Supplement 1

SUPPLEMENTARY TEXT

Extended Methods

Text S1. Suspended particulate organic material (SPOM) sampling and processing

At each underway station, 2 L of water were filtered onto 0.7 µm pore size, 25 mm pre-ashed Whatman® glass microfibre filters (grade GF/F) and rinsed with 0.2 µm filtered seawater. GF/F filters were combusted at 450°C for five hours and stored in tin foil prior to use, to minimise contamination from organic material and to achieve low blank values. Filter blanks were generated for each batch filtration, by placing a filter on the filtration manifold and rinsing with 0.2 µm filtered seawater, to replicate the rinsing of the 2 L water samples. All filters were placed in clean 6-well plates (Thermo Fisher Scientific, Auckland, New Zealand) and stored frozen at -20°C, until ready for analysis. On thawing, filters were fumed over concentrated hydrochloric acid (HCl) for two hours to remove any inorganic carbon and were then oven-dried at 60°C overnight. Laboratory tests found that this HCl fuming process had negligible effects on $\delta^{15}N$ values, which were within the analytical error of stable isotope analysis. Whole filters were sealed into tin capsules before analysis for carbon and nitrogen stable isotopes.

Text S2. Humpback whale biopsy sampling and tissue processing

A 6.3 m aluminium-hulled, inflatable Naiad (*Remora*) and a 6.3 m Gemini (*Beluga*) small vessel equipped with bow-sprits for tagging whales were used to tag and biopsy whales in 2010 (Gales 2010, Hull 2010, Schmitt et al. 2014a). In 2015, whales were tagged from the bow of the National Institute of Water & Atmospheric Research (NIWA) research vessel (RV) *Tangaroa*. On both voyages, samples were obtained using a biopsy dart propelled by a modified .22 calibre Larsen rifle (Larsen, 1998).

As soon as samples were brought on board, the skin/blubber biopsy was removed from the dart using clean metal forceps. Gloves were worn throughout the handling process. Each sample was placed in a glass petri dish and cut in half longitudinally using a new metal scalpel. All equipment was cleaned with ethanol and distilled water between the preparation of each sample. On the 2010 voyage (V2010), the two sample halves were placed in an Eppendorf® tube with one half stored in 70% ethanol at -80°C for stable isotope analysis and one half in RNA-later for genetic analysis. On the 2015 voyage (V2015) half of the sample was stored frozen at -20°C for stable isotope analysis and the other half was split three ways between storage in liquid nitrogen, All Protect (Qiagen), and ethanol for genetic analysis. Details of the molecular sexing of humpback whales sampled in 2010 and 2015 are given in Schmitt et al. (2014b) following methodology of Morin et al. (2005).

For whale biopsy samples stored in ethanol from V2010, the ethanol was evaporated off under a stream of nitrogen gas prior to sample preparation, whilst the 2015 frozen samples were thawed. For all samples, skin was then separated from the blubber and each skin sample was finely homogenised in a glass petri dish using a sharp metal scalpel. Samples were then freeze-dried overnight and stored in desiccator boxes. About 2 mg of freeze-dried material was prepared for lipid extraction for carbon stable isotope analysis as outlined below, and approximately 0.7 mg was weighed and sealed into tin capsules for bulk nitrogen stable isotope analysis. Tests were carried out on the V2015 biopsy samples, comparing bulk nitrogen stable isotope values, and lipid-extracted carbon stable isotope values between frozen- and ethanol-

stored samples. The isotopic variability was less than the analytical error of the stable isotope analytical method (See Section 2.3.1 in the manuscript). This is in line with Hobson et al. (1997) who found no isotopic difference between frozen tissues and those stored in 70% ethanol: V2010 and V2015 skin biopsy stable isotope data were therefore amalgamated with confidence in the compatibility of values.

Text S3. Tissue turnover rates and trophic discrimination factors

The rate at which a tissue turns over (i.e., the rate of replacement of the tissue, or in isotopic terms, the time for the stable isotope ratio of an animal's food sources to be fully assimilated into its tissue and reach a steady state) is a balance between accrual of new biomass (tissue growth) and catabolic tissue replacement (catabolic turnover) (Schoenheimer 1946, Bender 1975, Tieszen 1978). This rate depends on the metabolic activity of the tissue (high metabolic activity resulting in rapid turnover rates), which in turn determines the integration timeframe of the stable isotope value within that tissue. Quantification of a tissue turnover rate is therefore important to establish what timeframe the isotopic value of that tissue represents.

Identifying the temporal window over which a consumer's stable isotope value reflects its diet requires an understanding of elemental incorporation, which can vary from days to months across different species and tissue types (see reviews of Thomas & Crowther, 2015 and Vander Zanden et al. 2015). Tissue turnover rates have been traditionally measured via controlled diet experiments of animals in captivity (Bearhop et al. 2002, Cherel et al. 2005, Caut et al. 2011), with a few studies labelling sub-epidermal cells (Hicks et al. 1985, St. Aubin et al. 1990). Due to the impracticality of such studies being conducted on large cetaceans however, the cetacean stable isotope community uses tissue isotope incorporation rates measured in smaller cetaceans as a proxy for larger species.

Marine mammal skin has a relatively fast turnover rate compared to other tissues such as baleen plates or bones. Gavrilchuk et al. (2014) state that the stable isotope turnover time for baleen whale skin is unknown, but estimates of 70–75 days for beluga whales (*Delphinapterus leucas*, 3.5–5.5 m long: St. Aubin et al. 1990) and common bottlenose dolphins (*Tursiops truncates*, up to 4 m long: Hicks et al. 1985) led them to assume a turnover time for baleen whales (6–30 m long across species), of at least 75 days. Giménez et al. (2016) report a turnover time for bottlenose dolphins of 104, 35 (mean, ±1 SD) for $\delta^{13}C$ and 180, 71 days for $\delta^{15}N$, which is considerably longer than previously reported values for marine mammals of similar size (Hicks et al. 1985, St. Aubin et al. 1990, Alves-Stanley & Worthy, 2009). Busquets-Vass et al. (2017) estimated stable isotope incorporation rates of northern hemisphere blue whale (*Balenoptera musculus*) skin and baleen using natural gradients in baseline stable isotope values between whales foraging in different oceanic regions. They estimated mean $\delta^{15}N$ skin turnover times of 163, 91 days for these blue whales which grow up to 23–25 m long. Tissue turnover rates scale with organism size and growth rate (Fry & Arnold 1982, Hesslein et al. 1993, Lockyer 2007), so it is likely that humpback whales, which typically grow up to 15–16 m long, have longer tissue turnover rates than small cetaceans, but shorter than blue whales. The wide range of reported tissue nitrogen turnover times for bottlenose dolphins of 74 days (Browning et al. 2014 – converting a half-life of 17 days to 95% isotopic incorporation, following Giménez et al. (2016)), 73 days (Hicks et al. 1985) and 180 days (Giménez et al. 2016) indicates the large uncertainty associated with these estimates. With such variability in reported values, the best estimate for humpback skin turnover times is likely to be less than the blue whale estimates of 163 days (just over 5 months) (Busquet-Vass et al. 2017) given that humpback whales are on average around 10 m smaller than blue whales. In this study we have therefore assumed a skin

turnover time of between three to four months, by integrating tissue turnover times estimated for other tissues and cetaceans.

Skin shedding has been raised as a possible issue influencing tissue turnover rates. Skin shedding in whales seems to be a natural physical sloughing process of the outer layers of skin as that dies, and increases as whales migrate north, from colder to warmer waters (Pitman et al. 2020), removing Southern Ocean parasites. As skin shedding is a "mechanical" process, it seems unlikely to affect metabolic rates and tissue turnover times, as even without skin shedding, skin is still being constantly replaced. Since skin shedding is reduced in cold waters, it is unlikely to have affected tissue turnover rates in this study.

Text S4. Other faunal sampling and analytical preparation

During V2010 and V2015, mixed community zooplankton were sampled using a Multiple Opening Closing Net Environmental Sensing System (MOCNESS). The MOCNESS has a 1 m^2 opening with up to nine nets with a mesh size of approximately 230 µm. On the 2008 voyage (V2008), Antarctic krill (*Euphausia superba*) and fish (lantern fish - *Electrona carlsbergi, E. antarctica, Gymnoscopelus nicholsi, G. opisthopterus and G. braueri*); and Antarctic silverfish (*Pleuragramma antarctica*) were collected using three types of trawling: rough-bottom orange roughy trawl, oblique midwater trawl and targeted midwater trawl (Pinkerton et al. 2013). These fish species together are amongst the commonest encountered in the Southern Ocean (Koubbi et al. 2011, Woods et al. 2023). On V2015, these same fauna were sampled using the NIWA mesopelagic midwater trawl (O'Driscoll & Double 2015). This has a circular mouth opening of 12 m diameter, a cod-end mesh of 10 mm, and is rated to a maximum depth of 1200 m (Bury et al. 2008, Pinkerton et al. 2011, Escobar-Flores et al. 2020). Mark-identification trawls were targeted at acoustic marks of interest, detected using the multifrequency (18, 38, 70, 120 and 200 kHz) Simrad EK60 echosounders (Kongsberg Maritime, Melbourne, Australia) onboard RV *Tangaroa* (although 18 kHz was not available in 2008). The midwater trawl was towed for 20–30 minutes at 3–4 knots. An RBR temperature-depth logger (RBR Ltd., Ottowa, Canada) was attached to the midwater trawl on each deployment, which provided an accurate temperature and depth profile for each tow.

On V2008, individual zooplankton were hand-picked from filter papers following surface seawater filtration through 0.7 µm pore size Whatman® glass microfibre filters (grade GF/F). For V2015, zooplankton were stored and analysed either as a mixed homogenate, or as single samples. Antarctic krill samples were identified to species, with the tail muscle sampled and analysed individually. For fish, a small piece of muscle was extracted from between the dorsal spine and the head. All zooplankton, Antarctic krill and fish samples were stored frozen at -20ºC until analysis. Samples were then freeze-dried overnight, individually homogenised, and stored in desiccator boxes, prior to being weighed and sealed into tin capsules for bulk stable isotope analysis, or before being processed for lipid extraction prior to carbon stable isotope analysis.

Text S5. Lipid extraction of biological samples

All samples were freeze-dried prior to processing for lipid extraction. Freeze-dried material was sub-sampled and wrapped in Whatman® glass fibre filters (grade GF/C), labelled and secured using a stapler. Extraction of lipids was performed on an accelerated solvent extraction system (ASE) DIONEX 200 (Dionex Corporation, Sunnyvale, California, USA) at NIWA Hamilton. Samples were transferred to 22 mL stainless steel ASE cells and extracted three times with dichloromethane at 70ºC and 1500 psi for a static hold time of five minutes.

Following extraction, all samples were heated to 40°C in an oven overnight to evaporate any traces of solvent. Samples were then shipped back to NIWA Wellington and placed in a desiccator until weighing for carbon stable isotope analysis.

