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Temporal variation in isotopic composition and diet of Weddell seals in the western Ross Sea

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ABSTRACT

Weddell seals (*Leptonychotes weddellii*) are important predators in the Antarctic marine ecosystem, yet little is known about their diet. Previous studies have used scat and stomach content analyses to examine Weddell seal diet, however, these methods are biased towards prey with indigestible hard parts. To provide a more complete picture of their diet, we analyzed the stable isotope composition ($\delta^{13}C$ and $\delta^{15}N$ values) of red blood cells (RBC, $n=96$, representing a time scale of weeks to months) and vibrissae ($n=45$, representing months to a year) collected over a three year period (2010–2012). Our objectives were to (1) examine isotopic variation in relation to Weddell seal mass, sex, season, location, percent lipid, and age, and (2) quantify the contribution of prey items to overall diet. Body mass was a significant predictor of $\delta^{13}C$ and $\delta^{15}N$ values for both tissues, though the strength and direction of the relationship varied by year. The prey group consisting of *Pleurogramma antarcticum* and *Trematomus newnesi* was found to be an important dietary component, but its proportional contribution to Weddell seal diet varied with the timeframe represented by each tissue type [median RBC (range): 59.2% (40.2–8 1.1%); median mean vibrissae (range): 69.3% (43.9–89.6%)]. Results from mixing models ran for each seal indicate individual variation in diet. Overall, this study presents novel information on the isotopic variation and diet of Weddell seals over two time scales and provides insight into the feeding ecology of an important Antarctic predator.

1. Introduction

Within the Southern Ocean, the Ross Sea ecosystem is particularly unique due to its vast extensive continental shelf, significant polynyas, diverse topography, and vast ice shelf (Smith et al., 2007). Although the Ross Sea is the most productive region in Antarctica (Arrigo et al., 1997), the biodiversity of fish fauna in this region is relatively low compared to the Southern Ocean, composed of 95 species from 16 families, and is dominated by a single taxonomic group. The family Nototheniidae comprises 77% of all fish species and 91% of total fish biomass in the Ross Sea ecosystem (Eastman and Hubold, 1999; Lenky et al., 2012; Smith et al., 2012). Despite the relative simplicity of the fish community and the extensive ecological studies that have been conducted in this region, trophic linkages between top predators and their prey are not well understood.

Weddell seals (*Leptonychotes weddellii*) have the southernmost distribution of any mammal. While only about 32,000–50,000 of the estimated 730,000 Weddell seals in Antarctic waters inhabit the Ross Sea, they serve an important role in the trophic ecology of this unique ecosystem (Ainley, 1985; Laws, 1977). Ross Sea Weddell seals are the only air-breathing homeotherms to reside in the fast-ice near the Antarctic continent as well as in the offshore pack-ice, and remain on the continental shelf year round (1000 m bathymetric contour) (Goetz, 2015; Testa, 1994). Most seals leave McMurdo Sound and travel north throughout the austral winter. In fact, less than 10 of 63 satellite tracked animals stayed within McMurdo Sound, with the majority traveling distances up to 550 km towards the continental shelf break and diving, on average, to 125 m (range: 5–705 m) where they mostly foraged on pelagic prey (Goetz et al., unpublished data). Due to their presence in large numbers, high metabolic rates, and large biomass, Weddell seals consume a large proportion of prey and, therefore, have

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a large influence on the trophic dynamics of the Ross Sea ecosystem (Costa and Crocker, 1996).

During the austral spring (September–November), Weddell seals return to their breeding colonies along the coasts of Ross Island and Victoria Land where they give birth and breed (Stirling, 1969). Unlike many phocid species, lactating Weddell seals spend ~25% of their time diving, foraging within a limited range of the colony (Hindell et al., 2002). Seals remain in these colonies until completing their annual molt in January–February, after which they leave the colonies and forage intensively over the austral winter (Castellini et al., 1992; Testa, 1994). During the months that they are tied to the fast-ice, Weddell seals are easily accessible from research stations and are an ideal marine predator for monitoring ecosystem health and resilience.

Previous studies examining the diet of Weddell seals in the Ross Sea found that notothenid fish were the most common prey item based on the number of fish parts counted in both scat and stomach contents (Burns et al., 1998; Dearborn, 1965). By providing high resolution taxonomic descriptions of diet, these studies provided valuable insight into the foraging ecology of Weddell seals. However, in cases where hard parts are either not consumed or do not persist after the digestion process, prey items cannot be detected using stomach and scat content analyses. For example, because Weddell seals only consume the flesh of large Antarctic toothfish (*Dissostichus mawsoni*) (Ainley and Siniff, 2009; Davis et al., 2004; Kim et al., 2005; Kooyman, 2013; Ponganis and Stockard, 2007), this large fish predator has not been detected in the diet of Weddell seals using such analyses.

Stable isotope analysis is a powerful tool for studying the foraging ecology of animals. In addition, this method does not over-represent indigestible material or under-represent items that leave little or no visual trace (Bodey et al., 2011). By providing information on prey items that have been incorporated into the consumer's tissue (as opposed to only ingested), stable isotope analyses can be linked to an animal's physiological condition and, therefore, its ability to adjust to environmental perturbations that may impact reproduction and survival (Jakob et al., 1996). The predictable enrichment in ^{15}N (3–5‰) from herbivores to carnivores makes it a useful indicator of a consumer's trophic position relative to its prey (DeNiro and Epstein, 1978; Hammill et al., 2005; Hobson et al., 1996; Minagawa and Wada, 1984). In contrast, the relatively small enrichment in ^{13}C between trophic levels (0–1‰ in marine systems) makes it a better indicator of the habitat, allowing us to distinguish benthic from pelagic and nearshore from offshore environments (McConnaughey and McRoy, 1979; Zhao et al., 2004).

Although past studies have examined $\delta^{13}C$ and $\delta^{15}N$ isotope values in Weddell seals, these studies focused on inter-individual differences and only examined blood serum or plasma samples, which reflect diet consumed over a period of days to weeks (Burns et al., 1998; Zhao et al., 2004). In addition, because complex isotopic mixing models that can deal with many prey items have only recently been developed, the proportional contribution of prey items to the diet of Weddell seals has not previously been examined.

