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Feeding ecology and population delineation of south-eastern Atlantic and south-western Indian Ocean humpback whales using stable isotope analysis

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Abstract

1. Population delineation is vital for effectively managing and protecting populations of all at-risk species. Population boundaries of Southern Hemisphere humpback whales on their breeding and feeding grounds have not been fully resolved. A number of methods have been used to delineate breeding stocks of Southern Hemisphere humpbacks, but ecological characteristics determined via stable isotope analysis provide valuable information to contrast other data sources.
2. In this study, stable isotope analysis is used to investigate potential separation of humpback whale populations on Southern Hemisphere feeding grounds as evidenced by carbon and nitrogen isotope values in their skin as proxies of diet.
3. One hundred samples of whale skin obtained from biopsies in sampling localities off the coasts of Gabon, Mayotte (Mozambique Channel), and Madagascar were analysed for carbon (δ^{13}C) and nitrogen (δ^{15}N) stable isotope ratios. The results showed a statistically significant difference in the mean δ^{15}N values for whales between the populations from Gabon and Madagascar (7.0 ± 0.1‰ and 7.6 ± 0.1‰), and Gabon and Mayotte (7.6 ± 0.1‰ and 7.2 ± 0.1‰), indicating that these breeding stocks are potentially visiting different areas of the feeding grounds outside of the breeding season.
4. The results from this study indicate that at least some breeding stocks may show fidelity to separate feeding areas and do not widely mix with individuals from other breeding stocks while feeding.

KEYWORDS
ecological status, feeding, mammals, ocean

1 | INTRODUCTION

Eco-geochemical analysis of the stable isotope ratios of nitrogen (δ^{15}N) and carbon (δ^{13}C) of skin can be useful for tracking diets, animal movement patterns, and delineating metapopulations of marine mammals (Newsome, Clementz, & Koch, 2010). Stable isotopes of nitrogen and carbon in tissues such as skin, hair, and blood directly reflect the diets of individual animals in all ecosystems. In marine systems, variation in these isotope ratios comes from variation in the base of the marine food web (phytoplankton) due to factors such as upwelling, primary production, and the local dissolved inorganic carbon pool (McMahon, Ling Hamady, & Thorrold, 2013), which are then propagated up the food chain and enriched as trophic levels increase.

Marine mammals are ecosystem sentinels (Moore, 2008), and stable isotopic analysis of migratory whales is proving an effective means of gleaning information about the changing climate (Bengtson Nash et al., 2018; Seyboth et al., 2018) and human

impacts on the ocean (Das et al., 2017). Owing to their elusive nature (migratory, often do not feed at the surface), specific dietary information of whales is usually obtained infrequently through surface observation or gut contents during necropsy when a dead whale is located. Though these methods are useful, they are rare and provide only a snapshot of the diet at the time the sample was taken. Stable isotope analysis gives an integrated look at the assimilated diet of marine mammals (Newsome et al., 2010) and, using biopsy sampling from free-ranging whales, is a valuable analytical method that allows us to obtain this information with a non-lethal sampling technique (Brierley & Clapham, 2016; Hunt et al., 2013; Lambertsen, 1987; Todd, Ostrom, Lien, & Abrajano, 1997).

Humpback whales (*Megaptera novaeangliae*) are migratory baleen whales that spend winters at low-latitude breeding areas and migrate to higher latitude feeding areas during the summer. The diet of humpback whales is typically defined as generalist, with their prey composition likely being representative of the dominant prey types in the ecosystem (Fleming, Clark, Calambokidis, & Barlow, 2016). Changes in their isotope composition would, therefore, be expected to reflect environmental variability (Bengtson Nash et al., 2018). Witteveen, Worthy, and Roth (2009) found that humpback whales in the North Pacific could be assigned to feeding groups from a breeding group using stable isotopes. Other isotopic studies of whales off the Antarctic Peninsula show that temporal and spatial variation can be seen in the δ^{15}N and δ^{13}C of both krill and whale skin samples, indicating stable isotopes in marine mammals do detect this regional environmental variability (Seyboth et al., 2018).

Significant dietary differences can exist regionally and between different populations due to spatial and temporal separation on feeding areas (Fleming et al., 2016; Johnson & Wolman, 1984; Witteveen, Worthy, Foy, & Wynne, 2012). Humpback whales in the Northern Hemisphere typically have diets of both benthic and pelagic organisms, such as sardine, herring, krill, and other small schooling fish (Clapham, Leatherwood, Szczepaniak, & Brownell, 1997). In contrast, the Southern Ocean near Antarctica is a krill-based ecosystem, where krill functions as the major prey resource for large mammals like fin whales (*Balaenoptera physalus*) and humpbacks (Nicol, Worby, & Leaper, 2008). Whales in the Southern Hemisphere that feed in sub-Antarctic and Antarctic waters have a diet restricted almost entirely to krill (Nicol et al., 2008).

