bulk tissue δ¹³C values (Fig. 2b) for adult females differed significantly along whiskers (Kruskal–Wallis χ² = 64.8, df = 3, P < 0.001), and observed changes were associated with life-history events (piecewise linear regression SE = 1.0‰; df = 813; Adj. R² = 0.12; P < 0.001). δ¹³C values in whisker segments grown while fasting during the molt were significantly higher by ~0.8‰ in comparison to portions of the whisker grown during the post-molting foraging trip (P < 0.001; Table 2 and S2). Inter-individual variation in δ¹³C values (SD: 1.0–1.2‰; Table 2) occurred in the portion of the whisker grown during the post-molting foraging trip (Fig. 2b; S3–S4).

Overlapping adult female and foetal whisker bulk tissue δ¹⁵N and δ¹³C values

Adult female and foetal whisker growth overlap started 56.0 ± 7.0 mm from the tip of female whiskers based on whisker growth rates and the δ¹⁵N depletion associated with the onset of active foraging detected along the whiskers of 17 adult females (Fig. 2a, Supplementary Material). The slope of the increase in foetal δ¹⁵N (0.013‰/mm) was comparable to the slope of the decline in adult female δ¹⁵N (~0.012‰/mm), and on average female δ¹⁵N values declined by ~0.5‰ while foetal δ¹⁵N increased by ~0.5‰ throughout pregnancy (Fig. 2a). The pooled (unpaired) mother–offspring bulk whisker isotope data were grouped into two overlapping time points corresponding to the approach used to generate our amino acid dataset (T1/2, T3; Fig. 2a; Table 2). The
Figure 3: Bulk tissue $\delta^{15}N$ and $\delta^{13}C$ values measured sequentially along the length of whiskers sampled from four southern elephant seal (SES, *Mirounga leonina*) mother–offspring pairs. The predicted onset of the overlap between the foetal and maternal whisker growth is indicated by the vertical red line (SE $\pm$ 3.3 mm, black dashed lines). The segments of the whisker grown on land while fasting is indicated in grey. Overlapping sampled whisker growth segments T1/2 and T3 are indicated in the top left panel. The least trimmed squares robust regression models used to illustrate the correlation of the maternal and foetal isotope values, respectively (red dashed line), are detailed elsewhere (Table S2).

The mother–offspring $\Delta^{15}N$ and $\Delta^{13}C$ values of the overlapping 67 whisker segments during T1/2 and T3 increased on average by 0.8 and 1.2‰ respectively, from sampling period T1/2 to T3; T1/2 ($\Delta^{15}N_{\text{mother-offspring}}$: 0.9 ± 0.5‰; range: 0.0–1.7‰; T 3 $\Delta^{15}N_{\text{mother-offspring}}$: 1.7 ± 0.5‰; range: 0.9–2.7‰; T1/2 $\Delta^{13}C_{\text{mother-offspring}}$: −0.2 ± 0.6‰; range: −1.6–1.1‰; T3 $\Delta^{13}C_{\text{mother-offspring}}$: 1.0 ± 0.5‰; range: 0.0–1.9‰). $\Delta^{15}C_{\text{mother-offspring}}$ ranged from being $-1.6\%e$ to $+1.9\%e$ before parturition. The least trimmed squares robust regression models describing the temporally matched mother–offspring isotopic correlations are detailed in Table S4 and were all significantly and negatively correlated, except for the $\Delta^{15}N_{\text{mother-offspring}}$ values of Pair 1 ($P = 0.62$). Overall, the isotopic composition of mothers and offspring did not appear to be in isotopic equilibrium during gestation (Fig. 4a and b). Based on the three linear mixed-effect model used to describe the influence of the sampling period (T1/2 and T3) and individual pairs (Table S5), the model containing the random effects, *pair* and *period*, described the $\Delta^{15}N_{\text{mother-offspring}}$ ($\chi^2 = 13.07$, df = 5, $P = 0.001$, $R^2_{\text{GLMM(c)}} = 55.0$) and $\Delta^{13}C_{\text{mother-offspring}}$ ($\chi^2 = 52.45$, df = 5, $P < 0.001$, $R^2_{\text{GLMM(c)}} = 92.6$) relationship the best (Table 3), which is confirmed based on the model $AIC$ and $BIC$ values (Table S5). For $\Delta^{13}C_{\text{mother-offspring}}$...
both random effects significantly affected the model (log-likelihood ratio test; \( P < 0.001 \)). For \( \Delta^{15}N_{\text{mother-offspring}} \), the sample pair had a significant influence on the offspring \( \delta^{15}N \) values (\( P < 0.001 \)), while the influence of the sampling period were marginal (\( P = 0.059 \)). The maternal \( \delta^{15}N \) values were 0.6 ± 0.2%/o (SE; 95% CI: −0.9 to −0.2%) lower than their offspring’s \( \delta^{15}N \) values and significantly negatively correlated (\( P < 0.01 \); Table S6). The maternal \( \delta^{15}C \) values were 0.4 ± 0.1%/o (SE; 95% CI: −0.6 to −0.2%) lower than their offspring’s \( \delta^{15}C \) values and were also significantly negatively correlated (\( P < 0.001 \); Table S7).

