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FEATURE ARTICLE

# Trophic position and foraging ecology of Ross, Weddell, and crabeater seals revealed by compound-specific isotope analysis

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ABSTRACT: Ross seals *Ommatophoca rossii* are one of the least studied marine mammals, with little known about their foraging ecology. Research to date using bulk stable isotope analysis suggests that Ross seals have a trophic position intermediate between that of Weddell *Leptonychotes weddellii* and crabeater *Lobodon carcinophaga* seals. However, consumer bulk stable isotope values not only reflect trophic dynamics, but also variations in baseline isotope values, which can be substantial. We used compound-specific isotope analysis of amino acids (CSI-AA) to separate isotopic effects of a shifting baseline versus trophic structure on the foraging ecology of these ecologically important Antarctic pinnipeds. We found that Ross seals forage in an open ocean food web, while crabeater and Weddell seals forage within similar food webs closer to shore. However, isotopic evidence suggests that crabeater seals are likely following sea ice, while Weddell seals target productive areas of the continental shelf of West Antarctica. Our CSI-AA data indicate that Ross seals have a high trophic position equivalent to that of Weddell seals, contrary to prior conclusions from nitrogen isotope results on bulk tissues. CSI-AA indicates that crabeater seals are at a trophic position lower than that of Ross and Weddell seals, consistent with a krill-dominated diet. Our results redefine the view of the trophic dynamics and foraging ecology of the Ross seal, and also highlight the importance of quantifying baseline isotope variations in foraging studies.

KEY WORDS: Ross seal · Weddell seal · Crabeater seal · Compound-specific isotopes · Amino acids · Antarctica · Foraging ecology · Trophic dynamics

Weddell seal female and her pup in McMurdo Sound, Antarctica.
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## 1. INTRODUCTION

The Ross seal *Ommatophoca rossii* is one of the least studied marine mammals (Bengtson et al. 2011, Würsig et al. 2018). The total population estimate for this species is around 200 000, considerably less than the estimates for other Antarctic true seals, which are approximately one million individuals for Weddell seals *Leptonychotes weddellii* and 10 to 15 million individuals for crabeater seals *Lobodon carcinophaga* (Laws 1977, Bengtson et al. 2011, Würsig et al. 2018). All 3 of these pinniped species have circumpolar distributions (Laws 1977, Würsig et al. 2018). Ross seals have fairly small and narrow bodies (with body masses up to about 250 kg) with short snouts and small mouths. Weddell and crabeater seals are generally larger than Ross seals, having body masses up to about 600 kg (Würsig et al. 2018). Weddell seals have bulkier bodies with small heads relative to their body sizes and short snouts, while crabeater seals have slender bodies with long, upturned snouts. Ross seals are not commonly observed, which is likely because they spend most of their time at sea and in habitats that are logistically challenging to access (Laws 1977, Würsig et al. 2018). Thus, several key aspects of Ross seal biology remain poorly understood, including their preferred prey, foraging habitat, and behavior. In contrast, many studies have been conducted on crabeater and Weddell seals, and thus more information is available on their ecology (Hückstädt et al. 2012a, Arcalís-Planas et al. 2015, Goetz et al. 2017).

Diets of Antarctic predators, such as Ross, Weddell, and crabeater seals, may consist of various fish, cephalopods, and zooplankton species (Laws 1977, Pinkerton et al. 2010). Possible fish prey include Antarctic toothfish *Dissotichus mawsoni*, Antarctic silverfish *Pleuragamma antarcticum*, and cod icefishes *Trematomus* spp. Glacial squid *Psychroteuthis glacialis* is a cephalopod and known diet component of some Antarctic consumers. Antarctic krill *Euphausia superba* are key prey for an array of predators. Other krill species, such as ice krill *E. crystallorophias* may contribute to their diets. In the subsequent text, 'krill' refers to various species within the order Euphausiacea. Krill are crustaceans that are largely herbivorous and form the link between primary producers (phytoplankton in marine systems) and apex predators.

Conventional techniques for studying an animal's diet, such as scat and stomach content analysis, have significant limitations when applied to Antarctic pinnipeds. These methods capture only a snapshot of a predator's diet, perhaps 1 to 2 d (Dellinger & Trillmich 1988, Burns et al. 1998). In addition, soft tissues are more completely digested than hard tissues, resulting in biases towards prey with indigestible hard parts (Burns et al. 1998, Staniland 2002, Arim & Naya 2003, Yonezaki et al. 2003). Given these drawbacks, recent research on Antarctic seal ecology has often used bulk stable isotope values.

Measurements of bulk tissue stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values (i.e. the weighted average of all components within a tissue) have been used to indicate a predator's foraging region and trophic position (Boecklen et al. 2011). The $\delta^{13}C$ and $\delta^{15}N$ values of a consumer are the isotopic values of primary producers, the base of the food web, with modifications resulting from each trophic transfer, when energy is transferred from one trophic level to another (e.g. Lorrain et al. 2009, Graham et al. 2010, Jaeger et al. 2010, Brault et al. 2018). These modifications are predictable and described further below (Minagawa & Wada 1984). Stable isotope analysis has the advantage of providing an integrated view of an animal's diet over longer time scales (weeks to years depending on the tissue) than the traditional procedures (Vander Zanden et al. 2015). Carbon isotope values show little $^{13}C$-enrichment with trophic transfer. Thus, consumer $\delta^{13}C$ values are often thought to closely reflect values at the base of food webs, making them useful for studying the foraging areas of a predator. Spatial changes in the $\delta^{13}C$ of primary producers (often referred to as 'baseline' $\delta^{13}C$ values) may reflect variations in their environmental conditions, such as dissolved inorganic carbon $\delta^{13}C$ values, dissolved $CO_2$ concentrations, $CO_2$ drawdown, and temperature (reviewed in McMahon et al. 2013). They may also reflect physiological characteristics of the primary producers, including internal biological parameters (e.g. growth rate) and structure (e.g. cell size and geometry of phytoplankton) (McMahon et al. 2013). Provided the primary processes driving variation are known, bulk $\delta^{13}C$ values of an animal can give valuable information on its foraging habitat. The Southern Ocean is known to have considerable spatial variation in baseline $\delta^{13}C$ ($\delta^{13}C_{baseline}$) values (Rau et al. 1982, Quillfeldt et al. 2010, Brault et al. 2018). Several studies have observed decreasing $\delta^{13}C$ values with increasing latitude, with offsets between about 55 and 79° S of approximately 3‰, largely reflecting variations in sea surface temperature (Rau et al. 1982, Graham et al. 2010, Quillfeldt et al. 2010, Magozzi et al. 2017, Brault et al. 2018).

In contrast to carbon isotopes, trophic transfers have a considerable affect on an animal's $\delta^{15}N$ val-

ues. Since a consumer's tissues become enriched in $^{15}N$ by ~2–5‰ with each trophic transfer (e.g. primary producers to herbivores to carnivores) due to the preferential loss of $^{14}N$ during amino acid metabolism (Minagawa & Wada 1984), $\delta^{15}N$ values are often used to indicate an animal's trophic position. Yet variations in baseline (i.e. primary producer) $\delta^{15}N$ ($\delta^{15}N_{baseline}$) values also occur and are passed on, with additional change due to trophic transfers, to upper trophic level predators (McMahon et al. 2013). Nutrient source (e.g. nitrate, ammonium, or $N_2$ fixation), microbial transformations (e.g. denitrification), and extent of nitrogen pool drawdown in a given environment can all strongly impact primary producers and, therefore, the $\delta^{15}N_{baseline}$ values (reviewed in McMahon et al. 2013). If variations in these factors are well understood, then the bulk $\delta^{15}N$ values of a consumer can provide insights into its foraging region and trophic position (Post 2002). As with carbon, substantial spatial variations in $\delta^{15}N_{baseline}$ values occur in the Southern Ocean (DiFiore et al. 2006, 2009, Somes et al. 2010, Jaeger et al. 2010, Brault et al. 2018). Much of the Southern Ocean has low $\delta^{15}N_{baseline}$ values, likely due to less complete nutrient drawdown (Somes et al. 2010, Brault et al. 2018). However, areas near the Antarctic continent have extensive coastal open water polynyas, which are often productivity 'hot spots' (Arrigo & van Dijken 2003). These 'hot spots' experience more complete nutrient drawdown and, thus, higher $\delta^{15}N_{baseline}$ values (DiFiore et al. 2006, 2009, Brault et al. 2018). Indeed, coastal regions may have $\delta^{15}N_{baseline}$ values that are up to ~3‰ higher than those in open ocean regions (DiFiore et al. 2006, 2009, Brault et al. 2018). In summary, a gradient of increasing $\delta^{15}N_{baseline}$ values from open ocean to coastal Antarctic areas likely derives from increasing nutrient drawdown due to enhanced primary productivity, the main process influencing $\delta^{15}N_{baseline}$ values in the Southern Ocean since it is a high-nutrient–low-chlorophyll (HNLC) region (Brault et al. 2018).

