**Combining multiple stable isotope methods elucidates diet, trophic position and foraging areas of Southern Ocean humpback whales (*Megaptera novaeangliae*)**

Sarah J. Bury1\*, Katharina J. Peters2,3,4, Amandine J.M. Sabadel1,5, Katie St John Glew6, Clive Trueman6, Wunder, M.B.7, Matthew R.D. Cobain8, Natalie Schmitt9,10, David Donnelly9,11, Sarah Magozzi12, Kylie Owen13, Julie C.S. Brown1,14, Pablo Escobar-Flores1, Rochelle Constantine15, Richard L. O’Driscoll1, Mike Double9, Nick Gales9,16, Simon Childerhouse17,18, Matthew H. Pinkerton1

Author Affiliations:

1 National Institute of Water & Atmospheric Research (NIWA), Greta Point, Hataitai, Wellington 6021, New Zealand

2School of Earth, Atmospheric and Life Sciences, University of Wollongong, NSW 2522, Australia

3 Cetacean Ecology Research Group, School of Natural Sciences, Massey University, 0745 Auckland, New Zealand.

4Evolutionary Genetics Group, Department of Anthropology, University of Zurich, 8057 Zurich, Switzerland.

5 Department of Zoology, University of Otago, PO Box 56, Dunedin, New Zealand.

6 Ocean and Earth Sciences, University of Southampton Waterfront Campus, Southampton SO14 3ZH, United Kingdom.

7 Department of Integrative Biology Campus Box 171 P.O. Box 173364. Denver, CO 80217-3364.

8 Department of Biological and Environmental Science, University of Jyväskylä, P.O. Box 35, FI-40014, Finland.

9 Australian Antarctic Division, Department of Agriculture, Water and the Environment, Kingston, Tasmania 7050, Australia.

10 Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, Ontario, Canada.

11 Killer Whales Australia, 17 Eric Crescent, Mornington, Victoria, Australia 3931.

12 Department of Integrated Marine Ecology, Stazione Zoologica Anton Dohrn, Fano Marine Centre, 61032 Fano (PU), Italy.

13 Department of Environmental Research and Monitoring, Swedish Museum of Natural History, PO Box 50007, Stockholm, Sweden, 104 05.

14 Bloomsbury Environmental Isotope Facility, Department of Earth Sciences, University College London, London, United Kingdom.

14 School of Biological Sciences, University of Auckland - Waipapa Tuamata Rau, Private Bag 92019, Auckland, New Zealand.

15 30 Power Road, North Bruny, Tasmania 7150, Australia.

16 Cawthron Institute, Nelson 7010, New Zealand.

17 Environmental Law Initiative, Epworth House, 75 Taranaki Street, Te Aro, Wellington 6011.

\*Corresponding author: [sarah.bury@niwa.co.nz](mailto:sarah.bury@niwa.co.nz)

ORCID ID: 0009-0004-0871-5833

Running title: Southern Ocean humpback whale trophic ecology

**ABSTRACT**

Southern Ocean humpback whales (*Megaptera novaeangliae*) are capital breeders, breeding in the warm tropics/subtropics in the winter and migrating to nutrient-rich Antarctic feeding grounds in the summer. The classic feeding model is for the species to fast when migrating and breeding, surviving on blubber energy stores. Whilst northern hemisphere humpback whales are generalists, southern hemisphere counterparts are perceived as krill specialists, but for many populations, uncertainties remain regarding their diet and preferred feeding locations. This study used bulk and compound-specific stable isotope analyses and isoscape-based feeding location assignments to assess the diet, trophic ecology and likely feeding areas of humpback whales sampled in the Ross Sea region and around the Balleny Islands. Sampled whales had a mixed diet of plankton, krill and fish, similar to the diet of northern hemisphere humpback whales. Proportions of fish consumed varied but were often high (2-60%), thus challenging the widely held paradigm of Southern Ocean humpback whales being exclusive krill feeders. These whales had lower ẟ15N values and trophic position estimates than their northern hemisphere counterparts likely due to lower Southern Ocean baseline ẟ15N surface water values and a lower percentage consumption of fish respectively. Most whales fed in the Ross Sea shelf/slope and Balleny Islands high productivity regions, but some isotopically distinct whales (mostly males) fed at higher trophic levels either around the Balleny Islands and frontal upwelling areas to the north, or en-route to Antarctica in temperate waters off southern Australia and New Zealand. These results support other observations of humpback whales feeding during migration, highlighting the species’ dietary plasticity, which may increase their foraging and breeding success and provide them with greater resilience to anthropogenically-mediated ecological change. This study highlights the importance of combining *in-situ* field data with regional-scale isoscapes to reliably assess trophic structure and animal feeding locations, and to better inform ecosystem conservation and management of Marine Protected Areas.

**Keywords**: feeding ecology; δ15N; δ13C; amino acids; MixSIAR; isoscapes; Antarctica, UNSDG14 Life Below Water

1. **INTRODUCTION**

Humpback whales (*Megaptera novaeangliae*) are balaenopterid cetaceans, which are distributed throughout the world’s oceans and are the most extensively studied of all large cetaceans (Fleming & Jackson 2011). Southern hemisphere humpback whales complete annual migrations of over 8000 km between cold, nutrient-rich Southern Ocean feeding grounds in summer, and warmer calving grounds (also often referred to as breeding grounds) in low latitude waters in winter (Clapham 1966, Corkeron & Connor 1999, Clapham 2018). Apart from the gray whale (*Eschrichtius robustus*), they have one of the longest recorded mammalian migrations (Clapham 2001, Rasmussen et al. 2007, Stevick et al. 2011). They are capital breeders, traditionally thought to fast during migration and breeding (Lockyer & Brown 1981), surviving on energy or “capital” from their blubber stores built up from feeding on high density prey patches during the feeding season (Hain et al. 1981, Hazen et al. 2009, Cade et al. 2020). However, uncertainties remain surrounding humpback whale movement patterns and mixing of different populations, their diet, and their degree of feeding whilst migrating (Gales et al. 2009, Barendse et al. 2010, Owen et al. companion paper in review). Given the large body size (12-17 m), high energy requirements and the importance of humpback whales as consumers in Southern Ocean foodwebs (Witteveen et al. 2006) there is a need to better understand their diet and foraging ecology to effectively manage and conserve this species and their associated ecosystems. This is particularly relevant considering the commercial harvesting of Antarctic krill (*Euphausia superba*) in the Scotia Sea region and ecosystem shifts resulting from global climate (Kawaguchi et al. 2013, Stock et al. 2014, Schine et al. 2016) and oceanic change (Nicol et al. 2008).

Humpback whales feed by lunge feeding, advancing on prey with their mouths open engulfing large quantities of water, then closing their mouths forcing water out through their baleen plates to trap filtered prey (Dolphin 1988, Baraff et al. 1991, Owen et al. 2017). In the northern hemisphere, they are classified as generalists, feeding on a mixed diet of fish and krill (Christensen et al. 1990, 1992, Ryan et al. 2014, Wittteveen & Wynne 2016), however in the southern hemisphere, numerous studies have recorded humpback whales as feeding predominantly on krill (Matthews 1937, Chittleborough 1965, Kawamura 1994, Bannister & Hedley 2001, Paterson et al. 2001, Friedlaender et al. 2006, 2008, Waugh et al. 2012, Groß et al. 2020). Southern Ocean humpback whales were thought to confine their feeding to Antarctic waters, fasting whilst on their calving grounds and when migrating (Dawbin 1966, Lockyer 1981, Baraff et al. 1991). However, there is now increasing evidence of humpback whales feeding on high density krill patches and fish during migration, both in the southern hemisphere (e.g., Gill et al. 1998, Stamation et al. 2007, Gales et al. 2009, Barendse et al. 2013, Eisenmann et al. 2017, Andrews-Goff et al. 2018, Owen et al. companion paper in review) and northern hemisphere (Baraff et al. 1991, Swingle et al. 1993, Laerm et al. 1997, Visser et al. 2011). Indeed, four of the seven humpback whale breeding populations have been observed to feed along migration routes (International Whaling Commission, 2011) with feeding events lasting from days to weeks in highly productive temperate areas (Gales et al. 2009, Owen et al. 2015). Seasonal aerial surveys recorded regular aggregations of more than 20 humpback whales feeding on baitfish between 37-42°S off the coast of Australia during migration (D Donnelly, unpublished data). Due to the mobile nature of this species and the remote location of their foraging grounds, long-term tracking of individuals to establish feeding sites is logistically challenging and expensive. Standard techniques for tracking whale movements have included observations from whaling records and ‘Discovery’ tag data (Rayner 1939, Chittleborough 1959, Dawbin 1964), photo-identification (Garrigue et al. 2004, Constantine et al. 2014, Franklin et al. 2014), satellite tagging (Dalla Rosa et al. 2008, Riekkola et al. 2018) and genetic analysis (Constantine et al. 2014, Schmitt et al. 2014a,b, Steel et al. 2018). More recently, stable isotope analysis has emerged as a powerful tool to investigate the trophic ecology and foraging ranges of humpback whales by analysing the stable isotope values of their tissues and prey within their foraging environments (Eisenmann et al. 2016, Witteveen & Wynne 2016, Mackenzie et al. 2022).

The isotopic composition of phytoplankton at the base of the food chain (the isotopic baseline) is transferred to higher trophic levels with relatively predictable relationships (De Niro & Epstein 1978, 1981, Minagawa & Wada 1984, Vander Zanden & Rasmussen 1999). The bulk nitrogen isotope value (the ratio of 15N to 14N, expressed as ẟ15Nin ‰ units) in a consumer, such as a humpback whale, can be increased by 3-4 ‰ relative to its diet. This difference in isotopic composition, referred to variously as fractionation factor, tissue-diet (isotopic) spacing or trophic discrimination factor (TDF), makes nitrogen isotopes a valuable tool for trophic studies (Peterson & Fry 1987, Post 2002, Vander Zanden & Rasmussen 2001). However, TDFs can be variable within individuals, among species, and within different environments (Vander Zanden & Rasmussen 2001, McCutchan et al. 2003; see also Methods 2.5.1) so knowledge of the species and ecosystem is important. Baseline δ15N values can also vary greatly across space (Somes et al. 2010, McMahon et al. 2013, MacKenzie et al. 2014) and time (Schmittner & Somes, 2016, Espinasse et al. 2019, St John Glew & Espinasse et al. 2021), so the importance of mapping phytoplankton δ15N values to estimate the trophic position of consumers in food webs is widely recognised (Hobson & Welch 1992, Cabana & Rasmussen 1996, Jennings & Warr 2003). Complementary to baseline and consumer tissue bulk δ15N analysis, an additional assessment of trophic position (TP) can be made via compound-specific stable isotope analysis (CSIA) of nitrogen in amino acids (N-AA), using δ15N values of certain amino acids (δ15NAA) (McClelland & Montoya 2002, Chikaraishi et al. 2009, 2014). This approach is based on the fractionation between the so-called ‘source’ and ‘trophic’ amino acids in metabolic processes (Popp et al. 2007, Hannides et al. 2009). Source amino acids cannot be synthesised and must be acquired through diet, therefore their δ15N values reflect the isotopic baseline, whereas trophic amino acids can be acquired or synthesised, and their δ15N values reflect the isotopic baseline plus trophic and physiological effects. As 14N is preferentially excreted, exchange of nitrogen with the available nitrogen pool results in an enrichment in 15N in an organism’s tissue as biomass is transferred from one trophic level to another, thereby increasing ẟ15NAA values of trophic amino acids in secondary consumers (Hannides et al. 2009, Chikaraishi et al. 2014). Thus, a general equation based on δ15NGlx (glutamic acid, trophic) and δ15NPhe (phenylalanine, source) can be used to assess the TP (TPGlx/Phe) of any organism across different environments (Chikaraishi et al. 2009, 2014; see also Materials and Methods).

Carbon is less affected by trophic fractionation than nitrogen, with an approximate 0.4 – 0.8 ‰ increase per trophic level (Vander Zanden & Rasmussen 2001, Post 2002). This means that bulk carbon isotope values (the ratio of 13C to 12C, expressed as δ13Cin ‰ units) are more suitable to trace the source of carbon to an organism or system, where sources are isotopically distinct (Fry & Sherr 1984, Rounick & Winterbourne 1986, France & Peters 1997). Nevertheless, carbon isotopes also show variable trophic enrichment and highly dynamic baseline values. Pelagic suspended particulate organic matter (SPOM), a proxy for marine phytoplankton (which strictly speaking comprises phytoplankton *and* detritus) in open ocean waters, shows a positive relationship between water temperature and δ13C values (Sackett et al. 1965, Rau et al. 1989, Goericke & Fry 1994). The relationship is particularly strong in the Southern Ocean between 40°S and 80°S where persistent carbon isotopic gradients have been measured (Cherel & Hobson 2007, Quillfeldt et al. 2010, Espinasse et al. 2019). The predictable relationship between carbon isotope compositions of SPOM and spatially-determined environmental variables has enabled the development of both data- and process-based models of natural spatio-temporal variations in isotopic baselines, which are termed *isoscapes*. These models enable trophic levels and diet (St John Glew et al. 2018), feeding grounds (Cherel & Hobson 2007, Cherel et al. 2007, Jaeger et al. 2010) and movements or migrations of marine organisms (Graham et al. 2010, Hobson et al. 2010, Trueman et al. 2019) to be inferred from consumer tissue isotope data. Isoscape applications are now widespread and summarised in several review papers (Hobson 1999, Ramos & González-Solís 2012, Trueman et al. 2012, McMahon et al. 2013, Trueman & St John Glew 2019) and the recent development of large-scale oceanic modelled isoscapes for carbon (Magozzi et al. 2017, St John Glew & Espinasse et al. 2021) and nitrogen (Somes et al. 2010, Schmittner & Somes 2016, St John Glew & Espinasse et al. 2021) has increased confidence in large basin-scale animal movement inferences from tissue isotope values.

This study focussed on humpback whales feeding during the summer months in the vicinity of the Balleny Islands and the Ross Sea slope, East Antarctica, a known high-density feeding ground (Franklin et al. 2012, Harrison et al. 2020). Animals feeding in this region most likely belong to groups of humpback whales that migrate in the autumn to calving grounds in north-eastern Australian and New Caledonian waters (Constantine et al. 2014, Schmitt et al. 2014a, Riekkola et al. 2018), classified by the International Whaling Commission as the E1 breeding population (International Whaling Commission 2011). The hypothesis that “Southern Ocean humpback whales have a similar diet to northern hemisphere humpback whales, eating a mixed diet of fish and krill” was tested. Combining multiple stable isotope methods and data from three voyages to the Balleny Islands and Ross Sea, the TP, diet and likely feeding locations of humpback whales were determined. After examining the whale isotopic niche data, the hypothesis that “some whales may feed at higher trophic levels than others” was also explored through modelling the relative proportions of prey taken by whales occupying different isotopic niches and through CSIA analysis. A literature review of carbon and nitrogen stable isotope and trophic position values was completed to compare Southern Ocean humpback whales of this study with northern hemisphere populations.

1. **MATERIALS AND METHODS**

**2.1 Overview**

Surface water SPOM (used as a proxy for phytoplankton in this study) was sampled along transect lines from New Zealand to the Ross Sea to provide baseline data for estimates of whale TP and to generate field data to validate carbon and nitrogen isoscapes. A combination of bulk carbon and nitrogen stable isotope analyses of whale skin biopsies and muscle from potential whale prey, and CSIA of N-AA of the whale skin were used to determine the diet and TP of humpback whales sampled around the Balleny Islands and the Ross Sea slope. The TP of the whales was validated using a Bayesian estimate of trophic position (Quezada-Romegialli et al. 2018). Bayesian modelling was used to determine niche width and isotopic niche overlap to assess if whale clusters were isotopically distinct (Jackson et al. 2011). In addition, MixSIAR modelling (Stock et al. 2018) was applied to determine the proportions of prey that humpback whales were feeding on. Finally, data-derived and modelled carbon and nitrogen isoscapes (St Glew & Espinasse et al. 2021) were used to ascertain where the humpback whales were most likely to have been feeding over the integrated time period of their skin biopsy record.

**2.2 Study area and sampling**

**2.2.1 Study area**

Biological samples were collected during three oceanographic voyages from New Zealand to the Ross Sea on the Research Vessel (*RV*) *Tangaroa*: 1)International Polar Year – Census of Antarctic Marine Life voyage, January-March 2008 (Pinkerton et al. 2011); 2)Antarctic Whale Expedition voyage, February-March 2010 (Gales 2010, Hull 2010) and; 3) New Zealand-Australia Antarctic Ecosystems voyage, February-March 2015 (O’Driscoll & Double 2015). Henceforth the sampling trips are referred to respectively as: 1) V2008, 2) V2010 and 3) V2015, where “V” represents “voyage”. The vessel tracks for each voyage and the key sampling locations of SPOM and marine fauna are shown in Figs*.* 1and2. Whale skin and SPOM samples were collected during V2010 and V2015, whilst prey samples were collected on all three voyages. Details of the sample types and numbers taken on each voyage are provided in Table S1.