**Paired mother–offspring amino acid \( \delta^{15}N \) values**

Apart from Ala (and to a lesser extent Val), \( \delta^{15}N \) values of trophic and source amino acids were similar between paired offspring and mothers at T1/2 and T3 (Table S8; Fig. S5). Both Gly and Ser were significantly higher in offspring bulk tissue and highlight the mechanisms behind the offsets in mother–offspring pairs are constant during gestation and consistent amongst individuals (Auriol et al., 2006; Drago et al., 2010; Hindell et al., 2012; but see Table 1). In contrast to these assumptions and findings, temporally comparable whiskers sampled from SES mothers and their foetuses differ significantly in both \( \delta^{15}N \) and \( \delta^{15}C \) composition and are not predictably correlated. In 75% of the cases, both \( \delta^{15}N \) and \( \delta^{15}C \) values of SES mothers and their pups were negatively correlated during gestation (Figs 2–3; this study). While mother–offspring \( \Delta^{15}N \) and \( \Delta^{15}C \) were generally positive during the third trimester of pregnancy (T3), the magnitude of the offset varied by several per mil amongst pairs. Importantly, SES mother–offspring discrimination changed as gestation progressed (Fig. 3) as both foetal \( \delta^{15}N \) and \( \delta^{15}C \) values increased relative to the decreasing \( \delta^{15}N \) and \( \delta^{15}C \) values of their mothers (Fig. 3), which was also observed in the larger dataset for unrelated adult females and pups (Fig. 2). In the only other study to our knowledge that measured paired tissues of phocid mother and pups, the \( \delta^{15}N \) values measured in the *in utero* grown whiskers of bearded seal (*Erignathus barbatus*) pups similarly increased consistently from the distal (oldest) to basal (most recent) sections (Hindell et al., 2012).

Foetal \( \delta^{15}N \) and \( \delta^{15}C \) values were comparable to their mothers in the first and second trimester, but mean \( \Delta^{15}N \) and \( \Delta^{15}C \) offsets between mother and pup increased to +1.7 ± 0.5 and +1.0 ± 0.5%, respectively during the third trimester (T3) of pregnancy (Fig. 3). Since whiskers are assumed to be in isotopic equilibrium with blood plasma (Hiron et al., 2001; Newsome et al., 2010), changes in foetal bulk whisker \( \delta^{15}N \) and \( \delta^{15}C \) values suggest possible differential isotopic discrimination and/or routing of particular amino acids occurred across the placental barrier during pregnancy. Of particular interest is that the negative correlations between pup and mother \( \delta^{15}N \) and \( \delta^{15}C \) values over the course of gestation were pair-specific and influenced by the period represented (Table 3). This likely reflects differences in the amount of endogenous (stored) maternal protein and adipose fat catalyzed to support foetal development and may be related to the maternal body condition as gestation progressed (e.g. Fuller et al., 2004; De Luca et al., 2012). These biochemical mechanisms were further explored via amino acid \( \delta^{15}N \) and bulk tissue \( \delta^{15}C \) analysis.

**Differences in paired mother–offspring amino acid \( \delta^{15}N \) values**

Interestingly, when corrected for baseline effects by using the mother’s lysine and phenylalanine \( \delta^{15}N \) values of each mother-
Figure 4: Correlation between maternal and foetal bulk tissue $\delta^{15}N$ (a) and $\delta^{13}C$ (b) values measured sequentially along the length of whiskers during the first-to-second trimester of gestation (T1/2; solid fill symbols) and third trimester of pregnancy (T3; open symbols) of four southern elephant seal (*Mirounga leonina*) mother–offspring pairs. The dashed black line represents a 1:1 correlation and coloured dashed line represent fitted Loess smoothers for each mother–offspring pair. Details of fitted models are provided elsewhere (Table 3 and S3–S7).