There are seven genetically distinct 'breeding stocks' (termed BSA to BSG) of humpback whales managed by the International Whaling Commission (IWC) in the Southern Hemisphere (IWC, 2011; Rosenbaum et al., 2017). Table 1 defines all breeding stocks worldwide. It was originally assumed that most Southern Hemisphere humpback whale breeding stocks remained segregated on both breeding areas and corresponding feeding areas in sub-Antarctic and Antarctic waters, with limited dispersal between stocks (Friedlaender et al., 2006). As such, the IWC originally recognized six circumpolar Management Areas (I–VI) in the Southern Ocean that represent feeding areas reported by the whaling industry, in order to inform estimates of historical abundance and post-whaling recovery of the associated stock. However, more recent genetic analyses suggest humpback whales from geographically separate and genetically distinct breeding stocks and substocks converge and significantly mix with one another on feeding areas (Amaral et al., 2016; IWC, 2010; Kershaw, 2015; Schmitt et al., 2014). An alternative hypothesis, termed 'Allocation Hypothesis 1' (hereafter, AH1), was recently proposed by the IWC that better accounts for the biology and behaviour of the species (IWC, 2010). AH1 comprises a binary designation of two areas: 'Nucleus' areas, where 100% of catches are allocated to the associated breeding stock (i.e. discrete stock feeding areas), and 'Margin' areas, where 50% of catches are allocated to the adjacent stocks to the east and west (i.e. mixed stock feeding areas) (IWC, 2010; Figure 1).

TABLE 1 The locations of all breeding stocks around the world and their substock designations. List adapted from Rosenbaum et al. (2017)

Breeding stock (BS)	Substock
(A) South-western Atlantic Ocean	
(B) South-eastern Atlantic Ocean	B1 Gabon, Cabinda
	B2 West South Africa
(C) South-western Indian Ocean	C1 Mozambique, East South Africa
	C2 Mayotte and Geyser, Comoros
	C3 Madagascar
(ASHW) Northern Indian Ocean	
(D) South-eastern Indian Ocean	
(E1) South-western Pacific Ocean	
(E) Western Pacific Islands of Oceania	E2 New Caledonia
	E3 Tonga
(F) Western Pacific Islands of Oceania	F (CI) Cook Islands
	F (FP) French Polynesia
(G) South-eastern Pacific Ocean	

Breeding stocks in the south-east Atlantic Ocean (BSB) and western Indian Ocean (BSC), and the substocks therein (BSB1 and B2, and BSC1, C2, C3, and C4; however, no regional comparative genetic analysis has been carried out on BSC4 to date), have been shown to exhibit different patterns of fidelity and mixing on feeding areas (Albertson-Gibb et al., 2008; Amaral et al., 2016; Kershaw, 2015; Schmitt et al., 2014). This may indicate differences in the timing of establishment of feeding areas, as well as the contemporary mixing of different stocks and substocks (Amaral et al., 2016). Whales sampled from the breeding area off Gabon in the Gulf of Guinea (BSB1) exhibit relatively higher levels of genetic differentiation on feeding areas, and analyses support that the westward Margin and Nucleus areas for BSB do appear to represent the primary feeding areas for this breeding stock, as currently hypothesized under AH1 (Amaral et al., 2016; IWC, 2010; Kershaw, 2015). In contrast, whales observed migrating past and feeding off western South Africa (BSB2) and the substocks of BSC in the western Indian Ocean show relatively high levels of mixing across feeding areas (Kershaw, 2015), mirroring the high levels