Text S6. Bulk and lipid-extracted carbon and bulk nitrogen stable isotope analysis

Samples were combusted in a Flash 2000 elemental analyser (EA) (Thermo Fisher Scientific, Bremen, German) at 1020°C in a flow of oxygen and helium carrier gas. Oxides of nitrogen were converted to nitrogen (N_2) gas in a reduction furnace at 640°C. Carbon di-oxide (CO_2) and N_2 gases were separated on a Porapak Q gas chromatograph column before being introduced to the continuous flow isotope ratio mass spectrometer (CF-IRMS) detector via an open split Conflo interface (Thermo Fisher Scientific, Bremen, Germany). ISODAT software (Thermo Fisher Scientific, Bremen, Germany) calculated $\delta^{15}N$ values against an N_2 reference gas, relative to an atmospheric air international standard. Values of $\delta^{13}C$ were calibrated against a CO_2 reference gas, relative to the international standard NBS-19 (National Institute of Standards and Technology (NIST), Gaithersberg, MD, USA), which in turn, was calibrated against the original Pee Dee Belemnite (PDB) limestone standard and was then corrected for ^{17}O (Santrock et al. 1985). Stable isotope ratios were expressed as delta values (δ) in per mil units (‰), which represent the ratios of heavy to light isotopes within a sample (R_{sample}), relative to the ratio in an international standard ($R_{standard}$) as: $\delta = \left(\left(\frac{R_{sample}}{R_{standard}} \right) - 1 \right) \times 1000$. All estimates of variance were calculated to ±1 standard deviation (SD). IRMS linearity was always < 0.06 ‰/volt for a known span of CO_2 voltage, and the standard deviations of δ isotope values from ten peaks of N_2 and CO_2 standard gas on-off tests were always < 0.06 ‰. Carbon stable isotope data were corrected via a two-point normalisation process (Paul et al. 2007) using NIST 8573 (USGS40 L-glutamic acid; $\delta^{13}C$ = -26.39, 0.09 ‰ and NIST 8542 (IAEA-CH-6 Sucrose; $\delta^{13}C$ = -10.45, 0.07 ‰). Nitrogen stable isotope data were corrected using the same two-point normalisation process using NIST 8573 (USGS40 L-glutamic acid; $\delta^{15}N$ = -4.52, 0.12 ‰) and IAEA-N-2 (ammonium sulphate: $\delta^{15}N$ = +20.41, 0.20 ‰). At the start of each run, %C and %N values were calculated relative to a laboratory reference standard of DL-Leucine (DL-2-Amino-4-methylpentanoic acid, $C_6H_{13}NO_2$, Lot 127H1084, Sigma-Aldrich, Australia), which was also run every ten samples to monitor analytical precision and drift. Additional international standards NIST 8574 (USGS41 L-glutamic acid; $\delta^{13}C$ = +37.63, 0.10 ‰ and $\delta^{15}N$ = +47.57, 0.22 ‰), NIST 8547 (IAEA-N-1 ammonium sulphate; $\delta^{15}N$ = +0.43, 0.04 ‰) were run daily to check isotopic accuracy. Repeat analysis of standards produced data accurate to within 0.25 ‰ for both $\delta^{15}N$ and $\delta^{13}C$, and a precision of better than 0.30 ‰ for $\delta^{15}N$ and 0.24 ‰ for $\delta^{13}C$. Replicate analysis of a humpback whale skin sample, subsampled 25 times along the longitudinal length of the biopsy, gave a mean $\delta^{15}N$ bulk value of 6.75, 0.13 ‰ and a mean $\delta^{13}C$ lipid-extracted value of -26.17, 0.38 ‰.

Text S7. Wet chemistry method for chemical extraction, purification, and derivatisation of samples for compound-specific stable isotope analysis of nitrogen in amino acids

The Hannides et al. (2009) method was established using modifications of previously reported protocols (Metges et al. 1996, Macko et al. 1997, Vueger at al. 2005) based on methods of Ueda et al. (1989), Silfer et al. (1991) and Cowie & Hedges (1992). During the hydrolysis step, tryptophan and cystine are destroyed, asparagine is converted to aspartic acid and glutamate is converted to glutamic acid. An external standard "Amino Acid Mix" (AA Mix) and an in-house quality control sample "NIWA Squid" were derivatised at the same time as the whale biopsy samples following the same wet chemistry protocol. The AA Mix standard comprised a suite

of fourteen commercially available AA reference materials: alanine, aspartate, glutamic acid, glycine, isoleucine, leucine, lysine, methionine, norleucine, phenylalanine, proline, serine, threonine, valine. The $\delta^{15}N$ value of each individual AA in the AA Mix standard was determined by EA CF-IRMS as described under Text S6 above. The Hannides et al. (2009) method is reproduced below, with the variations applied by the NIWA laboratory indicated in italics text in brackets.

Hannides et al. (2009) Method

1 mL (*0.5 mL*) of 6N Hydrochloric acid (HCl) was added to 5 mg (*2–20 mg, with 10-12 mg being an optimum weight*) of homogenised sample in each reaction vial. Vials were flushed with N_2 gas and hydrolysed on a heating block at 150°C for 70 minutes, then evaporated to dryness at 55°C under a stream of N_2 for ~1–2 hours. The samples were redissolved in 1 mL 0.01N HCl then filtered with low-protein binding filters to remove particles. The hydrolysate was further purified using cation-exchange chromatography (Metges et al. 1996) with a 5-cm column of resin prepared in a glass Pasteur™ pipette. Amino acids on the column were eluted with repeated rinses of 2N ammonium hydroxide (NH_4OH), and the eluant was evaporated to dryness under a stream of N_2 at 80ºC. Finally, the samples were re-acidified with 2 mL (*5 mL*) 0.2N HCl, heated at 110ºC for 5 min and evaporated to dryness under a stream of N_2 at 110ºC (*55ºC*). Hydrolysed samples were esterified with 2.0 mL (*2.5 mL*) of 4:1 isopropanol (C_3H_8O) and acetyl chloride (CH_3COCl) mixture, heated to 110ºC for 60 min following a N_2 flush. The esterified samples were dried under a stream of N_2 at 60ºC, and 1 mL (*800 µL*) of 3:1 methylene chloride (CH_2Cl_2):trifluoracetic anhydride ($C_4F_6O_3$) was added. Samples were acylated by heating to 100ºC for 15 min after a N_2 flush (trifluoroacetylation step). The derivatised samples were further subject to purification by solvent extraction following Ueda et al. (1989). The trifluoroacetyl derivatives were evaporated at room temperature, under a stream of N_2 and redissolved in 3 mL 1:2 chloroform ($CHCl_3$):P-buffer (potassium phosphate (KH_2PO_4) + sodium phosphate (Na_2HPO_4) in Milli-Q water, potential Hydrogen [pH] 7), placing the vials in a vortex for 60 secs. This transfers the amino acids to the chloroform fraction with contamination going into the P-buffer.

After sonication and centrifugation for 10 min at 600 g, the chloroform fraction containing solely the acylated AA esters was removed and the solvent extraction process repeated. Finally, to ensure complete derivatisation, the chloroform was evaporated at room temperature (*under a stream of N_2*), and the acylation step was repeated. All samples were stored in 1 mL (*800 µL*) of 3:1 methylene chloride: trifluoracetic anhydride at 4ºC and analysed immediately where possible. If analysed immediately, samples were dried under a stream of N_2 and taken up in ethyl acetate ($C_4H_8O_2$) and diluted to the appropriate concentration for analysis on the gas chromatography-IRMS (GC-IRMS). With this above procedure, nine samples (*up to 27 samples/standards*) can be prepared for AA analysis in two (*three*) days. If it was not possible to analyse samples immediately after derivatisation, samples were re-derivatised just before analysis by drying the samples down under N_2, adding 0.5 mL of trifluoracetic anhydride and 0.5 mL of ethyl acetate, leaving them to stand at room temperature for an hour, then evaporating them to dryness at room temperature. The samples were then diluted with about 100 µL of ethyl acetate and run on the GC-IRMS immediately, i.e. within a few hours.

The full step by step CSIA sample preparation method is reproduced below for research scientists new to this procedure to be able to follow.

Equipment

Equipment (listed in order of use) assuming 9 samples to be processed	**Chemicals (listed alphabetically)**
Weighing paper	Acetyl chloride (CH_3COCl)
1 x spatula	Ammonium hydroxide (NH_4OH), 2N
Kimwipes	Chloroform ($CHCl_3$)
9 x round-bottom screw-top culture tubes (16 x 150 mm)	Dowex ion exchange resin 50WX8-400
9 x polytetrafluoroethylene (PTFE)-lined caps for culture tubes	Ethanol (C_2H_6O)
Heating block with thermometer	Ethyl acetate ($C_4H_8O_2$)
Reactivap	Hydrochloric acid (HCl), 0.01N
33 x pre-combusted (400ºC for 4 hours) Pasteur™ pipettes and glass wool to prepare cation exchange columns	Hydrochloric acid (HCl), 0.2N
Cation exchange column stand	Hydrochloric acid (HCl), 10% (10% concentrated HCl, 90% de-ionised (DI) water)
Compressed nitrogen	Hydrochloric acid (HCl), 6N
3 x 200 mL beakers	Isopropanol (C_3H_8O)
9 x syringe filters, non-protein binding	Methylene chloride (CH_2Cl_2)
9 x 0.22 µm Millex-GP syringe filter units	P-buffer (potassium phosphate [KH_2PO_4] + sodium phosphate [Na_2HPO_4] in Milli-Q water, potential Hydrogen [pH] 7)
9 x 5 mL Cadence Science™ Micro-Mate™ interchangeable borosilicate glass syringes	Potassium phosphate monobasic (KH_2PO_4)
27 x 8 mL vials with PTFE-lined lids	Sodium phosphate dibasic anhydrous (Na_2HPO_4)
4 x 5 mL graduated glass measuring cylinders	Trifluoroacetic anhydride ($C_4F_6O_3$)
1 x 20 mL graduated glass measuring cylinder	
Plastic tray filled with ice	**Equipment for making chemicals**
3 x 50 mL glass beakers	
2 x 25 mL glass beakers	1 x 100 mL volumetric flask
2 x 10 mL glass beakers	1 x 250 mL volumetric flask
Centrifuge	4 x 1L glass Schott DURAN® bottles
Permanent marker pen	1 x 250 mL glass Schott DURAN® bottle
Marking tape	1 x 400 mL glass Schott DURAN® bottle
9 x scintillation vials for column waste	pH paper
1 x 50 mL Erlenmeyer flask	
1 x gastight syringe exclusively for trifluoroacetic anhydride	

1 x gastight syringe for other solvents	
Glass wool	
Freezer	
Container for 10% HCl bath	
Secondary rinse container	
Furnace	
Aluminum foil	
Drying oven	
Labelled chemical waste container	

Equipment cleaning: acid clean glassware in 10% HCl for 4 hours and polytetrafluoroethylene (PTFE) lids for 2 hours maximum, then rinse three times with de-ionised (DI) water. Wrap glassware in aluminium foil, combust for >4 hours at 500°C in furnace. Dry PTFE-lined lids in oven at 60ºC. Store all items in sealed container before use.

Preparation of cation exchange columns: place a small amount of glass wool in a Pasteur™ pipette using another Pasteur™ pipette to push the glass wool down to where the pipette tapers. Combust in an oven at 400ºC for 4 hours.

Preparation of Amino Acid Mix standard: weigh 2 mg of commercially prepared high-purity AA powders (alanine, aspartate, glutamate, glycine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, valine) into a 400 mL Schott DURAN® bottle, recording each weight to two decimal places. Norleucine is also added as an internal standard if quantitative AA concentrations are required via analysis on a GC-IRMS. Add 200 mL of 0.1M HCl. Each individual AA should have a final solution concentration of about 1 mg mL^{-1}. Store the AA standard mix solution in a fridge at 4°C.

Preparation of reagents

10% HCl for acid washing (1L): add 100 mL concentrated analar HCl in small stages to DI water to make up to 1L.
0.01N HCl (500 mL): pipette 0.411 mL of 37% concentrated HCl into 500 mL DI water.
0.2N HCl (100 mL): pipette 1.642 mL of 37% concentrated HCl into 500 mL DI water.
6N HCl (500 mL): pipette 0.833 mL of 37% concentrated HCl into 500 mL DI water.
2N NH_4OH (500 mL): pipette 68.806 mL of NH_4OH into 500 mL DI water.
Once prepared, store all above reagents in labelled 1L Schott DURAN® bottles

P-Buffer solution: Prepare a fresh solution for each batch of CSIA AA-N wet chemistry.
1M KH_2PO_4 Solution: Make up 13.6 g of KH_2PO_4 in 100 mL DI water in 100 mL volumetric flask. Stir until dissolved (about 1 hr) using stirring plate.
1M Na_2HPO_4 Solution: Make up 35.5 g of Na_2HPO_4 in 250 mL DI water in 250 mL volumetric flask. Stir until dissolved (about 1 hr) using stirring plate.
Pour 25 mL of the 1M KH_2PO_4 solution into 250 mL glass Schott DURAN® bottle and slowly add the 1M Na_2HPO_4 solution until pH reaches 7.

This method and the quantities of chemicals suggested assumes that nine samples/standard are processed in one batch of wet chemistry, however up to 27 samples/standards can be processed (total samples limited by number of Reacti-Vap N_2 streaming ports) in one batch if chemical quantities are adapted. Carry out this process in a fume hood.