Although much remains unknown, especially regarding the behaviors and movements of the Ross seal, recent studies have furthered our understanding of Antarctic seal ecology. Isotopic measurements, coupled with traditional ecological methods, have indicated that Weddell seals forage near the top of the Antarctic food web, consuming diverse diets of fish, cephalopods, and invertebrates (Burns et al. 1998, Plötz et al. 2001, Lake et al. 2003, Goetz et al. 2017). Researchers have debated the contribution of upper trophic level prey species, in particular *D. mawsoni*, to Weddell seal diets (Goetz et al. 2017), with some studies suggesting that *D. mawsoni* may comprise nearly the entire daily diet of Weddell seals during the spring and summer in McMurdo Sound (Plötz 1986, Ponganis & Stockard 2007, Ainley & Siniff 2009, Goetz et al. 2017). Hard parts of *D. mawsoni* are not consumed and thus not detected via scat and stomach content analyses, techniques that have been used for much of the prior research on Weddell seal foraging ecology (see Goetz et al. 2017). Recently, Goetz et al. (2017) assessed Weddell seal foraging ecology with bulk $\delta^{13}C$ and $\delta^{15}N$ measurements of vibrissae and red blood cells from Ross Sea specimens. They reported considerable individual variability in diet, and that *P. antarcticum* and *Trematomus* spp. were the primary prey consumed by Weddell seals. Overall, *D. mawsoni* contribute less than 2% to the Weddell seal diet. However, *D. mawsoni* may become increasingly important with age and at certain times in the life cycle, such as during reproduction and molting, since this fish has a high fat content and energy density (Goetz et al. 2017). Additionally, Goetz et al. (2017) noted temporal diet shifts—likely in response to sea ice dynamics affecting prey abundances.

Crabeater seals occupy a much lower trophic level than Weddell seals, with diets dominated by *E. superba*, as evidenced by the results of both scat and stomach content analyses as well as bulk isotopic analysis (Laws 1977, Rau et al. 1992, Burns et al. 2004, 2008, Zhao et al. 2004, Aubail et al. 2011). Recent work by Hückstädt et al. (2012a) revealed temporal variability in crabeater seal diet composition via bulk $\delta^{13}C$ and $\delta^{15}N$ measurements of vibrissae. The *E. superba* contribution ranged from 81 to 95%, likely in response to climate shifts affecting its abundances. The authors also reported significant variation in $\delta^{13}C$ values with body mass (increasing $\delta^{13}C$ values with increasing body mass) and season (highest $\delta^{13}C$ values in the austral winter) that they suggested might result from changes in $\delta^{13}C_{baseline}$ values associated with temporal and/or spatial shifts between open ocean phytoplankton and sea ice diatoms (Hückstädt et al. 2012a). As sea ice diatoms have higher $\delta^{13}C$ values than those of open ocean diatoms, increasing $\delta^{13}C$ values with increasing crabeater seal body mass may indicate a greater use with age of a food web based on sea ice diatoms rather than open ocean diatoms (Hückstädt et al. 2012a). Likewise, high crabeater seal $\delta^{13}C$ values in the austral winter may show heavy use of a sea ice diatom based food web during this season (Hückstädt et al. 2012a).

Only a small number of studies have examined Ross seal foraging ecology. Dive records suggest that

these animals typically dive from 100 to 300 m (maximum depth of 792 m) in search of mesopelagic squid and fish, depths that are similar to those undertaken by Weddell seals and greater than those typically performed by crabeater seals (Bengtson & Stewart 1997, Blix & Nordøy 2007). Analysis of Ross seal stomach contents showed that *P. antarcticum* and *P. glacialis* were found in varying proportions (Skinner & Klages 1994). Arcalís-Planas et al. (2015) suggested little use of sea ice by Ross seals, based on telemetry and remote sensing data. Seals tracked by Arcalís-Planas et al. (2015) generally remained in the open ocean except for haul outs on ice to molt (from December to January) and breed (from late October to mid-November). During their open ocean period of February to mid-October, Ross seals remained an average of ~840 km (range 587 to 1282 km) seaward from the ice edge (Arcalís-Planas et al. 2015). Bulk $\delta^{13}C$ and $\delta^{15}N$ isotope measurements place the Ross seal a trophic level intermediate between Weddell seals and crabeater seals (Rau et al. 1992, Zhao et al. 2004, Aubail et al. 2011). Thus, bulk stable isotope results to date suggest that Ross seals consume mostly squid and fish, such as *P. antarcticum and P. glacialis*, but with a contribution from lower trophic level prey like *E. superba* (Rau et al. 1992, Zhao et al. 2004, Aubail et al. 2011).

While these bulk stable isotope approaches have shed new light on the foraging ecology of Antarctic pinnipeds, it is critical to remember that the Southern Ocean exhibits strong spatial gradients in both $\delta^{13}C_{baseline}$ and $\delta^{15}N_{baseline}$ values (DiFiore et al. 2006, 2009, Jaeger et al. 2010, Somes et al. 2010, Brault et al. 2018), suggesting that we likely need to explicitly tease apart the relative influences of trophic dynamics and baseline variation on seal isotope values. For instance, Ross seals may spend more time in the open ocean than other Antarctic seals (Arcalís-Planas et al. 2015), and thus forage in areas with lower $\delta^{15}N_{baseline}$ values than the nearshore regions used by Weddell and crabeater seals (DiFiore et al. 2006, 2009, Brault et al. 2018). If so, not accounting for spatial variation in the $\delta^{15}N_{baseline}$ values would result in Ross seals being assigned a lower trophic position than species foraging nearer the continent.

Compound-specific isotopic analysis of amino acids (CSI-AA) has opened new doors to studying the foraging ecology and trophic dynamics of marine predators (e.g. Graham et al. 2010). Since only certain amino acids become enriched in $^{15}N$ with increasing trophic level ('trophic' amino acids), while others ('source' amino acids) do not, impacts of $\delta^{15}N_{baseline}$ variation and trophic position on consumer $\delta^{15}N$ values can be disentangled using this technique (Ohkouchi et al. 2017). Glutamic acid + glutamine (Glu) and phenylalanine (Phe) are widely considered the most representative trophic and source amino acids, respectively, with Phe $\delta^{15}N$ values typically used as a proxy for baseline isotope values, and Phe and Glu $\delta^{15}N$ values ($\delta^{15}N_{Phe}$ and $\delta^{15}N_{Glu}$, correspondingly) of a consumer used together to estimate its trophic position (TP) based on the unique $\delta^{15}N_{baseline}$ value of its environment (Ohkouchi et al. 2017). Proline (Pro) has also been shown to be a reliable trophic amino acid, with less variability than Glu for trophic $^{15}N$-enrichment factors between organisms (McMahon et al. 2015). As a consequence, Pro and Phe may represent a new CSI-AA combination that provides more ecologically realistic TP estimates for higher trophic level consumers, including marine mammals, although we do not yet understand the underlying mechanism (McMahon & McCarthy 2016). As mentioned above, trophic discrimination factors (TDFs) using Pro appear to be less variable than those with Glu (McMahon et al. 2015). This difference may be a result of Pro having a more consistent metabolic pathway through the internal nitrogen cycle than Glu (McMahon & McCarthy 2016)

Here, we report the first CSI-AA data for modern Ross, Weddell, and crabeater seals to refine our understanding of the trophic dynamics and foraging ecology of these important Antarctic predators. In particular, CSI-AA allows us to directly examine changes in $\delta^{15}N_{baseline}$ values linked to these seals' diets, to gain information on their foraging regions in conjunction with their trophic dynamics. Comparison of amino acid isotope data from these 3 Antarctic seals will also further our understanding of present Antarctic food web structures, which will provide valuable ecosystem baselines in light of ongoing climate change (Atkinson et al. 2004, Ducklow et al. 2007, 2012, Montes-Hugo et al. 2009).

## 2. MATERIALS AND METHODS

### 2.1. Sample collection

Tissue samples from Ross (n = 15), Weddell (n = 38), and crabeater seals (n = 41) were collected along Western Antarctica from the West Antarctic Peninsula (WAP) to the Ross Sea during multiple field seasons in the austral summers of 2008/2009 and 2010/2011 aboard the RV 'Oden'. These animal captures were conducted in accordance with the regulations of the Swedish Polar Research Secretariat

(Registration No. 2010-112). All other samples were obtained from animal captures conducted under National Marine Fisheries Service permit No. 87-1851-00. In most cases, body mass, age class (juvenile, subadult, and adult), gender, and location were recorded for each sampled seal (Table S1 in the Supplement at www.int-res.com/articles/suppl/m611p001_supp.pdf). Additionally, the Institutional Animal Care and Use Committee (IACUC) at the University of Santa Cruz (UC Santa Cruz) approved all protocols for these samples.

Whole blood samples were obtained from most seals, and in some cases, clot (blood with serum removed), red blood cells (RBCs; whole blood exposed to an anticoagulant, heparin, before having plasma removed), and hair samples were also collected (Tables S1 & S2). The sampling protocols are described in Aubail et al. (2011) and Goetz et al. (2017). Whiskers were taken from crabeater seals during multiple cruises on the RV 'Lawrence M. Gould' along the WAP during fall 2001 (n = 7), winter 2001 (n = 7), fall 2002 (n = 15), winter 2002 (n = 14), and fall 2007 (n = 9). Plasma was also obtained from a few of the fall 2007 individuals (G105, G110, and G112) (Hückstädt et al. 2012a). In addition, serum or plasma was obtained from 2 Weddell seals during the fall 2007 sampling in this region, and whiskers were taken from 2 WAP Weddell seals in the austral summer of 2009/2010. Whisker samples were also collected from Weddell seals during the summer 2009/2010 (n = 11), summer 2010/2011 (n = 10), summer 2011/2012 (n = 18) (Goetz et al. 2017). Hückstädt et al. (2012a) described the procedure for sampling the whiskers, and Goetz et al. (2017) described the protocol used for collecting seal blood.