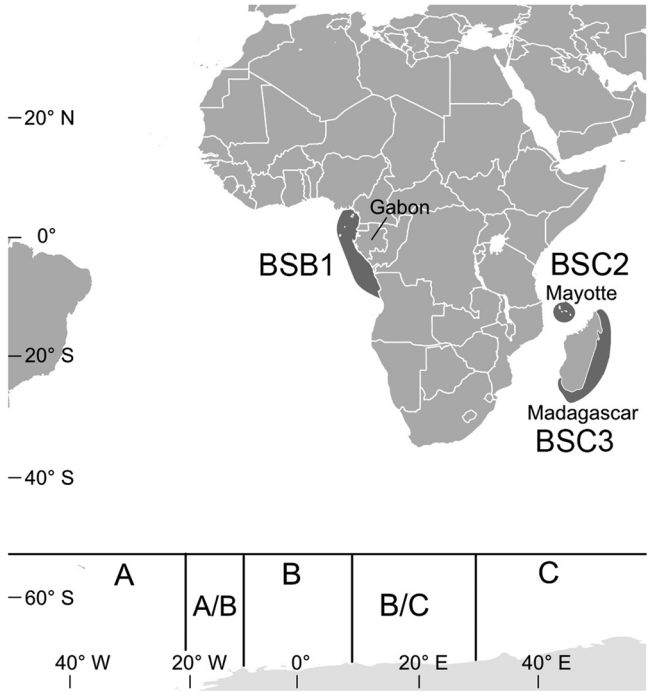

FIGURE 1 A map of the regions where the whale biopsies were collected, denoted by grey shaded areas and labelled with breeding substocks. Nucleus and Margin feeding areas as denoted by the International Whaling Commission under Allocation Hypothesis 1 (AH1) are labelled for the map area. Views of the wider breeding stock structure can be found in Rosenbaum et al. (2017)

of connectivity previously observed between the corresponding breeding areas for these substocks (Best et al., 1998; Carvalho, Brito, dos Santos, & Rosenbaum, 2011; Cerchio et al., 2016; Ersts et al., 2011; Fossette et al., 2014; Kershaw et al., 2017; Rosenbaum et al., 2009; Rosenbaum et al., 2017). BSC is broadly associated with Management Area III (Amaral et al., 2016), and assessments of population structure show that BSC1–BSC3 exhibit significant genetic differentiation from the BSB Nucleus feeding area but not the BSB/BSC Margin or BSC Nucleus (IWC, 2011). Satellite-tracked animals off BSC2 have also been observed to move south-eastwards towards the French sub-Antarctic Islands and Management Area III (0–70°E; IWC, 2013). Collectively, these findings indicate general support for the feeding area designation for BSC under AH1 (IWC, 2010).

Even in light of this existing research, few data exist to fully assess the distribution and significance of mixing of Southern Hemisphere breeding stocks on feeding areas. Prior to their protection by the IWC in 1963, some 215,000 humpback whales were hunted in the Southern Hemisphere as a result of 'open-boat' (i.e. 18th and 19th centuries) and more recent 20th-century modern whaling, including heavy illegal Soviet whaling (Findlay, 2001; Rocha, Clapham, & Ivashchenko, 2014). Resolving gaps in knowledge of the distribution of breeding stocks on feeding areas has direct relevance for informing estimates of pre-exploitation sizes and assessments of recovery of the species from whaling. These uncertainties regarding the delineation of humpback whale populations preclude a robust recovery assessment for all regions (Rosenbaum et al., 2017).

In this study, the aim is to better understand potential separation of humpback whale populations on Southern Hemisphere feeding areas by investigating differences in feeding ecology between BSB and BSC using stable isotope ratios of skin samples. This method of assessing regional differences of humpback whale diet has been successful in whale populations from the North Pacific (Filatova et al., 2012; Witteveen et al., 2012) and on numerous other species of cetaceans (Kiszka, Simon-Bouhet, Gastebois, Pusineri, & Ridoux, 2012; Mèndez-Fernandez et al., 2012), but it has never before been applied to Southern Hemisphere humpback whale populations in a comparative framework. Specifically, answers are sought to the following questions: (1) Is there a difference in stable isotope ratios of carbon and nitrogen between breeding stocks and/or between years of sampling? (2) Do stable isotope ratios of the whale skin reflect the estimated feeding area and diet composition? The results are interpreted in the context of the current understanding of the genetic structure of these breeding stocks in order to better understand how stable isotope analysis can be used alongside population genetics to inform marine mammal conservation and research.

2 | METHODS

2.1 | Sample collection

The humpback skin analysed for δ^{15}N and δ^{13}C was obtained via biopsy darts from populations in Gabon (BSB1), Mayotte (BSC2), and Madagascar (BSC3, Antongil Bay) (Figure 1). Skin tissues were collected as part of a larger sampling effort as detailed in Rosenbaum et al. (2009). The skin of cetaceans represents a time-averaged measurement of diet. A recent study of blue whale (*Balenoptera musculus*) skin found it has a mean isotopic incorporation time of 163 ± 91 days (Busquets-Vass et al., 2017). It is assumed that humpback whales will have similar isotopic incorporation times for their skin. One hundred biopsies were selected randomly to analyse for stable isotopes collected in the years 1998, 2000, 2004, and 2005. The samples were obtained following all appropriate regulations by permitting agencies in each location. The skin tissues are stored in tubes containing 95% ethanol in −20°C freezers at the Wildlife Conservation Society (Bronx, NY, USA).