Hydrolysis: Day 1

1. Pipette 1 mL AA Mix solution into a labelled 16 x 120 mm culture tube. Dry under a stream of N_2 at 60°C using a Reacti-Vap III Evaporator 27-port connected to a Reacti-Therm III (Thermo Fisher Scientific, Bremen, Germany). When dry, cap tube using a PTFE-lined lid.
2. Place weighed samples (2–20 mg range, but ideally aim for 10-12 mg) into labelled 16 x 120 mm culture tubes and seal the tubes using PTFE-lined lids.
3. Add 0.5 mL 6N HCl to each culture tube. Flush tubes with N_2 gas and seal with PTFE-lined caps.
4. Heat capped tubes in a heating block at 150°C for 70 minutes, then remove to cool. Uncap tubes and evaporate acid to dryness at 55°C under a stream of N_2 (~1–2 hours).
5. Add 1 mL 0.01N HCl to each tube to redissolve residue and cap the tube.
6. Take up 1 mL of sample into a 5 mL Cadence Science™ Micro-Mate™ interchangeable borosilicate glass syringe fitted with a 0.22 µm Millex-GP syringe filter unit. GENTLY push the sample through the filter into a clean newly-labelled culture tube.
7. Put another 1 mL of 0.01N HCl (using the same pipette used in step 5) into the culture tube and swirl the liquid around. Pour the liquid into the syringe. Plunge liquid through the filter into a labelled 8 mL vial with the first 1 mL of 0.01N HCl. Cap the vial with a PTFE-lined lid.
8. Freeze samples overnight.

Ion exchange columns: Day 2

1. Remove samples from freezer and thaw before processing.
2. Use a permanent marker pen to mark 5 cm from the top of the pipette of each cation exchange column and place in column stand with a scintillation vial under the bottom of each ion exchange column to collect the waste.
3. Using a glass rod, scoop some DOWEX 50WX8-400 ion exchange resin into a 200 mL beaker and add 5 mL of 0.01N HCl to create a thin slurry.
4. Using a glass pipette, fill the exchange columns with the DOWEX slurry up to the 5 cm mark.
5. Use a clean pipette to add 2 mL of the sample from the 8 mL sample vials (from stage 7, Day 1) into a column.
6. Add 1 mL of 0.01N HCl to the sample vials (using the 0.01N HCl pipette) as a rinse and add to the column (using the appropriate sample pipette).
7. When the 0.01N HCl stops dripping out of the bottom of the column, exchange the scintillation vial, collecting the waste with a new 12.5 mL culture sample vial labelled with the appropriate sample ID.
8. Pour ~36 mL 2N ammonium hydroxide into a clean and combusted 200 mL beaker.
9. Elute the AAs into a labelled 12.5 mL culture sample vial by adding 1 mL 2N ammonium hydroxide to each column. Add 3 mL more ammonium hydroxide to each column so that a total of 4 mL ammonium hydroxide is used to wash the AAs through the column. You should see a dark front of material migrate down the column. Collect every drop of the ammonium hydroxide. If you collect just the dark front of material, you will lose many of the AAs.
10. Dry the eluent under a stream of N_2 at 80ºC (~4 hrs).

Esterification and trifluoroacetylation: Day 2

11. Pour ~5 mL of 0.2N HCL into a 5 mL graduated cylinder.
12. Add 0.5 mL of 0.2N HCl to each 8 mL sample vial, flush with N_2, screw on the PTFE-lined cap, and heat to 110°C for 5 minutes. Leave the vials to cool.

Esterification and trifluoroacetylation: Day 3

1. Uncap the sample vials and dry the contents at 55°C under a stream of N_2.
2. Fill a plastic tray with ice. IMPORTANT: chemicals in the next few steps need to be handled with extra caution. Mixing acetyl chloride and isopropanol results in a very exothermic reaction.
3. Use a 20 mL combusted graduated cylinder to measure out 20 mL isopropanol. Pour into a 50 mL Erlenmeyer flask.
4. Place the 50 mL flask with isopropanol in the ice tray, embedding it in the ice to minimise the exothermic reaction.
5. Use a 5 mL graduated cylinder to measure out 5 mL of acetyl chloride.
6. Slowly add the acetyl chloride to the 50 mL flask containing isopropanol. If you mix them too quickly the solution boils and can shoot out of the top of the flask. TAKE EXTREME CARE WITH THIS STEP.
7. Using a clean glass pipette, add 2.5 mL of the 4:1 isopropanol and acetyl chloride mixture to each sample vial.
8. Flush each vial with N_2 and cap. Heat at 110°C for 60 minutes (esterification step).
9. Cool to room temperature then dry under a stream of N_2 at 60°C (~ 1–2 hours).
10. Pour 5.5 mL of methylene chloride (dichloromethane) into a 10 mL beaker. Using a transfer pipette, add 600 uL methylene chloride to each sample vial.
11. Pour ~2 mL of trifluoroacetic anhydride into a 10 mL beaker. Using a transfer pipette, add 200 uL of trifluoroacetic anhydride to each sample vial.
12. Flush the vials with N_2 and cap them. Heat vials at 100°C for 15 minutes (trifluoroacetylation step).
13. Remove vials from the heat and cool at room temperature.
14. Uncap vials and evaporate contents to dryness at room temperature under a stream of N_2 (<1 hour).
15. Pour ~20 mL of freshly-made P-buffer (see above) into a clean 25 mL beaker and 10 mL of chloroform into a separate clean 25 mL beaker.
16. Add 2 mL of P-buffer and 1 mL of chloroform to each sample vial.
17. Place sample vials in the vortex and mix the contents for 60 seconds. This transfers the AAs to the chloroform fraction with contamination going into the P-buffer.
18. Centrifuge the mixture at 600 g for ~5 minutes. The solvents will separate, with the chloroform layer remaining on the bottom.
19. For each sample, use a double glass pipette set-up to remove the chloroform layer and transfer it into a new labelled vial. It is better to leave a little chloroform than to transfer any P-buffer to the new vial.
20. Pour ~10 mL more chloroform into the chloroform beaker. Add 1 mL of chloroform to each of the vials with the P-buffer.
21. Put all sample vials in the vortex and mix the contents of the vials for 60 seconds.
22. Centrifuge mixture at 600 g for ~5 minutes. The solvents will separate again with the chloroform layer remaining on the bottom.
23. For each sample, use a double glass pipette set-up to remove the chloroform layer and transfer it into a new labelled vial with the first 1 mL of sample.
24. Evaporate the 2 mL of chloroform under a stream of N_2 to dryness at room temperature (1–1.5 hrs).

25. Add 600 uL methylene chloride and 200 uL of trifluoroacetic anhydride as directed above (Day 3, steps 10 and 11). Put the chemicals directly into the vials.
26. Flush the vials with N_2 and cap them. Heat vials at 100°C for 15 minutes (trifluoroacetylation step).
27. When cooled, store samples in capped vials in the freezer until you are ready to analyse them.
28. Immediately before analysing, dry down the sample at room temperature and add about 100 µL ethyl acetate to derivatise the sample. Check this volume with the CSIA IRMS analyst first. The ethyl acetate is the one chemical that is drawn from the flask and is not poured into a separate beaker. If samples sit in ethyl acetate for more than a few days, they will become un-derivatised and will need to be re-derivatised as below, so only proceed with this stage when the GC-IRMS is ready for analysis.

Re-derivatisation of samples

If samples need to be re-derivatised (i.e., if they have been in ethyl acetate for more than a few days before being analysed), the following procedure should be followed.

1. Dry the sample down under N_2 if the sample is in ethyl acetate.
2. Add 0.5 mL of trifluoroacetic anhydride and 0.5 mL of ethyl acetate to the sample vial. Do one sample at a time.
3. Allow to stand at room temperature for exactly 1 hour.
4. Evaporate to dryness at room temperature.

Dilute with about 100 µL of ethyl acetate and run on CSIA IRMS system immediately (within a few hours).

Text S8. Compound-specific stable isotope analysis of nitrogen in amino acids on the gas chromatograph isotope ratio mass spectrometer

Derivatised AAs were separated on an Agilent J&W DB-5ms column (60 m x 0.25 mm ID x 0.25 µm film thickness), then combusted/reduced at 1000°C in the GC Isolink furnace (Thermo Fisher Scientific, Bremen, Germany). The CO_2 from the combustion was removed with a liquid N_2 trap prior to sample introduction into the CF-IRMS via the ConFlo IV open split. Triplicate measurements of each sample were bracketed by the AA Mix external standard. The $\delta^{15}N_{AA}$ values in the external standard were calibrated against international standards NIST 8573 (USGS40 L-glutamic acid) and NIST 8574 (USGS41 L-glutamic acid) following analysis on the EA CF-IRMS system, enabling the correction of sample $\delta^{15}N_{AA}$ values. Correction of raw sample $\delta^{15}N_{AA}$ data was carried out by plotting the mean $\delta^{15}N_{AA}$ value of the standard measured on the GC-Isolink IRMS system, versus their "true" value measured on the EA CF-IRMS. Raw $\delta^{15}N_{AA}$ sample values were corrected using the fitting equation (see Fig. S1) where R^2 was always better than 0.98. Finally, an in-house quality control sample "NIWA squid" was used to monitor the reproducibility of the hydrolysis and derivatisation process and CF-IRMS analysis across the batches. Precision of $\delta^{15}N_{AA}$ values (across all measured AAs) of repeat runs of the AA Mix standard ranged from 0.4–1.0 ‰. Repeat runs of NIWA Squid produced $\delta^{15}N_{AA}$ values ranging from 0.03–0.89 ‰. For most amino acids, the $\delta^{15}N_{AA}$ replication values of triplicate analyses for each sample ranged between 0.05–0.70 ‰, with the exception of poorer precision for alanine, isoleucine, serine, threonine and valine which ranged from 0.10–3.00 ‰ (Table S2).

SUPPLEMENTARY TABLES

Table S1. Lipid-extracted or lipid-corrected $\delta^{13}C$ and bulk $\delta^{15}N$ values of humpback whale (*Megaptera novaeangliae*) skin, suspended particulate organic material (SPOM) (used as a proxy for phytoplankton), zooplankton, Antarctic krill (*Euphausia superba*), myctophids (*Electrona carlsbergi*, *E. antarctica*, *Gymnoscopelus nicholsi*, *G. opisthopterus* and *G. braueri*), and Antarctic silverfish (*Pleuragramma antarctica*) sampled in waters around the Balleny Islands (BI), South-East Balleny Islands (SEBI), Ross Sea Slope (RSS) and Ross Sea (RS) in 2008 (International Polar Year - Census of Marine Life voyage), 2010 (Antarctic Whale Expedition voyage) and 2015 (New Zealand-Australia Antarctic Ecosystems voyage). Abbreviations are as follows: *n* = number of samples, Min = minimum, Max = maximum, SD = ±1 standard deviation.