This is the first study to quantify Weddell seal diet over two time periods by comparing $\delta^{13}C$ and $\delta^{15}N$ isotope values from red blood cells (RBC) and vibrissae to those from a suite of potential prey items. Samples from the two tissues allowed us to examine the trophic ecology of Weddell seals over a period of weeks to months (RBC) and a longer period spanning months to a year (vibrissae) relative to multiple variables that may influence energy requirements and, therefore, diet. Overall, our objectives were to use isotopic results from both tissues to: (1) examine isotopic variation in relation to mass, sex, year, season, location, percent lipid as a measure of seal body condition, and age, and (2) quantify the contribution of prey items to overall diet. Results from these analyses provide important information on the foraging behavior of Weddell seals over short-term and long-term timeframes and provide insights into the position of these predators within the Ross Sea food web.

2. Materials and methods

2.1. Animal capture and sample collection

Between January and February over a three year period (2010–2012), 96 Weddell seals were captured near Ross Island and along the Victoria Land coast of the Ross Sea (Fig. 1). Because seals had been previously tagged, we were able to identify and recapture twenty animals in a second season, approximately nine months later. Seals were chemically immobilized with an initial dose of a tiletamine HCL/zolazepam HCL mixture (Telazol 0.5 mg/kg) administered intramuscularly. Approximately 12 min post-injection, animals were captured using a hoop net. Subsequent intravenous injections of a 2:1 mixture of ketamine hydrochloride (100 mg/ml Ketaset) and diazepam (5 mg/ml) were administered, when necessary, to maintain immobilization. While sedated, seals were weighed in a canvas sling suspended from a tripod using a Dyna-Link scale (1000 ± 1 kg). Seal body condition (lipid mass) was determined for each animal using the labeled water dilution technique as described in Shero et al. (2015, 2014). Because previous studies found large differences in mean $\delta^{13}C$ and $\delta^{15}N$ values between the root and the rest of the vibrissa and that the root was not composed of pure keratin (Hückstädt et al., 2012; Zhao et al., 2006), we opted to clip, rather than pluck, the longest vibrissa as close as possible to the muzzle of each animal. Finally, blood samples were collected from the extradural vein of each seal in heparinized blood tubes.

2.2. Stable isotope analysis

Following the methods of Hückstädt et al. (2012), all vibrissae were subjected to a two-step cleaning process: (1) lightly scrubbed

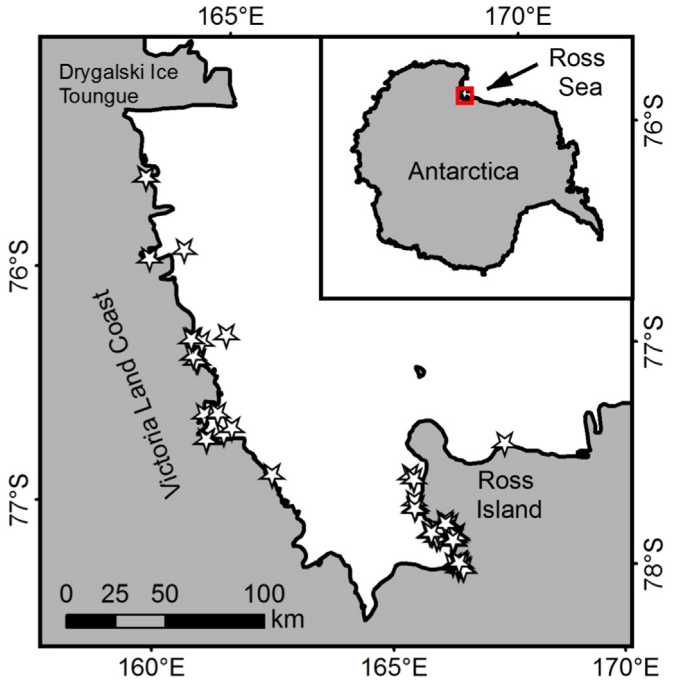

Fig. 1. Weddell seal (*Leptonychotes weddellii*) capture and sample (red blood cells and vibrissae) locations near Ross Island and along the Victoria Land coast, Antarctica.

using distilled water and detergent, and allowed to dry in a 60 °C oven for a minimum of 30 min; (2) submerged in petroleum ether and rinsed in an ultrasonic bath for 15–20 min. Vibrissae were then measured and cut into 0.5 cm segments. Samples (0.5 ± 0.05 mg) were cut from the proximal end of each segment. Whole blood samples were centrifuged to extract RBCs and stored in a −20 °C freezer. RBC samples were subsequently freeze-dried and 0.5 ± 0.05 mg subsamples were obtained.

Weddell seal prey species were collected in collaboration with concurrent projects to study Ross Sea fish (2010–2012) and stored in a −80 °C freezer. In preparation for stable isotope analysis, fish were thawed at room temperature and lightly rinsed in deionized (DI) water. Standard length and weight measurements were taken before homogenizing individual whole fish. Subsamples of the homogenate from each fish were freeze-dried for 48 h, mixed into a fine powder, and subsamples of 0.5 ± 0.05 mg was obtained for isotopic analysis.

Because many Antarctic fish species are rich in lipids (Clarke et al., 1984; Lenky et al., 2012), we lipid-extracted each sample in order to avoid the potential influences of lipids on $\delta^{13}C$ values (Pinnegar and Polunin, 1999; Post et al., 2007; Sweeting et al., 2006). Lipid extraction was performed by combining 0.1 g of homogenate with 5 ml of 2:1 chloroform:methanol and submerging the container in an ultrasonic bath for 30 min. This process was repeated 3–4 times, decanting the supernatant between rinses, until the liquid appeared clear. Finally, each sample was rinsed with 2 ml of DI water and freeze-dried for an additional 48 h. The dried homogenate was mixed into a fine powder and subsamples of 0.5 ± 0.05 mg were obtained.

Fish (original and lipid-extracted), vibrissae, and RBC subsamples were placed into tin capsules and analyzed at the Light Stable Isotope Laboratory at University of California, Santa Cruz using a Carlo Erba Elemental Analyzer interfaced with a Finnigan Delta Plus XP mass spectrometer in order to obtain $\delta^{13}C$ and $\delta^{15}N$ values. Standards of Pugel and Acetanilide were used to check for instrument drift and calibration throughout the sampling period.