**2.2.2 *Suspended particulate organic material (SPOM) sampling and processing***

SPOM samples were obtained by underway sampling of near-surface water at six-hourly intervals from 5.5 m beneath the RV Tangaroa via the Underway Flow Through System on V2010 and V2015 (Fig. 1). For sample processing details see Supplementary Material, Extended Methods, Text S1.

**2.2.3 Humpback whale biopsy sampling and tissue processing**

A total of 65 humpback whale biopsies were sampled for stable isotope analysis, genetics and sex determination (Table S1, Text S2) with 55 humpback whale biopsies obtained between12 February-8 March 2010, and 10 biopsies sampled between 7 February-2 March 2015 (Fig. 1). The arrival time of humpback whales on their Antarctic feeding grounds is between October-December (Chittleborough 1965, Dawbin 1966, Andrews-Goff et al. 2018). Given an estimated skin turnover rate of three to four months (Text S3), for skin biopsies taken in February/March the stable isotopic composition of a whale’s prey should be almost fully integrated into the whale skin after three to five months of feeding. The measured isotopic values of the whale skin were therefore expected to primarily reflect the Antarctic feeding ground signal.

In 2010, 31 biopsies were sampled in the vicinity of the Balleny Islands (hereafter BI: 162.0°E-166.0°E, 66.0°S-67.5°S), 22 south-east of the Balleny Islands (hereafter SEBI: 166.0°E-170.0°E, 67.5°S-70.0°S) and two along the Ross Sea slope (hereafter RSS: 175.0°E-165.0°W, 69.0°S-70.5°S). In 2015, seven humpback whales were sampled around BI and three along the RSS. For detailed sampling methods and processing information see Text S2.

**2.2.4 Prey sampling and analytical preparation**

Sampling locations of fish, Antarctic krill and mixed community zooplankton (which were not identified to species) are shown in Fig. 2, with sample numbers provided in Table S1 and methods and tissue processing details in Text S4. The fish sampled included five myctophid (lantern fish) species (Electrona carlsbergi, E. antarctica, Gymnoscopelus nicholsi, G. opisthopterus and G. braueri), and Antarctic silverfish (Pleuragramma antarctica). These species were the only prey species sampled that were deemed to be relevant as potential humpback whale prey and together are amongst the commonest encountered in the Southern Ocean (Koubbi et al. 2011, Woods et al. 2023).

**2.2.5 Lipid extraction of biological samples**

Lipid synthesis strongly discriminates against the 13C isotope (De Niro & Epstein 1977, 1978), leading to more negative δ13C values in lipid-rich tissues, relative to proteins and carbohydrates (Rounick & Winterbourn 1986). To reduce bias in stable isotope results due to “lipid contamination” (Hebert & Keenleyside 1995, Post et al. 2007, Mintenbeck et al. 2008) we followed the recommended method of analysing bulk (whole) samples for nitrogen content (%N) and δ15N values, and lipid-extracting samples to obtain accurate carbon content (%C) and δ13C values (Ricca et al. 2007, Logan et al. 2008) for all whale biopsy (V2010, V2015), krill (V2008), and myctophid (V2010, V2015) samples (methodological details in Text S5). Krill (V2015) and silverfish (V2008, V2015) were analysed as whole bulk samples, with a sub-set analysed lipid-extracted to derive a species-specific δ13C correction formula. Where bulk C:N mass ratios exceeded 3.5 we corrected the δ13C values using these derived formulae. For mixed community zooplankton samples, where sample material was limited, δ13C data were corrected for lipid content using C:N molar ratios following equations in Fry (2002).

**2.3 Stable isotope analysis**

**2.3.1 Bulk stable isotope analysis**

All bulk stable isotope analyses were carried out at the NIWA Environmental and Ecological Stable Isotope Analytical Facility in Wellington, New Zealand, using two intercalibrated elemental analyser (EA) continuous flow isotope ratio mass spectrometer (CF-IRMS) analytical systems. Most analyses were carried out using a MAS200 autosampler connected to a Flash 2000 EA coupled with a DELTA V Plus (Thermo Fisher Scientific, Bremen, Germany) CF-IRMS. A small number of samples were analysed using an AS200\_LS autosampler on an NA-1500 EA (Fisons Instruments, Rodano, Italy) linked to a DELTAPlus CF-IRMS. For details of analysis, standards used, isotopic calculations and normalisation, accuracy and precision refer to Text S6. All estimates of variance reported are given as ± 1 standard deviation (SD), reporting using the format “mean.

**2.3.2** ***Amino acid hydrolysis and derivatisation for compound-specific isotope analysis of whale biopsy samples***

Fourteen humpback whale skin samples were selected for CSIA of δ15NAA from cluster A and B (see 2.4.2), from samples where sufficient skin biopsy material was available to encompass the maximum range of bulk isotope values and both sexes (eight males and six females). One sample was analysed in duplicate providing replication of analysis (Table S2: sample 2010\_216a and b). Amino acids are non-volatile molecules that require hydrolysis and derivatisation prior to analysis. Whale biopsy samples were hydrolysed into individual amino acids with 6N hydrochloric acid, then derivatised using acetyl chloride-isopropanol followed by trifluoroacetic anhydride to produce trifluoroacetic amino acid esters (Macko et al. 1997). The hydrolysis and derivatisation method of Hannides et al. (2009) was closely followed with only minor deviations, which are reported in Text S7.

**2.3.3 *Compound-specific stable isotope analysis of nitrogen in amino acids***

Derivatised samples were transferred into ethyl acetate and diluted to the appropriate concentration for analysis on the gas chromatograph (GC) IRMS. Eleven amino acids were detected and reported: seven “trophic” amino acids (alanine, valine, leucine, isoleucine, proline, aspartic acid and glutamic acid), a “source” amino acid (phenylalanine), a “metabolic” amino acid (threonine) and two “intermediate” amino acids (glycine and serine). Glycine and serine cannot easily be classified as either “source” or “trophic” amino acids (Cherel et al. 2019) and are thus considered “intermediate” (Shen et al. 2021).

The CSIA of N-AA was carried out on a TRACE Ultra GC with GC IsoLink interface coupled via a ConFlo IV to a DELTA V Plus IRMS (Thermo Fisher Scientific, Bremen, Germany), with GC PAL autosampler (CTC Analytics, Switzerland). For details of analysis, standards used, raw data corrections, accuracy and precision refer to Text S8 and Fig. S1.

**2.4 Statistical analyses**

**2.4.1 *Comparing stable isotope values between locations, age groups and sexes***

Differences in δ15N and δ13C values between locations (BI, SEBI, RSS), age groups (adult, subadult, dependent young), and sexes were investigated using generalised linear models (GLMs) with a Gaussian distribution and an identity link function. Twelve models were built for δ15N and δ13C respectively, including all possible combinations of variables. Models were then ranked according to their Akaike’s information criterion corrected for small sample sizes (AICC) (Burnham et al. 2011) to select the model that best explained the data. Final models were checked for interactions between variables and homogeneity of variance, and residual distributions were checked for normality. All statistical analyses were completed in R (R Core Team 2020).

**2.4.2 *Niche comparison***

K-means cluster analysis (MacQueen 1967, Lloyd 1982) was used to define two clusters (A and B) of humpback whales based on their respective δ15N and δ13C values. K-means clustering is an established unsupervised machine learning algorithm for segregating a data set into *k* groups or clusters, where *k* represents the number of clusters pre-specified by the user (here, *k* = 2, chosen *a priori* after visual inspection of the data), and objects within the same cluster are as similar as possible.

Six different Layman metrics (δ15N range, δ13C range, total area (TA), mean distance to centroid (CD), mean nearest neighbour distance (MNND), and standard deviation of nearest neighbour distance (SDNND)) (Layman et al. 2007) were used to compare isotopic niches between clusters (Table S3). All Layman metrics were bootstrapped with replacement (*n* = 10,000, indicated with a subscript ‘boot’) based on the smallest sample size in the data set (*n* = 9) to enable statistical comparison between clusters (Manly 1997, Jackson et al. 2012). To further assess niche widths and isotopic niche overlap between clusters, standard ellipse areas (SEAs), the bivariate equivalent to standard deviation in univariate analyses, were calculated with correction for small sample size (SEAc, Jackson et al. 2011). In addition, Bayesian SEAs (SEAB) were calculated using 1000 posterior draws to statistically compare niche width and to estimate the niche overlap between clusters, calculated as the proportion of the total SEAB for each sex respectively. All metrics were calculated using the R package Stable Isotope Bayesian Ellipses in R (SIBER) (Jackson et al. 2011, R Core Team 2020).

**2.5 Prey apportionment modelling**

**2.5.1 *Selection of diet-tissue trophic discrimination factors***

Quantifying the diet of an organism using prey apportionment modelling requires knowledge of the isotopic enrichment (i.e., TDF) in the predator relative to prey (DeNiro & Epstein 1978, 1981). TDFs can be highly variable depending on species, physiology, ontogeny, habitat, and food type (see summary by Broecklen et al. 2011, Table 1 therein) and in large marine mammals they are difficult to measure. However, from a study of wild fin whales (*Balaenoptera physalus*) feeding exclusively on euphausiid krill (*Meganyctiphanes norvegica*) Borrell et al. (2012) calculated whale skin TDFs of 1.28, 0.38 ‰ for carbon and 2.82, 0.30 ‰ for nitrogen. The authors suggest that TDFs are relatively constant between taxonomically-close species and that fin whale values can be extrapolated to other cetaceans. We therefore used the Borrell et al. (2012) TDF values (hereafter referred to as “Borrell TDF”), but for comparison we also present data in the Supplementary Materials using the traditionally accepted Post (2002) TDF values of 0.39, 1.3 ‰ for carbon and 3.4, 0.98 ‰ for nitrogen (hereafter referred to as “Post TDF”).

**2.5.2 *Mixing Model***

The contribution of different prey sources to the humpback whale diet was estimated using two isotopic tracers (δ13C and δ15N), applying the Stock et al. (2018) isotopic mixing model, which incorporates uncertainties in isotope values of both sources and consumers, TDFs and tissue turnover rates. The model included δ13C and δ15N values from five potential prey (phytoplankton, mixed community zooplankton, Antarctic krill, myctophids, and silverfish), from three sampling locations (BI, RSS and Ross Sea (RS)). Phytoplankton were included as possible prey, as potentially significant proportions of phytoplankton can be entrained and consumed during the feeding filtration process, particularly in densely aggregated patches of food. These various combinations of prey isotope values and locations were grouped using Ward’s hierarchical cluster analysis based on the mean δ13C and δ15N values of the prey (Fig. S2) to minimise the number of sources within the mixing model (Phillips & Greg 2003, Moore & Semmens 2008, Parnell et al. 2010). Data were checked for normal distribution using Shapiro-Wilk T-test and visual inspection (Table S4). To test the hypothesis that some of the male humpback whales may have been feeding at higher trophic levels than the rest of the sampled males and most females (see Results 3.2 Niche comparison) data were modelled to work out the relative proportions of prey taken by whales in the two clusters A and B (see Methods 2.4.2 Niche comparison).

To test whether data met the point-in-polygon requirement for every consumer (i.e. “all consumer isotopic values must lie within a polygon bounded by the isotopic signatures of the sources” (Phillips & Gregg 2003)), a simulated mixing polygon was computed (Smith et al. 2013). No tissue correction factor was applied to whale skin isotope data when plotted with whale prey muscle isotope data, as previous analysis of necropsied Hector’s dolphin (*Cephalorhynchus hectori*), Māui dolphin (*C. h. maui*) and killer whales (*Orcinus orca*) cetacean skin and muscle samples showed minimal fractionation for carbon or nitrogen between these two tissue types (S Bury, unpublished data). Similar findings were reported for fin whales by Borrell et al. (2012) and for humpback whales by Todd et al. (1997), who reported differences of less than 0.4 ‰ between whale skin and muscle.

Two iterations of MixSIAR were run using firstly, the Post TDF (reported only in Supplementary Materials) and secondly, the Borrell TDF (reported in the Results). To test which factors were involved in predicting dietary proportions, for each TDF the null model including all whales with no clustering, was compared with the model including the variable ‘whale cluster’ as a fixed categorical effect with two levels (cluster A and cluster B) (Table S5). Models had a multiplicative error term (Stock & Semmens 2016) and specifications were three Markov chain Monte Carlo (MCMC) chains, 200,000 iterations as burn-in, and 100,000 iterations thinned by a factor of 100, providing a total of 3000 draws for estimating posterior distributions and credible intervals. Model diagnostics were checked to ensure convergence and models were evaluated by comparing their Akaike information criterion weights (*w*AIC) (Burnham & Anderson 2002) and approximate leave-one-out cross-validation information criterion (LOOic) (Vehtari et al. 2017).

**2.6 Estimation of humpback whale trophic position**

Due to the difficulty of directly measuring a TDF for humpback whales, three different methods of TP calculations were applied to corroborate the results:

1) simple mathematical TP estimates using SPOM, whale prey and whale δ15N data from this study, combined with best estimates of TDFs between the consumer and the prey, taken from the literature (details provided in Results);

2) a Bayesian estimation of TP from consumer stable isotope ratios using the R package tRophicPosition (Quezada-Romegialli et al. 2018), which enables within-population variability to be accounted for and considers uncertainties and error propagation of the calculations. For the Bayesian model, krill were used as the nitrogen isotopic baseline. Carbon data were not incorporated in the model, as δ13C values in this study were primarily driven by latitudinal feeding location, which would confound the TP estimates.

3) TP estimates from CSIA data. Using the CSIA data, Equation 1 (based on δ15N values for the “trophic” amino acid glutamic acid (δ15NGlx) and the “source” amino acid phenylalanine (δ15NPhe)), was used to assess the TP (TPGlx/Phe) of the humpback whales (Chikaraishi et al. 2009, 2014):

TPGlx/Phe = [δ15NGlx – δ15NPhe - *β* / TDF] + 1 (Equation 1)

where *β* represents the isotopic difference between δ15NGlx and δ15NPhe in primary producers (taken to be +3.4, 0.9 ‰ for marine algae). In a recent review, Ramirez et al. (2021) showed that *β* values are taxon- and tissue-specific, but they also note that variability in *β* values dissipates at higher trophic levels. We therefore used the widely accepted marine algal value of +3.4 ‰. The validity of the TPGlx/Phe estimate depends on the consistency of both *β* and TDF values (Chikaraishi et al. 2014, Ramirez et al. 2021). In this study, the humpback whale data-derived TDF value used for the TPGlx/Phe calculations was based on a whale TP of 3.32 (taken from (1) simple mathematical TP estimate above – see also Results, 3.6.1), where

TDFwhale = (Glx-Phe - 3.4)/(TPwhale-1), where TPwhale= 3.58 (Equation 2)

**2.7 Isoscapes**

Whale skin δ13C values for individuals from the whale clusters A and B (as defined by the K-means cluster analysis) were used to identify the spatially explicit posterior probability for the origin of food resources incorporated into skin tissue of each individual whale. Assignment methods followed Wunder (2010) and were implemented with the R package ASSIGNR (Ma et al. 2020) using carbon and nitrogen isoscape models for SPOM in the Southern Ocean (St. John Glew et al. 2021). Since whale tissue of known origin was not available, δ13C and δ15N whale tissue values were adjusted by assuming a constant offset between whale skin and the isoscape model for SPOM of 1.67 ‰ for carbon (0.39 ‰ phytoplankton-krill Post TDF + 1.28 ‰ krill-whale Borrell TDF) and 6.22 ‰ for nitrogen (3.40 ‰ phytoplankton-krill Post TDF + 2.82 ‰ krill-whale Borrell TDF).

Spatially-explicit posterior probability densities were estimated for each whale individually.

Assignments were first carried out using only δ13C values and were then repeated using both δ13C and δ15N data. For the single carbon-only isoscape assignments, the estimated variance model from St. John Glew et al. (2021) was used. For the dual-isoscape assignments, single isotope variances estimated by St. John Glew et al. (2021) were used for the diagonal of the variance-covariance matrix, and the off-diagonals were estimated from the expected values for δ15N and δ13C from the isoscape models for each raster cell (Ma et al. 2020). In both assignment model cases, the bounding box for the posterior densities ranged from 140°E to 220°E longitude and from 77.5°S to 39.5°S latitude. Probability densities were averaged across all whales in each of the two previously identified feeding clusters, A and B. The resultant probability densities are spatially-explicit representations for the average feeding origin of the whales in each cluster.