Region	Sampling Years	$\delta^{13}C$					$\delta^{15}N$				
		n	Min	Max	**Mean**	SD	*n*	Min	Max	**Mean**	SD
		Whale skin									
BI	2010, 2015	38	-26.77	-20.90	**-25.20**	1.13	36	6.49	9.58	**7.58**	0.75
SEBI	2010, 2015	22	-26.52	-22.60	**-25.32**	0.90	22	6.90	9.00	**7.54**	0.49
RSS	2010, 2015	5	-26.07	-23.83	**-25.12**	0.85	5	6.61	8.50	**7.65**	0.71
All regions	2010	55	-26.77	-20.90	**-25.26**	1.07	55	6.49	9.58	**7.61**	0.63
All regions	2015	10	-26.17	-23.83	**-25.09**	0.78	10	6.60	8.90	**7.38**	0.83
All regions	2010, 2015	65	-26.77	-20.90	**-25.23**	1.03	65	6.49	9.58	**7.57**	0.66
Cluster A	2010, 2015	56	-26.77	-23.93	**-25.57**	0.50	56	6.49	8.29	**7.38**	0.43
Cluster B	2010, 2015	9	-24.49	-20.90	**-23.66**	0.43	9	8.23	9.58	**8.81**	0.48
		SPOM/Phytoplankton									
BI	2010, 2015	55	-27.40	-21.87	**-24.05**	1.66	55	-2.04	3.93	**1.49**	0.95
RSS	2010, 2015	65	-30.12	-27.50	**-28.41**	1.01	65	-2.41	2.14	**0.39**	1.97
RS	2010, 2015	109	-31.34	-26.38	**-28.56**	0.98	109	-2.65	2.14	**0.06**	0.96
All regions	2010	81	-31.34	-21.87	**-26.60**	3.04	81	-3.52	3.93	**0.37**	1.34
All regions	2015	148	-32.02	-22.15	**-28.16**	1.63	148	-1.21	3.58	**0.50**	1.00
All regions	2010, 2015	229	-32.02	-21.87	**-27.54**	2.41	229	-2.65	3.93	**0.45**	1.14
		Mixed Community Zooplankton									
BI	2010	6	-24.88	21.87	**-23.39**	1.25	6	3.99	6.54	**4.81**	1.01
RSS	2015	4	-28.96	-26.86	**-27.45**	1.01	4	4.57	9.47	**7.03**	2.04
RS	2008, 2015	97	-30.98	-22.20	**-27.19**	1.53	80	-0.41	11.11	**5.29**	2.26
All regions	2008	92	-30.98	-22.20	**-27.20**	1.55	75	-0.41	11.11	**5.19**	2.25
All regions	2010	5	-25.23	-23.27	**-24.33**	0.83	5	4.12	6.54	**5.25**	1.01
All regions	2015	8	-25.48	-24.33	**-24.85**	0.44	6	6.56	9.47	**7.85**	1.33
All regions	2008, 2010, 2015	105	-30.98	-22.20	**-26.88**	1.69	86	-0.41	11.11	**5.38**	2.25
		Antarctic krill (*Euphausia superba*)									
BI	2015	80	-27.05	-21.73	**-24.39**	1.07	80	3.34	7.39	**4.96**	0.60
RSS	2015	46	-27.32	-24.99	**-26.23**	0.65	46	2.03	5.90	**4.11**	0.62
RS	2008, 2015	87	-27.32	-24.83	**-26.09**	0.59	87	2.03	6.30	**4.10**	0.62
All regions	2008	31	-26.61	-24.89	**-25.91**	0.44	31	2.94	6.30	**4.08**	0.67
All regions	2015	182	-27.32	-21.73	**-25.41**	1.24	182	2.03	7.39	**4.48**	0.73
All regions	2008, 2015	213	-27.32	-21.73	**-25.48**	1.17	213	2.03	7.39	**4.43**	0.74

		Myctophids (5 species)									
BI	2015	25	-27.26	-22.21	**-24.54**	0.98	25	8.25	10.48	**9.28**	0.52
RSS	2015	112	-32.43	-21.97	**-25.46**	1.93	109	7.25	10.75	**9.08**	0.61
RS	2008, 2015	259	-32.43	-21.97	**-25.49**	1.52	254	7.25	11.80	**9.57**	0.77
All regions	2008	56	-30.19	-23.50	**-25.24**	1.08	56	8.05	11.80	**9.91**	0.79
All regions	2015	340	-32.43	-21.97	**-25.46**	1.71	332	7.25	11.53	**9.33**	0.71
All regions	2008, 2015	396	-32.43	-21.97	**-25.43**	1.63	388	7.25	11.80	**9.42**	0.75
		Antarctic silverfish (*Pleuragramma antarctica*)									
RS	2008, 2015	170	-26.79	-23.57	**-25.11**	0.68	170	8.04	12.73	**10.24**	0.80
All regions	2008	140	-26.79	-23.57	**-25.01**	0.66	140	8.04	12.73	**10.34**	0.81
All regions	2015	30	-26.57	-24.63	**-25.59**	0.55	30	8.42	10.88	**9.81**	0.54
All regions	2008, 2015	170	-26.79	-23.57	**-25.11**	0.68	170	8.04	12.73	**10.24**	0.80

Table S2: Metadata for 14 humpback whale (*Megaptera novaeangliae*) skin samples selected for compound specific stable isotope analysis of nitrogen in amino acids: whale sampling location; gender and cluster information; bulk $\delta^{15}N$ values; mean and standard deviation (±1 SD of triplicate injections on the GC-Isolink) $\delta^{15}N$ values of alanine (Ala), glycine (Gly), threonine (Thr), serine (Ser), valine (Val), leucine (Leu), isoleucine (Iso), proline (Pro), aspartic acid (Asp), glutamic acid (Glx) and phenylalanine (Phe); whale trophic discrimination factor (TDF_{whale}); and trophic position (TP) estimates. There was replication of analysis for sample 2010_AWE_216. TDF_{whale} = (Glx – Phe – 3.4)/(TP_{whale} – 1), where TP_{whale} = 3.32 based on simple arithmetic TP calculation from bulk nitrogen stable isotope data (see section 2.6 in the manuscript and Table S11). Compound-specific stable isotope analysis (CSIA) TP = 1 + (Glx – Phe – 3.4)/ Avg $TDF_{whale,}$ where Avg TDF_{whale} = 3.58, 0.80. M = male, F = female, ND = no data.

Sample ID	Lat	Long	Sex	Cluster	Bulk $\delta^{15}N$ (‰)	Ala Mean ± 1 SD (‰)	Gly Mean ± 1 SD (‰)	Thr Mean ± 1 SD (‰)	Ser Mean ± 1 SD (‰)	Val Mean ± 1 SD (‰)	Leu Mean ± 1 SD (‰)	Iso Mean ± 1 SD (‰)	Pro Mean ± 1 SD (‰)	Asp Mean ± 1 SD (‰)	Glx Mean ± 1 SD (‰)	Phe Mean ± 1 SD (‰)	Glx-Phe	TDF_{whale}	CSIA TP
2010_033	-69.135	166.532	M	A	7.26	11.50, 0.28	-3.67, 0.30	-31.12, 2.56	5.94, 0.69	24.39, 0.93	11.61, 0.40	12.71, 1.56	11.95, 0.61	11.42, 0.69	13.35, 0.40	-0.27, 0.62	13.62	4.39	3.85
2010_216a	-67.545	168.447	M	A	7.19	13.55, 0.82	-0.44, 0.95	-30.84, 0.54	6.07, 0.13	23.82, 1.42	12.65, 0.12	17.83, 0.36	15.95, 0.16	11.22, 0.30	13.19, 0.33	1.33, 1.11	11.87	3.63	3.36
2010_216b	-67.545	168.447	M	A	7.19	13.51, 0.64	-0.30, 0.55	-30.14, 0.48	3.76, 0.58	16.40, 0.49	12.58, 0.51	18.18, 1.90	14.41, 0.69	10.82, 0.34	12.88, 0.12	1.20, 0.41	11.68	3.55	3.31
2010_220	-67.507	164.303	M	A	6.91	23.24, 1.77	4.98, 0.48	-21.11, 0.68	5.38, 2.32	ND	14.10, 0.09	21.66, 1.51	14.00, 0.12	9.70, 0.12	12.52, 0.23	1.73, 1.23	10.79	3.17	3.07
2015_010	-69.709	185.31	M	A	7.38	11.24, 0.07	-0.37, 0.58	-32.98, 0.23	ND	9.67, 0.67	13.20, 0.39	16.60, 0.63	16.89, 0.39	12.85, 0.20	13.73, 0.22	3.80, 0.46	9.93	2.8	2.82
2010_013	-66.988	164.913	M	B	8.42	13.67, 0.39	2.10, 0.65	-28.09, 0.44	5.96, 0.31	19.79, 2.74	14.01, 0.56	12.78, 0.47	16.14, 1.00	12.20, 0.10	15.24, 0.65	0.00, 0.72	15.24	5.08	4.31
2010_208	-66.887	164.197	M	B	9.58	22.38, 0.81	7.22, 0.39	-19.87, 1.66	8.32, 1.94	ND	14.58, 0.42	13.67, 0.35	16.04, 0.25	12.20, 0.36	15.03, 0.16	2.21, 0.49	12.82	4.04	3.63
2010_215	-67.54	168.429	M	B	9	15.61, 0.85	0.31, 0.31	-22.32, 3.00	7.39, 0.49	ND	ND	14.73, 1.33	14.14, 1.68	12.37, 0.22	15.81, 0.46	0.84, 1.71	14.96	4.96	4.23
2010_210	-66.579	163.041	F	A	6.49	23.06, 1.99	7.18, 0.24	-26.41, 0.83	4.08, 1.27	21.79, 0.69	13.42, 0.07	20.23, 0.65	14.33, 0.47	8.88, 0.09	12.23, 0.08	2.16, 0.89	10.07	2.86	2.86
2010_219	-67.508	164.279	F	A	8.29	22.12, 2.23	6.78, 0.44	-20.91, 1.53	9.24, 0.69	ND	15.58, 0.64	22.3, 2.41	15.72, 0.64	11.98, 0.17	13.64, 0.55	1.85, 0.98	11.8	3.6	3.35
2015_006	-66.769	163.582	F	A	6.82	12.46, 0.63	-1.44, 0.49	-26.81, 0.87	ND	11.66, 0.58	12.78, 0.50	16.87, 0.44	15.72, 0.16	11.60, 0.43	13.85, 0.25	3.08, 0.45	10.77	3.16	3.06
2015_011	-69.708	185.307	F	A	6.61	ND	-1.68, 0.53	-30.02, 0.25	1.09, 0.52	ND	12.07, 0.66	13.00, 0.66	14.26, 0.79	11.73, 0.94	12.25, 0.72	3.21, 0.49	9.04	2.42	2.58
2015_001	-66.648	163.338	F	B	8.9	17.21, 0.41	3.83, 0.39	-22.66, 0.27	9.04, 0.27	13.18, 0.67	16.64, 0.46	18.26, 0.79	18.42, 0.43	14.05, 0.25	15.56, 0.20	4.04, 0.74	11.52	3.48	3.27
2015_009	-69.696	185.703	F	B	8.5	14.18, 0.90	3.47, 0.18	-22.75, 0.62	ND	13.62, 0.38	14.15, 0.18	17.58, 0.10	17.63, 0.57	13.09, 0.28	14.44, 0.45	4.20, 0.39	10.24	2.94	2.91
Avg All					7.75, 1.00	16.44, 4.62	2.00, 3.60	-26.15, 4.47	6.02, 2.45	17.15, 5.48	13.64, 1.41	16.69, 2.91	15.40, 1.68	11.72, 1.33	13.84, 1.21	2.28, 1.31	11.74, 1.86	**3.58, 0.80**	**3.33, 0.52**
Avg Female					7.60, 1.08	17.81, 4.70	3.02, 3.86	-24.93, 3.40	5.86, 3.98	15.06, 4.56	14.11, 1.73	17.59, 2.57	16.01, 1.70	11.89, 1.75	13.97, 1.22	1.59, 1.20	12.61, 1.91	3.95, 0.82	**3.00, 0.28**
Avg Male					7.87, 1.00	15.59, 4.66	1.23, 3.45	-27.06, 5.15	6.12, 1.45	18.81, 6.05	13.25, 1.05	16.02, 3.13	14.94, 1.61	11.60, 1.02	13.66, 1.29	3.09, 0.95	10.57, 1.01	3.08, 0.44	**3.57, 0.53**
Avg Cluster A					7.13, 0.53	16.34, 5.43	1.23, 3.99	-27.82, 4.37	5.08, 2.51	17.95, 6.34	13.11, 1.18	17.41, 3.04	14.80, 1.45	11.13, 1.20	13.07, 0.63	2.30, 0.96	11.06, 1.35	3.29, 0.58	**3.14, 0.38**
Avg Cluster B					8.88, 0.46	16.62, 3.50	3.39, 2.55	-23.14, 3.01	7.68, 1.33	15.53, 3.70	14.85, 1.22	15.40, 2.41	16.47, 1.65	12.78, 0.80	15.22, 0.53	2.26, 1.87	12.96, 2.16	4.10, 0.93	**3.67, 0.60**

Table S3. Isotopic niche metrics (including the six Layman metrics) for the two humpback whale (*Megaptera novaeangliae*) clusters A and B (after Layman et al. 2007 and Jackson et al. 2011). CA = Cluster A, CB = cluster B.