Table 3: Results of the best-fit linear mixed-effect model fit by reduced maximum likelihood (REML, model $M_3$–$\delta^{15}N$/S3–S7) for predicting whisker $\delta^{15}N$/$\delta^{13}C_{\text{offspring}}$ values from their temporally overlapping mother whisker $\delta^{15}N$ values ($\delta^{15}N$/$\delta^{13}C_{\text{mother}}$), with ‘period’ and ‘pair’ as random effects.

| Model | $M_3$–$\delta^{15}N$ | $M_3$–$\delta^{13}C$ |
|-------|------------------|------------------|
| Predictors | $\delta^{15}N_{\text{offspring}}$ | $\delta^{13}C_{\text{offspring}}$ | $\delta^{15}N_{\text{offspring}}$ | $\delta^{13}C_{\text{offspring}}$ |
| (Intercept) | 15.06 | 11.62–18.50 | $<0.001$ | −28.50 | −33.13–−23.87 | $<0.001$ |
| $\delta^{15}N_{\text{mother}}$ | −0.53 | −0.91–−0.15 | **0.007** | −0.38 | −0.60–−0.17 | $<0.001$ |

**Random effects**

|  | $\sigma^2$ | 0.08 | 0.06  |
|  | $\tau_{00}$ | 0.05 pairs | 0.47 pairs |
|  | ICC | 0.49 | 0.92 |
|  | $n$ | $2_{\text{period}}$ | $2_{\text{period}}$ |
|  | $n$ | $4_{\text{pairs}}$ | $4_{\text{pairs}}$ |
| Observations | 67 | 67 |
| Marginal $R^2$/Conditional $R^2$ | 0.116/0.550 | 0.059/0.926 |

Residual variance = $\sigma^2$; random intercept variance, or ‘between-subject’ variance = $\tau_{00}$, intraclass-correlation coefficient = ICC; number of classes/groups = $n$. 
pup pair, most offsets in amino acid $\delta^{15}N$ values between mothers and pups were isotopically indistinguishable. Lysine and phenylalanine are thus likely routed from the maternal plasma to the foetus with minimal isotopic alteration. Exceptions were foetal valine and leucine $\delta^{15}N$ values, which were $\sim 1.4$ and $\sim 0.8\%$ higher than that of their mothers, respectively. Likewise, foetal serine and glycine $\delta^{15}N$ values were $>4\%$ higher than that of their mothers (Fig. 5). Given the amino acid composition of $\alpha$-keratin, which is primarily composed of half-cystine (13.1%), glutamic acid (11.1%), serine (10.8%) and glycine (8.6%) (Marshall et al., 1991), the combined isotopic offsets of glycine and serine can explain the majority of the observed offsets in bulk tissue $\delta^{15}N$ values between pups and their mothers (Fig. 2). The glutamate–glutamine and glycine–serine shuttles are likely the most dominant pathways in maternal–foetal amino acid transport (Kalhan, 1998; Fig. 6) and provide a mechanistic explanation for the observed mother–offspring $\delta^{15}N$ differences in valine, leucine, glycine, and serine.

Branched-chain amino acids (valine and leucine) are deaminated by the placenta to form glutamate, which is then transaminated to form glutamine and ultimately transferred to the foetus by the glutamate–glutamine shuttle between the mother and foetus (Fig. 6). Amine groups containing $^{14}N$ are preferentially removed during deamination, resulting in $^{15}N$-enrichment of the remaining pool of branch-chained amino acids. This isotopic fractionation may explain the $+1.4$ and $+0.8\%$ increase in foetal valine and leucine $\delta^{15}N$ values relative to maternal values during the third trimester (T3) of pregnancy. Furthermore, the abundance of glutamate/mine (Wu et al., 2015), along with the rapid rate of cycling of these two amino acids between the mother and foetus, likely eliminates any isotopic discrimination in glutamic acid $\delta^{15}N$ values between mother and foetus (Fig. 6); note that the derivatization method used converts both glutamate and glutamine into glutamic acid (Silfer et al., 1991; Whiteman et al., 2019).