**2.8 Factors to consider when interpreting isotope data**

Several factors should be considered when interpreting stable isotope data in the context of TP status, diet apportionment, and feeding location assignments (Gannes et al. 1997, Jardine et al. 2006, Inger & Bearhop 2008). Useful summaries are provided in Bearhop et al. (2004) and review papers of Martínez del Rio et al. (2009), Newsome et al. (2010), Boecklen et al. (2011) and Thomas & Crowther (2015). Briefly, factors include isotopic incorporation rates into an animal’s tissue and the metabolic activity of that tissue affecting its turnover rates (Text S3), tissue type correction factors (Cherel et al. 2005b), diet-tissue TDFs (Hobson & Clark 1992, Vander Zanden & Ramussen 2001, Caut et al. 2009, Text S3), nutritional stress (Fuller et al. 2005), the sex, age, reproductive and physiological status of the organism (Fuller et al. 2004, Cherel et al. 2005a), estimates of isotopic baselines (including the calculation of β values in the CSIA TP calculation, Ramirez et al. 2021), and isoscape baseline-organism spacing values (see section 2.7). Furthermore, isoscape-based animal assignments generally rely on isoscapes constructed using surface SPOM stable isotope values, which only provide an approximation of isotopic baselines, since SPOM stable isotope values vary with depth (Lourey et al. 2003) and both humpback whales and their prey undergo vertical diel movements. Varying degrees of uncertainty exist for all these issues, with some information still unknown, generating caveats that need to be acknowledged.

1. **RESULTS**

**3.1 Humpback whale skin isotopic variability between sampling locations, whale age groups and sexes**

Individual humpback whale skin δ13C values ranged from -26.77 to -20.90 ‰ and δ15Nvalues ranged from 6.49 to 9.48 ‰ across all sampling locations and years with an overall arithmetic mean (henceforth just referred to as “mean”) of -25.23, 1.03 ‰ and 7.57, 0.66 ‰, respectively (Figs. 3a and 3b, Table S1). A similar spread of δ13C and δ15Nvalues occurred across all sampled regions (BI, RSS, and RS) resulting in mean values overlapping between sampling regions and years. There was little isotopic variation in the mean and SD values of δ13C and δ15Nvalues between adult whales sampled in 2015 (*n* = 10), and adults (*n* = 44), subadults (*n* = 6) and dependent young (*n* = 5) sampled in 2010 (Fig. S3b, Table S6).

Genetic analysis identified 29 males and 26 females sampled in 2010, with one male and nine females sampled in 2015, giving a total of 30 male and 35 females sampled overall. The temporal sampling of males and females was evenly spread throughout the 2010 voyage. There was considerable overlap between male and female δ13C and δ15Nvalues (Fig. S4), but across the dataset, mean male values were slightly more enriched in 13C and 15N (ẟ13C -24.86, 1.27 ‰; ẟ15N 7.88, 0.65 ‰) than female values (ẟ13C -25.56, 0.62 ‰; ẟ15N 7.31, 0.55 ‰ (Table S7).

Generalised linear models showed that sex, age and sampling year were the main predictors of both δ13C and δ15N values (Table S8). The top models retained sex and age for higher δ13Cvalues, and sex and year for higher δ15N values. However, the second and third-best models for δ13C (retaining sex and year; and sex, age, and year) and the second-best models for δ15N (retaining sex and year; and sex and age) explained the data almost equally well as the respective top-ranked models (Table S8). Values of δ15N increased slightly with year, and dependent young had lower, and subadult had higher δ13C values. However, although age and year were retained in the final models for δ13C and δ15N values respectively, the effects were not significant (Table S9). While sex and age had comparable variable importance contributing to the overall model fit for δ13C values, the final model for δ15N values was mainly driven by sex (Fig. S5). The deviance explained was low for top-ranked models for both δ13C and δ15N values (18.9% and 17.9% respectively), indicating that part of the data variation is not explained by the predictor variables.

**3.2 Niche comparison**

Two clusters (A and B) of individual humpback whales were identified, based on their respective δ13C and δ15N values (Fig. 4). Cluster A whales had a mean δ13C value of -25.57, 0.50 ‰ and a δ15N value of 7.38, 0.43 ‰, whilst cluster B whales had a mean δ13C value of -23.66, 0.43 ‰ and a δ15N value of 8.81, 0.48 ‰ (Table S1). Cluster B whales had 1.90 ‰ higher mean δ13C values and 1.43 ‰ higher mean δ15N values than cluster A whales. Isotopic niche metrics varied between the two clusters (Table S3), with cluster B having a higher probability for larger bootstrapped values than Cluster A for all metrics, except MNND (97.2% cluster A > cluster B), and SDNND (79.2% cluster A > cluster B). Niche differentiation between the two clusters was further demonstrated by the negligible SEAB overlap for both clusters (SEAB overlap: cluster A = 0.4%, cluster B = 0.1%). Both A and B clusters included whales sampled from all locations (BI, SEBI and RSS) (Fig. S6). However, cluster B comprised predominantly male whales: seven males and two females, with the two females plotting in the lower range of δ13C and δ15N values (Fig. 4). This led to our hypothesis that some of the male humpback whales (those in cluster B) may have been feeding at higher trophic levels than the rest of the sampled males (those in cluster A), and most females (cluster A).

**3.3 Carbon and nitrogen stable isotope values of humpback whale prey**

Phytoplankton from all areas showed high variability in both δ13C and δ15N values and this wide isotopic variability was reflected up the food chain and observed in mixed community zooplankton, Antarctic krill, myctophids and Antarctic silverfish (Figs. 5a and 5b). BI phytoplankton had higher mean isotope values (δ13C -24.05, 1.66 ‰; δ15N 1.49, 0.95 ‰) than RSS (δ13C -28.41, 1.01 ‰; δ15N 0.39, 1.97 ‰) and RS phytoplankton (δ13C -28.56, 0.98 ‰; δ15N 0.06, 0.96 ‰), which were similar (Table S1). Antarctic krill from BI had higher isotope values (δ13C-24.39, 1.07 ‰; δ15N 4.96, 0.60 ‰) compared to Antarctic krill from RSS (δ13C -26.23, 0.65 ‰; δ15N 4.11, 0.62 ‰) and RS (δ13C 26.09, 0.59 ‰; δ15N 4.10, 0.62 ‰). The same pattern was also observed for BI mixed community zooplankton (δ13C -23.39, 1.25 ‰), which had higher δ13C values than mixed community zooplankton from the RSS (δ13C-27.45, 1.01 ‰) and RS (δ13C -27.19, 1.53 ‰). Notably, RSS mixed community zooplankton were enriched in 15N (δ15N 7.03, 2.04 ‰) compared to both RS (δ15N 5.29, 2.26 ‰) and BI (δ15N 4.81, 1.01 ‰) zooplankton. Myctophids from RSS and RS had similar δ13C values (-25.5 ‰) with BI values 1 ‰ higher, and all three locations had similar δ15N values, ranging from 9.08, 0.61 ‰ (RSS) to 9.57, 0.77 ‰ (RS). Myctophid carbon isotope values overlapped with Antarctic silverfish values (δ13C -25.11, 0.68 ‰), which had marginally higher nitrogen isotope values (RS δ15N 10.24, 0.8 ‰) than myctophids.

The isotopic prey polygon biplot (Fig. 5c) shows the isotopic means of the prey clusters 1-6 (as defined based on the δ13C and δ15N values in Fig. S2), with the prey cluster numerals plotted on Fig. 5b and 5c. Humpback whale skin means (±1 SD) for “all whales”, “cluster A whales” and “cluster B whales” are shown with the Borrell TDF subtracted (Fig. 5c). The simulated mixing prey polygon plot (Smith et al. 2013) (Fig. S7) validated the use of these prey cluster data in the MixSIAR prey apportionment mixing model (Stock et al. 2018) with the exemption of one outlier, which was removed from the dataset used for the MixSIAR model prior to analysis.

**3.4 MixSIAR Mixing Model Outputs: proportions of different prey ingested by humpback whales**

T-tests showed that all prey clusters 1-6 (Fig. S2) had significantly different means in one or both of the isotope ratios (Table S4). Myctophids and silverfish grouped together in cluster 6 and are collectively referred to as “fish” in the dietary discussion.

The Bayesian mixing model containing ‘whale cluster’ as a categorical variable was ranked highest (Table S5) and this model had lower multiplicative error terms (ξj) than the null model. The posterior distributions of the proportional contributions of each prey cluster to humpback whale diet for Borrell TDFs are shown in Fig. 6 and Table S10 (with results for Post TDFs provided for comparison in Fig. S8). Whilst the model outputs generate a range of possible prey proportions, the means of these ranges and the modes are also given in Fig. 6 and Table S10 to facilitate interpretation of the relative importance of each potential prey.

**3.4.1** **Cluster A whale diet**

The dominant prey item for cluster A whales were RSS and RS phytoplankton (prey cluster 1: mean 34, 6%; range 11-50%), followed by fish (prey cluster 6: mean 27, 7%; range 2-46%), and RSS and RS mixed community zooplankton (prey cluster 5: mean 25, 10%; range 1-57%) (Fig. 6a). Contributions from RSS and RS krill (prey cluster 3), and BI phytoplankton (prey cluster 2), mixed community zooplankton and krill (prey cluster 4) were minimal. Cluster A whales appear therefore to be sourcing most of their diet from the RSS and RS.

**3.4.2 Cluster B whale diet**

Cluster B whales had a more varied diet than cluster A, with the greatest proportion of their diet from BI mixed community zooplankton and krill (prey cluster 4: mean 41, 30%; range 0.1-83% with a bimodal result, meaning there were two plausible solutions for the proportion vector) (Fig. 6b). Fish (prey cluster 6: mean 34, 14%; range 3-60%, bimodal) and BI phytoplankton (prey cluster 2: mean 13, 16; range 0-46%, bimodal) mostly made up the rest of their diet. Contributions from RSS phytoplankton, RSS and RS krill, and mixed community zooplankton (prey clusters 1, 3 and 5 respectively) were small. The bimodal results suggest that either: a) the diet was approximately two thirds BI mixed community zooplankton and krill with around a quarter fish and other minor components, or b) that the diet was approximately 50% fish balanced by around one third BI phytoplankton and little BI mixed community zooplankton and krill. However posterior modal peak heights suggest that the former diet is more probable. These results indicated a relative importance of fish in the diet of both cluster A and B whales, and suggested that cluster B whales derived a high proportion of their diet from around the BI.

**3.5 Compound-specific stable isotope analysis of nitrogen in amino acids**

Of the 14 humpback whale skin samples analysed for CSIA of N-AA, nine were from cluster A (five males and four females) and five from cluster B (3 males and 2 females) (Table S2). Values of δ15NGlx and δ15NPhe, along with TP were plotted against bulk δ15N data (Figs. 7a and 7b). These plots illustrate a “flat” trend line for δ15NPhe with those values remaining relatively constant with increasing bulk δ15N values, compared to a steeper trendline for δ15NGlu, which showed 15N enrichment in glutamic acid as bulk δ15N values increased. The AA δ15N data were examined applying the amino acid “fasting” and “foraging” indicators outlined in Lübcker et al. (2020): mean ẟ15N alanine values were similar between cluster A (16.34, 5.43 ‰) and cluster B whales (16.62, 3.50 ‰) (Table S2), whilst ẟ15N threonine values were generally higher for cluster B than cluster A whales (mean -23.14, 3.01 ‰ compared to -27.82, 4.37 ‰ respectively), although high standard deviations indicate there is considerable overlap in these data. No consistent trends were observed in any of the other amino acids.

**3.6 Estimates of whale trophic position**

Humpback whale ẟ15N values ranged from 6.49-9.58 ‰ across the 65 skin biopsy analyses (Fig. 3, Table S1). This range of 3.1 ‰ corresponds to slightly more than one TP when applying the Borrell et al. (2012) TDF.

**3.6.1 Simple mathematical trophic position calculation**

A simple calculation of whale TP values (Table 1) was carried out using data presented in Table S11. Using bulk δ15N data averaged over years 2010 and 2015 for whales, and years 2008, 2010 and 2015 for prey from all sampled regions south of 66⁰S, the following was noted. If the mean Southern Ocean phytoplankton baseline δ15N value in regions where whales were most likely feeding is 0.45 ‰, the phytoplankton-krill TDF is 3.40 ‰ (Post 2002), and the krill-whale TDF is 2.82 ‰ (Borrell et al. 2012), then if whales were exclusively eating krill, one would expect their mean δ15N isotope value to be 0.45 + 3.40 + 2.82 = 6.67 ‰. However, their mean δ15N value was 7.57 ‰, which is 0.90 ‰ greater than predicted, confirming that humpback whales in this study are likely to be incorporating fish into their diet to elevate the bulk δ15N value in their tissue, which is also supported by the Bayesian Modelling output presented above. Assuming a fish–whale TDF of 2.82 (after Borrell et al. 2012), then that means that the 0.90 ‰ increase of measured versus predicted δ15N value for whales represents 0.32 of a TP (0.90/2.82). If whales consumed a pure krill diet, then their expected TP would be 3.00. The mean TP of these humpback whales is therefore likely to be 3.32. The same calculation was carried out for the two whale clusters A and B, and for all females, and all males. Cluster A had a mean TP of 3.25, whilst the cluster B mean TP was 3.76. Female mean TP was 3.23, whilst male mean TP was 3.43 (Table 1).

**3.6.2 *Modelled t*R*ophicPosition calculation***

The Quezada-Romegialli et al. (2018) tRophicPosition model gave humpback whale mean TP estimates of 3.07 using the Borrell TDF (Table 1). The female mean TP value was 2.98 compared to the male value of 3.18. Bayesian modelling posterior pairwise comparisons gave a probability of 99.8% that males had higher TP than females. The cluster A mean TP value was 3.00 compared to the cluster B value of 3.51. Bayesian modelling posterior pairwise comparisons gave a probability of 100% that cluster B whales had higher TP than cluster A whales.

**3.6.3 *Compound-specific stable isotope analysis trophic position and trophic discrimination factor calculation***

A biplot of δ15NGlx versus δ15NPhe values over trophic isoclines showed that male humpback whales generally had higher TP values than females, supporting the TP calculations presented above, with male TPs ranging from 2.82-4.23 compared to female values of 2.58-3.35 (Fig. 8, Table S2). Male mean TP values were 3.57, 0.53 compared to female mean TP values of 3.00, 0.28. Two male whales in cluster B had the highest TP of above 4.2, with cluster B whales having a mean TP of 3.67, 0.60, compared to whales in cluster A with a mean TP of 3.14, 0.38. The mean TP of the sub-set of 14 whales analysed for CSIA was 3.33, 0.52.

**3.6.4 *Comparison of the three methods of trophic position calculation***

The simple mathematical calculation using mean bulk δ15N data from humpback whales and prey produced a mean whale TP of 3.32, which was very close to the CSIA-calculated value of 3.33, whilst the tRophicPosition model produced a mean value of 3.07.

**3.7 Isoscapes**

When assigning whales to the most probable feeding locations based on comparisons between whale isotope data and isoscapes, the mean posterior probability density for the 56 whales in cluster A was strongly bimodal when using only δ13C data. This suggested that some individual cluster A whales were feeding north of the BI, between ~53°S and ~63°S between the Subantarctic Front and the Southern Antarctic Circumpolar Current Front (Fig. 1), whereas another subset were feeding further south along the edge of and within the RS at ~75°S latitude (Fig. 9a). When using both δ13C and δ15N values for the cluster A whale assignments, the mean posterior probability was multi-modal: the density was much greater for regions around the RS, with lower density assignments between ~61°S and ~67°S around the BI and just north of the islands within the Southern Antarctic Circumpolar Current Front, and a few assignments around 50°S to the south-east of New Zealand (Fig. 9c). The mean posterior probability density of assignments based on δ13C values for the nine whales in cluster B, by contrast, suggested a more diffuse bimodal distribution that extended further north, from ~50°S to ~60°S with low probability density to the southwest and east of New Zealand (Fig. 9b). When using both δ13C and δ15N data for the cluster B assignments, the mean posterior probability was still multi-modal, however the density was much greater for regions off the south-western and south-eastern coasts of New Zealand and around Tasmania, with an additional lower density mode along the edge of the RS (Figs. 9d and S9).

1. **DISCUSSION**

This study applied multiple stable isotope methods to better constrain and understand Southern Ocean humpback whale trophic ecology. The primary hypothesis that Southern Ocean humpback whales have a similar diet to northern hemisphere humpback whales, eating a mixed diet of fish and krill was confirmed. The secondary hypothesis, that some of the male humpback whales (those in cluster B) were feeding at higher trophic levels than the rest of the sampled males (those in cluster A), and most females (cluster A) was also validated.

**4.1 Comparison of humpback whale bulk stable isotope values, trophic position and diet with other published studies**

The tRophicPosition model calculations gave results consistent with the simple arithmetic and CSIA TP estimates providing confidence in these methods. To place the bulk isotope values, TP calculations and MixSIAR dietary conclusions from this study in context, a literature review of baleen whale stable isotope and TP data, and likely consumed prey was carried out (Table 2). Whilst it is acknowledged that there is no control or correction made for any variability in ẟ15N baselines for the data collated in the table, there are some interesting general trends. For example, for whales sampled in similar locations, whales that fed mainly on zooplankton generally had lower ẟ15N values compared to whales sampled in the same area feeding predominantly on fish.