Isotopic composition is expressed in δ (delta) notation, as follows:

$$\delta hX = \frac{R_{sample} - R_{standard}}{R_{standard}} \times 1000$$

where h is the atomic mass of the heavy isotope, X is C or N, R_{sample} is the ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$, and $R_{standard}$ is Vienna PeeDee Belemnite (VPDB) limestone for carbon and nitrogen isotope values. Units are expressed as parts per thousand differences from the standard or per mil (‰). Experimental precision across runs, estimated by the standard deviation of replicated standards (Pugel), was ±0.2‰ and ±0.1‰ for stable carbon and nitrogen isotopes, respectively.

For all dietary analyses, $\delta^{13}C$ values from lipid extracted fish samples, and $\delta^{15}N$ values from untreated samples were used. This was necessary as ^{15}N becomes enriched during the lipid extraction process, which may be related to the composition of essential and non-essential amino acids (Logan et al., 2008; Pinnegar and Polunin, 1999; Sweeting et al., 2006).

2.3. Statistical analysis – isotopic variation

We evaluated the relationship between $\delta^{13}C$ and $\delta^{15}N$ values and the following variables: sex, year, season, tagging location, mass, and seal lipid mass (as % total body mass). Animal mass [mean (range): 328.2 kg (181.0–502.0 kg)] and percent lipid [33.04% (21.98–45.49%)] were continuous variables while sex, year, season (spring or summer) and tagging location were factor variables (Table 1). Tagging location was categorized as south (around Ross Island) or north (Victoria Land Coast). Using the 'lme4' package (Bates et al., 2015) within the statistical software R (R Development Core Team, 2013), we ran linear mixed models on a global model (including interaction terms) for both $\delta^{13}C$ and $\delta^{15}N$ values as a response variable. Separate models were run for each tissue type and vibrissae $\delta^{13}C$ and $\delta^{15}N$ values were calculated by taking the mean isotopic value for all segments. The 'drop1'command was used to drop variables in turn, and the differences between the full model and the models with single term deletions were compared to the full model using a chi-square distribution. We used Akaike's Information Criterion corrected for finite sample sizes (AICc) to assess model performance. Variables were removed in a step-wise fashion and models were refit until the lowest AICc score was obtained. To account for animals that were sampled twice, we included individual as a random effect. We also used linear mixed models to test for intra-individual differences in RBC and mean vibrissae $\delta^{13}C$ and $\delta^{15}N$ values for animals that were sampled in both the January–February and October–November field seasons.

Table 1
Number of Weddell seal tissue samples (RBC: red blood cells, and vibrissae) per variable (sex, age, location, year, season, mass and % lipid mass) used in general linear mixed models.

Variable	Category	RBC	Vibrissae
Sex	Male	16	16
	Female	80	29
Age	Known	38	21
	Unknown	58	24
Location	North	32	15
	South	64	30
Year	2010	33	11
	2011	38	16
	2012	25	18
Season	Summer	54	25
	Spring	42	20
Mass	Known	95	43
	Unknown	1	2
% Lipid	Known	91	40
	Unknown	5	5
	Total	96	45

Finally, as a result of a long-term population study on Weddell seals in the McMurdo region, age was known for a subset of the animals. Due to the relatively small sample size (RBCs: $n=30$, vibrissae: $n=19$), general linear models were run separately for each isotope and tissue type in relation to age [mean (range): 3 yrs (10–25 yrs)], excluding duplicates from January. Models were validated by examining residuals for normality and homoscedasticity.

2.4. Statistical analysis – diet and foraging ecology

As Weddell seals in the Ross Sea are known to be primarily piscivorous, we limited our analysis to fish species that have been previously documented as prey items through (1) scat and stomach content analyses: *P. antarcticum*, *Trematomus bernacchii* and other *Trematomus* spp., and (2) observations: *D. mawsoni*, *P. borchgrevinki* and *N. ionah*. Although amphipods, mysids, and polychaetes have been found in scat and stomach contents, they are thought to be a result of secondary ingestion by fish known to forage on these items (Burns et al., 1998; Dearborn, 1965; Eastman,

1985; Eastman and DeVries, 1985). Similarly, euphausiids do not appear to compose a large proportion of the Weddell seal diet in the Ross Sea likely due to their absence in large numbers from the ecosystem (Eastman, 1985).

We used a Bayesian isotope mixing model to determine the percent contribution of each prey species to the diet of Weddell seals as a population and individually. The "siar" package in R was used to fit the mixing models, using a Bayesian framework based upon a Gaussian likelihood (Parnell, 2013). We calculated mean and standard deviation of $\delta^{13}C$ and $\delta^{15}N$ values for six potential prey species, and used a student's t-test assuming unequal variance to determine if species were isotopically distinct at the $P=0.05$ significance level. Prey species that were not significantly different from each other in both $\delta^{13}C$ and $\delta^{15}N$ values were combined into a single prey group. Unless otherwise stated, the percent contribution of prey items to Weddell seal diet is presented as median (range).

Weddell seal diet was examined on two timescales, through the use of both RBC and vibrissa isotope values. RBCs allowed us to examine diet over the most recent weeks to months (Hilderbrand et al., 1996; Vander Zanden et al., 2015), whereas vibrissae offered insight into diet on the timescale of months to a year depending on vibrissae growth rate. We examined diet both on a population-level by pooling all individuals of a given tissue type into one mixing model and on an individual-level by running separate models for each Weddell seal. For vibrissa mixing models, mean isotope values were used for population-level analyses and values from all segment were incorporated into individual-level models. A trophic enrichment factor (TEF) of 1.7 ± 0.12‰ and 2.8 ± 0.12‰ was used for blood and vibrissae $\delta^{15}N$ values (Hobson et al., 1996).

The carbon trophic discrimination is not obvious in the Antarctic ecosystem due to considerable overlap in $\delta^{13}C$ values throughout the food web (Nyssen et al., 2002). Thus we used a TEF specific to the Ross Sea region (-0.7 ± 0.08‰ for RBC and 0.8 ± 0.12‰ for vibrissae) (Pinkerton et al., 2014). The lower TEF for RBC reflects a 1.5‰ difference in discrimination factors between RBC and vibrissae. Standard deviations were provided by Hobson (pers. comm.) in reference to the data published in Hobson et al. (1996). Note that there are many caveats associated with the interpretation of isotopic data (see electronic Supplementary material).