Several blood samples were obtained from Weddell seals in the McMurdo Sound region, Ross Sea, Antarctica over multiple field seasons. A total of 12 whole blood samples were taken from juvenile Weddell seals near Inexpressible Island (74.9° S, 163.7° E) during the austral summer of 2010/2011. Whole blood samples were taken from Weddell seals in the austral summer of 2010/2011 (n = 5) and austral spring of 2012 (n = 5). RBCs were sampled in the austral summer of 2009/2010 (n = 5), austral summer of 2011/2012 (n = 5), and austral spring of 2012 (n = 5). Whole blood, plasma, and serum were obtained from 5 Weddell seals sampled in the austral spring of 2015, and whole blood from an additional 7 Weddell seals was also acquired during this time. Goetz et al. (2017) described the sampling protocol for these Weddell seals.

Lastly, a few samples were obtained from crabeater seals in McMurdo Sound. Hair samples were taken from 3 recently deceased juvenile crabeater seals that were found on the seasonal pack ice around Cape Royds in the austral summer of 2009/2010. Whole blood was sampled, using the protocol of Goetz et al. (2017), from a male adult crabeater seal found in Erebus Bay during the austral summer of 2010/2011.

### 2.2. Sample preparation

After sample collection, all samples were kept frozen at −20°C. Blood samples were freeze-dried with a Labconco Freeze Dry System (Lyph Lock 4.5) and homogenized manually prior to isotope analysis. Lipid extraction was not performed on the blood samples, as blood has a relatively low lipid content. A test set of blood samples with and without lipid extraction revealed no significant effect of lipid extraction on blood $\delta^{13}C$ values (Table S3), though lipid extraction did have an undesired impact on $\delta^{15}N$ values (Table S3).

Since hair and whisker samples are known to have surficial contaminants that may affect $\delta^{13}C$ values, these samples were lipid extracted as described in Hückstädt et al. (2012a). These samples were washed with Milli-Q water (Thermo Fisher Scientific) and then rinsed 3 times in an ultrasonic bath with petroleum ether for 15 min.

### 2.3. Bulk stable isotope analysis

For all blood and hair samples, ~1 mg was weighed into tin cups (Costech; 3 × 5 mm) for bulk stable carbon and nitrogen isotope analysis. For hair, the follicle was removed since prior work has shown it to have a different biochemical and isotopic composition than the rest of the hair (Hückstädt et al. 2012b). This analysis was performed at the Stable Isotope Lab (SIL) of the University of California, Santa Cruz (UCSC) on a Carlo Erba EA 1108 elemental analyzer coupled to a Thermo-Finnigan Delta$^{Plus}$ XP isotope ratio mass spectrometer. The $\delta^{13}C$ values were referenced to the V-PDB standard, and $\delta^{15}N$ values were referenced to air. PUGel and Acetanilide standards were analyzed in each instrument session in order to correct for variations in mass across samples and instrument drift. Across 10 analytical sessions, the standard deviations were 0.1‰ (n = 139) for $\delta^{13}C$, 0.1‰ (n = 139) for $\delta^{15}N$, and 0.1 (n = 139) for C/N (atomic) for PUGel and 0.2‰ (n = 38) for $\delta^{13}C$, 0.2‰ (n = 38) for $\delta^{15}N$, and 0.4 (n = 38) for C/N (atomic) for acetanilide.

### 2.4. Compound-specific isotope analysis

To obtain the $\delta^{15}N$ values of amino acids from modern seal tissues, CSI-AA was performed at UCSC via gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS). All samples were prepared for GC-C-IRMS analysis using the methods described in McCarthy et al. (2007, 2013). In brief, samples (~1 mg) were hydrolyzed (6 N HCl for 20 h at 110°C) and converted to trifluoro-acetylated isopropyl amino acid derivatives. Samples were stored at −20°C in a 1:3 TFAA:DCM (trifluoroacetic anhydride: methylene chloride) solution until analysis. Immediately before the analysis, the TFAA/DCM mixture was evaporated under $N_2$ and samples were diluted in ethyl acetate.

Amino acid $\delta^{15}N$ values were measured on a Thermo Trace GC coupled to a Thermo-Finnigan Delta$^{Plus}$ XP isotope-ratio-monitoring mass spectrometer (oxidation furnace at 980°C and reduction furnace at 650°C) using an SGE Analytical Science BPX5 column (60 m by 0.32 mm with a 1 µm film thickness). The injector temperature was 250°C with a split He flow of 2 ml min$^{-1}$. The GC temperature program was as follows: initial temperature of 70°C for 1 min; ramp 1 = 10°C min$^{-1}$ to 185°C, hold for 2 min; ramp 2 = 2°C min$^{-1}$ to 200°C, hold for 10 min; ramp 3 = 30°C min$^{-1}$ to 300°C, hold for 6 min. Directly measured amino acid $\delta^{15}N$ values were corrected based on bracketed external standards of amino acids with known isotopic composition, as described in McCarthy et al. (2013). The $\delta^{15}N$ values of 11 amino acids were quantified: alanine (Ala), glycine (Gly), threonine (Thr), serine (Ser), valine (Val), leucine (Leu), isoleucine (Iso), Pro, aspartic acid + asparagine (Asp), Glu, Phe, and lysine (Lys). We note that hydroxyproline (Hpro) may have co-eluted with Pro during our amino acid $\delta^{15}N$ analysis. However, Hpro was either not present in, or composed a small portion of (<1%), the tissues analyzed in this study (Stein & Moore 1949, 1954, Mahan & Shields 1998).

### 2.5. Data analysis

Most samples were whole blood. Since bulk stable isotope values can vary across different tissues, species-specific corrections were applied to account for isotopic offsets between different types of samples. We show the data used to derive these isotopic offsets and the resulting correction factors in Tables S4–S7. These corrections were applied to all bulk stable isotope data from tissues other than whole blood that had significant isotopic offsets from whole blood. An isotopic offset >0.2‰ was considered significant, since the instrument error is ≤0.2‰. Bulk stable isotope data pre- and post-application of correction factors are presented in Table S8. No correction factors were applied to CSI-AA data since these values are for individual amino acids, isolated from different tissues, typically whole blood (Table S2).

All data analyses were performed in R statistical software (R Core Team 2014). Tests of normality and equal variance were conducted to assure test assumptions were met. In a few cases, an assumption was violated and a data transformation was applied, as noted in 'Results'. Bulk stable isotope values of the 3 different seal species were compared with a 1-way analysis of variance (ANOVA) and post hoc Bonferroni pairwise comparisons. Data were Box-Cox transformed (Box & Cox 1964). A 4-way ANOVA with post hoc Bonferroni pairwise comparisons was used to test for significant effects of gender, sampling period, age class, and region (WAP, Amundsen Sea, and Ross Sea) on the bulk isotopic values of Ross, Weddell, and crabeater seals. Both Weddell and crabeater seal data were Box-Cox transformed. Linear regression analyses were used to test for significant relationships between bulk $\delta^{13}C$ and $\delta^{15}N$ values and body mass for each species.

A 1-way ANOVA with post hoc Bonferroni pairwise comparisons was used to test for significant differences in the $\delta^{15}N$ values of each amino acid among the 3 seal species. This same procedure was conducted to compare the $\delta^{15}N$ values for each category of amino acid (i.e. source or trophic) among the different seal species. For both Weddell and crabeater seals, a 2-tailed Student's *t*-test was used to compare the $\delta^{15}N$ values of Pro, Glu, and Phe for the WAP to those of a combined Amundsen and Ross Sea region ('Amundsen/Ross Sea' in the subsequent text). Amino acid $\delta^{15}N$ values for seals from the Amundsen/Ross Seas were combined by species (crabeater or Weddell) since both species had bulk $\delta^{15}N$ values that were similar between these 2 regions. This analysis was not done for Ross seals since this species was almost exclusively sampled in the Amundsen Sea. A 2-tailed Student's *t*-test was used to compare the $\delta^{15}N_{Phe}$ values of Weddell seals to those of crabeater seals for the WAP, and a 1-way ANOVA with post hoc Bonferroni pairwise comparisons was conducted to assess variation between the $\delta^{15}N_{Phe}$ values of Ross, Weddell, and crabeater seals from the Amundsen/Ross Sea region.

TP estimates using the CSI-AA data ($TP_{CSI-AA}$) were calculated with a modified version of the equation

originally proposed by Chikaraishi et al. (2009). Here, we substituted Pro for Glu as the trophic amino acid, as suggested by the comparative synthesis of $TP_{CSI-AA}$ methods in McMahon & McCarthy (2016), because Pro trophic discrimination appears to be less variable across variations in diet. McMahon & McCarthy (2016) suggest that this new equation will likely produce more ecologically realistic TP estimates for marine mammals. $TP_{CSI-AA}$ was, therefore, calculated as follows:

$$TP_{CSI-AA} = 1 + [(\delta^{15}N_{Pro} - \delta^{15}N_{Phe} - \beta_{Pro/Phe}) / TDF_{Pro-Phe}]$$

where $\delta^{15}N_{Pro}$ is the seal Pro $\delta^{15}N$ value, $\beta_{Pro/Phe}$ is the isotopic difference between Pro and Phe in marine phytoplankton (3.1‰; Chikaraishi et al. 2009), and $TDF_{Pro-Phe}$ is the trophic discrimination between diet and consumer for Pro minus the same for Phe ($\Delta^{15}N_{Pro} - \Delta^{15}N_{Phe}$ = 4.5‰; McMahon & McCarthy 2016). Differences in $TP_{CSI-AA}$, as well as the offsets between $\delta^{15}N_{Pro}$ and $\delta^{15}N_{Phe}$ ($\delta^{15}N_{Pro-Phe}$) values, among the 3 seal species were determined with a 1-way ANOVA with post hoc Bonferroni pairwise comparisons using data for individuals only from the Ross and Amundsen Seas to reduce the effect of location on our findings. Differences in $TP_{CSI-AA}$ and $\delta^{15}N_{Pro-Phe}$ values between the WAP versus Amundsen and Ross Seas were determined with a 2-tailed Student's *t*-test for Weddell and crabeater seals. For all statistical analyses, a result was considered significant if $p < 0.05$.