**4.1.1 *Southern hemisphere humpback whale* ẟ15N *values***

The mean humpback whale ẟ15N value of 7.5, 0.7 ‰ from this study, was relatively close to Southern Ocean humpback whale supplementary feeders of E1 breeding stock sampled off east Australia (7.1, 1.0 ‰), feeding on Antarctic krill and Antarctic or temperate fish (Eisenmann et al. 2016). It was slightly higher than the mean value of 6.8, 0.4 ‰ reported for the E1 breeding stock in Groß et al. (in press), but close to the mean values measured for population D (7.1, 0.5 ‰) and E2 (7.3, 0.5 ‰) in the same study (see Table 2 for descriptions of whale breeding stocks). Bengtson Nash et al. (2018) obtained ẟ15N values closely aligned to this study for humpback whales sampled off Moreton Bay, south-east Queensland, Australia, with values ranging from 7.8, 1.2 ‰ for males and 7.4, 1.6 ‰ for females, reported as eating Antarctic krill. They too observed higher values for males than females. Owen et al. (companion paper in review) recorded ẟ15N values of 7.4, 0.2 ‰ to 8.0, 0.1 ‰ for whales sampled off south-east Australia in the sub-tropics. In contrast, whales sampled in temperate waters off south-east Australia, observed to be eating temperate krill (*Nyctiphanes australis*) and pilchards (*Sardinops sagax*), had higher values of 8.1, 0.2 ‰ to 9.2, 0.2 ‰ (Owen et al. companion paper in review). Southern Ocean whales reported as being exclusive Antarctic krill ‘classical feeders’ of D breeding stock had lower ẟ15N values of 5.4, 0.7 ‰, and ‘classical feeders’ of E1 breeding stock had values of 6.0, 0.7 ‰ (Eisenmann et al. 2016). These whales, which feed purely on Antarctic krill, had ẟ15N values that were 1-2 ‰ lower than whales in the same study that were supplementing their diet with fish, and were between 1.5-2 ‰ lower than whales sampled around the BI in this study. These comparisons provide confidence in the Borrell MixSIAR modelling results, which indicated that the Southern Ocean humpback whales of this study had high contributions of fish in their diet (posterior means: cluster A 27%; cluster B 35%). These proportions are plausible in the context of ẟ15N values and dietary information from other studies presented in Table 2.

**4.1.2 *Comparison of Southern Ocean and northern hemisphere humpback whale ẟ15N values***

The wide range of ẟ15N values reported for northern hemisphere humpback whales (Table 2) suggests that these whales are flexible generalists, feeding across multiple trophic levels or varying isotopic baselines (Christensen et al. 1992, Filatova et al. 2013, MacKenzie et al. 2022). North-west Atlantic humpback whales (Gavrilchuk et al. 2014) and North Pacific Ocean humpback whales (Witteveen et al. 2008, 2009b, 2011, Fleming et al. 2011, Filatova et al. 2013, Wright et al. 2015, 2016),) which consume a high proportion of fish in their diet, have much higher ẟ15N values (13.0 - 14.7 ‰) than southern hemisphere humpback whales. This prevails even for those whales reported as predominantly eating krill, with some fish contributing to their diet (e.g., ẟ15N values of 12.3 -13.1 ‰: Fleming et al. 2011, Witteveen et al. 2011, Mackenzie et al. 2022). These ẟ15N values are around 5 ‰ higher than Southern Ocean humpback whales in this study, thought to have a similar diet. This difference is likely driven by higher nitrogen isotopic baseline values in northern hemisphere oceanic regions where whales were feeding compared to Southern Ocean feeding areas, in addition to potentially higher percentage fish contributions to the whales’ diet. Mechanistic models of ẟ15N baseline values in Somes et al. (2010) give values of 2-4 ‰ in the North Pacific Ocean and 4-8 ‰ in the north-east Pacific Ocean, whereas Southern Ocean spatial statistical models of compiled measured ẟ15N data suggest baseline values of 0-2 ‰ (Somes et al. 2010) and -3 to 2 ‰ (St John Glew & Espinasse et al. 2021). Mean SPOM ẟ15N values measured in this study (Table S1) ranged from 0.1-1.5 ‰, which were considerably lower than northern hemisphere values given in Somes et al. (2010) and could explain the lower southern hemisphere ẟ15N values of humpback whales.

**4.1.3 *Nitrogen isotope values and diets of other baleen whale species***

Like humpback whales, fin whales have diets that range from almost exclusively foraging on copepods and krill, to supplementation of zooplankton with fish. Various studies of northern hemisphere fin whales report ẟ15N values ranging from around 9.5-11 ‰ for whales with a predominantly zooplankton diet (Aguila et al. 2014, Silva et al. 2019, MacKenzie et al. 2022), to 12-15 ‰ for fin whales that have fish in their diet (Gendron et al. 2001, Gavrilchuk et al. 2014, Ryan et al. 2014, Witteveen & Wynne, 2016, Wild et al. 2018). Northern hemisphere blue whales (*B. musculus*), feeding exclusively on zooplankton, have ẟ15Nvalues ranging from 9-10 ‰ (Ostrom et al. 1993, Gavrilchuk et al. 2014, Silva et al. 2019, MacKenzie et al. 2022). These values for northern hemisphere fin and blue whales with pure zooplankton diets are around 1.5 ‰ higher than values for the Southern Ocean humpback whales in this study, shown to have a mixed diet of zooplankton and fish. A Southern Ocean blue whale skin biopsy obtained from the 2015 voyage had a ẟ15Nvalue of 6.9 ‰ (Bury, unpublished data, Table 2) which was 2-3 ‰ lower than northern hemisphere blue whale values. Although it is only a single blue whale ẟ15N value, this measurement lends support to the concept that higher nitrogen baseline values in the northern hemisphere oceans drive up the ẟ15N values of whales feeding in those areas. The humpback whale mean ẟ15Nvalue of this study was 0.6 ‰ higher than the 2015 blue whale skin value, likely indicating that most of this study’s humpback whales were feeding at a higher trophic level than blue whales, further supporting fish being a marked component of the humpback whale diet.

**4.1.4 *Trophic position comparison of southern and northern hemisphere humpback whales***

The mean simple arithmetic and CSIA TP values of 3.3, and the tRophicPosition Borrell model value of 3.1 for humpback whales in this study (Table 1) agree with other published Southern Ocean humpback whale TP estimates (Table 2). For southern hemisphere whales, Groß et al. (in press) calculated a mean TP of 3.0, 0.1 for the E1 humpback whale population, and a value of 3.1, 0.1 for the E2 population, which from fatty acid concentration data, they concluded included some higher TP prey in their diet. Haro et al. (2020) derived a TP of 3.4 from an ecosystem model for south-east Pacific humpback whales in the Magellan Strait.

In contrast, in the northern hemisphere, North Pacific Ocean humpback whales eating a diet of predominantly zooplankton and fish had TP values of between 3.1 and 3.3 (Hirons 2001, Witteveen et al. 2011, Wright et al. 2015, 2016). North Pacific Ocean humpback whales from the northern Gulf of Alaska that predominantly ate fish had a higher TP of 3.7 (Hirons 2001, Witteveen et al. 2008, 2011, Wright et al. 2015), whilst whales sampled from California to southern British Columbia with a similarly high fish content in their diet had a mean TP of 3.9 (Miller, 2006. Witteveen et al. 2011). Pauly et al. (1998) used stomach content analysis to derive a mean TP value of 3.6 for humpback whales from a variety of northern hemisphere locations estimating a diet of 55% zooplankton and 45% fish, which is much in line with the 3.7 TP value for cluster B whales in this study consuming a posterior mean of 34% fish estimated from the MixSIAR Borrell model. A high TP value of 4.1 was assigned to European Arctic humpback whales by MacKenzie et al. (2022) who reported whales consuming a diet of euphausiids and fish. The highest value of 4.5 was reported by Ostrom et al. (1993) for humpback whales feeding off the coast of Newfoundland consuming a diet of zooplankton, euphausiids, crustaceans, small fish and small squid, with squid likely elevating the TP of these whales.

**4.1.5 *Trophic position and diet of other baleen whale species***

Interestingly, fin whales consuming a similar diet to Southern Ocean humpback whales had TP values aligned to a value of 3.3 in this study (TP 3.4. Pauly et al. 1998; TP 3.0, MacKenzie et al. 2022). The higher fin whale TP value of 4.5 reported by Haro et al. (2020) for whales feeding in the Magellan Strait off Chile, can be explained by a diet of higher trophic level organisms including fish and cephalopods. Blue whale TP values ranged from 3.0 (MacKenzie et al. 2022) to 3.2 (Ostrom et al. 1993, Pauly et al. 1998) being slightly lower than the TP for whales in this study. These TP comparisons provide further evidence that the Southern Ocean humpback whales of this study were consuming a diet predominantly of zooplankton, supplemented with a small proportion of fish.

**4.2 Isotopic niche space and diet of cluster A and B humpback whales**

An organism’s isotopic niche space provides insight into its ecological range, resource use and geographical diversity, where niche parameters can respond rapidly to changes in prey abundance and intra- and interspecific competition (Bearhop et al. 2004, Newsome et al. 2007, Fry & Davis 2015). The smaller isotopic niche area of cluster A compared to cluster B whales in this study, suggested that cluster B individuals might be more specialised, have more similar ecological behaviour, and be more vulnerable to change (Newsome et al. 2012). The higher isotopic values and TP of males and cluster B whales compared to females, suggested the former were either feeding at a higher trophic level or in areas where baseline δ15N values were elevated. The CSIA “flat” trend line for δ15NPhe which remained relatively constant with increasing bulk δ15N values, indicated that the elevated δ15N values of male and cluster B humpback whales were most likely due to trophic influences, rather than isotopic baseline effects. In addition, δ15N values of threonine were generally higher for cluster B whales, than cluster A whales, suggesting foraging at a higher trophic level (Lübcker et al. 2020) of the cluster B whales.

**4.3 Feeding locations of the whales**

There was good agreement on feeding locations of the humpback whales surmised from the MixSIAR modelling data and from the isoscape-based whale feeding location assignments. MixSIAR modelling data indicated that cluster B whales likely fed closer to the highly productive BI, than along the RSS and in the RS, feeding on more fish than the cluster A whales, whilst cluster A whales fed mostly in the RSS and RS area. In both cases*,* whales consumed varying but sometimes high proportions of fish (2-60%), contrary to the paradigm of them being exclusive krill feeders (Chittleborough 1965, Bannister & Hedley 2001, Waugh et al. 2012). These data are consistent with more recentobservations that Southern Ocean humpback whales consume fish as part of their diet during migration (Eisenmann 2016, Groß et al. 2020, Owen et al. companion paper in review) and that they are seemingly quite plastic in their feeding behaviour (Gavrilchuk et al. 2014, Haro et al. 2016).

The strong latitudinal gradient of SPOM ẟ13C values (Fig. 10), enabled the feeding areas of cluster A and B whales to be estimated using the isoscape-based whale assignment method (Wunder 2010, St John Glew & Espinasse et al. 2021). From the δ13C and δ15N assignments, cluster A and cluster B whales likely fed in different locations: cluster A whales probably fed further south, mainly in the RSS and RS region with lower density assignments around and to the north of the BI, whilst cluster B whales, seem to have predominantly fed south-east of Australia and south-west and south-east of New Zealand, with one or two individuals assigned to the RS.

The assignments using both δ13C and δ15N data (Figs. 9c and d) produced results that seem more plausible than assignments using only δ13C data (Figs. 9a and b). This is likely because the field-measured δ15N SPOM values more closely matched those predicted by the δ15N isoscape model, than the equivalent field- and isoscapes-modelled δ13C values (Figs. 10 and S10). Trends in measured SPOM ẟ13C values (Transect SPOM) and modelled data from the St John Glew & Espinasse et al. (2021) isoscape with latitude were quite closely aligned between 40-60°S (Fig. 11). However, there were clear anomalies, which likely reflect localised regional variability in baseline carbon isotope values due to enhanced primary productivity within the Subantarctic Front, Polar Front and Southern Antarctic Circumpolar Current Front regions, areas of enhanced upwelling, such as around the BI, and the ice-melt regions of the RSS and RS. These small-scale high productivity areas, such as around the BI and other localised highly productive frontal regions are not captured in large-scale spatial modelling (such as St John Glew & Espinasse et al. (2021).

The elevated δ13C values of cluster B whales indicate that these whales were either predominantly feeding: 1) further north than the other whales; 2) in more productive waters and/or closer inshore; 3) on benthic or ice-associated fauna; or 4) off the shores of eastern Australia or New Zealand during migration. The assignment results support 1) and 4), but 2) and 3) could be equally plausible explanations. During the whale sampling periods in 2010 and 2015, high productivity around the BI produced locally high δ13C phytoplankton values and large swarms of krill and silverfish (O’Driscoll & Double 2015, Harrison et al. 2020). It is known from observational data (Gales 2010) that the BI is an important feeding ground for Southern Ocean humpback whales that primarily originate from east Australia (Andrews-Goff et al. 2018, Constantine et al. 2012) and the data from this study support this.

Cetacean distributions generally reflect patterns of oceanographic processes, such as fronts, generating regional biological activity (Bost et al. 2009, Riekkola et al. 2019). Southern Ocean humpback whales have been observed to largely feed along the marginal ice zone, the RSS and slope, and seamount/island areas characterised by local hotspots of productivity (Kaschner 2008, Harrison et al. 2020, Bestley et al. 2019). Seamounts and plateaus represent key feeding areas for cetaceans (Skov et al. 2008, Johnston et al. 2008, Morato et al. 2010) and are important for the New Caledonia humpback whale breeding stock and the E1 population (Garrique et al. 2015) and Western Australian humpback whales, which also feed along the western boundary current (Bestley et al. 2019). Surface waters around oceanic islands and plateaus often have high nutrient and iron concentrations due to upwelling effects (Blain et al. 2001, Schallenberg et al. 2018), high chlorophyll *a* concentrations (Sokolov & Rintoul 2007) and elevated primary productivity. These phenomena, collectively recognised as the Island Mass Effect (Doty & Aguri 1956, Blain et al. 2001), generate increased δ13C and δ15N SPOM values (Bidigare et al. 1997, Popp et al. 1998), resulting in higher isotopic values in humpback whales feeding in these areas.

Key predictors of foraging behaviour are water temperature (Owen et al. 2018), distance from the ice edge, ice melt rate, and variability in ice concentration two months prior to arrival on the feeding grounds (Nicol et al. 2008, Andrews-Goff et al. 2018, Riekkola et al. 2019). Humpback whale feeding is thus sustained by a biological cascade, in which new biological productivity is triggered by ice melting, which in turn supports phytoplankton grazers such as krill and other zooplankton, and ultimately whales. Humpback whales can show persistent space use and site fidelity (Tynan 1998, Branch 2011, Bombosch et al. 2014), with whales moving through the same location or occupying adjacent habitats over consecutive austral summers (Andrews-Goff et al. 2018). The similarity between 2010 and 2015 whale skin isotopic values in this study supports this observation.

**4.4 Potential factors affecting stable isotope values of Southern Ocean humpback whales**

**4.4.1 *Whale sex and pregnancy***

Although this study found no isotopic difference between humpback whale age groups, sampling location or year, males had slightly higher ẟ13C and ẟ15N values than females. Several studies of humpback whales have found no difference in ẟ13C or ẟ15N values between sexes (Witteveen et al. 2011, Gavrilchuk et al. 2014, Fleming et al. 2016, Bengston Nash et al. 2018). In addition, a study of southern hemisphere humpback whale blubber fatty acid profiles showed no clear nutritional status separation between females and males (Eisenmann 2016). The mean isotopic difference between males and females in this study, was not large (Tables S2 and S7) and may be due to a small sub-set of male whales feeding at a higher trophic level (Figs. 4 and S4). However, there are other factors that should also be considered.

Differences in size, time spent on feeding grounds and associated fasting duration between male and female humpback whales, plus the reproductive status of females, could contribute to the isotopic difference in their skin tissues superimposed on diet effects. Adult female humpback whales range in length from 12-17 m and are between 40-150 cm longer than males. Considering the potential positive relationship between size and ẟ15N value (Jennings et al. 2002, 2008) one might expect higher ẟ15N values in females than males. Unfortunately, there were no size-based data within this study to assess if size had any effect on stable isotope ratios. Movement modelling studies of humpback whales migrating from Western Australia to the Southern Ocean showed that females moved faster than males during resident and transit periods, which Bestley et al. (2019) attributed to either their larger size or different energetic requirements. A difference in metabolism and energetic requirements between males and females could also contribute to sex-related isotopic differences.