3. Results

3.1. Isotopic variation

We analyzed $\delta^{13}C$ and $\delta^{15}N$ values of both RBC samples from each Weddell seal ($n=96$) and vibrissa samples from a smaller sublet of animals ($n=45$). There was considerably less population-level variation in $\delta^{13}C$ and $\delta^{15}N$ values for RBCs than vibrissae (Fig. 2). Overall, $\delta^{13}C_{RBC}$ and $\delta^{15}N_{RBC}$ values ranged from -25.8 to -24.4‰ (mean $\pm$ SD: -25.2 ± 0.0‰) and 11.4–13.1‰ (12.0 ± 0.3‰), respectively. Mean $\delta^{13}C_{vibrissa}$ values ranged from -24.3 to -22.5‰ (-23.4 ± 0.4‰) and mean $\delta^{15}N_{vibrissa}$ values spanned from 12.2 to 13.8‰ (13.0 ± 0.4‰). Using resampling to ensure equal sample size between RBC and vibrissa segments, we found that the variation in $\delta^{13}C_{vibrissa}$ values (0.20‰) was double the variation of $\delta^{13}C_{RBC}$ values (0.10‰) and that the variation in $\delta^{15}N_{vibrissa}$ values (0.27‰) was nearly triple the variation of $\delta^{15}N_{RBC}$ values (0.10‰). Isotopic variation along the length of individual vibrissae is provided in Supplementary material (Fig. S1).

Because seal mass and/or seal lipid was not always measured, the dataset was reduced to 91 animals for RBCs and 41 for vibrissae for all linear models. Fourteen of the individuals in each dataset were sampled twice, once each season. Seal mass, capture

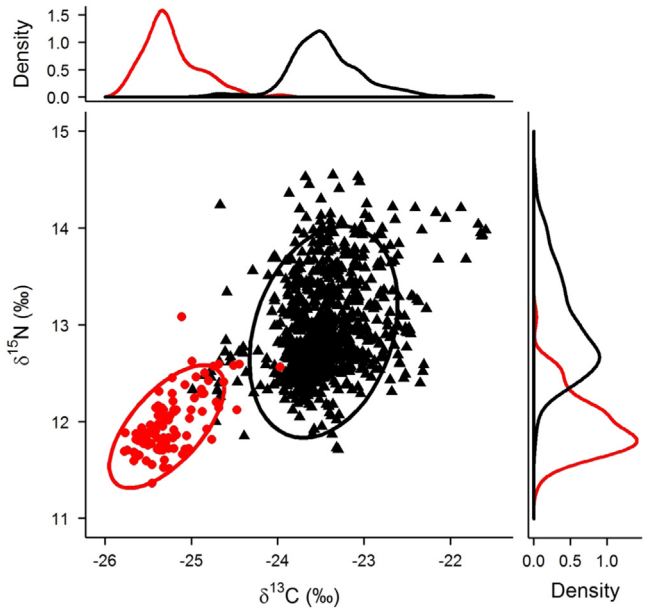

Fig. 2. Isotopic values ($\delta^{13}C$ and $\delta^{15}N$) for red blood cells (RBC; circles) and vibrissae segments (every 0.5 cm; triangles). Ellipses indicate the 95% confidence interval and density plots show less variation in $\delta^{13}C$ and $\delta^{15}N$ values for RBC than vibrissae. RBC and vibrissa isotopic values have not been corrected relative to one another and therefore cannot be directly compared.

location, and study year best explained the variability in $\delta^{13}C_{RBC}$ values (Table 2). Values for $\delta^{13}C_{RBC}$ increased with mass, though the intercept varied by year; $\delta^{13}C_{RBC}$ values in 2010 were 0.2‰ higher than 2011 and 2012. For a given mass and year, $\delta^{13}C_{RBC}$ values of Weddell seals tagged along the Victoria Land coast were 0.3‰ higher than seals tagged near Ross Island. For a given tagging location and year, an increase in mass of 100 kg is associated with a 0.2‰ increase in $\delta^{13}C_{RBC}$ values. In contrast, mass was the only significant predictor of $\delta^{13}C_{vibrissa}$ values with a 0.1‰ increase in $\delta^{13}C_{vibrissa}$ values for every 100 kg increase in mass.

For both RBCs and vibrissae, mass, and year best explained the variation in $\delta^{15}N$ values. However, the relationship between $\delta^{15}N$ values and mass varied among years. In 2010 and 2011, $\delta^{15}N_{RBC}$ values increased 0.2‰ and 0.1‰, respectively, for every 100 kg increase in mass but, in 2012, $\delta^{15}N_{RBC}$ values decreased <0.1‰ for every 100 kg increase in seal mass. We observed a different relationship between $\delta^{15}N_{vibrissae}$ values among years than for $\delta^{15}N_{RBC}$ values. In both 2010 and 2012, $\delta^{15}N_{vibrissae}$ values increased $\sim$0.1‰ for every 100 kg increase in mass. However, in 2011 $\delta^{15}N_{vibrissa}$ values decreased by 0.2‰ for every 100 kg increase in mass.

Both $\delta^{13}C_{RBC}$ and $\delta^{15}N_{RBC}$ values increased significantly with Weddell seal age ($\delta^{13}C = -25.54 + 0.03$ (age), $R^2_{adj} = 0.19$, $P=0.01$; $\delta^{15}N = 11.7 + 0.3$ (age), $R^2_{adj} = 0.19$, $P=0.01$) (Fig. 3). The relationship between $\delta^{15}N_{RBC}$ values and age suggests that Weddell seals may be consuming a larger proportion of higher trophic level prey within a period of approximately 10–12 years. There was no relationship between either $\delta^{13}C_{vibrissae}$ values or $\delta^{15}N_{Vibrissae}$ values and age ($\delta^{13}C_{vibrissae}$: $R^2_{adj} = 0.00$, $P=0.94$; $\delta^{15}N_{vibrissae}$: $R^2_{adj} = 0$, $P=0.39$) (Fig. 3).

Results from linear mixed models showed no significant intra-individual differences in $\delta^{15}N_{RBC}$ values between January and October ($P=0.23$). However, $\delta^{13}C_{RBC}$ values from samples collected in October were significantly higher than samples collected from the same individuals in January ($P=0.01$). There was no significant difference in either mean $\delta^{13}C_{Vibrissae}$ ($P=0.88$) or mean $\delta^{15}N_{Vibrissae}$ ($P=0.23$) values for individuals sampled in January and October.