Lastly, color-shaded contour maps were produced in Ocean Data View (ODV) v.4.7.4 (Schlitzer 2015) using Data Interpolating Variational Analysis (DIVA) gridding software (Barth et al. 2010) to show spatial patterns in bulk $\delta^{13}C$ and $\delta^{15}N$ values, as well as $\delta^{15}N_{Phe}$ and $\delta^{15}N_{Pro}$. The DIVA gridding is highly optimized, relying on a finite-element resolution that accounts for the distance between analysis and data (observation constraint), the regularity of the analysis (smoothness constraint) and physical laws (behavior constraint).

## 3. RESULTS

### 3.1. Bulk $\delta^{13}C$ and $\delta^{15}N$ values of Ross, Weddell, and crabeater seals

Multiple tissue types for Antarctic seals were used to measure bulk $\delta^{13}C$ and $\delta^{15}N$ values. Corrections were applied to all bulk stable isotope data from tissues that were not whole blood, the most common sample type, that had significant isotopic offsets from whole blood (see 'Materials and methods' and Tables S4–S7 for further details). Bulk $\delta^{13}C$ and $\delta^{15}N$ values varied significantly among the 3 species (Fig. 1, Tables 1 & S8). Ross seals had significantly higher $\delta^{13}C$ values than both Weddell seals and crabeater seals (Bonferroni $p < 0.001$; Fig. 1, Table 1). All seals had $\delta^{15}N$ values significantly different from each other: crabeater seals < Ross seals < Weddell seals (Bonferroni $p < 0.001$; Fig. 1, Table 1). We found no consistent relationships between bulk stable

Table 1. Measurements, mean ± SD (n), for Antarctic seals from study sites in West Antarctica. WAP: West Antarctic Peninsula; Phe: phenylalanine; Pro: Proline; $TP_{CSI-AA}$: trophic position estimates using compound-specific isotope analysis of amino acids; –: data not available

| Measurement | Region | Ross seal | Weddell seal | Crabeater seal |
|---|---|---|---|---|
| Bulk $\delta^{13}C$ (‰) | All | −23.8 ± 0.3 (15) | −25.0 ± 0.6 (125) | −25.0 ± 1.4 (97) |
| | WAP | – | −22.9 ± 0.9 (4) | −24.0 ± 1.1 (52) |
| | Amundsen Sea | −23.8 ± 0.3 (14) | −24.7 ± 0.4 (21) | −26.1 ± 0.4 (35) |
| | Ross Sea | −23.8 (1) | −25.1 ± 0.5 (100) | −26.1 ± 0.5 (10) |
| Bulk $\delta^{15}N$ (‰) | All | 9.1 ± 0.4 (15) | 12.3 ± 0.6 (125) | 7.2 ± 0.8 (97) |
| | WAP | – | 11.4 ± 0.7 (4) | 6.8 ± 0.6 (52) |
| | Amundsen Sea | 9.1 ± 0.4 (14) | 12.3 ± 0.9 (21) | 7.6 ± 0.6 (35) |
| | Ross Sea | 8.6 (1) | 12.3 ± 0.5 (100) | 8.0 ± 1.4 (10) |
| Phe $\delta^{15}N$ (‰) | All | 2.7 ± 0.7 (6) | 5.7 ± 0.5 (6) | 5.2 ± 1.0 (6) |
| | WAP | – | 5.7 ± 0.4 (3) | 4.3 ± 0.4 (3) |
| | Amundsen/Ross Sea | 2.7 ± 0.7 (6) | 5.7 ± 0.7 (3) | 6.0 ± 0.7 (3) |
| Pro $\delta^{15}N$ (‰) | All | 17.2 ± 0.5 (6) | 20.0 ± 1.4 (6) | 15.6 ± 0.6 (6) |
| | WAP | – | 19.1 ± 1.5 (3) | 15.9 ± 0.5 (3) |
| | Amundsen/Ross Sea | 17.2 ± 0.5 (6) | 20.9 ± 0.5 (3) | 15.4 ± 0.6 (3) |
| $TP_{CSI-AA}$ | All | 3.5 ± 0.2 (6) | 3.5 ± 0.4 (6) | 2.6 ± 0.3 (6) |
| | WAP | – | 3.3 ± 0.4 (3) | 2.9 ± 0.1 (3) |
| | Amundsen/Ross Sea | 3.5 ± 0.2 (6) | 3.7 ± 0.1 (3) | 2.4 ± 0.2 (3) |

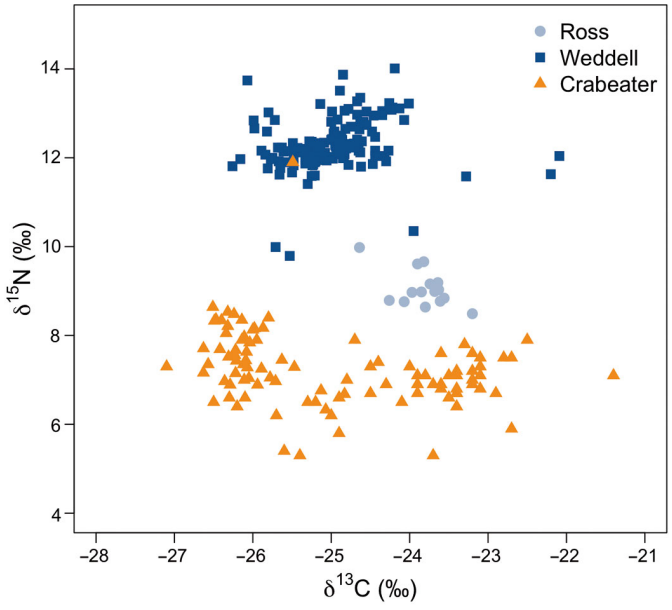


Fig. 1. Bulk $\delta^{15}N$ values versus $\delta^{13}C$ values for Ross, Weddell, and crabeater seals in the West Antarctic. The bulk $\delta^{15}N$ values are from whole blood, with the application of stable isotope corrections for isotope values from tissues that were not whole blood

isotope values of seals and measures of sampling period, gender, age class, or body mass (see the Supplement).

### 3.2. Spatial patterns of bulk $\delta^{13}C$ and $\delta^{15}N$ values for Antarctic seals

Both Weddell and crabeater seals showed significant spatial variation in their $\delta^{13}C$ values. Weddell seal $\delta^{13}C$ values were significantly less in the Ross Sea and the Amundsen Sea than in the WAP (Bonferroni $p \leq 0.002$ for all tests; Fig. 2a, Table 1). Likewise, the $\delta^{13}C$ values of crabeater seals along the WAP were significantly higher than those in the Amundsen Sea and Ross Sea (Bonferroni $p < 0.001$ for both tests; Fig. 3a, Table 1).

Unlike their bulk $\delta^{13}C$ values, Weddell seals showed no significant differences in bulk $\delta^{15}N$ values across the 3 regions (Fig. 2b, Table 1). In contrast, crabeater seals from the WAP had significantly lower $\delta^{15}N$ values than those from the Amundsen Sea and the Ross Sea (Bonferroni $p < 0.001$ for both tests; Fig. 3b, Table 1). Note that spatial variation in bulk $\delta^{13}C$ and $\delta^{15}N$ values of Ross seals across West Antarctica could not be examined because all but one of the individuals were from the Amundsen Sea (Fig. S1).

### 3.3. Compound-specific $\delta^{15}N$ values of Ross, Weddell, and crabeater seals

As mentioned above, CSI-AA data were derived from multiple tissue types; see Tables S1, S2 & S9 for details. We did not apply corrections to these data as with bulk stable isotope data since these isotopic values were from individual amino acids, isolated from different tissues, most commonly from whole blood. The nitrogen isotope values were significantly different among at least 2 of the 3 seal species for all amino acids, except Gly (Figs. 4 & S2, Tables S9 & S10). For most trophic amino acids (Glu, Ala, Ile, Leu, Pro, and Val), the $\delta^{15}N$ values differed significantly among all 3 species, with Weddell seals > Ross seals > crabeater seals (Tables S8 & S9). For example, the $\delta^{15}N_{Pro}$ values of Weddell seals were significantly greater than those of Ross and crabeater seals (Bonferroni $p < 0.001$ for both tests; Table 1). The $\delta^{15}N_{Pro}$ values of Ross seals were significantly greater than those of crabeater seals (Bonferroni $p = 0.035$; Table 1). For the trophic amino acid Asp, crabeater seals had significantly lower $\delta^{15}N$ values (mean ± SD: 10.1 ± 0.6‰, n = 6) than Weddell (16.2 ± 2.2‰, n = 6) and Ross seals (14.1 ± 1.0‰, n = 6) (Bonferroni $p < 0.001$ for both tests). Additionally, the $\delta^{15}N$ values among all trophic amino acids were significantly different among all 3 seal species (Bonferroni $p < 0.001$ for all tests) with these values decreasing in the manner: Weddell seals (21.1 ± 2.7‰, n = 42) > Ross seals (17.7 ± 1.9‰, n = 41) > crabeater seals (13.5 ± 1.9‰, n = 42).