Pregnancy is also known to affect the stable isotope composition of females, such that their isotopic values do not exclusively reflect diet (Clark et al. 2016). Pregnant mammals often have lower ẟ15N values as they become net anabolic (i.e., as they increase protein synthesis) and decrease the excretion of nitrogenous waste (Fuller et al. 2004, Martínez Del Rio et al. 2009, Kurle et al. 2014). Carbon isotope values also decrease as pregnant females mobilise lipid stores to meet the energetic demands of pregnancy (Kelly 2000, Kurle & Worthy 2001). The existence and magnitude of these effects vary among species (Kurle 2002, Habran et al. 2010, Newsome et al. 2010). There was no information relating to the pregnancy status of females in this study. However, Southern Ocean humpback whale pregnancy rate estimates of 37% (Chittleborough 1965), 18-48% (Clark et al. 2016), and 30-86% (mean 52%) (Pallin et al. 2023) in the Western Antarctic Peninsula, and an estimate of 57% obtained from whales sampled around the Kermadec Islands (Riekkola et al. 2018), indicate that pregnancy could have accounted for the overall lower mean ẟ13C and ẟ15N values in females compared to males. For future studies, it would be useful to carry out progesterone analysis of biopsied blubber to obtain information on the proportion of pregnant females in the population. Without this information, pregnancy-related changes in stable isotope values are difficult to assess and complicate interpretations of data to infer diet and migration (Newsome et al. 2010, Clark et al. 2016).

**4.4.2 *Time spent on feeding grounds and feeding and fasting effects on whale stable isotope values***

Another factor contributing to humpback whale isotopic variability is the length of time spent on the feeding grounds. Male and female humpback whales arrive at and depart from Antarctic feeding grounds at different times: the first whales to arrive from October onwards are pregnant females, then males, then lactating females with a calf, with whales departing around April in the order of lactating females with a calf, males, then pregnant females (Chittleborough 1965, Dawbin 1966, 1997). In addition, Riekkola et al. (2018) noted that Oceania humpback whales migrated to different feeding grounds based on their life history stages, with all tagged females with calves in their study migrating to the Ross Sea region. Some non-pregnant females also overwinter in Antarctica to improve body condition for the next reproductive cycle (Clark et al. 2016). For the whole population, if around 20-60% of whales are pregnant, then between approximately one fifth to two thirds of the females may arrive before males and the remaining proportion of females (those that are lactating) after them. Differences in arrival time may therefore not account for male/female differences in isotope values, since arrival times for males/females over the whole population could be averaged out to be quite similar, however differences in arrival time could contribute to greater isotopic variability within the population.

Later arrival on the feeding grounds could mean that those whales spend longer migrating and fasting. Some studies have shown that fasting increases ẟ15N values of some organisms by up to 1 ‰ (Hobson et al. 1993, Cherel et al. 2005a), due to protein synthesis using 15N-enriched amino acids derived from catabolism of endogenous protein (Hatch 2012). Other studies, however observed that ẟ15N values declined during periods of reduced feeding, reflecting changing nitrogen baselines between summer and winter feeding grounds, rather than tissue metabolism effects (Matthews & Ferguson 2015, Pomerleau et al. 2018). Furthermore, several studies suggest that ẟ15N values are not affected by fasting (Hobson & Schell 1998, Ben David et al. 1999, Williams et al. 2007, Gomez-Campos et al. 2011, Aguilar et al. 2014, Owen et al. companion paper, in review). For humpback whales this is likely because they have evolved to endure long predictable periods of fasting, so the effects of fasting are not equivalent (in terms of metabolic and biochemical processes) to starvation; i.e. they do not experience ‘nutritional stress’ during these times, due to physiological adaptation (Kempster et al. 2007, Witteveen et al. 2009a). In addition, Riekkola et al. (2020) surmise from humpback whale tracking and energetic studies that even extreme long-distance migration does not appear to adversely affect the energetic expenditure of these animals.

Blubber stores are likely to be the primary source of energy until stages of extreme nutritional stress are reached (Aguilar et al. 2014). This is because the quantity of lipid reserves stored by an organism influences the degree to which protein versus lipid is catabolised for energy (Elia et al. 1999). Large fat reserves mean the whales are less likely to metabolise 15N-enriched proteins during fasting (Polishuk et al. 2001, Aguilar et al. 2014). Evidence to support minimal effect of fasting on ẟ15N values is provided by Bengtson Nash et al. (2018) who sampled E1 breeding stock humpback whales off the south-east Queensland coast and measured very similar ẟ15N values in southward-migrating “fasting” whales (7.5, 1.3 ‰) compared to the northward migrating “post-feeding” whales (7.7, 1.8 ‰). These southward-migrating “fasting whales” not only had similar ẟ15N values, but also similar ẟ13C values (-24.9, 1.0 ‰) to humpback whales in this study, which had been feeding in Antarctica for several months (ẟ13C -25.2, 1.0; ẟ15N 7.6, 0.7). Owen et al. (companion paper, in review) also noted that southward migrating whales in the sub-tropics had similar isotope values to whales feeding in the Antarctic, supporting the notion that there is little change in isotopic values due to whale fasting. Furthermore, alanine δ15N values (which if higher, are indicative of fasting: Lübcker et al. 2020) were similar between cluster A and B whales, indicating fasting was likely not affecting nitrogen isotope values.

A further consideration is that delayed arrival on the feeding grounds could mean that those whales had more time for migratory feeding on fish and krill in temperate waters (Gales et al. 2009, Andrews-Goff et al. 2018, Owen et al. companion paper in review) which would increase their ẟ15N values. Stable isotope analysis of baleen whale plates showed that supplementary feeding may be a common strategy for about 30% of east Australian humpback whales (Eisenmann, 2016). It is likely that the cluster B whales with higher isotope values were late arrival males engaged in feeding in temperate waters off south-east Australia or southern New Zealand during south-ward migration (corroborated by the whale assignment locations in Figs. 9d and S9), while migrating en-route to Antarctica supporting observations of Gales et al. (2009). This supplementary feeding whilst migrating may be an indicator that the Southern Ocean ecosystem does not meet the energetic requirements of the humpback whales during the summer feeding season as suggested by Riekkola et al. (2018), or it could just be opportunistic feeding behaviour.

**4.4.3 *The effects of* *quality, abundance, and location of prey on humpback whale stable isotope values***

Humpback whales are largely opportunistic foragers that depend on quality, high lipid-content, energy-dense prey to maximise fat deposition in their blubber layers, providing a sustained energy source (Worthy & Edwards 1990, Koopman et al. 2002, Witteveen et al. 2011). At low latitudes, humpback whales can lose between a third to half of their body mass (Dawbin 1966, Lockyer 1981, Baraff et al. 1991) due to lipids being catabolised during migration and periods of limited nutrient intake (Lockyer 1986, Castellini & Rea 1992, Parrish 1997). As has been shown for other animals (Urton & Hobson 2005, Inger et al. 2006), prey choice for humpback whales can therefore significantly impact survival, migration and reproductive success (Witteveen et al. 2011). In addition, baleen whales need to feed on aggregated prey above a threshold density to ensure positive net energy gain from a feeding event (Piatt & Methven 1990, Hazen et al. 2009, Goldbogen et al. 2011). It is thus possible that the consumption of a higher quality diet (McCutchan et al. 2003, Vanderklift & Ponsard 2003) was a contributor to the higher ẟ15N values of cluster B whales of this study.

As confirmed by this study, Southern Ocean humpback whales are dependent on Antarctic krill as a major dietary component. Krill are strongly associated with the sea ice extent and abundance of associated sea ice algae (Atkinson et al. 2004, Loeb et al. 1997) and are thus vulnerable to climate change (Flores et al. 2012). They are recruited at the sea ice edge and disperse northwards following sea ice melt (Ward et al. 1990, Brierley et al. 1999). Their abundance is dependent on high levels of primary productivity, which in turn is affected by ocean circulation, bathymetry, coastline morphology, localised upwelling and frontal dynamics (Bathman et al. 1997, Strutton et al. 2000, Martinson et al. 2008). These factors, combined with climate change, exert important controls on isotopic baselines and prey availability for humpback whales (Gross 2005, Fountain et al. 2012, Pallin et al. 2023). Higher encounter rates of humpback whales tend to be observed in the northern RS (65-70°S), where this study was located, which is a region of rapid ice retreat and high chlorophyll productivity (Moore & Abbott 2000, Nicol et al. 2006). Clarke and Tyler (2008) observed that, whilst most post-larval Antarctic krill populate the upper 150 m of the water column, some can be found in abyssal waters as deep as 3500 m, where omnivory is increased with resultant higher ẟ15N values (Cresswell et al. 2009, Bengtson Nash et al. 2018). This could explain the wide variability observed in krill ẟ15N values in this study, ranging from 2.03-7.39 ‰ (Table S1), which is reflected up the food chain in myctophids and Antarctic silverfish.

Since there is lower abundance of Antarctic krill in the RS region compared to the Scotia Sea (Atkinson et al. 2004, 2017), it is likely that humpback whales feeding in the RS area have lower dependence on krill than those elsewhere in the Southern Ocean. Due to ocean acidification, a complete collapse of Antarctic krill populations is forecasted by 2300 if current CO2 emissions are not mitigated (Kawaguchi et al. 2013). In this scenario, humpback whales may avoid the threat of starvation by being highly responsive to environmental oscillations through dietary diversification (Bengtson Nash et al. 2018). Diversity in the interannual feeding strategies of humpback whales from eastern Australian E1 breeding stocks demonstrates plasticity in prey selection and migratory behaviour of this species (Eisenmann et al. 2016), which provides hope for adaptive strategies and their long-term survival. The more diverse diet of Southern Ocean humpback whales confirmed by this study provides further evidence for possible dietary plasticity in the face of potential anthropogenically-mediated changes to the trophic structure and prey abundance and distributions within the Southern Ocean.

Humpback whales in the north-east Pacific Ocean have shown temporal and geographical variability in diet driven by changes in prey abundance (Fleming et al. 2016). Changes at lower trophic levels are often amplified at higher trophic levels due to non-linear responses of biological communities and predatory interactions (Friedland et al. 2012, Stock et al. 2014). Such top-predator responses are due to the dynamic interactions and cumulative effects between changing oceanographic conditions, mid-trophic level prey dynamics, and predator foraging behaviour (Hilty & Merenlender 2000, Abraham & Sydeman 2004, Sydeman et al. 2013). Although our data show an isotopic difference between males and females this difference is not marked (<1‰ between female and male mean values for both ẟ13C and ẟ15N), with humpback whale isotope values primarily reflecting consumption of the dominant prey types in the ecosystem. Changes in prey abundance likely drive changes in whale diet at the population level (males and females combined), such that humpback whale prey composition can be an indicator of dominant prey types in the ecosystem (Fleming et al. 2016). Thus, multi-decadal changes in foraging behaviour of humpback whales could be a useful synoptic indicator of changing oceanographic and ecological conditions.

The variety and complexity of factors that can influence humpback whale carbon and nitrogen stable isotope values makes it difficult to provide a definitive assessment of the relative importance of different drivers determining their isotopic composition. Additionally, biopsy collection could be affected by sampling biases, since animals that spend more time at the surface, are more easily sighted and sampled and may therefore be overrepresented in the sampling. Cows with calves may be more readily sighted and sampled as they are often more active at the surface than non-nursing females or males. Whales that spend less time at the surface and perhaps feed in deeper waters may be underrepresented, such that the full dietary range of isotopic values is not captured by biopsy sampling. Nonetheless, dietary intake and feeding location are likely to be the dominant determinants of isotopic values, with other factors potentially also contributing to isotopic variation.

1. **CONCLUSIONS**

This study combined multiple stable isotope methods to quantify diet and trophic position and to identify foraging areas of Southern Ocean humpback whales. It confirmed the hypothesis that Southern Ocean humpback whales sampled around the Balleny Islands and in the Ross Sea had a mixed diet of plankton, krill and fish, similar to the diet of northern hemisphere humpback whales. The percentage of fish consumed varied (2-60%), but proportions were often high, thus challenging the widely held paradigm of Southern Ocean humpback whales being exclusive krill feeders and re-enforcing the notion that they have dietary plasticity. Southern Ocean humpback whales had lower ẟ15N values than northern hemisphere populations and lower trophic position values likely due to a combination of lower baseline ẟ15N surface water values in the Southern Ocean compared to the northern hemisphere and a lower percentage consumption of fish respectively.

MixSIAR prey apportionment modelling and trophic position calculations using the Borrell et al. (2012) trophic discrimination factor produced data that closely aligned with trophic information from other studies, hence placing more confidence on the Borrell et al. (2012) factors than the Post (2002) values. It is recommended that future studies of humpback whale trophic ecology utilise the Borrell et al. (2012) trophic enrichment factors.

The majority of the whales sampled in this study appeared to be foraging in the Ross Sea, along the Ross Sea Shelf and in the vicinity of the Balleny Islands. An isotopically-distinct subset of male humpback whales with higher trophic position was identified through bulk carbon and nitrogen and compound specific isotope analysis of nitrogen in amino acids. Isoscape-based whale assignments combined with regional isotopic baseline field measurements indicated these whales were either taking higher quality food sourced from productivity hotspots such as the Balleny Islands and/or frontal upwelling areas, or that they had fed en-route to Antarctica in temperate waters off south-eastern Australia, south-west or south-east New Zealand.

Since Antarctic ecosystems are particularly vulnerable to climate change, warming, freshening, ocean acidification and shifts in primary production patterns leading to variable krill abundance, an improved understanding of ocean biogeochemistry and trophic interactions is becoming increasingly important, both for predicting change and for robust ecosystem management of the Ross Sea region. It is hoped that the development of seasonal and regional smaller-scale isoscapes combined with improvements in remote sensing techniques and oceanic modelling will enhance the applicability of isotopic tools to better understand and manage such ecosystems. As demonstrated by this study, *in-situ* field data are essential to inform and rationalise these models to improve data interpretations, and to assist managerial decisions and policy implementation to better protect and conserve Southern Ocean humpback whales and their environment, particularly within the Ross Sea region Marine Protected Area. The revelation of a more diverse Southern Ocean humpback whale diet has implications for predicting the impact of future ecosystem changes on the foraging and hence breeding success of humpback whales. It also highlights the need for further dietary studies on other marine predators in the Southern Ocean that are thought to be highly dependent on Antarctic krill.

**ACKNOWLEDGEMENTS**

We are grateful to the masters and crew of *RV Tangaroa* on the 2008, 2010 and 2015 voyages and to the following people for their sampling assistance: Paul Sagar, Stéphane Gauthier and Dylan Amyes for underway phytoplankton sampling in 2010; Dave Paton, Curt Jenner, Jean-Benoit Charrassin, and Sarah Laverick for whale biopsy sample collection in 2010; Owen Anderson, Darren Stevens, Blake Hornblow, and Zac Penman, for biological sampling in 2015; and Andrea Polanowski for sample freighting. Animal ethics permit #AEC195 and SC544 were obtained to biopsy whales in 2010 and 2015 respectively. Samples were held in New Zealand under permits #NO/2009/05, #NO/2010/01 and WE-34273\_MAR. Many thanks to the NIWA analytical team Andrew Kingston, Thomas Max, Brittany Graham, Greg Olsen, Anna Kilimnik, Josette Delgado, Jaret Bilewitch, and Rahul Peethambaran who assisted with bulk and compound specific stable isotope analysis and to Jeff Foreman who carried out post-voyage processing of whale prey. Thanks to Leigh Torres, Jill Schwarz and Moira Decima for helpful discussions in the early stages of this project and to Kim Goetz for assistance with whale field work on V2015. Helpful advice and improvements to the manuscript from the associate editor and two anonymous reviewers are gratefully acknowledged.

**FUNDING INFORMATION**

Funding for this work was provided through the following projects: New Zealand’s International Polar Year Census of Antarctic Marine life; the Ministry for Primary Industries (MPI) *Management of Ross Sea Toothfish Fishery*; MPI *Isoscapes for Trophic and Animal Movement Studies*; MPI *Humpback whale connectivity - determining the migration path and Antarctic feeding grounds of New Zealand's humpback whales*; the Ministry of Business, Innovation and Employment (MBIE) *Protecting the structure and function of Ross Sea Ecosystems*; MBIE Ross Sea Climate and Ecosystem; Land Information New Zealand Ocean Survey 2020; MBIE Ross Sea Research and Monitoring Programme (Ross-RAMP, C01X1710); Antarctic Science Platform, Project 3 (ANTA1801); Antarctica New Zealand; Australian Antarctic Division and NIWA. KJP was supported by a postdoctoral grant from the University of Zurich.