Table 2
Predictors of $\delta^{13}C$ and $\delta^{15}N$ values from two Weddell seal tissues (RBC: red blood cells ($n=91$), and vibrissae ($n=41$) obtained from linear mixed models).
$\delta^{13}C$ estimate (95% CI)

Carbon RBCs	**$\delta^{13}C$ estimate (95% CI)**	**SE**	***t*-Value**	***P***
Intercept	−2.55E+01 (−2.58E+01 to −2.52E+01)	1.18E−01	−215.46	< 0.001
Mass	1.66E−03 (8.47E−04 to 2.47E−03)	4.21E−04	3.94	< 0.001
Year 2011	−1.80E−01 (−3.12E−01 to −4.82E−02)	6.82E−02	−2.64	0.008
Year 2012	−2.34E−01 (−3.85E−01 to −8.21E−02)	7.86E−02	−2.97	0.003
Location South	−2.65E−01 (−4.01E−01 to −1.29E−01)	7.05E−02	−3.76	< 0.001
Carbon Vibrissae				
Intercept	−2.37E+01 (−2.40E+01 to 2.33E+01)	−1.93E−01	122.94	0.000
Mass	8.51E−04 (−2.74E−04 to 1.98E−03)	5.69E−04	1.50	0.135
Nitrogen RBCs	**$\delta^{15}N$ estimate (95% CI)**	**SE**	***t*-Value**	***P***
Intercept	1.13E+01 (1.09E+01 to 1.16E+01)	1.71E−01	65.84	0.000
Mass	2.07E−03 (9.84E−04 to 3.17E−03)	5.64E−04	3.66	< 0.001
Year 2011	4.50E−01 (−6.99E−02 to 9.69E−01)	2.70E−01	1.67	0.096
Year 2012	9.24E−01 (3.38E−01 to 1.50E+00)	2.95E−01	3.13	0.002
Mass: Year 2011	−1.18E−03 (−2.82E−03 to 4.66E−04)	8.54E−04	−1.38	0.168
Mass: Year 2012	−2.44E−03 (−4.08E−03 to −7.49E−04)	8.52E−04	−2.86	0.004
Nitrogen Vibrissae				
Intercept	1.23E+01 (1.17E+01 to 128E+01)	2.76E−01	44.52	0.000
Mass	1.55E−03 (−1.44E−04 to 3.36E−03)	8.88E−04	1.75	0.080
Year 2011	1.30E+00 (4.27E−01 to 2.20E+00)	4.53E−01	2.87	0.004
Year 2012	2.43E−01 (−5.60E−01 to 1.09E+00)	4.19E−01	0.58	0.562
Mass: Year 2011	−3.28E−03 (−6.10E−03 to −5.53E−04)	1.41E−03	−2.33	0.020
Mass: Year 2012	3.27E−05 (−2.52E−03 to 2.45E−03)	1.25E−03	0.03	0.979

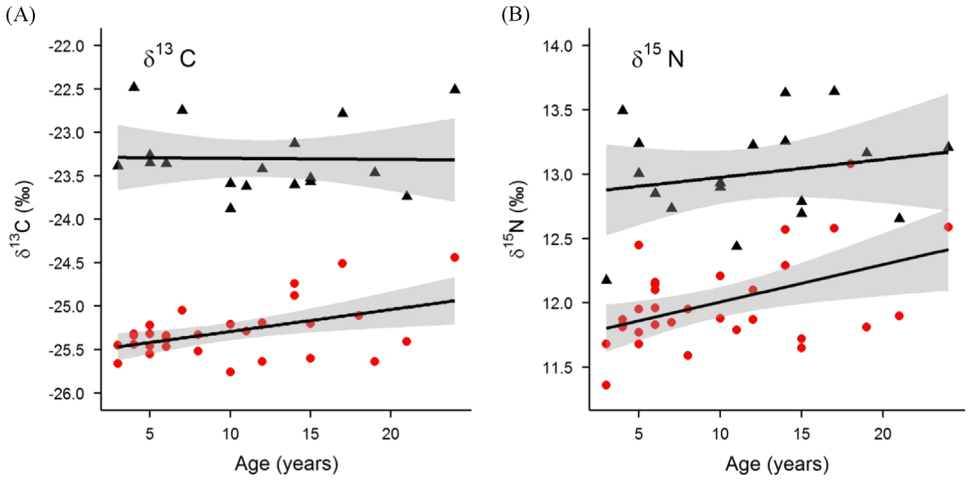

Fig. 3. Results from general linear models showing the variation in (A) $\delta^{13}C$ values and (B) $\delta^{15}N$ values for both red blood cell (RBC) and vibrissae with known age of Weddell seals. Lines of best fit are shown as solid black lines with RBC values shown as circles and mean vibrissae values shown as triangles ($\delta^{13}C_{RBC}$: $R^2_{adj}=0.20$, $P=0.01$; $\delta^{13}C_{Vibrissae}$: $R^2_{adj}=0$, $P=0.94$; $\delta^{15}N_{RBC}$: $R^2_{adj}=0.18$, $P=0.01$; $\delta^{15}N_{Vibrissae}$: $R^2_{adj}=0$, $P=0.39$).

3.2. Diet and foraging ecology

One of the limitations of using stable isotopes to reconstruct diet is that this method does not allow for prey identification and, therefore, prey items considered must be isotopically distinguishable (see electronic Supplementary material for more information). Because several species in our study were isotopically indistinguishable from others, several species were combined into prey groups (Fig. 4, Table 3).

Mixing model results from RBC isotopic values showed that over 75% of the Weddell seal diet, on a population-level, consisted of two species/species groups: group *d* (*Pleurogramma antarcticum* and *Trematomus newnesi*, median: 59.2%, range: 40.2–81.0%) and *e* (*Neopagetopsis ionah*, median: 22.1%, range: 3.0–35.2%) (Fig. 5a). Similarly, mixing model estimates from mean vibrissae isotopic values showed that prey group *d* (median: 69.3%, range: 43.9–89.5%) contributed the most to the diet of seals. Prey group *c* contributed nearly the same proportion to Weddell seal diet over both a short-term period (*Pagothenia borchgrevinki*, *T. Nicolai*, *T. pennellii*, and *T. bernacchii*; median RBC: 14.1%, range (0.0–24.8%)) and a long-term period (median vibrissae: 17.9%, range: 0.0–34.4%) (Fig. 5b).