The placenta also converts maternal serine to glycine, which is actively transported across the foetal-placenta barrier along with alanine by transport System A (Narkewicz et al., 2002; Kalhan, 2016; Fig. 6). Some foetal serine is transferred back to the placenta to form the glycine–serine shuttle, while the remaining serine and glycine can be used for foetal tissue or pyruvate synthesis. The glycine–serine shuttle and demethylation of glycine by the foetal liver provide essential one-carbon units (via $\alpha$-adenosylmethionine) required for nucleotide synthesis and foetal growth (Lindsay

Figure 5: Maternal phenylalanine and lysine $\delta^{15}N$ (baseline) corrected amino acid–specific $\delta^{15}N$ values measured during the first-to-second trimester of gestation (T1/2; open symbols) and third trimester of pregnancy (T3; closed symbols) along the length of whiskers sampled from five mother–offspring pairs. Adult females (red); intrauterine offspring whisker (blue). T/S = trophic or source amino acid. * $P < 0.05.
Figure 6: Maternal, placenta and foetal amino acid transfer portraying the mechanism responsible for isotopic enriched foetal $\delta^{15}N$ values. The isotopically light nitrogen ($^{14}N$) alanine formed when alanine aminotransferase catalyzes the synthesis of alanine from pyruvate, are transferred to the foetus (glucose–alanine cycle). The pathways by which $^{14}N$ are delivered to the foetus are indicated in red. Blue lines depict the glycine and serine placenta to foetal interaction (glycine–serine shuttle) while the glutamate–glutamine shuttle is indicated in green. Together with the catabolism of branched-chain amino acids (BCAA), the glutamate–glutamine shuttle delivers carbon skeletons and amino acids required for foetal development and are involved in the process of enriching the foetal $\delta^{15}N$ values. Abbreviation explanation for amino acids can be found in the Materials and methods section.

et al., 2015; Kalhan, 2016). The significantly higher (>4%) foetal serine and glycine $\delta^{15}N$ values relative to maternal values (Fig. 5) suggest that the amine groups containing $^{14}N$ are preferentially deaminated to form pyruvate during this process, which leaves the remaining serine and glycine that are used to build foetal tissues $^{15}N$-enriched. Ammonia containing deaminated $^{14}N$ is excreted from the placenta into the mother's bloodstream (plasma), leaving the heavier isotope in the foetus. This process, akin to Rayleigh distillation, could cause the observed systematic increase in foetal tissue $\delta^{15}N$ values over time (Fig. 6). It is possible that $^{14}N$-enriched ammonia excreted by the foetus contributes to the preservation of maternal amino acid homeostasis (Kalhan, 1998; De Luca et al., 2012) but is more likely that this ammonia pool gets incorporated into the mother’s urea cycle and is excreted via urine. Although many of these placental dynamics have primarily been described in humans (Kalhan, 2016), we expect that they are common to all placental mammals.

Glucose–alanine cycle explains mother–offspring amino acid $\delta^{15}N$ offsets

Foetal $\delta^{15}N$ alanine values were depleted on average 1.4% relative to their mothers, possibly driven by the glucose–alanine (Cahill) cycle. When SES are in a catabolic (fasting) state associated with negative nitrogen balance (Lübcker et al., 2020), the waste nitrogen produced by amino acid catabolism in extrahepatic tissues is combined with glucose-derived carbon, yielding alanine (Felig et al., 1970). This alanine is then transferred to the liver, providing a safe means of shuttling what would otherwise be dangerous nitrogen atoms (i.e. ammonium ions). Because this waste nitrogen is expected to have relatively low $\delta^{15}N$ values relative to the amino acid pool from which it originated, the newly synthesized alanine is likewise expected to have low $\delta^{15}N$ values (Lübcker et al., 2020). The depleted $^{14}N$-alanine in the plasma is then incorporated in tissues that are vital to maintain but will also be transferred to the foetus by transport System A. The low foetal alanine $\delta^{15}N$ values observed here therefore suggests that pregnant females are in an anabolic–catabolic physiological state during gestation (e.g. Fuller et al., 2004; Habran et al., 2019).

Lastly, foetal and maternal lysine and phenylalanine $\delta^{15}N$ values were similar and remained consistent during gestation. Thus, comparison of $\delta^{15}N$ in trophic (glutamic acid) and source (phenylalanine) amino acids could still provide an accurate estimate of trophic level assuming beta values and TDF for amino acids are known and do not appreciably vary...
amongst individuals (Chikaraishi et al., 2015). The increase in foetal threonine δ15N values (+1.3‰) was offset by a decrease in maternal threonine δ15N values of similar magnitude (−1.1‰). Threonine can be converted to glycine by the enzyme threonine dehydrogenase and could contribute to the maternal-to-foetal nitrogen flux (Anderson et al., 1997).