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Table 1. Trophic position (TP) estimates using three different methods, 1) simple mathematical calculation, 2) the Quezada-Romegialli et al. (2018) tRophicPosition model, and 3) compound-specific stable isotope analysis (CSIA) TP calculation for humpback whales (*Megaptera novaeangliae*)for a) all whales, b) females, c) males, d) cluster A whales and e) cluster B whales. The tRophicPosition model was run applying Borrell et al. (2012) trophic discrimination factors, using Antarctic krill (*Euphausia superba*) as the nitrogen isotopic baseline. TP estimates are shown as the median with 95% confidence levels (CL). The whole dataset was used for the simple mathematical calculation and the tRophicPosition model, whilst a sub-set of samples (*n* = 14) was used for the CSIA calculations as indicated.

|  |  |  |  |
| --- | --- | --- | --- |
| Whale Groups | **Trophic Position Calculation Method** | | |
| Simple Arithmetic | tRophicPosition Model | CSIA |
| **a)   All (*n* = 65, CSIA *n* = 14)** |  |  |  |
| **Mean** | **3.32** | **3.07** | **3.33** |
| Lower 95% CL |  | 2.99 |  |
| Median |  | 3.07 |  |
| Upper 95% CL |  | 3.15 |  |
|  |  |  |  |
| **b)  Females (*n* = 35, CSIA *n* = 6)** |  |  |  |
| **Mean** | **3.23** | **2.98** | **3.00** |
| Lower 95% CL |  | 2.89 |  |
| Median |  | 2.98 |  |
| Upper 95% CL |  | 3.06 |  |
|  |  |  |  |
| **c)   Males (*n* = 30, CSIA *n* = 8)** |  |  |  |
| **Mean** | **3.43** | **3.18** | **3.57** |
| Lower 95% CL |  | 3.08 |  |
| Median |  | 3.18 |  |
| Upper 95% CL |  | 3.28 |  |
|  |  |  |  |
| **d)  Cluster A (*n* = 56, CSIA *n* = 9)** |  |  |  |
| **Mean** | **3.25** | **3.00** | **3.14** |
| Lower 95% CL |  | 2.94 |  |
| Median |  | 3.00 |  |
| Upper 95% CL |  | 3.06 |  |
|  |  |  |  |
| **e)    Cluster B (*n* = 9, CSIA *n* = 5)** |  |  |  |
| **Mean** | **3.76** | **3.51** | **3.67** |
| Lower 95% CL |  | 3.36 |  |
| Median |  | 3.51 |  |
| Upper 95% CL |  | 3.67 |  |