Examining mixing model results of RBC and vibrissae isotopic values separately for each seal, we found evidence of variation in the proportional contribution of prey items to the diet of individuals. For example, for prey groups *d* and *e* which contributed the highest proportion to the diet of Weddell seals on a population level, over a period of weeks to months, individual median values ranged from 21.6% to 63.7% for prey group *d* and from 9.7% to 36.3% for prey group *e* (Table S1). Prey group *d*, which contributed the most to Weddell seal diet over a period of weeks to a year also showed individual variation ranging between 23.3% and 84.6% (Table S2). Despite prey groups *a* (*D. mawsoni*) and *b* (*T. hansoni*)

contributing only 1.6% (0–10.3%) and 2.0% (0–11.5%) to the diet of Weddell seals on a population level, over a short-term and long-term period, respectively, the relative contribution of these prey groups varied between individuals. The median percent contribution of prey group *a* varied individually between 2.3% and 19.3% (RBC) and between 0.8% and 15.8% (vibrissae) while prey group *b* ranged between 3.2% and 20.4% (RBC) and 1.1% and 17.3% (vibrissae). Mixing model results for all individuals are provided in Supplementary material (Tables S1 and S2).

4. Discussion

4.1. Isotopic variation

This is the first study to quantify isotopic variation in $\delta^{13}C$ and $\delta^{15}N$ for two different tissues (RBCs and vibrissae), allowing us to quantify isotopic variation and the diet of Weddell seals over two time scales. The increase in $\delta^{13}C_{RBC}$ values with mass suggests that larger animals were diving to or near the benthos or staying closer to the coast than smaller animals during the weeks before blood samples were collected (France, 1995). However, the relationship between $\delta^{13}C_{RBC}$ values and mass depended on year and sampling location; $\delta^{13}C$ values were 0.2‰ lower in both 2011 and 2012 compared to 2010 and, after accounting for mass and year; animals along the Victoria Land coast were more ^{13}C enriched than seals closer to Ross Island. In contrast, neither year nor location were significant predictors of $\delta^{13}C_{vibrissa}$ values, which is likely due to the integration of $\delta^{13}C$ values over the longer period of vibrissa growth (months to a year).

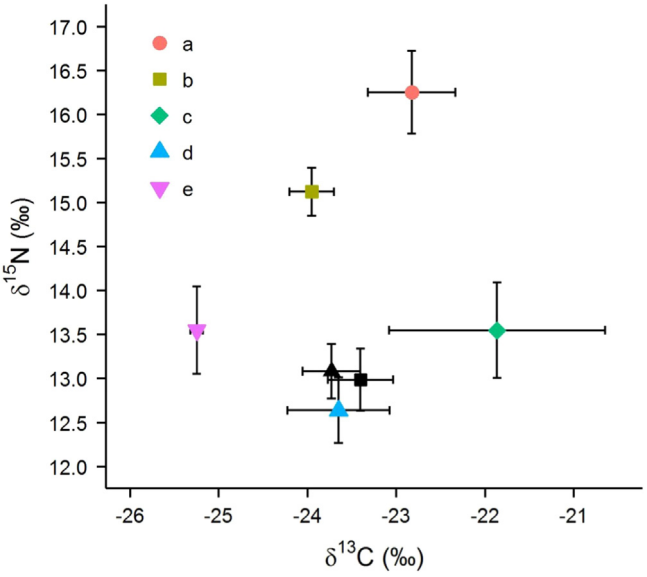

Fig. 4. Means and standard deviations of $\delta^{13}C$ and $\delta^{15}N$ values from two Weddell seal tissues (red blood cells (RBC) and vibrissae) and five prey species or species groups. RBC and vibrissa isotopic values for Weddell seals are shown as a black triangle and square. Isotopic values for RBCs were adjusted to vibrissae by adding 1.5‰ to $\delta^{13}C$ and 1.1‰ to $\delta^{15}N$ values. Isotopic values for prey sources were adjusted to the predator by adding 0.5‰ and 2.8‰ to $\delta^{13}C$ and $\delta^{15}N$ values, respectively. Prey species are defined as follows: *a*: *Dissostichus mawsoni*; *b*: *Trematomus hansoni*; *c*: *Pagothenia borchgrevinki*, *T. nicolai*, *T. pennellii*, and *T. bernacchii*; *d*: *Pleurogramma antarcticum* and *T. newnesi*; *e*: *Neopagetopsis ionah*.

Differences in the relationship between $\delta^{13}C_{RBC}$ values and Weddell seal mass among years may be related to the annual variability of ice conditions in the Ross Sea. During the three years of this study, the sea-ice extent in the Ross Sea was more reduced in 2011 than either 2010 or 2012 (Przyborski, 2015). The presence of sea-ice alters the plankton distribution and composition at the base of the food web (Smith et al., 2012). In the heavy sea-ice years 1997–1998 and 2000–2001, phytoplankton blooms were greatly reduced with chlorophyll-*a* concentration peaking two months later than reduced sea-ice years (Arrigo and van Dijken, 2004). While the exact changes in biota related to fluctuations in sea-ice concentration and extent are difficult to predict, they will undoubtedly cause a shift in the prey base, propagating up to the consumer (Lorrain et al., 2009). The observed differences in $\delta^{13}C$ values over a short time period (RBCs) appear to reflect differences in these dynamic ice conditions, whereas evidence suggests that $\delta^{13}C$ values integrated over a much longer time period (vibrissae) does not reflect the highly dynamic nature of sea ice and its effects on the food web.

RBC $\delta^{15}N$ and $\delta^{15}N_{vibrissa}$ values changed with mass, but the direction of the relationship varied by year and was not consistent between tissue types. Because $\delta^{15}N$ values of animals are strongly associated with trophic position (Hobson et al., 1996; Post, 2002), these results suggest that diet varies as a function of a seal's mass over a period of weeks as well as over a longer time period. However, the inter-annual difference in $\delta^{15}N$ values in relation to mass suggests that seals shift their diet between years and is likely the result of changing environmental conditions that influence prey availability. The differences in $\delta^{15}N$ values between the two tissues are likely the result of life history characteristics in which Weddell seals have a limited foraging range while tied to the breeding colonies (October–January). Wheatley et al. (2008) found that larger females fed more during the lactation period in October–November than smaller females and suggests that larger animals are able to exploit different prey resources due to their physiological capacity to dive longer. Our results support this finding and further suggest that larger adult animals forage on higher trophic-level prey than smaller adult animals.