It has been shown that ethanol-preserved cetacean epidermis biopsy samples can be utilized in general trophic studies, but there is some concern of alteration of δ^{13}C values after storage (Kiszka, Lesage, & Ridoux, 2014). Ethanol preservation does not seem to impact nitrogen isotopes, but it has more of an impact on carbon isotopes. It has been calculated that this difference can be up to 0.5‰ (Kiszka et al., 2014). However, in this study, mixing models are not used and there is no fine-scale trophic differentiation where small differences in stable isotope values are absolutely critical; thus, it will not have a deleterious effect on the conclusions.

2.2 | Stable isotope analysis

Small pieces of the preserved skin biopsy (~5 mg) were subsampled for isotopic analysis. Stable isotope analysis was conducted at the Environment and Natural Resources Institute Stable Isotope Laboratory at the University of Alaska, Anchorage (AK, USA). Skin samples were analysed with lipid extraction treatment using a modified Bligh and Dyer (Logan & Lutcavage, 2008) method. Skin samples were dried and homogenized and then immersed in a 2:1 ratio of chloroform/methanol with a solvent volume approximately three to five times the sample volume. Samples were mixed for 30 s, left to sit for 30 min, centrifuged for 10 min at 3,400 rpm, and the supernatant containing the solvent and the lipids was removed. This process was repeated until the supernatant was clear and colourless following centrifugation; samples were then redried at 50°C for 24 h to remove any remaining solvent.

Extracted samples were analysed for δ^{13}C and δ^{15}N using an ECS 4010 elemental analyser interfaced to a ThermoFinnigan Delta V Advantage continuous-flow isotope ratio mass spectrometer. This instrument was calibrated against international reference standards from the International Atomic Energy Agency and the United States Geological Survey. Internal standards, including purified methionine, homogenized peach leaf, and homogenized bowhead whale baleen, were used for sample quality control. Stable isotope ratios were reported in delta notation as per mil determined using the equation $\delta = (R_{sample}/R_{standard}) - 1$, where R is the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio. Records of internal standards yield an analytical precision of 0.2‰ for δ^{15}N values and 0.1‰ for δ^{13}C values. Data were examined for normality using a Shapiro–Wilkes test, and δ^{15}N values are normally distributed (W = 0.9888, P = 0.5674) but δ^{13}C are not (W = 0.9679, P = 0.01536), so non-parametric tests were used for comparing means between groups for both isotope systems. Kruskal–Wallis and non-parametric post hoc tests were performed using the R package pgirmess (Giraudoux, 2016) to compare the means of different years and sampling locations. Plots were created in R using ggplot2 (Wickham, 2009).

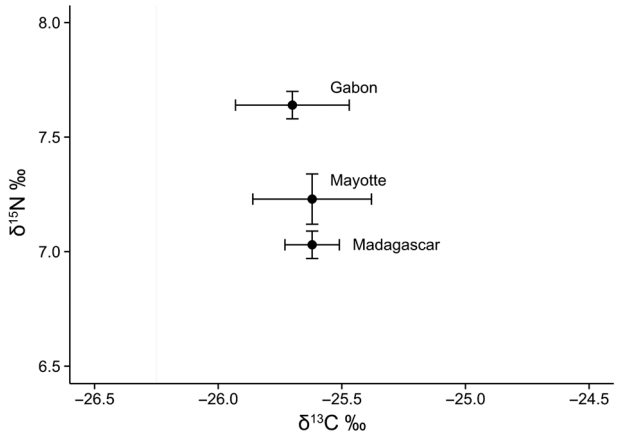

FIGURE 2 Bivariate plot of mean nitrogen and carbon isotope values for all years combined for each sampling region (mean ± 1 *SE*)