Table 2. Summary of published carbon and nitrogen stable isotope data, trophic position (TP) calculations and dietary information of northern and southern hemisphere cetaceans. Stable isotope data were obtained from skin biopsy analyses, unless specified in the “Reference Source” column with + for blubber analysis, # for baleen analysis and ^ for muscle analysis. Unless otherwise indicated, all ẟ13C data were either lipid-corrected or obtained from lipid-extracted samples. IWC = International Whaling Commission, SD = ±1 standard deviation, TP = trophic position, ND = no data. The International Whaling Commission (International Whaling Commission 2011) identifies southern hemisphere humpback whales (*Megaptera novaeangliae*) into breeding populations and subpopulations: D population breeds off the west coast of Australia, E1 breeds off the east coast of Australia, and E2 breeds off New Caledonia. Where specified in the reference, information on prey species is provided, otherwise generic groups are given. Humpback whale dietary items are listed in order of relative importance with % contribution provided where known. At the first mention of a species, the common and latin name are provided and thereafter the common name is used. In the location columns, N = north, S = south, W = west, E =east. The equations used to calculate TPs are provided at the base of the table.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Whale Species** | **Reference Source: IWC whale breeding stock\*, movement and feeding details and sex where known** | ***n* =** | **ẟ13C  (‰)** | | **ẟ15N (‰)** | | **TP** | **TP calcul-ation method/ equation number** | **Dietary information supplied in publication** | **Sampling Details** | | | |
| **Mean** | **SD** | **Mean** | **SD** | **General Oceanic Location** | **Regional Location** | **Location Type** | **Sampling Year(s)** |
| Humpback whale  (*Megaptera novaeangliae*) | This study, E1 | 65 | -25.2 | 1.0 | 7.6 | 0.7 | 3.3 | CSIA, Simple arithmetic | Zooplankton and Antarctic krill (*Euphausia superba*) (approx. 70% combined), fish (approx. 30%) | Antarctic | Southern Ocean, Ross Sea shelf, slope and Balleny Islands | Oceanic, oceanic islands | 2010, 2015 |
| Eisenmann et al. (2016) # D, classical feeders | 184 | -25.1 | 0.6 | 5.4 | 0.7 | ND | ND | Antarctic krill | SW Pacific | W Australia | Coastal, oceanic | 1940-2015 |
| Eisenmann et al. (2016) # E1, classical feeders | 154 | -24.9 | 0.7 | 6.0 | 0.7 | ND | ND | Antarctic krill | SW Pacific | E Australia | Coastal, oceanic | 1940-2015 |
| Eisenmann et al. (2016) # Mostly E1, supplementary feeders | 191 | -24.9 | 0.9 | 7.1 | 1.0 | ND | ND | Antarctic krill, plus Antarctic and temperate water fish | SW Pacific | E Australia | Coastal, oceanic | 1940-2015 |
| Eisenmann et al. (2016) # Temperate zone feeders, may or may not feed in Antarctica | 93 | -20.1 | 2.3 | 10.2 | 1.9 | ND | ND | Mainly temperate water prey e.g., temperate krill (*Nyctiphanes australis*) and fish | SW Pacific | E Australia | Coastal, oceanic | 1940-2015 |
| Bengtson Nash et al. (2018) + Northward migration, post feeding | 50 | -25.3 | 0.9 | 7.7 | 1.8 | ND | ND | Antarctic krill | SW Pacific | E Australia, SE Queensland, Moreton Bay | Coastal, oceanic | 2008, 2009, 2011, 2013, 2014, 2015 |
| Bengtson Nash et al. (2018) + Southward migration, "fasting" | 110 | -25.0 | 1.1 | 7.5 | 1.3 | ND | ND | Antarctic krill | SW Pacific | E Australia, SE Queensland, Moreton Bay | Coastal, oceanic | 2008, 2009, 2011, 2013, 2014, 2015 |
| Bengtson Nash et al. (2018) + Southward migration 2015 only | 65 | -24.9 | 1.0 | 7.5 | 0.5 | ND | ND | Antarctic krill | SW Pacific | E Australia, SE Queensland, Moreton Bay | Coastal, oceanic | 2015 |
| Bengtson Nash et al. (2018) + Males | 86 | -25.1 | 1.0 | 7.8 | 1.2 | ND | ND | Antarctic krill | SW Pacific | E Australia, SE Queensland, Moreton Bay | Oceanic | 2008, 2009, 2011, 2013, 2014, 2015 |
| Bengtson Nash et al. (2018) + Females | 63 | -25.1 | 1.0 | 7.4 | 1.6 | ND | ND | Antarctic krill | SW Pacific | E Australia, SE Queensland, Moreton Bay | Oceanic | 2008, 2009, 2011, 2013, 2014, 2015 |
| Owen et al. (in review) Subtropical, 2011 | 22 | -26.0 | 0.2 | 8.0 | 0.1 | ND | ND | Antarctic krill | SW Pacific | E Australia, SE Queensland, off Peregian Beach, Sub-tropical Site | Coastal, oceanic | 2011 |
| Owen et al. (in review) Subtropical, 2012 | 8 | -25.9 | 0.2 | 7.4 | 0.2 | ND | ND | Antarctic krill | SW Pacific | E Australia, SE Queensland, off Peregian Beach, Sub-tropical Site | Coastal, oceanic | 2012 |
| Owen et al. (in review) temperate, 2011 | 19 | -25.1 | 0.3 | 9.2 | 0.2 | ND | ND | Antarctic krill, temperate krill, pilchard (*Sardinops sagax*) | SW Pacific | E Australia, New South Wales, off Eden, Temperate Site | Coastal, oceanic | 2011 |
| Owen et al. (in review) Temperate, 2012 | 19 | -25.2 | 0.2 | 8.1 | 0.2 | ND | ND | Antarctic krill, temperate krill, pilchard | SW Pacific | Temperate Site, off Eden, New South Wales, Australia | Coastal, oceanic | 2012 |
| Groß et al. (in press) D breeding stock | 40 | -25.5 | 0.5 | 7.1 | 0.5 | 2.8, 0.1 | 1 | Mainly krill | SW Pacific | W Australia, 21º55’S, 114º10’E | Coastal, oceanic | 2019 |
| Groß et al. (in press) E1 breeding stock | 21 | -25.5 | 0.5 | 6.8 | 0.4 | 3.0, 0.1 | 1 | Mainly krill | SW Pacific | E Australia, 27º26’S, 153º34’E | Coastal, oceanic | 2019 |
| Groß et al. (in press) E2 breeding stock | 26 | -25.3 | 0.6 | 7.3 | 0.5 | 3.1, 0.2 | 1 | Mainly krill, some fish | SW Pacific | New Caledonia, 22º36’S, 167º00’E | Coastal, oceanic | 2019 |
| Haro et al. (2020) | N/A | N.D. | N.D. | N.D. | N.D. | 3.4 | Ecopath plus Ecosim | Coastal sources of lobster krill (*Munida gregaria*), euphausiids (*Euphausia lucens*), amphipods, Fuegian sprat (*Sprattus fueguensis*) | SE Pacific | SE Pacific, Magellan Strait, off Chile | Coastal, oceanic | N/A |
| Haro et al. (2020) | 33 | -16.3 | 0.6 | 14.7 | 1.0 | ND | ND | Coastal sources of lobster krill, euphausiids, amphipods, Fuegian sprat | SE Pacific | SE Pacific, Magellan Strait, off Chile | Coastal, oceanic | 2011, 2012 |
| Haro et al. (2021) | 64 | -15.9 | 1.2 | 14.4 | 0.9 | ND | ND | Coastal sources of lobster krill, euphausiids, amphipods, Fuegian sprat | SE Pacific | SE Pacific, Magellan Strait, off Chile | Coastal, oceanic | 2011, 2012, 2017 |
| Witteveen et al. (2009a) | 597 | -17.8 | 0.0 | 12.9 | 0.1 | ND | ND | Unspecified | N Pacific | Bering Sea and Coastal areas off: W & E Aleutian Islands, W & N Gulf of Alaska, SE Alaska, N British Columbia, California, Oregon, Washington | Coastal, oceanic | 2004, 2005, 2006 |
| Witteveen et al. (2009b) | 1105 | -17.6 | 0.0 | 13.2 | 0.0 | ND | ND | Fish, zooplankton | N Pacific | Bering Sea and Coastal areas off: Russia, W & E Aleutian Islands, W & N Gulf of Alaska, SE Alaska, N British Columbia, California, Oregon, Washington | Coastal, oceanic | 2004, 2005 |
| Witteveen et al. (2011) | 1105 | ND | ND | 13.2 | 0.0 | 3.6, 0.02 | 2 | Fish: Pacific herring (*Clupea pallasii),* capelin (*Mallotus villosus),* zooplankton (Copepods: *Neocalanus* spp., *Calanus* spp.), Crustaceans - krill | N Pacific | All: Bering Sea and Coastal areas off: Russia, W & E Aleutian Islands, W & N Gulf of Alaska, SE Alaska, N British Columbia, California, Oregon, Washington | Coastal, oceanic | 2004, 2005 |
| Witteveen et al. (2011) Recalculated TP values from Wright et al. (2015) | 81 | ND | ND | 12.3 | 0.2 | 3.1, 0.1 | 2 | Predominantly zooplankton, some fish | N Pacific | W Aleutian Islands and Russia (WEST) | Oceanic islands | 2004, 2005 |
| Witteveen et al. (2011), Hirons (2001) Recalculated TP values from Wright et al. (2015) | 282 | ND | ND | 12.6 | 0.1 | 3.3, 0.0 | 3 | Predominantly zooplankton (e.g., *Neocalanus* spp., *Calanus* spp.), euphausiids, some fish | N Pacific | W Gulf of Alaska, E Aleutian Islands, and Bering Sea (CENT) | Coastal & oceanic islands | 2004, 2005 |
| Witteveen et al. (2008, 2011), Hirons (2001) Recalculated TP values from Wright et al. (2015) | 199 | ND | ND | 13.6 | 0.1 | 3.7, 0.0 | 3 | Predominantly fish e.g., capelin, some zooplankton (*Calanus* spp.) | N Pacific | N Gulf of Alaska (NGOA) | Coastal, oceanic | 2004, 2005 |
| Witteveen et al. (2011) | 227 | ND | ND | 12.7 | 0.1 | 3.4, 0.03 | 2 | Predominantly zooplankton (euphausiids), some fish | N Pacific | SE Alaska (SEAK) | Coastal, oceanic | 2004, 2005 |
| Witteveen et al. (2011)  Recalculated TP values from Wright et al. (2015) | 135 | ND | ND | 13.0 | 0.1 | 3.3, 0.0 | 3 | Predominantly zooplankton (euphausiids), some fish | N Pacific | N British Columbia (NBC) | Coastal, oceanic | 2004, 2005 |
| Witteveen et al. (2011), Miller (2006) | 181 | ND | ND | 14.7 | 0.1 | 3.9, 0.03 | 2 | Predominantly Fish, some zooplankton (*Calanus* spp.) | N Pacific | California, Oregon, Washington, and S British Columbia (COW) | Coastal, oceanic | 2004, 2005 |
| Witteveen et al. (2012) | 93 | -17.9 | 0.6 | 13.3 | 0.9 | ND | ND | Predominantly euphausiids (*Thysanoessa* spp. and *Euphausia pacifica*), plus juvenile walleye pollock (*Gadus chalcogrammus),* capelin*,* and Pacific sand lace (*Ammodytes hexapterus*) | N Pacific | Kodiak Island, Alaska | Oceanic islands | 2004-2006 |
| Wright et al. (2015) (2016) | 63 | -18.0 | 0.6 | 13.7 | 0.8 | 3.3, 0.1 | 3 | Predominantly zooplankton (euphusiids, dominated by *Thysanoessa inermis*) 39%, forage fish e.g., capelin *27%,* walleye pollock 12%, Pacific sand lace 10%, eulachon (Thaleichtys pacificus) <5%, Pacific herring <5%, Pacific sandfish (Trichodon trichodon) <5% | N Pacific | Kodiak Island, Alaska: N feeding ground | Oceanic islands | 2004-2013 |
| Wright et al. (2015) (2016) | 55 | -17.9 | 0.7 | 13.0 | 0.8 | 3.0, 0.1 | 3 | Predominantly zooplankton (euphausiids, dominated by *Thysanoessa inermis) 66%*, some forage fish e.g., capelin <10%, walleye pollock <5%, Pacific sand lace <5%, eulachon <5%, Pacific herring <5%, Pacific sandfish <5% | N Pacific | Kodiak Island, Alaska: S feeding ground | Oceanic islands | 2004-2013 |
| Witteveen & Wynne (2016) | 145 | -17.9 | 0.6 | 13.4 | 0.9 | ND | ND | Krill (dominated by *Thysanoessa inermis*), copepods, juvenile capelin | N Pacific | Gulf of Alaska, Kodiak Island | Oceanic islands | 2003, 2005 |
| Witteveen & Wynne (2016) | 86 | -18.3 | 0.7 | 13.2 | 0.7 | ND | ND | Zooplankton, small fish e.g., juvenile capelin | N Pacific | Gulf of Alaska, Shumagin Islands | Oceanic islands | 2004, 2005 |
| Wild et al. (2018) | 1 | -17.5 | 0.3 | 12.4 | 0.8 | ND | ND | Krill, herring, small schooling fish | N Pacific | Gulf of Alaska | Coastal, oceanic | 2010 |
| Filatova et al. (2013) | 47 | -18.7 | 0.1 | 10.4 | 0.1 | ND | ND | Zooplankton (deep oceanic areas), likely euphausiids (*Thysanoessa raschii, T. inermis, T. spinifera, T. longipes*, and *Euphausia pacifica*) | NW Pacific | Russian Far E whale feeding grounds: Commander Islands | Oceanic islands | 2004, 2005, 2009, 2010, 2011 |
| Filatova et al. (2013) | 48 | -17.2 | 0.1 | 12.7 | 0.2 | ND | ND | Fish (neritic areas) Pacific sand lance, likely euphausiids (Thysanoessa raschii, T. inermis, T. spinifera, T. longipes, and Euphausia pacifica) | NW Pacific | Russian Far E whale feeding grounds: Karaginsky Gulf | Coastal, oceanic | 2004, 2005, 2009 |
| Filatova et al. (2013) | 16 | -17.8 | 0.1 | 14.0 | 0.4 | ND | ND | Fish (neritic areas), likely euphausiids (Thysanoessa raschii, T. inermis, T. spinifera, T. longipes, and Euphausia pacifica) | NW Pacific | Russian Far E whale feeding grounds: Anadyr Gulf | Coastal, oceanic | 2005 |
| Fleming et al. (2011) | 204 | -18.4 to -17.7 | ND | 12.8 to 13.1 | ND | lower | ND | Predominantly euphausiids | NE Pacific | California current system, Negative N Pacific Gyre Oscillation | Coastal, oceanic | 1993-2003, 2010-2012 |
| Fleming et al. (2011) | 155 | -16.4 | ND | 14.2 | ND | higher | ND | Predominantly Californian anchovy (*Engraulis mordax*), pilchard | NE Pacific | California current system, Positive N Pacific Gyre Oscillation | Coastal, oceanic | 2004-2006 |
| Clark et al. (2016) | 62 | -17.3 to -17.8 | ND | 12.5 to 13 | ND | ND | ND | Unspecified | NE Pacific | Monterey Bay, California coast | Coastal, oceanic | May-Nov 2011 |
| Clark et al. (2016) | 64 | -17.8 to -18.2 | ND | 12.3 to 12.9 | ND | ND | ND | Unspecified | NE Pacific | Monterey Bay, California coast | Coastal, oceanic | April-Jul 2012 |
| Pauly et al. (1998) | Not given | ND | ND | ND | ND | 3.6 | 4 | Large zooplankton (55%), miscellaneous fishes (30%), small pelagic fish (15%) | N Pacific, NW Atlantic, NE Atlantic, N Atlantic/ Norwegian Sea & unspecified | Unspecified | Unspecified | NW Atlantic & Unspecified |
| MacKenzie et al. (2022) | 6 | -19.4 | 1.1 | 12.7 | 1.5 | 4.1 | 5 | Euphausiids (*Thysanoessa inermis*), fish e.g., capelin | European Arctic | Near Svalbard and Barents Sea | Oceanic islands | 2013-2018 |
| Ostrom et al. (1993) ^ | 1 | -18.7 | ND | 13.4 | ND | 4.5 | 6 | Zooplankton, euphausiids, crustaceans, small fish, small squid | NW Atlantic | Waters off Newfoundland | Coastal, oceanic | 1986-1990 |
| Todd et al. (1997) | 4 | -18.8 | 0.1 | 14.2 | 0.1 | ND | ND | Unspecified | NW Atlantic | Newfoundland | Coastal, oceanic | 1992-1994 |
| Gavrilchuk et al. (2014) | 97 | -18.7 | 0.4 | 14.3 | 0.6 | ND | ND | Zooplankton, northern krill (*Meganyctiphanes norvegica*) and American sandlace (48%), capelin and Atlantic herring (*Clupea harengus*) (44%), Arctic krill (*Thysanoessa raschii)* (10%) | NW Atlantic | Gulf of St Lawrence, Canada | Coastal, oceanic | 1992-2010 |
| Ryan et al. (2014) | 4 | -17.8 | 0.3 | 12.9 | 0.7 | ND | ND | More fish e.g., Atlantic herring, less northern krill (*Meganyctiphanes norvegica* and *Nyctiphanes couchii*) (<10%) | NE Atlantic | Celtic Sea | Coastal, oceanic | 2009-2011 |
| Fin whale (*Balaenoptera physalus*) | Haro et al. (2020) | N/A | ND | ND | ND | ND | 4.5 | Ecopath plus Ecosim | Fish, cephalopods | SE Pacific | SE Pacific, Magellan Strait, off Chile | Coastal, oceanic | N/A |
| Witteveen & Wynne (2016) | 6 | -18.6 | 1.0 | 12.5 | 0.8 | ND | ND | Copepods, krill | N Pacific | Gulf of Alaska, Kodiak Island | Oceanic islands | 2003, 2005 |
| Witteveen & Wynne (2016) | 9 | -18.3 | 0.7 | 12.5 | 1.2 | ND | ND | Copepods, krill | N Pacific | Gulf of Alaska, Shumagin Islands | Oceanic islands | 2004, 2005 |
| Wild et al. (2018) | 1 | -20.2 | 0.2 | 12.1 | 0.4 | ND | ND | Euphausiids, copopods, small schooling fish | N Pacific | Gulf of Alaska | Coastal, oceanic | 2016 |
| Gendron et al. (2001) | 2 | -16.0 | 0.6 | 15.4 | 1.1 | ND | ND | Euphausiids (e.g., *Nyctiphanes simplex*), small pelagic fish e.g., pilchard | Central E Pacific | Gulf of California | Coastal, oceanic | 1995, 1996 |
| MacKenzie et al. (2022) | 27 | -19.5 | 0.8 | 10.7 | 0.7 | 3.4 | 5 | Pelagic crustacea e.g., krill, amphipods, plus fish e.g., Atlantic herring, capelin | European Arctic | Near Svalbard and Barents Sea | Oceanic islands | 2012-2020 |
| Pauly et al. (1998) | Not given | ND | ND | ND | ND | 3.4 | 4 | Large zooplankton (80%), small squid (5%), small pelagics (5%), mesopelagics (5%), miscellaneous fishes (5%) | N Atlantic/ Norwegian Sea & unspecified | Unspecified | Unspecified | Unspecified |
| Gavrilchuk et al. (2014) | 69 | -18.6 | 0.4 | 12.4 | 1.3 | ND | ND | Zooplankton, Arctic krill (56%), northern krill (*Meganyctiphanes norvegica*) and American sandlace (40%) | NW Atlantic | Gulf of St Lawrence, Canada | Coastal, Oceanic | 1992-2010 |
| Aguila et al. (2014) | 55 | ND | ND | 9.8 | 0.4 | ND | ND | Northern krill | NE Atlantic | NW Spain | Coastal, Oceanic | 1983-1985 |
| Ryan et al. (2014) | 21 | -18.2 | 0.5 | 12.1 | 1.1 | ND | ND | Northern krill and *Nyctiphanes couchii* about 50% | NE Atlantic | Celtic Sea | Coastal, oceanic | 2009-2011 |
| Silva et al. (2019) | 42 | -19.4 | 0.8 | 9.5 | 0.7 | ND | ND | Euphausiids (37-81%), e.g., Northern krill and Arctic krill, epipelagic and mesopelagic schooling fish | Central N Atlantic | Azores | Oceanic islands | 2002-2014 |
| Blue whale  (*Balaenoptera musculus*) | Bury (unpublished data) | 1 | -25.5 |  | 6.9 | ND | ND | ND | Zooplankton | Antarctic | Ross Sea Shelf | Oceanic | 2015 |
| Gendron et al. (2001) | 2 | -18.2 | 0.6 | 12.9 | 0.3 | ND | ND | Euphausiids (e.g., *Nyctiphanes simplex*) | Central E Pacific | Gulf of California | Coastal, oceanic | 1995, 1996 |
| Pauly et al. (1998) | Not given | ND | ND | ND | ND | 3.2 | 4 | Large zooplankton (100%) | E Pacific, Norwegian Sea & unspecified | Unspecified | Unspecified | Unspecified |
| MacKenzie et al. (2022) | 21 | -18.7 | 1.3 | 9.4 | 0.6 | 3.0 | 5 | Pelagic crustacea e.g., Arctic krill, amphipods | European Arctic | Near Svalbard and Barents Sea | Oceanic islands | 2014-2019 |
| Ostrom et al. (1993) \* | 1 | -20.1 | ND | 9.6 | ND | 3.2 | 6 | Zooplankton | NW Atlantic | Waters off Newfoundland | Coastal, oceanic | 1986-1990 |
| Gavrilchuk et al. (2014) | 22 | -18.7 | 0.4 | 9.9 | 1.4 | ND | ND | Zooplankton, Arctic krill (70%), northern krill + American sandlace (26%) | NW Atlantic | Gulf of St Lawrence, Canada | Coastal, oceanic | 1992-2010 |
| Silva et al. (2019) | 17 | -18.7 | 1.0 | 9.1 | 0.7 | ND | ND | Euphausiids, e.g. Northern krill, Arctic krill and *Thysanoessa inermis* | Central N Atlantic | Azores | Oceanic islands | 2002-2014 |
| Minke whale  (*Balaenoptera acutorostrata*) | MacKenzie et al. (2022) | 17 | -19.4 | 0.4 | 12.2 | 1.3 | 3.9 | 5 | Generalist diet | European Arctic | Near Svalbard and Barents Sea | Oceanic islands | 2009-2019 |
| Ostrom et al. (1993) ^ | 1 | -18.3 | ND | 12.3 | ND | 4.1 | 6 | Zooplankton (euphausiids, crustaceans) small fish, small squid | NW Atlantic | Waters off Newfoundland | Coastal, offshore | 1986-1990 |
| Gavrilchuk et al. (2014) | 53 | -18.6 | 0.4 | 13.0 | 1.4 | ND | ND | Northern krill + fish (57%), capelin and Atlantic herring (22%) | NW Atlantic | Gulf of St Lawrence, Canada | Coastal, offshore | 1992-2010 |
| Pauly et al. (1998) | Not given | ND | ND | ND | ND | 3.4 | 4 | Large zooplankton (65%) | N Atlantic, NE Atlantic, Norwegian Sea, W Pacific, Antarctic & unspecified | Unspecified | Unspecified | Unspecified |
| Sei Whale  (*Balaenoptera borealis*) | Silva et al. (2019) | 36 | -17.5 | 1.0 | 9.0 | 0.6 | ND | ND | Predominantly calenoid copepods, also euphausiids | Central N Atlantic | Azores | Oceanic islands | 2002-2014 |
| Pauly et al. (1998) | Not given | ND | ND | ND | ND | 3.4 | 4 | Large zooplankton (80%), small squid (5%), small pelagics (5%), mesopelagics (5%), miscellaneous fishes (5%) | NE Atlantic/ Norwegian Sea & unspecified | Unspecified | Unspecified | Unspecified |
| Bryde's Whale  (*Balaenoptera edeni*) | Gendron et al. (2001) | 2 | -18.1 | 1.5 | 15.8 | 0.6 | ND | ND | Small pelagic fish, euphausiids | Central E Pacific | Gulf of California | Coastal | 1995, 1996 |
| Pauly et al. (1998) | Not given | ND | ND | ND | ND | 3.7 | 4 | Large zooplankton (40%), small pelagics (20%), mesopelagics (20%), higher invertebrates (20%) | NE Pacific, Arctic & unspecified | Unspecified | Unspecified | Unspecified |
| Northern right whale (*Eubalaena glacialis*) | Pauly et al. (1998) | Not given | ND | ND | ND | ND | 3.2 | 4 | Large zooplankton (100%) | NW Atlantic, N Atlantic & unspecified | Unspecified | Unspecified | Unspecified |
| Southern right whale (*Eubalaena mysticetus*) | Pauly et al. (1998) | Not given | ND | ND | ND | ND | 3.2 | 4 | Large zooplankton (100%) | Antarctic & unspecified | Unspecified | Unspecified | Unspecified |
| Best & Schell (1996) # | 11 | -26 to -16 | ND | 6 to 11 | ND | ND | ND | Antarctic krill, post-larval lobster krill, zooplankton | SE Atlantic | Off coast of S Africa | Coastal, oceanic | 1963-1994 |
| Bowhead whale  (*Balaena mysticetus*) | Hoekstra et al. (2002) | 84 | -21.0 | 0.3 | 13.2 | 0.4 | 2.9 | 7 | Zooplankton (calenoid copepods, euphausiids), pelagic & epibenthic crustaceans and invetebrates | N Pacific, Arctic | Bering–Chukchi–Beaufort Sea | Oceanic | 1997-2000 |
| Lee et al. (2005) | 27 | -20.7 | 0.6 | 13.2 | 0.7 | ND | ND | Zooplankton (copepods, euphausiids, amphipods, mysids) | N Pacific, Arctic | Bering–Chukchi–Beaufort Sea, Barrow and Kaktovik | Oceanic | 1986-1988 |
| Lee et al. (2005) | 25 | -19.5 | 0.5 | 14.3 | 0.8 | ND | ND | Zooplankton (copepods, euphausiids, amphipods, mysids) | N Pacific, Arctic | Bering–Chukchi–Beaufort Sea, Barrow and Kaktovik | Oceanic | 1997-1999 |
| Pomerleau et al. (2018) # | 4 | -19.0 | 0.7 | 14.2 | 1.5 | ND | ND | Zooplankton, mainly calenoid copepods | N Pacific, Arctic | Bering–Chukchi–Beaufort Sea | Oceanic | 1988-1996 |
| Pomerleau et al. (2018) # | 4 | -17.7 | 0.9 | 13.2 | 1.0 | ND | ND | Zooplankton, mainly calenoid copepods | N Pacific, Arctic | E Canadian Arctic/W Greenland | Oceanic | 1988-1996 |
| Pauly et al. (1998) | Not given | ND | ND | ND | ND | 3.2 | 4 | Large zooplankton (80%), benthic invertebrates (20%) | NE Pacific, Bering Sea, Arctic & unspecified | Unspecified | Unspecified | Unspecified |
| Matthews & Ferguson (2015) # | 14 | -18.2 | 0.3 | 13.6 | 0.3 | ND | ND | Pelagic copepods (primarily *Calanus spp*.) and euphausiids, epibenthic invertebrates | NW Atlantic | Eastern Canadian Arctic/West Greenland (Hudson Bay, Baffin Bay, Davis Strait) | Coastal, oceanic | 1998-2011 |
| Matthews & Ferguson (2015) # | 14 | -18.9 | 0.4 | 12.8 | 0.3 | ND | ND | Pelagic copepods (primarily *Calanus spp.*) and euphausiids, epibenthic invertebrates | NW Atlantic | E Canadian Arctic/W Greenland (Hudson Bay, Baffin Bay, Davis Strait) | Coastal, oceanic | 1998-2011 |
| Pygmy right whale  (*Caperea marginata*) | Pauly et al. (1998) | Not given | ND | ND | ND | ND | 3.2 | 4 | Large zooplankton (100%) | Unspecified | Unspecified | Unspecified | Unspecified |
| Sperm whale  (*Physeter macrocephalus*) | Wild et al. (2018) | 1 | -16.8 | 0.5 | 17.2 | 0.4 | ND | ND | Deep sea fish, including sablefish (*Anoplopoma fimbria*) and halibut (*Hippoglossus stenolepis*) from long-line fisheries, and squid | N Pacific | Gulf of Alaska | Coastal, offshore | 2015 |
| Wild et al. (2018) | 28 | -17.5 | 0.6 | 16.8 | 0.8 | ND | ND | Deep sea fish, including sablefish and halibut from long-line fisheries, and squid | N Pacific | Gulf of Alaska | Coastal, offshore | 2003-2017 |
| Pauly et al. (1998) | Not given | ND | ND | ND | ND | 4.4 | 4 | Large squid (60%), miscellaneous fishes (15%), small squid (10%), benthic invertebrates (5%), small pelagics (5%), mesopelagics (5%) | NE Atlantic, Norwegian Sea, NE Pacific, SE Pacific, W Pacific, SW Pacific & unspecified | Unspecified | Unspecified | Unspecified |
| MacKenzie et al. (2022) | 5 | -16.9 | 1.4 | 13.8 | 0.3 | 4.4 | 5 | Pelagic-benthic/coastal-offshore/sympagic open water prey sources | European Arctic | Near Svalbard and Barents Sea | Oceanic islands | 2020 |
| Ostrom et al. (1993) ^ | 1 | -22.8 | ND | 11.1 | ND | 3.7 | 6 | Mainly squid | NW Atlantic | Waters off Newfoundland Canada | Coastal, offshore | 1986-1990 |
| Troina et al. (2021) | 6 | -17.2 | 0.2 | 14.4 | 0.6 | 5.3, 0.5 | 8 | Deep water squid | SW Atlantic | Off SE coast of Brazil, S America | Coastal, offshore | 2009-2015 |
| Beluga Whale (*Delphinapterus leucas*) | Hoekstra et al. (2002) | 22 | -18.7 | 0.2 | 16.6 | 0.1 | 3.8 | 7 | Benthic and pelagic invertebrates, small fish | N Pacific, Arctic | Bering–Chukchi–Beaufort Sea | Coastal, oceanic | 1998-1999 |
| Lesage et al. (2001) Males | 11 | -16.7 | 0.2 | 15.8 | 0.6 | 4.6 | 9 | Benthic or demersal prey, invertebrates, small estuarine and pelagic fish, higher trophic level prey than females | NE Pacific | Gulf of St Lawrence, Canada | Coastal, estuarine | 1988-1990 |
| Lesage et al. (2001) Males | 4 | -16.2 | 0.3 | 16.3 | 1.1 | 4.8 | 9 | Benthic or demersal prey, invertebrates, small estuarine and pelagic fish, higher trophic level prey than females | NE Pacific | Gulf of St Lawrence, Canada | Coastal, estuarine | 1997 |
| Lesage et al. (2001) Females | 16 | -17.3 | 0.2 | 15.1 | 0.4 | 4.4 | 9 | Benthic or demersal prey, invertebrates, small estuarine and pelagic fish, in summer feed in 13C-depleted upper estuary | NE Pacific | Gulf of St Lawrence, Canada | Coastal, estuarine | 1988-1990 |
| Lesage et al. (2001) Females | 6 | -16.7 | 0.2 | 15.3 | 0.5 | 4.5 | 9 | Benthic or demersal prey, invertebrates, small estuarine and pelagic fish, in summer feed in 13C-depleted upper estuary | NE Pacific | Gulf of St Lawrence, Canada | Coastal, estuarine | 1997 |
| MacKenzie et al. (2022) | 10 | -17.2 | 0.5 | 17.5 | 0.5 | 5.7 | 5 | Coastal and benthic prey, preferred prey are ice-reliant gadoids | European Arctic | Near Svalbard | Coastal | 2013-2016 |
| Ostrom et al. (1993) ^ | 1 | -17.6 | ND | 13.6 | ND | 4.6 | 6 | Zooplankton, euphausiids, crustaceans, small fish, small squid | NW Atlantic | Waters off Newfoundland | Coastal | 1986-1990 |
| Pauly et al. (1998) | Not given | ND | ND | ND | ND | 4.0 | 4 | Miscellaneous fishes (40%), benthic invertebrates (20%), small pelagics (20%), mesopelagics (10%), large squid (5%), small squid (5%) | Unspecified | Unspecified | Unspecified | Unspecified |

**Trophic Position Equations**

1) TP = λ + (ẟ15Nconsumer - ẟ15Nbase)/∆n where λ = TP of the organisms used to estimate ẟ15Nbase and ∆n = enrichment of 15N per trophic level. A λ of 2 was assigned to Antarctic krill (*Euphausia superba*) using an enrichment of ∆n of 3.4 ‰.