Significantly increasing $\delta^{15}N_{RBC}$ values with age suggests that older adult Weddell seals consume higher trophic level prey compared to younger adult animals. In addition, increasing $\delta^{13}C_{RBC}$

Table 3
Species identification, assigned prey group, number of individuals (*n*), standard length, mass and isotopic values ($\delta^{13}C$ and $\delta^{15}N$) of Weddell seal prey species collected opportunistically from the Ross Sea 2010–2012. Isotopic values are presented as mean ± standard deviation. Due to indistinguishable isotopic differences, species belonging to the same prey group were combined before using in mixing models to estimate Weddell seal diet.

Species	Prey Group	*n*	Length (cm) (range)	Mass (g) (range)	$\delta^{13}C \pm SD$	$\delta^{15}N \pm SD$
Dissostichus mawsoni	a	9	98.7 (67.0–123.0)	17744.4 (5,500.0–37,000.0)	−23.6 ± 0.5	13.5 ± 0.5
Trematomus hansoni	b	6	20.6 (16.9–27.2)	160.4 (77.4–374.4)	−24.8 ± 0.2	12.3 ± 0.3
Pagothenia borchgrevinki	c	8	16.1 (11.4–19.3)	62.6 (20.7–97.3)	−23.3 ± 0.5	10.4 ± 0.3
Trematomus nicolai	c	11	14.8 (11.7–17.6)	74.3 (27.7–114.7)	−23.0 ± 0.8	10.5 ± 0.5
Trematomus bernacchii	c	26	17.2 (12.0–21.9)	114.5 (38.6–236.8)	−22.3 ± 1.5	11.0 ± 0.5
Trematomus pennellii	c	3	12.2 (10.8–14.6)	41.29 (22.4–76.6)	−22.7 ± 0.4	10.7 ± 0.9
Trematomus newnesi	d	11	15.5 (14.8–17.6)	73.1 (49.4–123.0)	−24.5 ± 0.5	10.0 ± 0.3
Pleurogramma antarcticum	d	3	11.5 (6.4–18.0)	15.0 (1.8–33.2)	−24.3 ± 0.9	9.4 ± 0.2
Neopagetopsis ionah[a]	e	2	–	–	−26.1 ± 0.1	11.1 ± 0.7

[a] Isotopic values for *Neopagetopsis ionah* are from Jo et al. (2013).

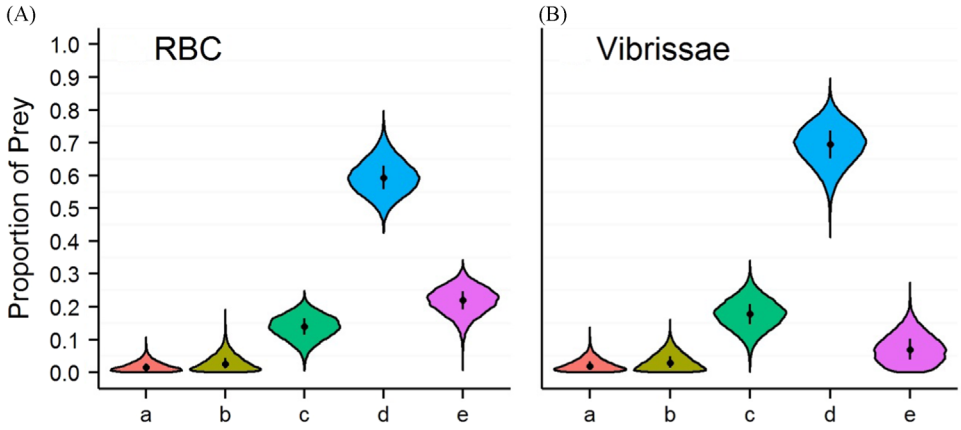

Fig. 5. The proportional contribution of five fish species or species groups to the diet of Weddell seals in the western Ross Sea, Antarctica, as determined by (A) red blood cells (RBC) and (B) mean vibrissae $\delta^{13}C$ and $\delta^{15}N$ values. Points and lines show median and interquartile range, respectively. The distribution of the data for each prey species is indicated by the shape, representing kernel density plots. Prey species are defined as follows: *a*: *Dissostichus mawsoni*; *b*: *Trematomus hansoni*; *c*: *Pagothenia borchgrevinki*, *T. nicolai*, *T. pennellii*, and *T. bernacchii*; *d*: *Pleurogramma antarcticum* and *T. newnesi*; *e*: *Neopagetopsis ionah*.

values with age suggest either the older animals forage more coastally or consume a higher proportion of benthic/demersal prey than younger animals. Zhao et al. (2004) found that adult Weddell seals had higher $\delta^{13}C$ and $\delta^{15}N$ values than subadults, suggesting that adults were feeding at a higher trophic level and were possibly engaged in more benthic foraging than subadults. However, Zhao et al. (2004) did not have known age data for animals in their study, thus precluding comparisons with our study. Research on other marine mammal species have showed similar patterns between isotope values and known age. For example, Hanson et al. (2009) examined $\delta^{13}C$ and $\delta^{15}N$ values of Antarctic fur seal (*Arctocephalus gazelle*) teeth and found that animals tended to forage on higher trophic level prey as they aged and Marcoux et al. (2012) found this same relationship between age and isotope values in beluga whales (*Delphinapterus leucas*) and suggested that the differences in isotope values between age groups may be explained by physiological states associated with reproduction and body condition. Although $\delta^{15}N_{vibrissa}$ values were also higher in older than younger Weddell seals, the difference was not significant, likely due to the integration of diet over many months.