3 | RESULTS

For all years pooled per location (Figure 2), mean values plus/minus *SE* for δ^{13}C and δ^{15}N are: −25.7 ± 0.2‰, 7.6 ± 0.1‰ (BSB1: Gabon, n = 43), −25.6 ± 0.2‰, 7.2 ± 0.1‰ (BSC2: Mayotte, n = 13), and −25.6 ± 0.1‰, 7.0 ± 0.1‰ (BSC3: Madagascar, n = 44). A summary of all descriptive statistics for each year and region is presented in Table 2. Full descriptions of Kruskal–Wallis test results are found in Table 3. Results from pairwise post hoc tests divided by year are presented in Table 4. Results of post hoc tests divided only by region are presented in Table 5. Statistical analysis (Kruskal–Wallis) indicates there is a difference in mean δ^{13}C between years in Gabon (df = 2, χ^2 = 10.0882, P = 0.0064), and pairwise post hoc tests indicate this region shows a difference in mean δ^{13}C between sampling years 2004 and 2005. The δ^{13}C mean in Madagascar remained indistinguishable across all three sampling years (df = 2, χ^2 = 5.9772, P = 0.0504). For δ^{15}N, analysis of variance shows there is no difference between years in Madagascar (df = 2, χ^2 = 1.9899, P = 0.3697) or Gabon (df = 2, F = 0.5728, P = 0.751). When sampling years are combined and data

TABLE 2 Stable isotope values. The column heading *n* represents the number of skin samples analysed for each year and regional category. Values in italics for Madagascar and Gabon are a summary of all three years of isotope data

			δ^{13}C (‰)			δ^{15}N (‰)		
Region	**Year**	***n***	**Mean**	***SE***	**Min, max**	**Mean**	***SE***	**Min, max**
Madagascar	2000	15	−25.3	0.2	−26.6,−24.1	7.1	0.1	6.2,8.0
Madagascar	2004	14	−25.6	0.2	−26.5,−23.3	7.1	0.1	6.5,7.5
Madagascar	2005	15	−26.0	0.1	−26.7,−24.9	6.9	0.1	6.3,7.4
Madagascar (BSC3)	*All*	*44*	*−25.6*	*0.1*	*−26.7,−23.3*	*7.0*	*0.1*	*6.2, 8.0*
Gabon	2000	14	−25.4	0.5	−28.4,−21.8	7.7	0.1	6.9,8.6
Gabon	2004	14	−26.6	0.3	−28.5,−24.3	7.6	0.1	7.1,7.9
Gabon	2005	15	−25.1	0.3	−26.8,−23.0	7.7	0.1	6.9,8.6
Gabon (BSB1)	*All*	*43*	*−25.7*	*0.2*	*−28.5,−21.8*	*7.6*	*0.1*	*6.9,8.6*
Mayotte (BSC2)	1998	13	−25.6	0.2	−27.38,-23.8	7.2	0.1	6.6,7.8

TABLE 3 Results from non-parametric Kruskal–Wallis tests done on different data groupings. Significant *P*-values are in bold

Variable	*df*	*F*	*P*-value
δ^{13}C between all years	6	19.0511	**0.004078**
δ^{15}N between all years	6	41.2401	**2.60×10^{-7}**
δ^{13}C between regions	2	0.352	0.8386
δ^{15}N between regions	2	40.1558	**1.91×10^{-9}**
δ^{13}C Madagascar	2	5.9772	0.05036
δ^{13}C Gabon	2	10.0882	**0.006447**
δ^{15}N Madagascar	2	1.9899	0.3697
δ^{15}N Gabon	2	0.5728	0.751

are pooled by region, there is no difference in δ^{13}C (df = 2, χ^2 = 0.352, P = 0.8386) but there is a significant difference between δ^{15}N (df = 2, χ^2 = 40.1558, $P = 1.91 \times 10^{-9}$), with significant differences between Gabon and Madagascar and Gabon and Mayotte. Post hoc difference tests following the Kruskal–Wallis test of the regional data divided by year indicate differences in δ^{15}N in all pairwise year comparisons between Gabon and Madagascar. These same regional data divided by year for δ^{13}C show a difference in means between Madagascar 2000 and Gabon 2004, and Gabon 2004 and Gabon 2005. All isotope values measured and the code used for statistical analysis can be found in the Supplementary Information.

4 | DISCUSSION

The results reveal a relationship between stable isotope values and breeding stock and substock delineations. Similarity between isotopic values and mitochondrial haplotypes and kinship estimators observed in southern right whales sampled on the Península Valdés wintering ground (Valenzuela, Sironi, Rowntree, & Seger, 2009), and Australia (Carroll et al., 2015), indicates a common thread of maternally directed fidelity and migratory culture as potential influences of genetic structure of baleen whales on feeding areas. Though we have only sampled 100 whales, which is similar to other studies (e.g. Valenzuela et al., 2009), the observed differences between groups are significant and indicate this is a method that can and should be used on other whale populations for future study. This study is limited to only comparing years and no other variables, so future studies can and should compare isotope ratios on the breeding grounds by other factors, like sex