2) TP = 2 + (ẟ15Nspecimen - ẟ15Nprimary consumer)/2.4, where 2 is the TP of the primary consumer and 2.4 is the mean ẟ15N enrichment per trophic level for marine mammals (Hobson et al., 1994; Post, 2002). Mean TP values for each feeding group were calculated by averaging the TP of individuals within feeding groups.

3) TP = 2 + (ẟ15Nhumpback whale - ẟ15Nscallop)/2:8, where 2 was the assumed TP of weathervane scallop (*Patonopecten caurinus*), which was used to define the baseline nitrogen isotope value. The trophic discrimination factor (TDF) of 2.8 was that measured between euphausiids and Fin whale skin (Borrell et al, 2012).

4) TL assigned from stomach contents/observation diet composition data. Diet composition (DCij) Trophic Levels (Tli) were computed for each whale species i*.* feeding on 8 prey types j, using the Pauly et al. (1998) equation:

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5) TP = (δ15Nspecies − δ15Nbase Δ15NT−D) + rTPbase where δ15Nspecies and δ15Nbase are the mean δ15N values of the species of interest and of the species used as a proxy baseline, respectively, Δ15NT-D is the TDF value and rTPbase is the relative TP of the proxy baseline species. Blue whales were assigned as the rTPbase with a value of 3, and Δ15NT-D had a value of 3 as a relative scaling factor (Hobson and Welch 1992; Hobson et al. 1996; Hoekstra et al. 2002; e.g., Matthews et al. 2020).

6) TP assigned from ẟ15N, using a TDF of 3 ‰ and defining basking shark as an operational TP 1. Since basking shark consume zooplankton and small crustaceans 2 TP were added to each value to bring their estimates in line with usual TP definitions.

7) TP = 2 + (δ15Nconsumer – δ15N*Calanus spp.*)/3.8, TDF was taken to be 3.8 ‰ following Hobson & Welch (1992).

8) TP estimated using 15N in trophic (Tr) and source (Sr) amino acids (AAs), using a multiple amino acids equation. weighted mean δ15N values of the trophic AAs (Tr-AA) Alanine, Aspartate, Glutamate, Isoleucine (Ile), Leucine and Valine (δ15NTr-AA) and the source AAs (Sr-AA) Phenylalanine and Lysine (δ15NSr-AA). A TDF of 3.1, 0.4 ‰ (Ruiz-Cooley et al., 2021) and a β of 3.4, 0.9 ‰ (Nielsen et al. 2015) was used.

TPTr− Sr = (δ15NTr− AA − δ15NSr− AA − β)/TDF) +1

9) TP = 2 + (Dm – POM – TDFmmt)/TDF, where Dm = δ15N value in a consumer’s tissue, TDFmmt is the marine mammal TDF, POM = δ15N value of particulate organic matter, and TDF is 2.4, calculated from 15N values of muscle from captive seal feeding experiments (Hobson et al, 1996)

**FIGURES**

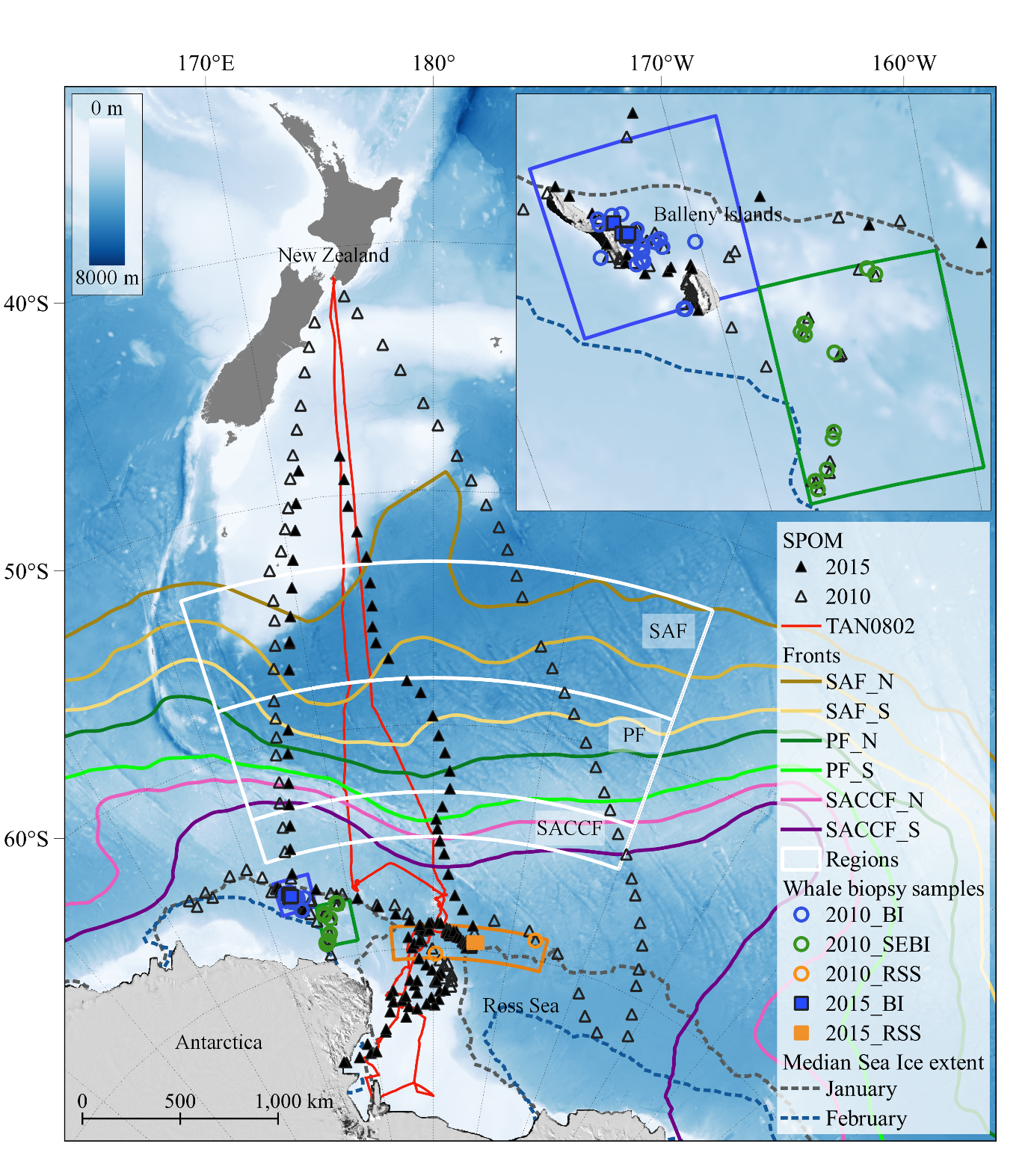
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Fig. 1. Map showing the coverage of the International Polar Year-Census of Marine Life voyage (TAN0802, January-March 2008), the Antarctic Whale Expedition voyage (TAN1002, February-March 2010) and the New Zealand-Australia Antarctic Ecosystems voyage (TAN1502, February-March 2015). The locations of suspended particulate organic material (SPOM) samples are shown as open black triangles for the 2010 voyage, and filled black triangles for the 2015 voyage, which also indicate the ship’s tracks in 2010 and 2015. The ship’s track in 2008 is marked by the solid red line (TAN0802) – no SPOM samples were taken on this voyage. The location of humpback whale (*Megaptera novaeangliae*) skin biopsies sampled during 2010 (open circles) and 2015 (filled squares) are shown, with finer scale detail of sampling locations around the Balleny Islands illustrated in the insert map. Whale skin sample location abbreviations are: BI (Balleny Islands), SEBI (south-east Balleny Islands), RSS (Ross Sea slope). The location of the major oceanographic fronts (after Sokolov and Rintoul, 2009) are marked as SAF\_N (Subantarctic Front, northern boundary), SAF\_S (Subantarctic Front, southern boundary), PF\_N (Polar Front, northern boundary), PF\_S (Polar Front, southern boundary), SACCF\_N (Southern Antarctic Circumpolar Current Front, northern boundary) and SACCF\_S (Southern Antarctic Circumpolar Current Front, southern boundary). The location of the median sea ice extent between 1981 and 2010 is also included for the months of January and February using data from the National Snow and Ice Data Centre (Fetterer et al., 2017). Bathymetry was generated from the General Bathymetric Chart of the Oceans (GEBCO), sourced online at <https://www.gebco.net/data_and_products/gridded_bathymetry_data/>.

A map of the united states

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Fig. 2. Map showing the sampling locations of potential humpback whale (*Megaptera novaeangliae*) prey samples taken during the 2008, 2010 and 2015 voyages. ZOO = zooplankton, EUS = Antarctic krill (*Euphausia superba*), MYC = myctophids (5 *spp*.: *Electrona carlsbergi*, *E. antarctica, Gymnoscopelus nicholsi*, *G. opisthopterus and G. braueri*), ANS = Antarctic silverfish (*Pleuragramma antarctica*). BI denotes Balleny Islands and RS Ross Sea. The colour of the species symbols match those in Fig. 5.

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Fig. 3. Lipid-extracted carbon and bulk nitrogen stable isotope biplot of 2010 and 2015 humpback whale (*Megaptera novaeangliae*) skin biopsy samples, showing sampling year and location for panel a) all values, and panel b) mean and standard deviation (±1 SD) values. Abbreviations in the legend are: BI Balleny Islands, SEBI south-east Balleny Islands, RSS Ross Sea slope, and *n*= denotes the number of samples taken on each voyage in each region.

A diagram of a cluster of clusters

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Fig. 4.Lipid-extracted carbon and bulk nitrogen stable isotope biplot showingstandard ellipse area corrected for small sample size (SEAc, solid line ovals) and convex hull area (TA, area within dotted lines) for humpback whales (*Megaptera novaeangliae*) (see Table S3 for definitions). Ellipse areas hold 40% of the data. A niche comparison using **“**K minus means cluster analysis” (MacQueen 1967; Lloyd 1982) defined two clusters of individual humpback whales based on their respective δ15N and δ13C values: cluster A (open triangles) and cluster B (filled triangles). Males and females are depicted as red and blue symbols respectively. See Figs. 3 and S3 to view year and location of whale biopsy samples for each of the isotopically segregated clusters A and B.

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Fig. 5. Panel a) Lipid-extracted or lipid-corrected carbon and bulk nitrogen stable isotope biplot of humpback whale (*Megaptera novaeangliae*) skin and muscle tissue of potential prey sampled in 2008, 2010 and 2015 from waters around the Balleny Islands (BI), the Ross Sea slope (RSS) and the Ross Sea (RS); panel b) carbon and nitrogen stable isotope biplot of humpback whale skin means (±1 SD) from 2010 and 2015 voyages, and muscle tissue means (±1 SD) of potential prey sampled in 2008, 2010 and 2015 from waters around BI, RSS and RS. Bold numbers on the plot refer to the prey clusters identified in the dendogram of Fig. S2; panel c) carbon and nitrogen stable isotope prey polygon biplot showing meanss (±1 SD) of potential humpback whale prey clusters (as determined in dendrogram, Fig. S2). Humpback whale skin means (±1 SD) for “all whales”, “cluster A” and “cluster B” whales are shown with the Borrell et al. (2012) trophic discrimination factors (TDFs: 1.28, 0.38 ‰ for δ13C, and 2.82, 0.30 ‰ for δ15N) subtracted. Error bars for whale skin isotope values are shown in panel b) but have been omitted in panel c) for image clarity. Colours represent prey clustering used in Ward’s Hierarchical analysis (Fig. S2) and MixSIAR Bayesian mixing model output results (Fig. 6).

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Fig. 6. The posterior distributions of the proportional contributions of each prey cluster to the diet of humpback whales estimated using MixSIAR (Stock et al., 2018) applying the Borrell et al. (2012) 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 95, 90, 75 and 50% are plotted for each prey cluster with decreasing bar thickness and colour intensity. 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.

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Fig. 7. Amino acid δ15N values (δ15NAA) of humpback whale (*Megaptera novaeangliae*)skin and whale trophic position (TP) plotted against bulk δ15N values. Glutamic acid (“trophic amino acid”) is shown in green, phenylalanine (“source amino acid”) in orange and TP in black: panel a) depicts males (filled triangles) and females (open circles); panel b) differentiates humpback whales in cluster A (open triangles) and cluster B (filled triangles).

Chart, scatter chart

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Fig. 8. Nitrogen isotope values of glutamic acid (“trophic amino acid”, δ15NGlx) plotted against phenylalanine values (“source amino acid”, δ15NPhe) for humpback whale (*Megaptera novaeangliae*)skin samples. Trophic isoclines with a slope of 1.0 and y-intercept intervals of 3.58 ‰ represent different trophic positions (TPs = 2, 3, 4 and 5). These isoclines are calculated according to CSIA data-derived trophic discrimination factor of the whale: TDFwhale = (Glx-Phe - 3.4)/(TPwhale-1) = 3.58, where TPwhale is 3.32 based on simple arithmetic TP calculation from bulk nitrogen isotope data (see Methods: Estimation of humpback whale trophic position, and Table S11).

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Fig. 9. Mean posterior probability densities for spatial assignment (c.f. Wunder 2010) of individual humpback whale (*Megaptera novaeangliae*) foraging areas using spatial models (isoscapes) for δ13Cand δ15N in suspended particulate organic matter of the Southern Ocean (St. John Glew & Espinasse et al. 2021). Panels a) and b) are the assignments for cluster A and B whales respectively, using only carbon isotope data. Panels c) and d) are the assignments for cluster A and B whales respectively, using both carbon and nitrogen isotope values. Posterior probability densities for the spatial assignment of individual cluster B humpback whale foraging regions are provided in Fig. S9.

A screenshot of a map

Description automatically generatedFig. 10: Southern Ocean spatial variability of ẟ13C (panel a) and ẟ15N (panel c) values of suspended particulate organic material (SPOM) derived from the St John Glew & Espinasse et al. (2021) isoscapes model (baseline map) and field-measured SPOM ẟ13C and ẟ15N values (filled circles). Panels on the right show the ẟ13C (panel b) and ẟ15N (panel d) modelled isoscape for SPOM and field data for the regional area around the Balleny Islands. The letters A and B in panels b) and d) indicate the locations of cluster A and cluster B whales when skin biopsies were sampled.

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Fig. 11: Variation of ẟ13C values in surface suspended particulate organic matter (SPOM) with latitude. “Isoscape model SPOM” are the “no interactions” modelled SPOM isoscape values from St John Glew & Espinasse et al. (2021); “Transect SPOM” are field measured ẟ13C SPOM values from 2010 and 2015 voyages on transects from New Zealand to the Ross Sea; “Balleny Islands SPOM” are ẟ13C SPOM data from the same voyages measured in the vicinity of the Balleny Islands. The Borrell et al. (2012) ẟ13C trophic enrichment factor was subtracted from all plotted humpback whale (*Megaptera novaeangliae*)skin values to enable comparison to SPOM ẟ13C data: the grey solid line is the median ẟ13C value of cluster A humpback whale skin, whilst the black solid line is the median ẟ13C value of skin from Cluster B whales. The white and black filled histograms are the frequency distributions of ẟ13C values for cluster A and cluster B whales respectively.

SUPPLEMENTARY MATERIALS are supplied as a separate electronic file.