Intra-individual differences in RBC $\delta^{13}C$ values are most likely a reflection of Weddell seal life history characteristics and the rate of tissue turnover. The samples in January–February (summer) were collected after the seals had spent at least two months breeding and molting on the fast ice. While at the breeding colony, Weddell seals engage in short foraging trips and the enriched ^{13}C values are probably an indication of an individual's coastal, and possibly benthic, foraging behavior. During this time, animals were also losing mass, thus RBCs might reflect a mix of dietary and endogenous tissue contribution. The RBC samples from October–November (spring) were collected shortly after animals arrived at the breeding colony and $\delta^{13}C$ values were probably a reflection of the overwinter foraging trip during which many animals travel long distances towards the continental shelf break. Individual differences in mean vibrissae $\delta^{13}C$ values were not observed and, as stated previously, were likely due to the integration of $\delta^{13}C$ values over a longer period of time.

The similarity in both RBC and vibrissae $\delta^{15}N$ values within individuals collected at different time periods suggest that individual Weddell seals were foraging at the same trophic level during both the summer molting season and the winter foraging/spring breeding season. The lack of intra-individual differences in $\delta^{15}N$ values for either tissue implies that individuals either have similar diets across seasons, or that they specialize on prey items in a sequential fashion in which the integration of $\delta^{15}N$ values over the time appeared consistent.

4.2. Diet and foraging ecology

Consistent with previous studies, mixing models identified *P. antarcticum* and *Trematomus species* (including *P. borchgrevinki*, previously known as *T. borchgrevinki*) as important prey items for Weddell seals (Burns et al., 1998; Davis et al., 1999; Dearborn, 1965; Fuiman et al., 2002; Zhao et al., 2004); however, the percent contribution of these prey items differed by foraging timescale, represented by each tissue type. Isotopic values for RBCs indicated that *N. ionah* contributed more to Weddell seal diet over the most recent weeks–months than over a longer period of months to a year. While past diet studies do not identify *N. ionah* (family Channichthyidae) as an important prey item, in January 2012 we observed Weddell seals in a large ice hole feeding on what is most likely this species (species identification from photograph by Dr. B. Buckley of Portland State University, pers. comm.). While most channichthyids are benthic, *N. ionah* has a bentho-pelagic lifestyle, traveling into pelagic waters at night to feed in the water column (Eastman and Hubold, 1999; La Mesa et al., 2004).

Results from mixing models using vibrissae $\delta^{13}C$ and $\delta^{15}N$ values indicate that Weddell seals were feeding on different species and in different proportions over a period of months to a year compared to a period of weeks to months reflected in RBCs. Species in groups *c* (*P. borchgrevinki*, *T. nicolai*, *T. bernachii*, and *T. pennellii*) and *d* (*P. antarcticum* and *T. newnesi*) were the two dominant prey groups in the diet of Weddell seals over a period of months to a year. *P. borchgrevinki* is a cryopelagic species, associated with the undersurface of the ice, while *T. nicolai* is benthic and inhabits shallow waters 30–50 m deep, often close to anchor ice (Eastman and DeVries, 1982, 1985; La Mesa et al., 2004). These results agree with Burns et al. (1998) in which Weddell seals were found to feed primarily on *P. antarcticum*, *P. borchgrevinki*, and other *Trematomus* species; however, results from Burns et al. were obtained using isotopic values from blood plasma rather than vibrissae and *N. ionah* was not considered as a potential prey item. Animal-borne cameras have also documented Weddell seals hunting *P. borchgrevinki* in the platelet ice by blowing air into subice crevices (Davis et al., 1999).

Overall, this study identified the prey group consisting of *P. antarcticum* and *T. newnesi* as an important prey item in the diet of Weddell seals over a period of weeks to a year and is likely a result of the species' abundance and unique life history characteristics. *P. antarcticum* is the dominant fish in the Ross Sea, representing over 90% of both the number and biomass of all fish species (Dewitt, 1970; Hubold, 1985), and likely plays an important role in the mid-water shelf ecosystem where krill are less abundant (Hempel,

1985). Indeed, scat and stomach content analysis have confirmed the presence of *P. antarcticum* in the diet of most Weddell seals (Burns et al., 1998; Castellini et al., 1992). By attaching video cameras to Weddell seals foraging under the sea-ice, Fuiman et al. (2002) showed that seals forage on loose aggregations of *P. antarcticum* which exhibit diel vertical migration at mean depths of 252 m at night and 346 m during the day. The diel migration of both *P. antarcticum* and *N. ionah* match the diel diving pattern of Weddell seals, diving deeper during the day and shallower at night, observed previously (Kooyman, 1981; Kooyman, 1975) and during this study (Goetz et al., unpublished data).

While *N. ionah,* and the species in prey groups *c* and *d* were found to have the highest contribution to the diet of Weddell seals in the western Ross Sea, it is important to recognize that there are also individual differences in diet. Prey groups *c* and *d* were the most variable in their contribution while *D. mawsoni*, and *T. hansoni*, were the least variable in the degree of their contribution to the diet of individual seals. Because the hard parts of *D. mawsoni* are not consumed, and therefore are not detected in scat and stomach content analyses, there has been considerable debate regarding the importance of this species in the diet of Weddell seals (Ainley and Siniff, 2009; Burns et al., 1998; Davis et al., 1999; Fuiman et al., 2002; Kim et al., 2011; Ponganis and Stockard, 2007).

Although the contribution of *D. mawsoni* to the diet of Weddell seals was low, it has the highest fat content (% wet mass) and energy density (kJ g^{-1} wet mass) of the six notothenid species analyzed (Lenky et al., 2012). We hypothesize that Weddell seals increase their consumption of higher trophic level prey as they age. For example, given their large size, it would seem logical that some degree of experience is needed to capture a toothfish. In addition, the need for more energy rich prey may be driven by the substantial loss in body condition during the reproduction and molting season. Although additional data are needed to confirm this hypothesis, our data suggest that a small proportion of the animals with known age consume a higher proportions of higher trophic level prey (*a*: *D. mawsoni*, *b*: *T. hansoni* and prey group *c*) before their peak reproductive age of 14–15 years than afterwards (Fig. S2). In addition, the data suggest that Weddell seals consume relatively constant or increasing proportion of lower trophic level fish such as *P. antarcticum* and *N. ionah* with age. Due to the abundance of *P. antarcticum*, they may be easier to capture by Weddell seals, especially for older animals exhibiting tooth wear as a result of ice reaming. However, due to the small sample size, more research is needed to verify our hypothesis. By providing the first quantitative estimates of isotopic variation and diet of Weddell seals, this study provides novel insight into the feeding ecology of this important Antarctic predator and its role within the Ross Sea ecosystem.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.dsr2.2016.05.017.

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