TABLE 4 Multiple comparison post hoc pairwise tests after Kruskal–Wallis between each sampling locality divided by year for δ^{13}C and δ^{15}N comparisons. Significant differences are denoted as TRUE and are in bold

	δ^{13}C			δ^{15}N		
	Difference			Difference		
	Observed	Critical	Different?	Observed	Critical	Different?
Mayotte–Gabon2000	1.631868	33.94804	FALSE	26.8296703	33.94804	FALSE
Mayotte–Gabon2004	27.93956	33.94804	FALSE	25.8296703	33.94804	FALSE
Mayotte–Gabon2005	11.512821	33.39875	FALSE	27.6820513	33.39875	FALSE
Mayotte–Madagascar2000	9.446154	33.39875	FALSE	9.5179487	33.39875	FALSE
Mayotte–Madagascar2004	1.510989	33.94804	FALSE	8.5989011	33.94804	FALSE
Mayotte–Madagascar2005	12.687179	33.39875	FALSE	18.5179487	33.39875	FALSE
Gabon2000–Gabon2004	29.571429	33.31344	FALSE	1	33.31344	FALSE
Gabon2000–Gabon2005	9.880952	32.75351	FALSE	0.852381	32.75351	FALSE
Gabon2000–Madagascar2000	7.814286	32.75351	FALSE	**36.347619**	**32.75351**	**TRUE**
Gabon2000–Madagascar2004	3.142857	33.31344	FALSE	**35.4285714**	**33.31344**	**TRUE**
Gabon2000–Madagascar2005	14.319048	32.75351	FALSE	**45.347619**	**32.75351**	**TRUE**
Gabon2004–Gabon2005	**39.452381**	**32.75351**	**TRUE**	1.852381	32.75351	FALSE
Gabon2004–Madagascar2000	**37.385714**	**32.75351**	**TRUE**	**35.347619**	**32.75351**	**TRUE**
Gabon2004–Madagascar2004	26.428571	33.31344	FALSE	**34.4285714**	**33.31344**	**TRUE**
Gabon2004–Madagascar2005	15.252381	32.75351	FALSE	**44.347619**	**32.75351**	**TRUE**
Gabon2005–Madagascar2000	2.066667	32.18384	FALSE	**37.2**	**32.18384**	**TRUE**
Gabon2005–Madagascar2004	13.02381	32.75351	FALSE	**36.2809524**	**32.75351**	**TRUE**
Gabon2005–Madagascar2005	24.2	32.18384	FALSE	**46.2**	**32.18384**	**TRUE**
Madagascar2000–Madagascar2004	10.957143	32.75351	FALSE	0.9190476	32.75351	FALSE
Madagascar2000–Madagascar2005	22.133333	32.18384	FALSE	9	32.18384	FALSE
Madagascar2004–Madagascar2005	11.17619	32.75351	FALSE	9.9190476	32.75351	FALSE

TABLE 5 Multiple comparison post hoc pairwise test of all years sampled pooled by sampling localities for δ^{13}C and δ^{15}N comparisons. Differences in pairs are noted as TRUE and in bold

	Observed difference	Critical difference	Different?
δ^{13}C			
Mayotte–Gabon	4.549195	21.98258	FALSE
Mayotte–Madagascar	1.585664	21.92451	FALSE
Gabon–Madagascar	2.963531	14.89325	FALSE
δ^{15}N			
Mayotte–Gabon	26.80143	21.98258	**TRUE**
Mayotte–Madagascar	12.29371	21.92451	FALSE
Gabon–Madagascar	39.09514	14.89325	**TRUE**

and month of sampling, to see whether there are other patterns of variation in stable isotope ratios.

4.1 | Isotopic differentiation of breeding stocks

The majority of the stable isotope ratio differentiation occurs between the δ^{15}N values of the breeding stocks and substocks investigated in this study (Tables 3 and 4). There is a significant difference between mean δ^{15}N values of Gabon (BSB1) and Madagascar (BSC3) for each year (Table 3). The δ^{15}N of Gabon (BSB1) is different from each year of the Mayotte (BSC2) samples except for in 2004. The δ^{13}C means are not different except for one pair of years. Since δ^{13}C of plankton varies based on the local dissolved inorganic carbon pool (McMahon, Ling Hamady, & Thorrold, 2013) this could mean that these whales are feeding in areas with similar dissolved inorganic carbon inputs, but this does not necessarily mean they are the same location. The latitudinal gradient in δ^{13}C of plankton in the surface ocean (McMahon et al., 2013) means whales could be feeding at similar latitudes but not on the exact same feeding grounds.