The $\delta^{15}N$ values were significantly different between at least 2 seal species for both commonly defined source amino acids: Lys and Phe (Fig. 4, Tables S9 & S10). For Lys, Ross seals had significantly lower $\delta^{15}N$ values (2.8 ± 0.7‰, n = 6) than Weddell (5.2 ± 1.5‰, n = 6) and crabeater seals (5.0 ± 0.4‰, n = 6) (Bonferroni $p = 0.005$ and 0.009, respectively). Likewise, Ross seals had significantly lower $\delta^{15}N_{Phe}$ values than Weddell and crabeater seals (Bonferroni $p < 0.001$ for both tests; Table 1).

While Gly and Ser are challenging to accurately categorize in terms of conventional trophic and source groupings (McMahon & McCarthy 2016), we do note that Ser $\delta^{15}N$ values of Weddell seals (8.7 ± 1.4‰, n = 6) were significantly higher than those of both crabeater (4.2 ± 1.9‰, n = 6) and Ross seals (5.3 ± 0.6‰, n = 6) (Bonferroni $p < 0.001$ and 0.002, respectively; Fig. 4, Tables S9 & S10). No significant differences among the 3 seal species occurred for Gly $\delta^{15}N$ values (Fig. 4, Tables S9 & S10). Ross, Weddell, and crabeater seals had Gly $\delta^{15}N$ values of 4.9 ± 1.1‰ (n = 6), 5.4 ± 2.9‰ (n = 6), and 3.8 ± 2.8‰ (n = 6), respectively.

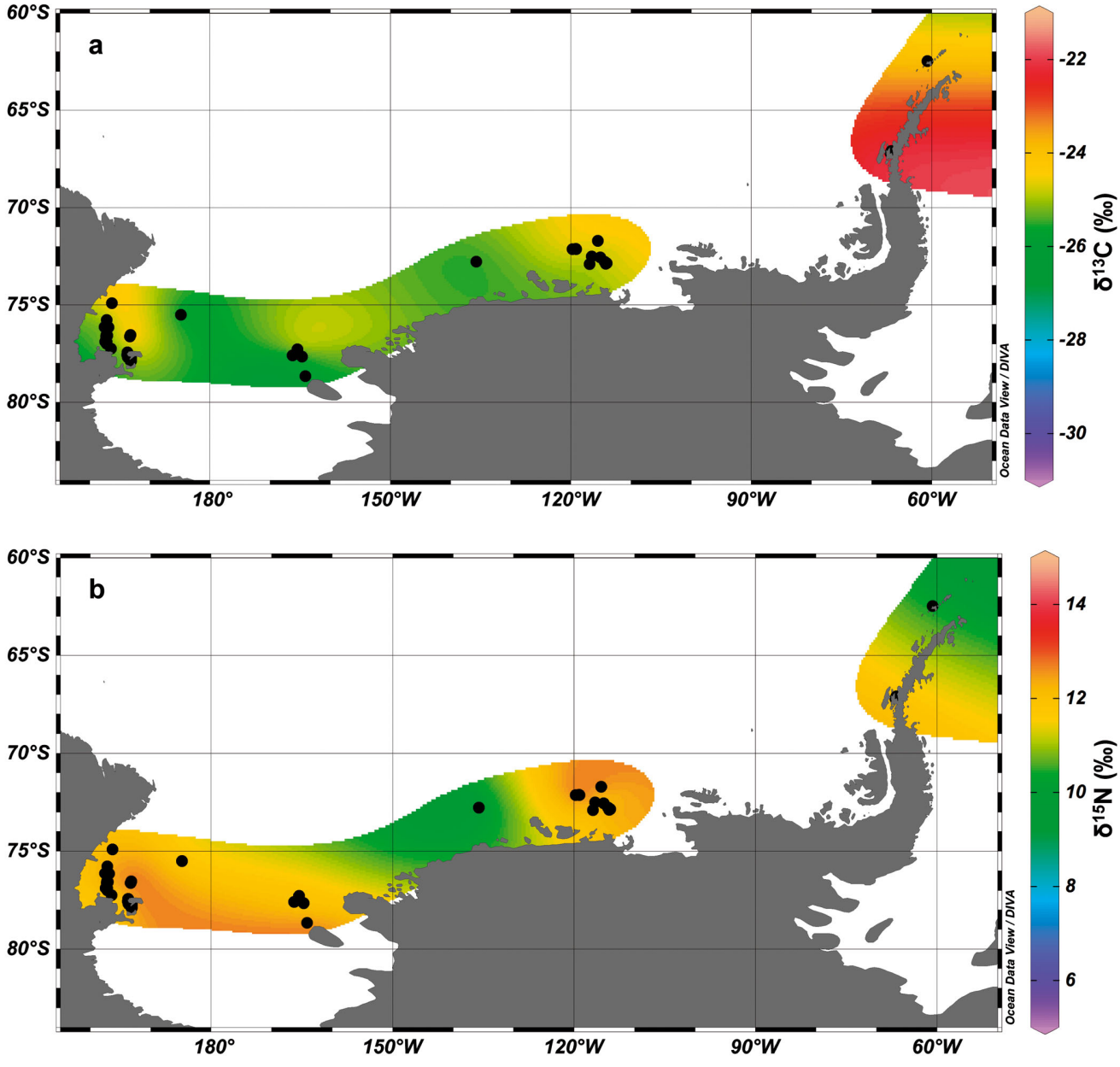


Fig. 2. Spatial variation in the bulk (a) $\delta^{13}C$ and (b) $\delta^{15}N$ values of Weddell seals. Black dots: sampling locations. Figures were produced in Ocean Data View v.4.7.4 (Schlitzer 2015)

### 3.4. Spatial patterns of Phe, Pro, and Glu $\delta^{15}N$ values for Antarctic seals

The $\delta^{15}N_{Phe}$, $\delta^{15}N_{Pro}$, and $\delta^{15}N_{Glu}$ values of Weddell seals did not differ significantly between the Amundsen/Ross Sea region and the WAP (Fig. S3, Tables 1, S9 & S10). In contrast, crabeater seals had significantly lower $\delta^{15}N_{Phe}$ values in the WAP relative to those from the Amundsen/Ross Sea region (2-tailed Student's *t*-test p = 0.037; Figs. 5 & 6, Table 1). The $\delta^{15}N_{Pro}$ and $\delta^{15}N_{Glu}$ values of crabeater seals were not significantly different between the WAP (15.9 ± 0.5 and 15.0 ± 0.2‰, respectively, n = 3 for both) and the Amundsen/Ross Sea region (15.4 ± 0.6 and 14.8 ± 0.4‰, correspondingly, n = 3 for both; Table 1). As with the bulk stable isotope values, spatial variation in the $\delta^{15}N$ values of source amino acids for Ross seals could not be examined since all but one individual were from the Amundsen Sea (Fig. S4).

Crabeater and Weddell seals had similar $\delta^{15}N_{Phe}$ for animals sampled in the Amundsen/Ross Sea region, but they were significantly higher than the $\delta^{15}N_{Phe}$ for