Metrics	Cluster A	Cluster B	Probability CB > CA (%)
n	56	9	
SEA	0.67	1.10	
SEA_c	0.68	1.38	
SEA_B	0.74	0.84	97.90
$\delta^{13}C$ range	2.84	3.59	
$\delta^{13}C$ $range_{boot}$	1.47	2.84	100.00
$\delta^{15}N$ range	1.80	1.35	
$\delta^{15}N$ $range_{boot}$	1.21	1.66	99.50
TA	3.29	2.30	
TA_{boot}	0.99	3.16	100.00
CD	0.58	0.96	
CD_{boot}	0.54	0.58	65.30
MNND	0.14	0.51	
$MNND_{boot}$	0.30	0.15	2.80
SDNND	0.15	0.44	
$SDNND_{boot}$	0.23	0.15	20.80

Abbreviations and definitions

n = number of samples

SEA = Standard Ellipse Area

SEA_c = SEA Corrected for small sample size

SEA_B = Bayesian SEA

$\delta^{13}C$ range = distance between the highest and lowest $\delta^{13}C$ values (i.e., max $\delta^{13}C$ – min $\delta^{13}C$). "Estimates the diversity of basal resources".

$\delta^{15}N$ range = distance between the highest and lowest $\delta^{15}N$ values (i.e., max $\delta^{15}N$ – min $\delta^{15}N$). "Measure of trophic length of the community".

TA = Total Area of the convex hull comprising all data points. "Measure of the total amount of niche space occupied and indication of niche width".

CD = mean Distance to Centroid. Mean Euclidean distance of each sample to the centroid. "Measure of niche width and sample spacing".

MNND = Mean Nearest Neighbour Distance, which is the mean of the Euclidean distances to each sample's nearest neighbor. "Measure of density and clustering of individuals".

SDNND = Standard Deviation of Nearest Neighbour Distance. "Measure of the evenness of spatial density and packing of individuals". Low SDNND values indicate more even distribution of trophic niches.

Subscript 'boot' indicates that the value (mean) has been generated via bootstrapping.

Table S4. Results of the T test analysis of differences in $\delta^{13}C$ and $\delta^{15}N$ values between prey clusters 1 and 6 as defined in Fig. S2: *n* = number of samples in cluster; Mean is the mean value of the cluster; SD is the ±1 standard deviation; pSW is the Shapiro-Wilk probability (normality test) for the cluster (low, less than 0.05 means unlikely to be normally distributed); Tstat is the T-test (difference in means with equal variances between the clusters assumed); Sig is the probability of null hypothesis (equal means, equal variance), where a low value <0.05, means there is a difference; Tstat_unequal is the T-test (difference in means with unequal variances between clusters assumed); Sig_unequal is the probability of null hypothesis (equal means with equal variance between the clusters assumed), where a low value of <0.05 means there is a difference.

Lipid-extracted $\delta^{13}C$ Results													
Cluster Comparison		Parameters for cluster in first column				Parameters for cluster in second column				Test results			
		n	Mean	SD	pSW	*n*	Mean	SD	pSW	Tstat	Sig	Tstat_ unequal	Sig_ unequal
1	2	156	-28.371	1.072	1.31E-09	41	-24.057	1.685	0.001	-20.094	0	-15.5819	1.40E-20
1	3	156	-28.371	1.072	1.31E-09	133	-26.136	0.611	0.049	-21.274	0	-22.1574	0
1	4	156	-28.371	1.072	1.31E-09	84	-24.380	1.052	0.933	-27.686	0	-27.8453	0
1	5	156	-28.371	1.072	1.31E-09	101	-26.992	1.622	0.136	-8.2103	1.11E-14	-7.54541	3.47E-12
1	6	156	-28.371	1.072	1.31E-09	566	-25.331	1.422	0.000	-24.824	0	-29.0652	0
2	3	41	-24.057	1.685	0.001	133	-26.136	0.611	0.049	11.9553	2.30E-24	7.74152	1.06E-09
2	4	41	-24.057	1.685	0.001	84	-24.380	1.052	0.933	1.31109	0.19227	1.12428	0.265718
2	5	41	-24.057	1.685	0.001	101	-26.992	1.622	0.136	9.65897	3.15E-17	9.50352	2.53E-14
2	6	41	-24.057	1.685	0.001	566	-25.331	1.422	0.000	5.46504	6.77E-08	4.71861	2.41E-05
3	4	133	-26.136	0.611	0.049	84	-24.380	1.052	0.933	-15.547	4.99E-37	-13.8872	1.22E-26
3	5	133	-26.136	0.611	0.049	101	-26.992	1.622	0.136	5.58711	6.45E-08	5.0372	1.66E-06
3	6	133	-26.136	0.611	0.049	566	-25.331	1.422	0.000	-6.3879	3.07E-10	-10.076	7.64E-22
4	5	84	-24.380	1.052	0.933	101	-26.992	1.622	0.136	12.6969	6.71E-27	13.1848	6.12E-28
4	6	84	-24.380	1.052	0.933	566	-25.331	1.422	0.000	5.89149	6.15E-09	7.34685	1.85E-11
5	6	101	-26.992	1.622	0.136	566	-25.331	1.422	0.000	-10.575	2.90E-24	-9.64802	6.55E-17
Bulk $\delta^{15}N$ Results													
1	2	156	0.269	1.014	0.015	41	1.483	0.958	0.000	-6.8971	7.21E-11	-7.13268	9.69E-10
1	3	156	0.269	1.014	0.015	133	4.104	0.617	0.000	-38.023	0	-39.4469	0
1	4	156	0.269	1.014	0.015	84	4.969	0.628	0.000	-38.656	0	-44.2473	0
1	5	156	0.269	1.014	0.015	82	5.390	2.289	0.506	-23.867	0	-19.2855	3.90E-35
1	6	156	0.269	1.014	0.015	558	9.669	0.852	0.166	-116.61	0	-105.796	0
2	3	41	1.483	0.958	0	133	4.104	0.617	0.000	-20.646	0	-16.503	5.14E-22
2	4	41	1.483	0.958	0	84	4.969	0.628	0.000	-24.364	0	-21.1932	8.44E-29
2	5	41	1.483	0.958	0	82	5.390	2.289	0.506	-10.463	1.23E-18	-13.301	3.04E-25
2	6	41	1.483	0.958	0	558	9.669	0.852	0.166	-58.841	0	-53.2084	4.00E-42
3	4	133	4.104	0.617	0	84	4.969	0.628	0.000	-9.9951	1.46E-19	-9.95499	9.09E-19
3	5	133	4.104	0.617	0	82	5.390	2.289	0.506	-6.1341	4.11E-09	-4.97582	3.18E-06
3	6	133	4.104	0.617	0	558	9.669	0.852	0.166	-70.981	0	-86.2677	0
4	5	84	4.969	0.628	0	82	5.390	2.289	0.506	-1.6233	0.106452	-1.60643	0.111575
4	6	84	4.969	0.628	0	558	9.669	0.852	0.166	-48.58	0	-60.7137	0
5	6	82	5.390	2.289	0.506	558	9.669	0.852	0.166	-31.739	0	-16.757	1.44E-28

Table S5. Model comparison for analyses of contributions of potential prey groups to the diet of humpback whales (*Megaptera novaeangliae*) derived from MixSIAR analysis of the null model, with the model including whale cluster as a fixed factor for two trophic discrimination factors (TDFs) (Post, 2002; Borrell et al., 2012, see Section 2.5.2 in manuscript). Models were evaluated by LOOic and *w*AIC. ΔLOOic is the difference between each model and the model with the lowest LOOic. The top-ranked model (shown in bold) for both TDFs included whale cluster as a fixed factor, as it had the highest proportion of *w*AIC and the lowest LOOic. This model also had the lowest multiplicative error term ξ_j,. LOOic = leave-one-out cross validation information criterion, SE = standard error, ΔLOOic = difference between LOOic of current and top-ranked model; *w*AIC = Akaike Information Criterion weight; ξ_j = multiplicative error term for carbon and nitrogen.

#	Model	TDF	LOOic	SE (LOOic)	ΔΛΟΟιχ	SE (DLOOic)	*w*AIC	ξ_X	ξ_N
1	**Cluster**	**Post (2002)**	**-46.5**	**18.0**	**0**	**-**	**1**	**0.3**	**0.4**
2	Null	Post (2002)	57.8	29.0	104.3	21.9	0	1.0	0.9
1	**Cluster**	**Borrell et al. (2012)**	**-44.0**	**19.7**	**0**	**-**	**1**	**0.4**	**0.4**
2	Null	Borrell et al. (2012)	58.5	27.8	102.5	19.5	0	1.1	0.7

Table S6. Lipid-extracted $\delta^{13}C$ and bulk $\delta^{15}N$ mean and standard deviation (±1 SD) values of humpback whale (*Megaptera novaeangliae*) skin sampled from adults, sub-adults and dependent young: *n* = number of whales sampled.

Year of sampling and humpback whale ontogeny	*n*	$\delta^{13}C$ (‰)		$\delta^{15}N$ (‰)	
		Mean	SD	Mean	SD
2010 Adult	44	-25.29	0.85	7.54	0.58
2010 Sub-adult	6	-24.45	2.2	8.06	1.01
2010 Dependent young	5	-25.98	0.34	7.67	0.4
2015 Adult	10	-25.09	0.78	7.38	0.83

Table S7. Lipid-extracted $\delta^{13}C$ and bulk $\delta^{15}N$ mean and standard deviation (±1 SD) values of humpback whale (*Megaptera novaeangliae*) skin sampled from males and females during voyages in 2010 and 2015: *n* = number of whales sampled.

Year of sampling	Sex	*n*	$\delta^{13}C$ (‰)		$\delta^{15}N$ (‰)	
			Mean	SD	Mean	SD
2010	Male	29	-24.84	1.29	7.9	0.66
2010	Female	26	-25.73	0.43	7.28	0.40
2015	Male	1	-25.40		7.38	
2015	Female	9	-25.06	0.82	7.38	0.88
2010, 2015	Male	30	-24.86	1.27	7.88	0.65
2010, 2015	Female	35	-25.56	0.62	7.31	0.55

Table S8. Results of generalised linear models for a) lipid-extracted $\delta^{13}C$ and b) bulk $\delta^{15}N$ values of all humpback whale (*Megaptera novaeangliae*) skin sampled during voyages in 2010 and 2015. Significant variables in each model are given in bold. K = number of estimated parameters for each model, LL = log-likelihood; % DE = % deviance explained; Δ AIC_C = difference in Akaike's Information Criterion corrected for small sample sizes (AIC_C) of the current and top-ranked model; and $wAIC_C$ = AIC_C weight.

Model	K	ΔAICc	wAICc	LL	% DE
a) $\delta^{13}C$					
~ **Sex** + Age]	5	-	0.31	-87.22	18.9
~ **Sex** + Year	4	0.16	0.29	-88.48	21.3
~ **Sex** + Age + Year	6	0.38	0.26	-86.19	19.2
b) $\delta^{15}N$					
~ **Sex** + Year	4	-	0.38	-58.12	17.9
~ **Sex** + Age	5	0.4	0.31	-57.15	14.6
~ **Sex** + Location	5	2.15	0.13	-58.02	20.4

Table S9. Results of final generalised linear models for a) lipid-extracted $\delta^{13}C$ and b) bulk $\delta^{15}N$ values of humpback whale (*Megaptera novaeangliae*) skin sampled during voyages in 2010 and 2015: SE = standard error. P-value = probability value.