The finding of differentiation between BSB1 and both BSC2 and BSC3 by the stable isotope results is generally consistent with previous genetic findings. Collectively, Amaral et al. (2016) and Kershaw (2015) indicate that there appears to be genetic differentiation of BSB1 on feeding areas, and more extensive mixing of individuals from BSB2 and the substocks of BSC. The analysis of stable isotopes from the breeding stocks and substocks sampled in this study indicates a potential for dietary overlap between Mayotte (BSC2) and Madagascar (BSC3), but most likely not between Gabon (BSB1) and Madagascar (BSC3). Acoustic study of these same breeding stocks shows song sharing between those from Madagascar (BSC) and Gabon (BSB), although in some years they may be exchanging songs more than in others (Rekdahl et al., 2018). This shows that stable isotope analysis is an extremely useful tool that can corroborate genetic and song exchange analyses and provide insights into the distribution of demographic populations over more recent timescales. Conducting stable isotope analyses for each breeding stock and then comparing with genetic and acoustic data would enhance our understanding of the distribution of breeding stocks and substocks on feeding areas and where and when they are comingling.

Within regions, there is no difference in either isotope measured between years except between one pair of years in Gabon for δ^{13}C values (2004 and 2005). Although this does not necessarily mean the breeding stocks are returning to the exact same location every year to feed, it does ostensibly indicate there is no drastic change in the location of the feeding ground between years, such as significant latitudinal changes. In the Gabon BSB1, the difference between the δ^{13}C values between years 2004 and 2005 suggests they are moving and changing where they feed between years, or it is possible that the primary δ^{13}C value of the feeding ground has shifted between years. Significant seasonal variability in the base of the marine food web can be influenced by temperature, phytoplankton productivity, and many other factors; in fact, it has been shown that seasonal variation in δ^{13}C values at the base of the food web is greatest at high latitudes (McMahon et al., 2013). Another hypothesis is that samples in 2004 and 2005 may have been collected from distinct groups of whales that feed in different locations on their migration route. Thus, it is possible that sampled animals on the breeding grounds do have some potential feeding ground preferences, and that was detected in field sampling across years and reflected in the subset of samples chosen for our stable isotope analyses. This is supported by previous studies: Whales travelling from BSB1 may undertake a coastal route south and possibly temporarily or extensively feed off west South Africa (e.g. Findlay et al., 2017), whereas others may undertake an offshore route and predominantly fast until they reach the Southern Ocean (Barendse, Best, Carvalho, & Pomilla, 2013; Elwen et al., 2014; Rosenbaum, Maxwell, Kershaw, & Mate, 2014). Further investigation into the genetic structure and feeding ecology of the BSB2 substock could potentially reveal more fine-scale dietary variation between whales that take inshore versus offshore migratory routes within this stock.

Mayotte (BSC2) and Madagascar (BSC3) have the highest amount of overlap in isotopic ratios of any of the substocks analysed here; they are not significantly different for either δ^{13}C or δ^{15}N (Table 4). These breeding substocks are also not genetically distinct based on genetic differentiation indices for nuclear microsatellite or mitochondrial markers (Kershaw et al., 2017; Rosenbaum et al., 2009). Corollary evidence from photo-identification, genotypic matching (Ersts et al., 2011), and satellite telemetry data (Cerchio et al., 2016; Fossette et al., 2014) indicate that BSC2 may represent a migratory stream that comprises, at least in part, wide-ranging animals from coastal Africa and Madagascar (as originally speculated by Best et al., 1998). If BSC2 and BSC3 are, at least partially, comprised of the same overall breeding population, it could stand to reason that whales from both breeding stocks may be following similar migration patterns to feeding grounds and feeding in close proximity.

4.2 | Feeding ecology and δ^{15}N values

Although mean δ^{13}C values seem to indicate these whales are feeding in the mid to high latitudes of the southern Atlantic Ocean, their low δ^{15}N values seem to indicate feeding at a low trophic level, which is characteristic of a diet consisting of a majority of krill. Stable isotopic analysis of BSC4, which breeds off La Reunion Island in the western Indian Ocean, has revealed comparable δ^{15}N values to this study (7.8 ± 0.4%; Das et al., 2017), indicating the substocks of BSC share a primarily krill-based diet. These nitrogen and carbon diet values are similar to the average krill and copepod values (δ^{15}N = 3.6‰; δ^{13}C = −25.6‰) in the areas of the southern Atlantic these whales are expected to feed in between the Polar Front and 55°S (Schmidt et al., 2003). A study utilizing satellite tags has also tracked two females from BSB1 to sub-Antarctic feeding grounds off Bouvet Island (55°S), providing direct evidence of feeding location (Rosenbaum et al., 2014). Recent satellite telemetry data also indicate that individuals tagged off west South Africa (presumably originating from BSB2) make broad-scale west and eastward movements between approximately 55 and 65°S (Seakamela et al., 2015). The isotope data are therefore in concordance with satellite tag data, strengthening the conclusion that the isotope values of whale skin are good indicators of general latitudinal feeding area location.