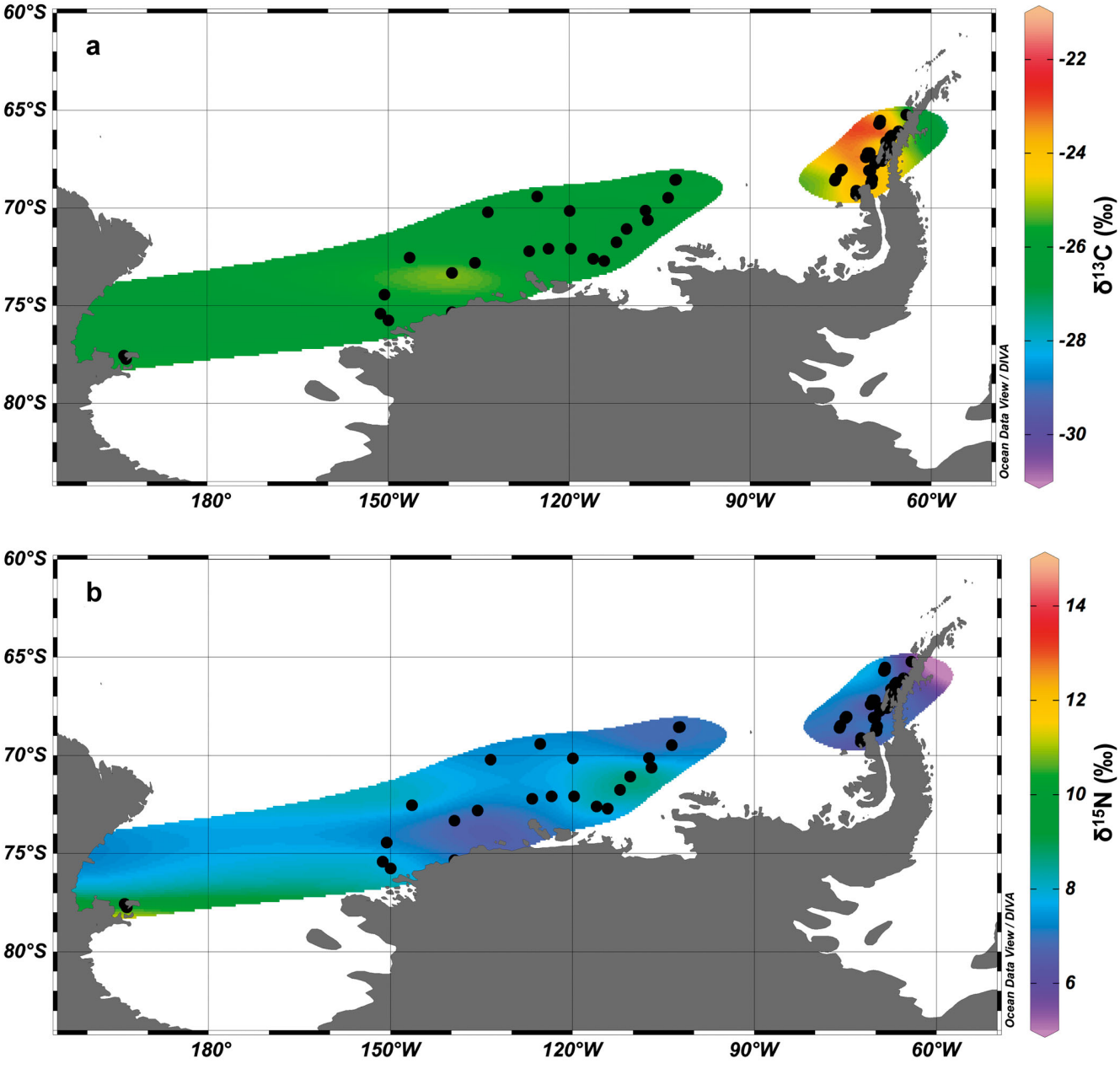


Fig. 3. Spatial variation in the bulk (a) $\delta^{13}C$ and (b) $\delta^{15}N$ values of crabeater seals. Black dots: sampling locations. Figures were produced in Ocean Data View v.4.7.4 (Schlitzer 2015)

Ross seals (Bonferroni $p < 0.001$ for all tests; Table 1). However, crabeater seals had significantly lower $\delta^{15}N_{Phe}$ than Weddell seals for the WAP (2-tailed Student's *t*-test $p = 0.011$; Table 1).

### 3.5. TPs of Ross, Weddell, and crabeater seals

Offsets between $\delta^{15}N_{Pro}$ and $\delta^{15}N_{Phe}$, indicative of TP, of Ross (14.5 ± 0.7‰, n = 6) and Weddell (15.2 ± 0.7‰, n = 3) seals were significantly greater than that of crabeater seals (9.4 ± 0.8‰, n = 3) (Bonferroni $p < 0.001$ for all tests, restricted to the Amundsen/Ross Sea region where all species were collected). Likewise, both Ross seals and Weddell seals were over a full trophic level higher than crabeater seals (Bonferroni $p < 0.001$ for both tests, restricted to the Amundsen/Ross Sea region where all species were collected) (Fig. 7, Table 1).

Within species, Weddell seals sampled in the WAP (13.4 ± 1.9‰, n = 6) had similar $\delta^{15}N_{Pro\text{-}Phe}$ values to

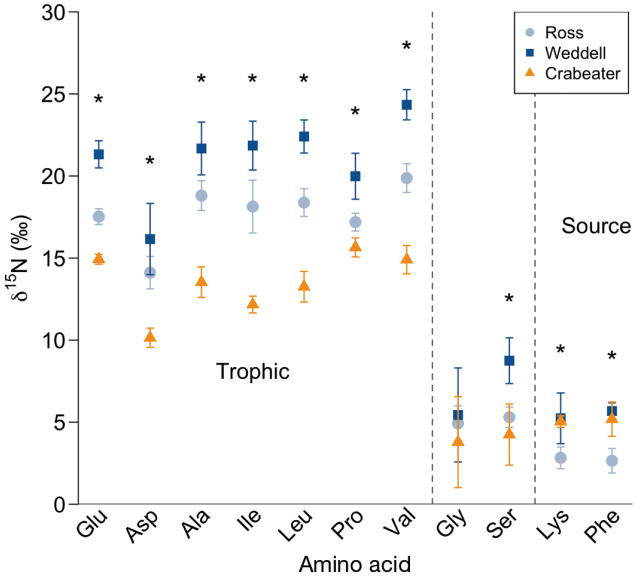


Fig. 4. Mean (±1 SD) $\delta^{15}N$ values of amino acids for Ross, Weddell, and crabeater seals. Significant differences ($p < 0.05$) among species for an amino acid are indicated with asterisks. Glu: Glutamic acid + glutamine; Asp: aspartic acid + asparagine; Ala: alanine; Ile: isoleucine; Leu: leucine; Pro: proline; Val: valine; Gly: glycine; Ser: serine; Lys: lysine; Phe: phenylalanine. Amino acids are divided into trophic and source amino acids, with Gly and Ser categorized separately as their classification for marine mammals is uncertain (McMahon & McCarthy 2016)

those of Weddell seals sampled in the Amundsen/Ross Sea region (15.2 ± 0.7‰, n = 6). Thus, Weddell seals from both regions also had comparable $TP_{CSI-AA}$ values for the WAP and Amundsen/Ross Sea region (Table 1). In contrast, the $\delta^{15}N_{Pro-Phe}$ values of crabeater seals were significantly greater for individuals sampled in the WAP (11.6 ± 0.3‰, n = 6) than those sampled in the Amundsen/Ross Sea region (9.4 ± 0.8‰, n = 6) (2-tailed Student's *t*-test p = 0.033). Correspondingly, crabeater seals had significantly higher $TP_{CSI-AA}$ values in the WAP than the Amundsen/Ross Sea region (2-tailed Student's *t*-test p = 0.032; Table 1). For crabeater seals, note that one subadult was included along with 5 adult seals in the CSI-AA sample set. Although some significant variation in the bulk $\delta^{15}N$ values was observed across different age classes for this species (see the Supplement), the $TP_{CSI-AA}$ value of the subadult from the Amundsen/Ross sea region (2.3) was indistinguishable from those of the adults from this region (2.4 ± 0.2, n = 2). Finally, we note that there were some significant differences in the bulk $\delta^{15}N$ values of Weddell seal age classes (discussed in the Supplement), but only samples from adults were used in our CSI-AA subset.

## 4. DISCUSSION

Bulk stable isotope values that have been reported in earlier work on Ross, Weddell, and crabeater seals support the data in our study (Burns et al. 1998, Zhao et al. 2004, Aubail et al. 2011, Goetz et al. 2017, Lehnert et al. 2017, Botta et al. 2018), after correction for isotopic offsets for different tissue types (Table S11). Our new bulk stable isotope results are especially valuable for Ross seals since very little isotopic measurements exist to date. Our bulk $\delta^{15}N$ values, like those of prior studies (Rau et al. 1992, Burns et al. 1998, Zhao et al. 2004, Aubail et al. 2011, Cipro et al. 2012, Hückstädt et al. 2012a), all point to Ross seals being at an intermediate TP between those of Weddell and crabeater seals. In the following discussion we explore the trophic positions, diets, and foraging habitats of these 3 seal species using our bulk stable isotope and CSI-AA data. Our CSI-AA data sets per species are more limited than their corresponding bulk stable isotope data sets since—in contrast to bulk stable isotope analysis—CSI-AA requires extensive analytical processing (see 'Materials and methods'). Below, we first describe a pattern established with a robust bulk stable isotope data set and then use our novel CSI-AA data to interpret it.

### 4.1. Spatial patterns in seal bulk and amino acid isotope values

Both Weddell and crabeater seals showed spatial patterns in their bulk $\delta^{13}C$ values. Weddell and crabeater seals had significantly higher $\delta^{13}C$ values in the WAP than the Amundsen and Ross Seas. As this carbon isotope gradient occurs in Weddell and crabeater seals at different trophic levels, it is likely driven by changes in the $\delta^{13}C_{baseline}$ values. Prior research has shown that $\delta^{13}C$ values decrease with increasing latitude in the West Antarctic as a result of increasing $CO_2$ solubility with decreasing sea surface temperatures (Cherel & Hobson 2007, Quillfeldt et al. 2010, Brault et al. 2018). Thus, the spatial variation in the bulk $\delta^{13}C$ values of these 2 species likely predominantly reflects a latitudinal sea surface temperature gradient in the West Antarctic, with colder temperatures in the higher latitude Amundsen and Ross Sea compared to the warmer, lower latitude WAP. The difference in sea surface temperatures of the WAP and Ross Sea (Ducklow et al. 2007, 2012, Smith et al. 2014) contributes to about a 2‰ decrease in the $\delta^{13}C_{baseline}$ values from the WAP to the Ross Sea (Brault et al. 2018), similar to the offset between the