Nutritional pool supporting foetal development

While SES foetal δ13C values were similar to their mother’s at the onset of gestation, the δ13C-enrichment of foetal whiskers relative to mother throughout pregnancy further confirms that foetal development is reliant on endogenous maternal protein reserves rather than maternal adipose tissue, which has significantly lower δ13C values than proteinaceous tissues by 6–8‰ (DeNiro and Epstein, 1976; Tieszen et al., 1983). Foetal development depends on the steady supply of glucose and catabolized endogenous maternal nitrogen (tissue proteins) from the onset of gestation. The maintenance of the maternal anabolic–catabolic state ensures a constant nutrient supply to the foetus, buffered from acute fluctuations in the mother’s nutritional status during gestation. This makes sense from a maternal fitness, and perhaps evolutionary, perspective given that foetal development requires a reliable and constant supply of nutrients.

Although lactate is considered to be the main gluconeogenic precursor in fasting elephant seals and influences carbon cycling (Houser and Crocker, 2004; Champagne et al., 2005; Crocker et al., 2017), the glucose–alanine cycle also contributes to the maintenance of elephant seal glucose and nitrogen homoeostasis, as described above and evident through the decrease in alanine δ15N values (Lübcker et al., 2020). The glucose-lactate (Cori) cycle and the glucose–alanine (Cahill) cycle differ from each other in the three carbon (C3) molecules used as intermediates and recycled to produce glucose (Dashty, 2013). The Cori cycle returns carbon to the liver as pyruvate (C3H4O3), whereas the glucose–alanine cycle returns carbon to the liver as alanine (C3H7NO2; Felig et al., 1969). The effects of the glucose–alanine cycle on the δ15N values of pregnant elephant seals have not been investigated. Potential alanine synthesis by the tricarboxylic acid (TCA) cycle is likely inadequate to replenish the plasma alanine concentrations (Felig et al., 1969). It would be of interest to assess if the reported de novo synthesis of amino acids (e.g. glycine, serine) in fasting seal tissues could have misrepresented the actual contribution of amino acids to their glucose cycling/amount of protein sparing (e.g. Houser and Costa, 2001).

Implications and conclusions

Our study is the first to combine bulk tissue and amino acid analysis to assess whether the isotopic composition of pup tissues can be used as proxies for their mothers’ isotopic composition during gestation. Contrary to the assumption that mother–offspring isotope values are positively and linearly correlated, whiser δ15N and δ13C values of paired, temporally overlapping mother–offspring SES were negatively correlated during gestation. It is hypothesized that the magnitude of both the nitrogen (Δ15N) and carbon (Δ13C) offset relates to foraging success and associated maternal body condition while pregnant, and caution is advised when using bulk tissue isotope values of offspring as a proxy for inferring the trophic ecology of their mothers. The observed increases in δ15N values of branched-chain amino acids (valine and leucine), glycine and serine in offspring relative to their mothers, and the concurrent depletion of offspring alanine δ15N values indicates that pregnant females are in a constant catabolic-anabolic state from at least the onset of gestation. Our findings shed new light on foetal amino acid metabolism, and the patterns in δ13C values amongst mother and foetus confirm that foetal development primarily relies on endogenous maternal proteinaceous sources throughout gestation rather than adipose tissue. Keratinous tissue (e.g. hair) sampled from human mother–offspring pairs can similarly provide longitudinal data of foetal amino acid metabolism during pregnancy, especially when isotopically labelled one-carbon metabolites are supplemented to improve DNA methylation and promote foetal development (Kalhan, 2016).

Stable isotope analysis of offspring tissue is increasingly used to infer maternal diet selection and habitat use in free-ranging animals (Table 1). If a constant offset is assumed between offspring and maternal tissue, our results indicate that researchers could erroneously conclude that females substantially shift their resource and/or habitat use. Such misrepresentation of species ecology could mislead managers and have consequences for conservation strategies. The ecological inferences made from over two dozen studies that applied bulk tissue δ15N and δ13C values measured in tissues sampled from offspring of ~30 mammal species might require reconsideration (Table 1) and potentially have serious conservation consequences. Lastly, physiological method validations that advocate minimally invasive sampling designs such as the collection of tissues from pups as proxies of their mothers’ ecology should include a description of the possible limitations of this approach that are highlighted by our study.

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**Data Accessibility**

Data will be made available on the Dryad Digital Repository.

**Authors Contributions**

All authors conceived the ideas and designed methodology and enabled sample analyses. N.I., S.D.N. and J.P.W. analyzed the samples and processed the data. P.J.N.dB. maintained and enabled sample analyses. N.L., S.D.N. and J.P.W. analyzed the fieldwork programme, facilitating access to sampling. All authors assisted with the writing of the manuscript.

**Supplementary material**

Supplementary material is available at *Conservation Physiology* online.

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