Term	Estimate	SE	Statistic	P-value
a) **$\delta^{13}C$**				
(Intercept)	-25.510	0.167	-153.031	0.000
Sex: Male	0.608	0.245	2.484	0.016
Age: Dependent young	-0.717	0.447	-1.605	0.114
Age: Subadult	0.548	0.423	1.296	0.200
b) **$\delta^{15}N$**				
(Intercept)	-2.438	88.102	-0.028	0.978
Sex: Male	0.579	0.159	3.650	0.001
Year	0.005	0.044	0.111	0.912

Table S10. Proportional (mean, range and 95% credible interval) and posterior (modes, 95% highest probability density intervals (HPDI)) distributions of each prey cluster to humpback whale (*Megaptera novaeangliae*) diet (for whale clusters A and B), using either Post (2002) or Borrell et al. (2012) trophic discrimination factors (TDFs), inferred from the MixSIAR Bayesian stable isotope mixing model (Stock et al., 2018). The prey clusters which make up the greatest contributions to humpback whale diet based on proportional distributions are indicated in bold text. Where posterior distributions are multi-modal, the highest peak is denoted in bold text. Prey Cluster Key: (1) Phytoplankton Ross Sea Shelf (RSS), Ross Sea (RS); (2) Phytoplankton Balleny Islands (BI); (3) Antarctic Krill (*Euphausia superba*), RSS, RS; (4) Zooplankton BI, Antarctic Krill BI; (5) Zooplankton RSS, RS; (6) Myctophids (*Electrona carlsbergi*, *E. antarctica*, *Gymnoscopelus nicholsi*, *G. opisthopterus* and *G. braueri*) BI, RSS, RS, Antarctic Silverfish (*Pleuragramma antarctica*) RS.

Whale cluster	TDF	Prey cluster	Mean ±1 SD	Range	95% credible interval	Modes	95% HPDI
A	Post (2002)	**1**	**0.224, 0.064**	0.0220–0.4240	0.098–0.344	0.2165	0.0965–0.3470
		2	0.069, 0.049	0.0002–0.2800	0.003–0.181	**0.0255**, 0.0645	0.0000–0.1630
		3	**0.201, 0.142**	0.0005–0.7100	0.006–0.519	**0.0455**, 0.1160	0.0000–0.4710
		4	**0.268, 0.077**	0.0180–0.5350	0.117–0.414	0.2760	0.1160–0.4180
		5	0.141, 0.086	0.0003–0.4710	0.006–0.321	**0.1230**, 0.1855	0.0000–0.2965
		6	0.097, 0.055	0.0002–0.2900	0.007–0.216	0.0920	0.0000–0.1980
B		1	0.021, 0.014	0.0007–0.1050	0.003–0.056	0.0135	0.0965–0.3470
		2	0.021, 0.040	0.0000–0.4920	0.000–0.083	**0.0040**, 0.0590	0.0000–0.1630
		3	0.031, 0.029	0.0001–0.2390	0.001–0.111	0.0095	0.0000–0.4710
		4	**0.848, 0.098**	0.0090–0.9920	0.676–0.96	0.8825	0.1160–0.4180
		5	0.026, 0.024	0.0000–0.2020	0.001–0.091	0.0085	0.0000–0.2965
		6	0.053, 0.054	0.0000–0.4820	0.001–0.168	0.0110	0.0000–0.1980
A	Borrell et al. (2012)	**1**	**0.335, 0.060**	0.1120–0.5040	0.214–0.442	0.3335	0.2185–0.4495
		2	0.025, 0.020	0.0000–0.1320	0.001–0.081	0.0055	0.0000–0.0725
		3	0.065, 0.060	0.0001–0.4330	0.002–0.221	0.0145	0.0000–0.1930
		4	0.058, 0.040	0.0004–0.2200	0.002–0.141	**0.0095**, 0.0590, 0.0830	0.0000–0.1290
		5	**0.250, 0.100**	0.0140–0.5720	0.057–0.435	0.2815	0.0500–0.4330
		6	**0.267, 0.070**	0.0220–0.4600	0.139–0.392	0.2640	0.1360–0.3945
B		1	0.039, 0.020	0.0020–0.3160	0.008–0.095	0.0255	0.0025–0.0855
		2	0.132, 0.160	0.0000–0.4560	0.000–0.401	**0.0065**, 0.3480	0.0000–0.0840, 0.2415–0.4355
		3	0.023, 0.030	0.0000–0.2940	0.000–0.094	0.0055	0.0000–0.0765
		4	**0.410, 0.310**	0.0001–0.8260	0.001–0.754	0.0140, **0.6540**	0.0000–0.1385, 0.4580–0.8205
		5	0.053, 0.040	0.0010–0.2900	0.007–0.155	0.0255	0.0000–0.1310
		6	**0.343, 0.140**	0.0250–0.6000	0.168–0.560	**0.2375**, 0.5230	0.1400–0.3425, 0.4375–0.5915

Table S11. Simple mathematical estimations of trophic position (TP) of humpback whales (*Megaptera novaeangliae*) using measured bulk $\delta^{15}N$ data (mean over 2010 and 2015 for whales, and mean over 2008, 2010 and 2015 for prey from all sampled regions south of 66º S) and reported trophic discrimination factors (TDFs) from the literature. Prey include Antarctic krill (*Euphausia superba*), myctophids (*Electrona carlsbergi*, *E. antarctica*, *Gymnoscopelus nicholsi*, *G. opisthopterus* and *G. braueri*), and Antarctic silverfish (*Pleuragramma antarctica*). "Fish" refers to the mean value of all sampled myctophids and Antarctic silverfish, SPOM = suspended particulate organic material. Calculations of the values (a)-(e) are given in the footnote.

Approx-imate TP	Mean measured bulk $\delta^{15}N$ (±1 SD) value across years and SO regions (‰)	Organism	TDF (Reference source)	TDF value	Predicted whale isotope value if whales are at TP 3	Difference between measured and predicted whale isotope value (‰)	Fraction of TP measured minus predicted whale isotope value (%)	Mean estimated TP of whales
	Value (a)				**Value (b)**	**Value (c)**	**Value (d)**	**Value (e)**
3 to 4	10.24, 0.80	Silverfish						
3 to 4	9.42, 0.75	Myctophids						
			Whale – Fish TDF (Borrell et al. 2012)	2.82				
3	7.57, 0.66	Mean All Whales			6.67	0.90	0.32	3.32
3	7.31, 0.55	Mean Female Whales			6.67	0.64	0.23	3.23
3	7.88, 0.65	Mean Male Whales			6.67	1.21	0.43	3.43
3	7.38, 0.43	Mean Cluster A Whales			6.67	0.71	0.25	3.25
3	8.81, 0.48	Mean Cluster B Whales			6.67	2.14	0.76	3.76
			Whale – Krill TDF (Borrell et al. 2012)	2.82				
2	4.43, 0.74	Antarctic Krill						
			Krill – Phytoplankton TDF (Post, 2002)	3.40				
1	0.45, 1.14	Phytoplankton/ SPOM						

Calculations for values (a) to (e)

(a) = Mean measured bulk $\delta^{15}N$ value as provided in the data column

(b) = Mean SPOM/Phytoplankton value + (Krill – Phytoplankton TDF) + (Whale – Krill TDF)

(c) = (a) – (b)

(d) = (c) – Borrell et al. (2012) TDF

(e) = TL value of 3 + (d)

SUPPLEMENTARY FIGURES

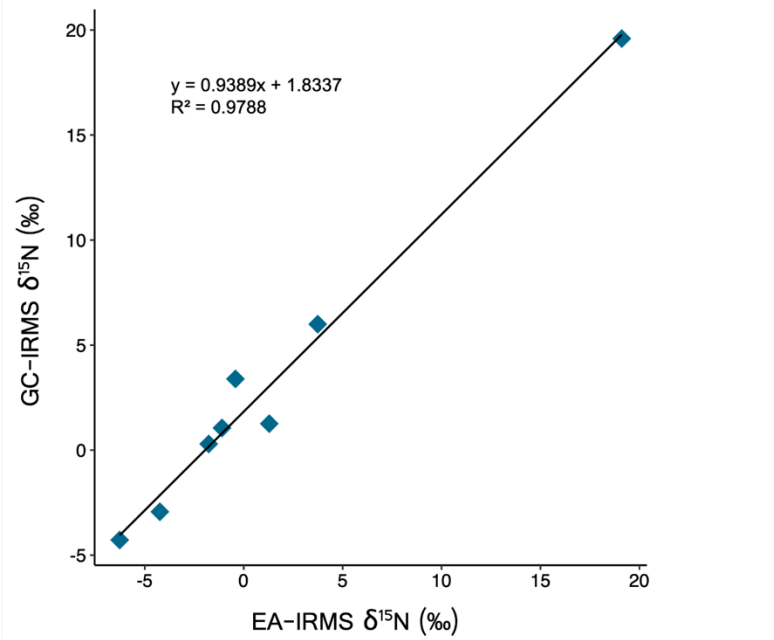

Fig. S1. Example of a standard calibration for the suite of amino acid standards used for $\delta^{15}N$ corrections of values for each standard measured using elemental analyser isotope ratio mass spectrometry (EA-IRMS) *versus* gas chromatography isotope ratio mass spectrometry (GC-IRMS) values of each standard.

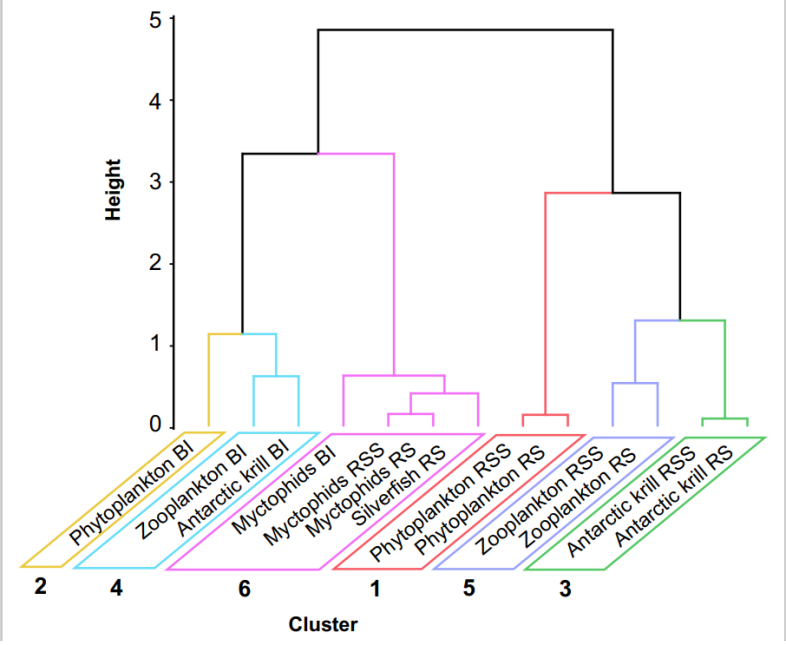

Fig. S2. Dendrogram showing clustering of potential prey species of Southern Ocean humpback whales (*Megaptera novaeangliae*) based on the mean lipid-extracted or lipid-corrected carbon and bulk nitrogen stable isotope values of the prey. Ward's correlation coefficient = 0.716. Height indicates the cophenetic distance between members. Abbreviations for locations are as follows: BI = Balleny Islands, RSS = Ross Sea Slope, RS = Ross Sea. Specific prey species, where known, are Antarctic krill (*Euphausia superba*), myctophids (*Electrona carlsbergi*, *E. antarctica*, *Gymnoscopelus nicholsi*, *G. opisthopterus* and *G. braueri*), and Antarctic silverfish (*Pleuragramma antarctica*).