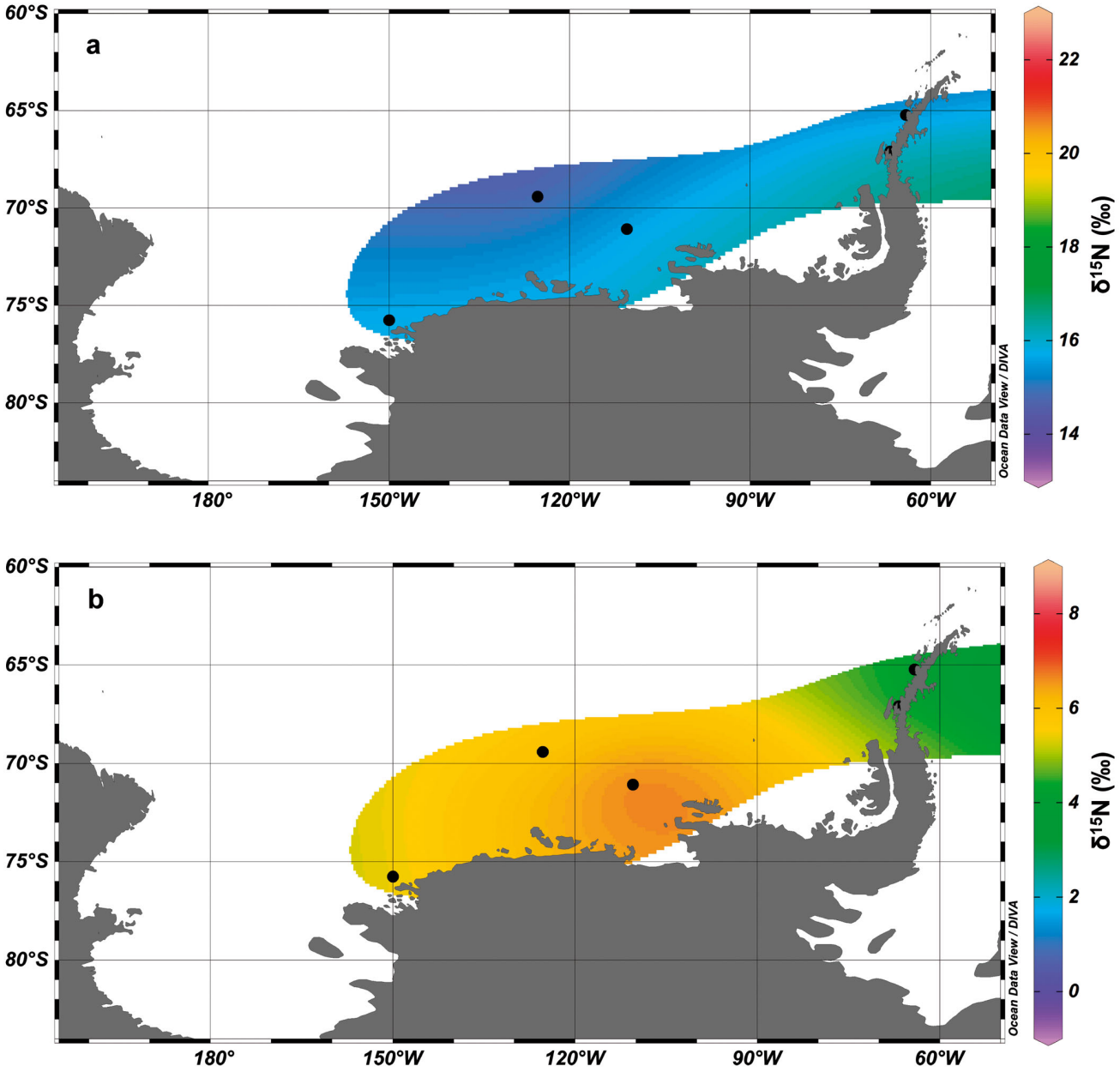


Fig. 5. Spatial variation in the $\delta^{15}N$ values of (a) proline and (b) phenylalanine for crabeater seals. Black dots: sampling locations. Figures were produced in Ocean Data View v.4.7.4 (Schlitzer 2015)

WAP and Ross Sea bulk $\delta^{13}C$ values that we observed for Weddell and crabeater seals (2.0 and 2.1‰, respectively).

Weddell and crabeater seals showed different spatial patterns in their bulk $\delta^{15}N$ values across West Antarctica. Weddell seals showed no spatial patterns in their bulk $\delta^{15}N$ values across the study area, whereas crabeater seals had significantly lower bulk $\delta^{15}N$ values in the WAP than the Amundsen and Ross Seas. As mentioned above, a spatial gradient in the $\delta^{15}N_{baseline}$ values of the Southern Ocean has been detected by previous research, and likely reflects changes from the open ocean to coastal regions in nutrient utilization and primary productivity (DiFiore et al. 2006, 2009, Brault et al. 2018). A lower $\delta^{15}N_{baseline}$—by about 2‰—in the WAP compared to the Amundsen and Ross Seas was found in a study of zooplankton (Brault et al. 2018); this is similar to the difference observed here between WAP and Amundsen/Ross Sea crabeater seal $\delta^{15}N_{Phe}$ values (1.7‰). This difference in the $\delta^{15}N_{baseline}$ values appears to reflect the relative proportion of open ocean versus

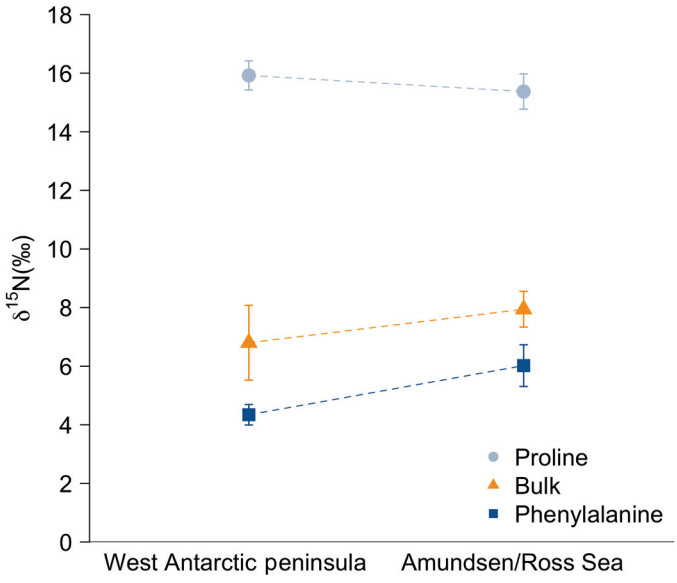


Fig. 6. Mean (±1 SD) proline $\delta^{15}N$, bulk $\delta^{15}N$, and phenylalanine $\delta^{15}N$ values for crabeater seals. The bulk $\delta^{15}N$ values are from whole blood, with the application of stable isotope corrections for isotope values from tissues that were not whole blood

coastal production across the West Antarctic (DiFiore et al. 2006, 2009, Brault et al. 2018). The $\delta^{15}N_{baseline}$ values likely increase from open ocean to coastal areas due to increasing productivity and nutrient drawdown towards the continent in the summer (DiFiore et al. 2006, 2009, Brault et al. 2018). The WAP, with its narrow shelf, likely has a greater influence from open ocean waters beyond the continental margin, whereas the Amundsen Sea and Ross Sea have wider, more productive shelf systems consistent

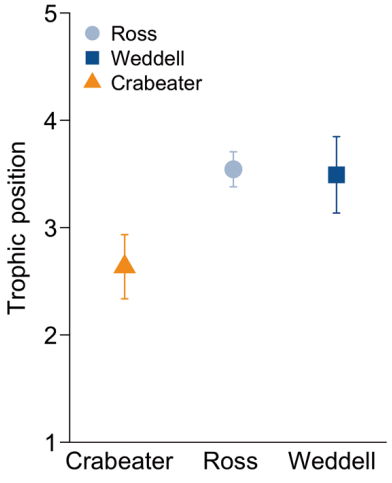


Fig. 7. Mean (±1 SD) trophic position estimates for crabeater, Ross, and Weddell seals from the Amundsen and Ross Seas. Trophic positions were calculated based on the proline and phenylalanine $\delta^{15}N$ values

with higher $\delta^{15}N_{baseline}$ values (Arrigo et al. 1998, 2008, DiFiore et al. 2006, 2009, Smith & Comiso 2008, Alderkamp et al. 2012, Brault et al. 2018).

Continental shelves in Antarctica are especially productive areas compared to offshore waters since both light and iron become available for phytoplankton blooms at times of coastal polynya formation and increased iron inputs from various sources (e.g. melting glaciers) (Gordon et al. 2000, Alderkamp et al. 2012, Arrigo et al. 2015). Although annual production in the Amundsen and Ross Seas exceeds that of the WAP (Arrigo et al. 1998, 2008, Smith & Comiso 2008, Alderkamp et al. 2012), localized regions in the WAP may experience high rates of primary productivity comparable to those within the Amundsen and Ross Seas. For example, Schmidt et al. (2003) found that Marguerite Bay in the WAP can be a 'hot spot' of productivity, as revealed by high phytoplankton and zooplankton $\delta^{15}N$ values.