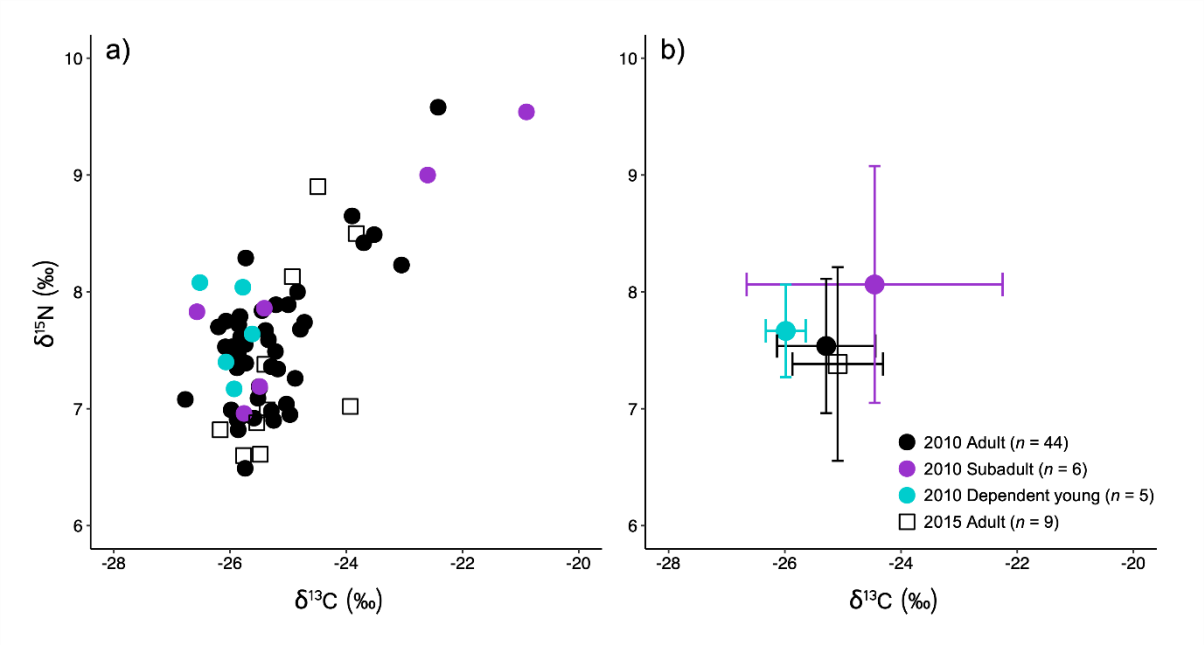

Fig. S3. Lipid-extracted $\delta^{13}C$ and bulk $\delta^{15}N$ values of humpback whale (*Megaptera novaeangliae*) skin biopsies from adults, sub-adults and dependent young sampled in February-March 2010 (filled circles) and Feb-March 2015 (open squares), showing in panel a) all values, and in panel b) mean and standard deviation (±1 SD) values, where *n* = number of whales sampled.

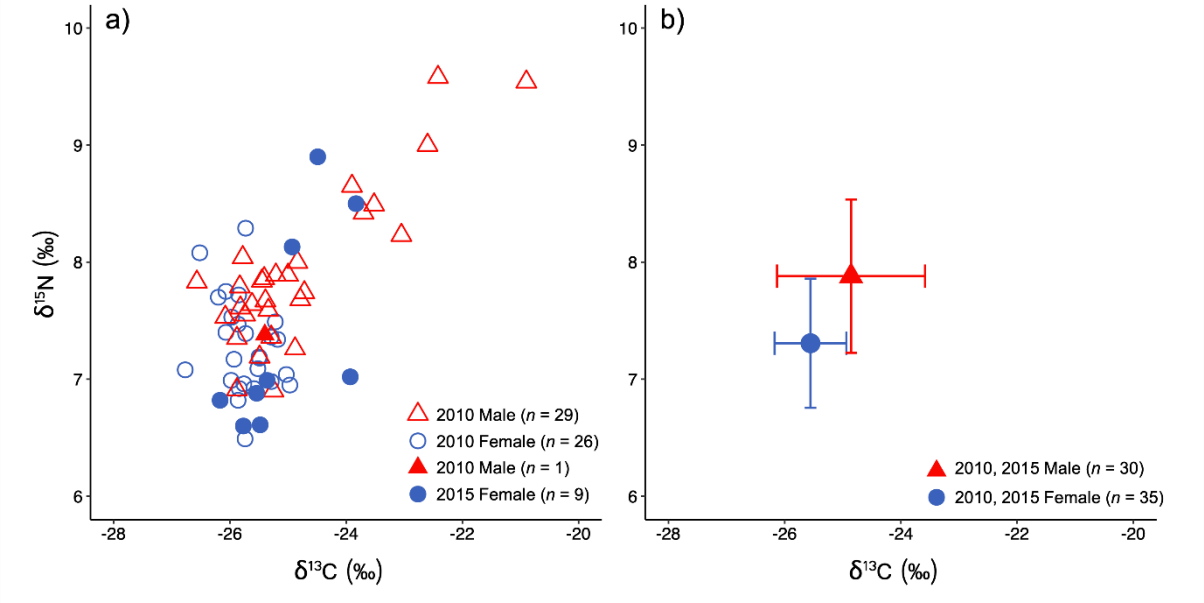

Fig. S4. Panel a) Lipid-extracted $\delta^{13}C$ and bulk $\delta^{15}N$ values of humpback whale (*Megaptera novaeangliae*) skin biopsies from males (red triangles) and females (blue circles) sampled in February-March 2010 (open symbols) and Feb-March 2015 (filled symbols). Panel b) shows the lipid-extracted $\delta^{13}C$ and bulk $\delta^{15}N$ mean and standard deviation (±1 SD) values averaged across both voyages for males (filled red triangle) and females (filled blue circle), where *n* = number of whales sampled.

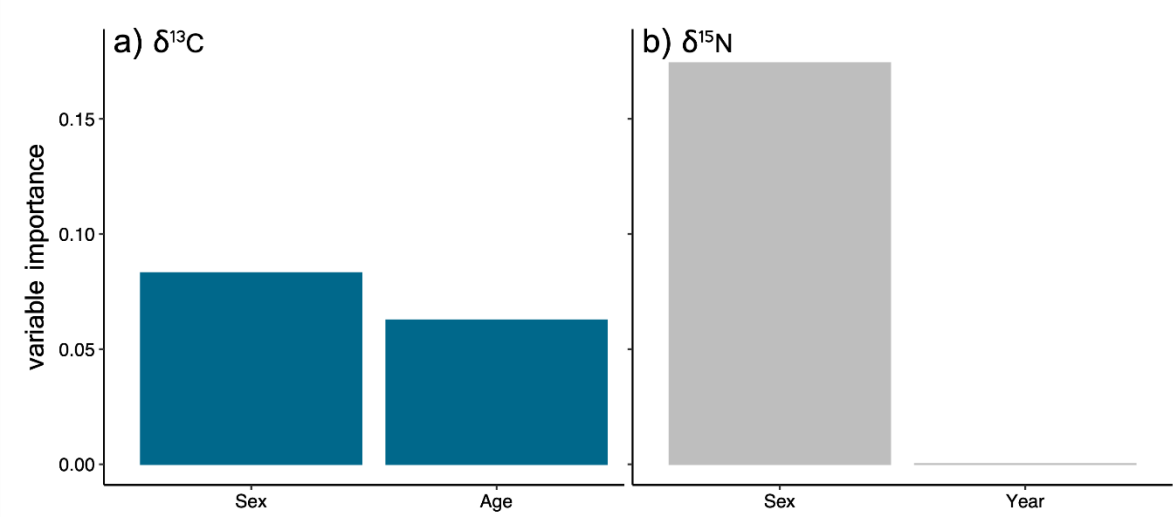

Fig. S5. Generalised Linear Model results illustrating the variable importance of a) sex and age for the $\delta^{13}C$ model, and b) sex and year for the $\delta^{15}N$ model.

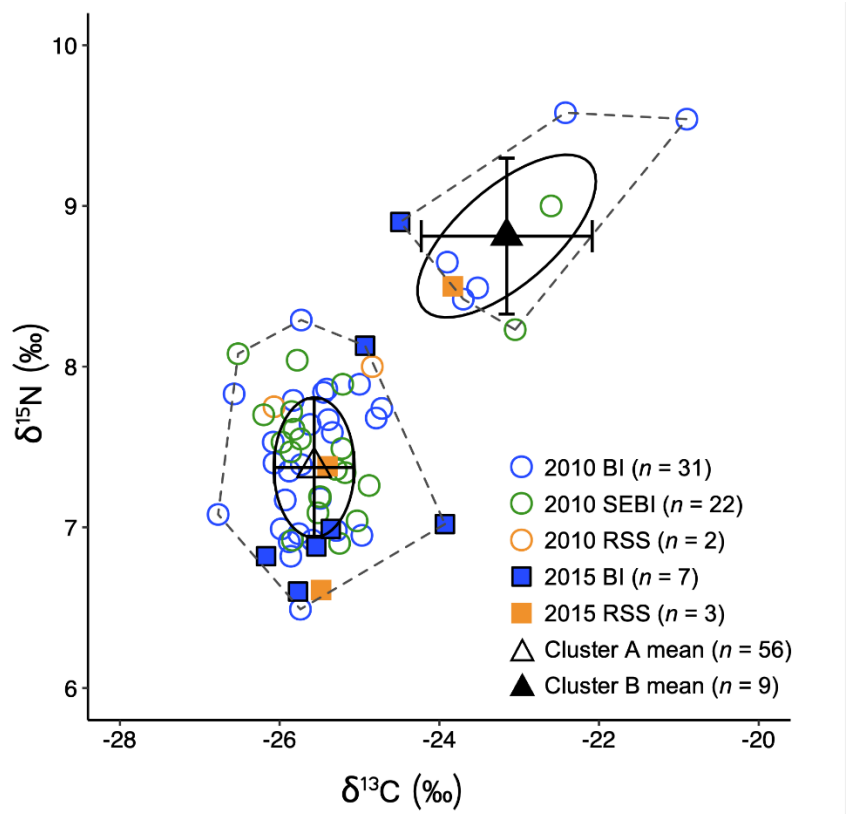

Fig. S6. Lipid-extracted $\delta^{13}C$ and bulk $\delta^{15}N$ biplot of 2010 and 2015 humpback whale (*Megaptera novaeangliae*) skin biopsy samples, showing sampling year and location for each of the isotopically segregated clusters A and B. The mean (±1 SD) values of cluster A and B are also shown. Locations are abbreviated as follows: Balleny Islands (BI), South East Balleny Islands (SEBI), Ross Sea Slope (RSS) and Ross Sea (RS), and *n* = number of whales sampled.

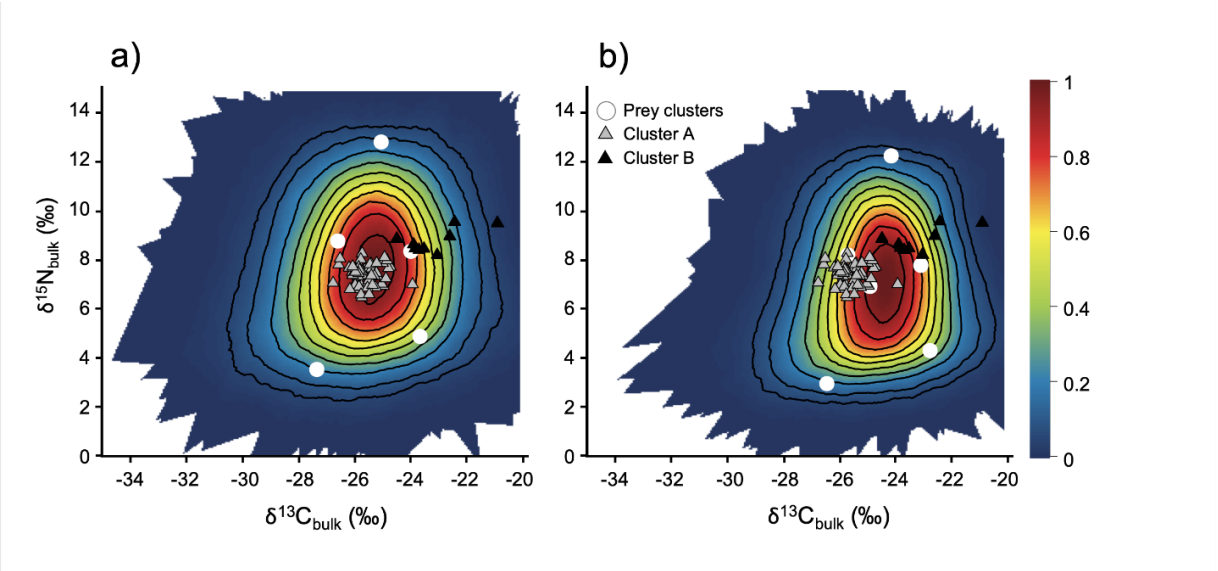

Fig. S7. Simulated mixing prey polygon plot (Smith et al. 2013) applying trophic discrimination fractionation factors from Post (2002) in panel a) and Borrell et al. (2012) in panel b) to lipid-extracted $\delta^{13}C$ and bulk $\delta^{15}N$ values of humpback whale (*Megaptera novaeangliae*) skin. The colour scale indicates the probability (1 being high) that the proposed mixing model can calculate source contributions to explain the consumer's stable isotope value. The single outlier is not used in the MixSIAR (Stock et al. 2018) Bayesian prey apportionment model.