While it is probable that the observed spatial differences in the Weddell and crabeater seal bulk $\delta^{15}N$ values reflect $\delta^{15}N_{baseline}$ gradients, spatial patterns in the seal bulk $\delta^{15}N$ values could be related to shifts in diet, the $\delta^{15}N_{baseline}$ values, or both. The $\delta^{15}N_{Phe}$ values of Ross, Weddell, and crabeater seals, on the other hand, reflect the $\delta^{15}N_{baseline}$ values—likely driven by spatial gradients in nutrient utilization and primary productivity—without the confounding factor of trophic fractionation. As such, the spatial changes in the $\delta^{15}N_{baseline}$ values in West Antarctica are useful for deducing the relative foraging habitats of Weddell and crabeater seals. Weddell seals from the WAP to the Ross Sea consistently had the highest $\delta^{15}N_{Phe}$ values (5.7 ± 0.4‰ for the WAP and 5.7 ± 0.7‰ for the Amundsen/Ross Sea region, n = 3 in both cases) within the overall range of $\delta^{15}N_{Phe}$ for all Antarctic seals (1.90 to 6.81‰). This suggests that Weddell seals throughout West Antarctica follow a similar foraging behavior in which they predominantly target coastal and, likely, productive areas (i.e. with the highest $\delta^{15}N_{baseline}$ values; Brault et al. 2018) given our understanding of West Antarctic $\delta^{15}N_{baseline}$ gradients. Satellite tracking data of different seal species in this region support this hypothesis. Costa et al. (2010) used satellite data to show that in the WAP, Weddell seals (unlike crabeater seals) moved minimally, foraging almost exclusively in the likely highly productive coastal fjords (Schmidt et al. 2003, DiFiore et al. 2006, 2009). Goetz (2015), likewise, found that Weddell seal movements did not extend beyond the Ross Sea continental shelf.

In contrast to Weddell seals, the crabeater $\delta^{15}N_{Phe}$ values varied significantly across West Antarctica,

with higher values in the Amundsen and Ross Seas versus the WAP. These data indicate that crabeater seals have greater foraging habitat flexibility than Weddell seals, and thus may use a wider range of productivity regimes. Weddell and crabeater seals also had significantly different $\delta^{15}N_{baseline}$ values for the WAP, which was not observed for the Amundsen/Ross Sea region, pointing to environmental heterogeneity in the WAP. Our current knowledge of crabeater seals indicates that this seal heavily consumes *E. superba* (Laws 1977, Rau et al. 1992, Burns et al. 2004, 2008, Zhao et al. 2004, Aubail et al. 2011, Hückstädt et al. 2012a), a krill species with a distribution linked to that of sea ice. As a consequence, crabeater seals likely move on and off the continental shelf, depending on the *E. superba* life cycle stage and seasonal ice cover (Nicol 2006). We hypothesize that crabeater seals move across the heterogeneous environments of the WAP, at times of the year incorporating the lower off-shelf $\delta^{15}N_{baseline}$ values, in contrast with Weddell seals. Such a movement pattern by crabeater seals is supported by the tracking results of Costa et al. (2010). Since crabeater seals in the Amundsen/Ross Sea region have $\delta^{15}N_{Phe}$ values more similar to those of Weddell seals from this area, the same interpretation would suggest that crabeater seals in this region stay within the wide and productive continental shelf areas of the Amundsen and Ross Seas. Overall, our results indicate that Weddell seals have a strong preference for productive coastal sites, whereas crabeater seals use more diverse habitats in West Antarctica, but that their foraging patterns vary by region.

Ross seals sampled in the Amundsen/Ross Seas had significantly lower $\delta^{15}N_{Phe}$ values than both Weddell and crabeater seals from this area (Fig. 4). This result suggests that Ross seals are foraging in a different region than the other 2 species. These low $\delta^{15}N_{baseline}$ values coupled with our current understanding of the West Antarctic $\delta^{15}N_{baseline}$ gradient (Brault et al. 2018) strongly suggest that Ross seals are likely feeding much further offshore than Weddell and crabeater seals, largely in the open ocean which experiences low nutrient drawdown and low productivity relative to coastal areas (DiFiore et al. 2006, 2009, Jaeger et al. 2010, Somes et al. 2010).

Prior research supports our hypothesis that Ross seals are largely open ocean feeders. Blix & Nordøy (2007) examined the foraging behavior of Ross seals via satellite-linked dive recorders. The tags tracked the movements of 10 adult Ross seals captured off Queen Maud Land (East Antarctica) just after their molt in February 2001. The animals migrated 2000 km north to the open ocean south of the Antarctic Polar Front. These Ross seals stayed in that area until October when they traveled south into the pack ice (Blix & Nordøy 2007). Similarly, Arcalís-Planas et al. (2015) showed via satellite-tracking data that Ross seals minimally use sea ice, hauling out for only short periods each year to molt (December to January) and breed (late October to mid-November). They reported that Ross seals moved from 587 to 1282 km off the ice edge during much of the year (Arcalís-Planas et al. 2015). The low $\delta^{15}N_{baseline}$ values measured in our study represent independent evidence that Ross seals are indeed spending the majority of each year foraging in less productive, open ocean waters, separate from the more coastal food webs of the crabeater and Weddell seals.

### 4.2. Reevaluating trophic dynamics of Antarctic seals

Differences in regional foraging habitat utilization among seals, as indicated by the variation in the $\delta^{15}N_{phe}$ values of Ross seals versus crabeater and Weddell seals (~3.0‰), suggest the need for a reevaluation of the TP and associated food web ecology for Ross seals. Our compound-specific approach allowed us to calculate TPs for these Antarctic seals that accounted for variation in $\delta^{15}N_{baseline}$ values. We found that Ross seals had $TP_{CSI\text{-}AA}$ values similar to those of Weddell seals, both of which were significantly higher than crabeater seals. This result was novel in that it differed from previous conclusions based on the bulk $\delta^{15}N$ values, which suggested that Ross seals were at an intermediate TP between crabeater and Weddell seals (Rau et al. 1992, Zhao et al. 2004, Aubail et al. 2011). Our results suggest that, like Weddell seals, Ross seals are predominantly feeding on high trophic level prey, such as mid-to-deep water fish and squid, and that lower trophic level prey (e.g. *E. superba*) are not a major part of their diets. This conclusion is supported by dive records, which indicate that Ross seals forage at depths associated with capturing mesopelagic squid and fish (Bengtson & Stewart 1997, Blix & Nordøy 2007), and corroborate limited stomach content analyses that have reported *Pleuragamma antarcticum* and *Psychroteuthis glacialis* comprising their diets in varying proportions (Skinner & Klages 1994).

Our compound-specific isotope approach to trophic dynamics also revealed significant spatial variation in the trophic dynamics of crabeater seals across West Antarctica. Following spatial variation in the

$\delta^{15}N_{Pro\text{-}Phe}$ values of crabeater seals, the $TP_{CSI\text{-}AA}$ values of this species decrease by approximately 0.5 from the WAP to the Amundsen/Ross Sea region, which is substantial for a low trophic level consumer like the crabeater seal that is thought to specialize on krill. Variations of this range have been shown to be significant in many past studies (e.g. McCarthy et al. 2007, Batista et al. 2014), with recent ecological work showing that $TP_{CSI\text{-}AA}$ variation as small as 0.2 to 0.3 indicates real ecological change (Ostrom et al. 2017). For instance, such $TP_{CSI\text{-}AA}$ shifts as those of crabeater seals in this study may be in response to a restructuring of a food web (e.g. changes in the composition of trophic levels and/or trophic linkages) (Ostrom et al. 2017). This pattern is not likely driven by differences in age class across regions since all but one crabeater seal from both regions were adults. Crabeater seals respond quickly to changes in food availability as also documented in long-term fluctuations in their mean age of sexual maturity (Hårding & Härkönen 1995). The WAP has been experiencing increased krill fishing pressure, a resurgence in baleen whales (competitors for krill), and dramatic reductions in sea ice extent as a function of rapid regional warming, all of which have negative effects on *E. superba* abundance (Ducklow et al. 2007, 2012, Trivelpiece et al. 2011, Nicol et al. 2012). Perhaps these environmental changes have decreased the availability of krill for crabeater seals in this area, causing crabeater seals to supplement their diet with fish in the WAP relative to the Amundsen/Ross Sea region.

Alternatively, the regional differences in crabeater seal $TP_{CSI\text{-}AA}$ values could be the result of bottom-up effects. Prior research has shown omnivorous behaviors by *E. superba* in the WAP and Drake Passage regions, possibly contributing to a higher euphausiid TP in this region than other areas of the Antarctic (Schmidt et al. 2006). However, euphausiid omnivory in the Ross Sea has also been suggested (Hopkins 1987, Pinkerton et al. 2010), and it is possible that omnivory by this taxon varies in time and space (Schmidt et al. 2006). Given these uncertainties, it is not possible to definitively assess the relative roles of shifts in crabeater seal diet versus krill omnivory to our observed spatial gradient of the crabeater seal trophic position as estimated by our compound-specific stable isotope analysis.

## 5. CONCLUSIONS

Our work using bulk and amino acid isotope analyses revealed significant variability in the foraging habitats and trophic dynamics of 3 important seal species in the West Antarctic. Ross seals are foraging in a low productivity, open ocean food web far offshore from that of crabeater and Weddell seals. Crabeater and Weddell seals are foraging within similar food webs closer to shore, but isotopic data suggest that crabeater seals are likely following sea ice to capture *Euphausia superba*, whereas Weddell seals appear to forage primarily in the most productive, near-shore areas within the Western Antarctic. In addition, our CSI-AA data revealed that Ross seals occupy a higher TP than originally thought, equivalent to Weddell seals and greater than that of crabeater seals. Additionally, as the TP estimates based on bulk stable isotope analysis are unable to account for varying $\delta^{15}N_{baseline}$ values, we hypothesize that the strong baseline changes across the environments inhabited by these species are responsible for this underestimate of TP. Our study reframes our understanding of Ross seal foraging ecology, while also demonstrating solutions to the challenges posed by interpreting stable isotope data from top-level consumers across isotopically disparate marine habitats.

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