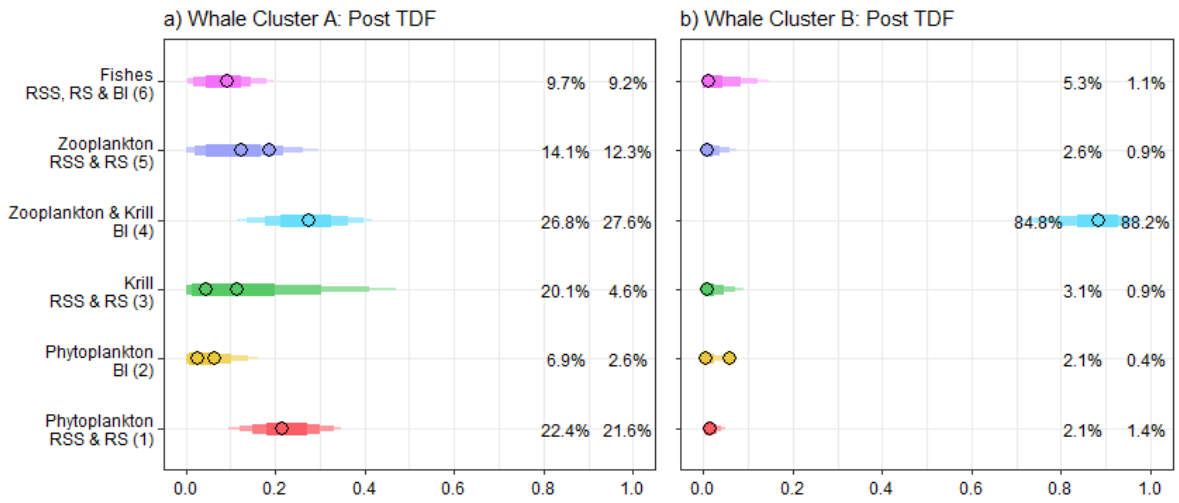

Fig. S8. Posterior distributions of the proportional contributions of each prey cluster (1-6, in parentheses) to the diet of humpback whales estimated using MixSIAR (Stock et al., 2018) applying the Post (2002) trophic discrimination factors (TDFs). Diets were estimated separately for cluster A whales (left hand panel a)) and cluster B whales (right hand panel b). Posteriors are plotted as the Highest Probability Density Intervals (HPDIs), which represent the shortest interval width containing the desired credibility range, and are more appropriate when posteriors are skewed or multimodal compared to equal-tailed credible intervals. HPDIs of 50, 75, 90 and 95% are plotted for each prey cluster with decreasing bar thickness and colour intensity (95% being the thinnest, longest bar). Posterior peaks (modes) are plotted separately as filled circles. The posterior means and highest posterior peaks are given as percentages at the right-hand side of each panel for each prey cluster, with the mean given first on the left. Abbreviations in the axis labels are as follows: RSS = Ross Sea slope; RS = Ross Sea; BI = Balleny Islands; Krill = Antarctic Krill (*Euphausia superba*); Fishes = Myctophids (5 *spp*.: *Electrona carlsbergi*, *E. antarctica, Gymnoscopelus nicholsi*, *G. opisthopterus and G. braueri*) plus Antarctic silverfish (*Pleuragramma antarctica*). Numbers in brackets for the y axis labels relate to Ward's hierarchical prey cluster numbers depicted in the dendrogram of Fig. S2.

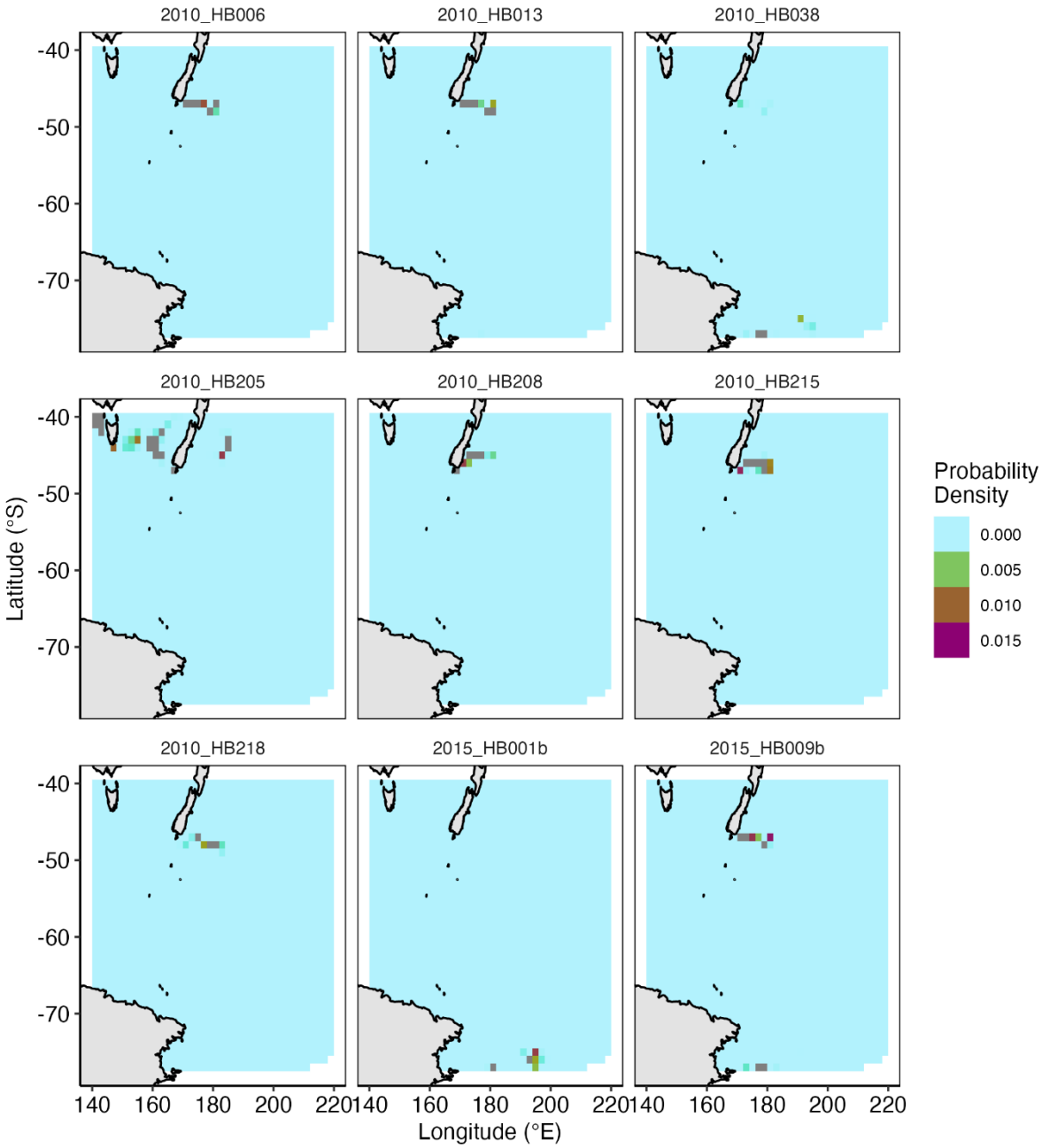

Fig. S9. Posterior probability densities for the spatial assignment (c.f. Wunder 2010) of individual cluster B humpback whale (*Megaptera novaeangliae*) foraging regions using spatial models (isoscapes) for $\delta^{13}C$ and $\delta^{15}N$ in suspended particulate organic matter of the Southern Ocean (St. John Glew & Espinasse et al. 2021).

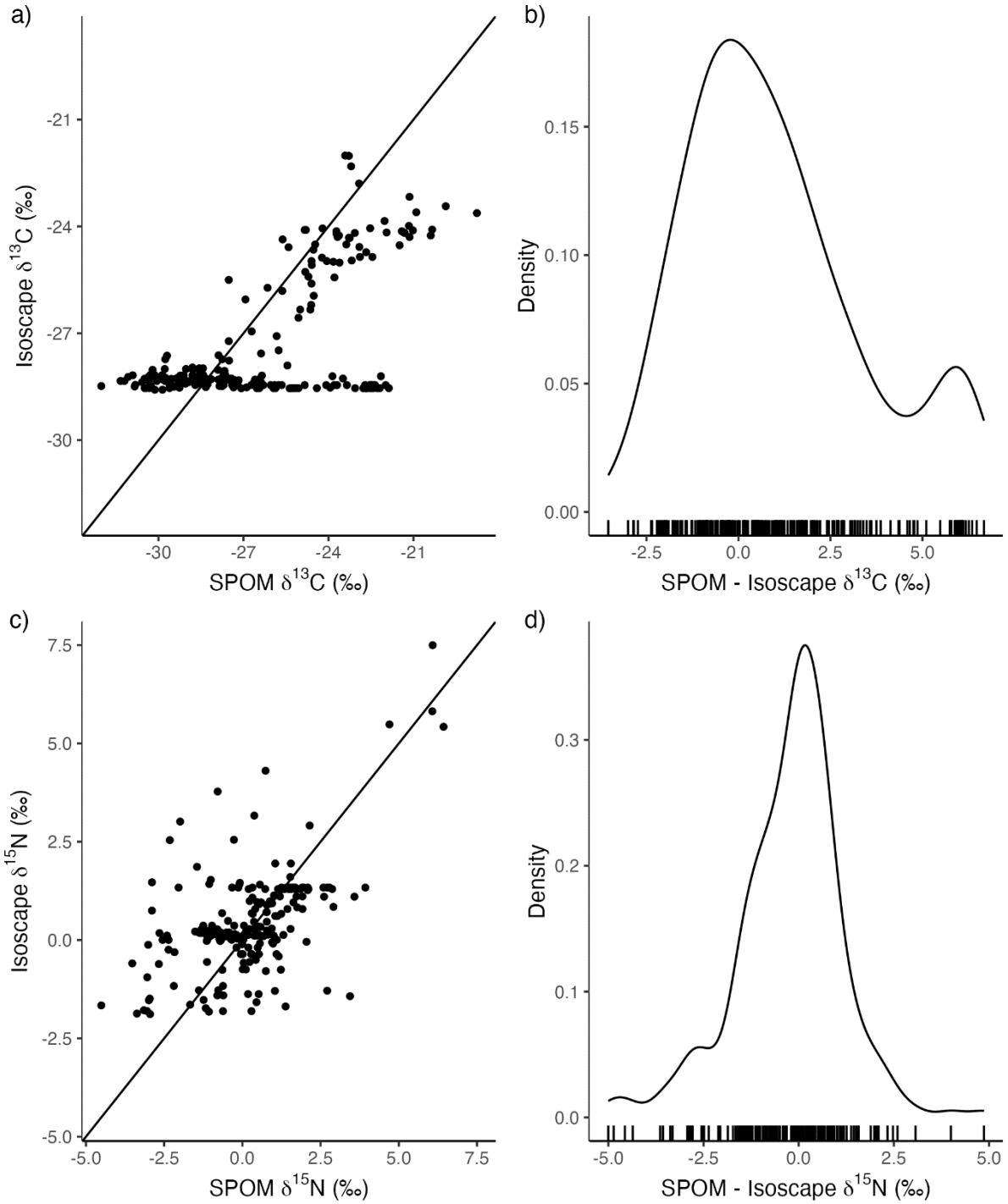

Fig. S10. Suspended particulate organic material (SPOM) $\delta^{13}C$ values (panel a) and $\delta^{15}N$ values (panel c) derived from the St John Glew & Espinasse et al. (2021) isoscapes model plotted against field-measured SPOM $\delta^{13}C$ and $\delta^{15}N$ values. The density versus $\delta^{13}C$ and $\delta^{15}N$ SPOM isoscape values are shown in panel b) and d) respectively.

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