It was previously noted that Southern Hemisphere humpbacks consume diets of primarily krill (Nemoto, 1959) whereas Northern Hemisphere whales include a larger proportion of fish in their diets (Clapham et al., 1997). This difference is generally borne out in stable isotope values when the whales from this study are compared with humpback whale isotope values from other studies that focused on the Northern Hemisphere. Both carbon and nitrogen values (−17.9 ± 0.57‰ and 13.3 ± 0.86‰ respectively) from humpbacks sampled in the North Pacific by Witteveen et al. (2012) reflect whales feeding at higher trophic levels in more coastal environments. Stable isotope values of whale skin can clearly indicate where whales are positioned in the trophic structure of different environments, and in the case of the Northern and Southern Hemisphere humpbacks there is a clear difference in trophic position on feeding grounds.

Owing to the fact that humpback whales are typically not feeding during breeding and migration, with some notable exceptions (e.g. Findlay et al., 2017), stable isotope ratio differences in breeding substocks indicate that either these three substocks do not all feed in the same location and/or at the same time, or they systematically feed on different prey that exhibit different isotope ratios in the same feeding area. These two hypotheses are difficult to tease out, but humpback whales in the Southern Hemisphere appear to primarily feed in surface and subsurface waters ('bubble-netting' behaviour, for example), and engulf as many prey as possible at one time (Friedlaender et al., 2011). As mentioned previously, this is a krill-based ecosystem ideal for bulk feeders, where the Antarctic krill, *Euphasia superba*, is a keystone species (Marrari, Daly, & Hu, 2008), although other species of krill do occur in high abundance. Substantial blooms of *Euphasia crystallorophias* and *Thysanoessa macrura* can also exist in the Southern Ocean (Herr et al., 2016). On the one hand, it may seem unlikely that an animal that feeds using this method could distinguish between different species of krill and consistently achieve differing δ^{15}N values between populations, but a recent study by Herr et al. (2016) showed clear horizontal niche partitioning off the western Antarctic Peninsula. Fin whales and humpback whales, while all feeding in a relatively small area off the peninsula, were congregating by species in areas of different species of krill schools. It is possible, therefore, that the breeding groups with different δ^{15}N values could be feeding close by, but on blooms of krill with different isotope values.

Another recent study on humpback whales in California illustrates shifts in their diets over the years, as it is clear these whales were switching their diets from krill to fish based on availability and ocean currents (Fleming et al., 2016). This study reveals the importance of temporal variability in isotope studies, which may occur at such a fine scale that it is difficult to trace. Abundances and diversity of krill are not monitored on the same detailed spatial and temporal scales as whales (Herr et al., 2016). In this study, a small (~0.6‰) but significant difference in δ^{15}N between breeding substocks has been found that likely represents changes in the base of the food web on small spatial and/or temporal scales rather than a full trophic-level shift (Seyboth et al., 2018). Fleming et al. (2016) found that humpback whales off the coast of California shifted their diets from krill to fish, but this change was reflected by a difference of over 1.5‰ δ^{15}N, which is indicative of an entire trophic-level shift. δ^{15}N sources vary due to a number of factors, such as atmospheric deposition, nitrogen fixation by cyanobacteria, and denitrification (McMahon et al., 2013). As there is evidence δ^{15}N of plankton can vary temporally, even within weeks (Cifuentes, Sharp, & Fogel, 1988), the possibility of δ^{15}N differences in whales reflecting temporal separation on feeding grounds is a possibility. Without sampling prey from the feeding ground region at the same time as collecting the whale skin biopsies, it cannot be said for certain what is driving the difference in δ^{15}N between breeding stocks. The fact that there is a significant difference between the two stocks indicates there is a degree of either spatial or temporal separation during feeding times.

4.3 | Conservation implications

A wide range of contemporary anthropogenic impacts currently affect humpback whale populations (Bettridge et al., 2015; Rosenbaum et al., 2014), and the reassessment of the species' conservation status is ongoing, such as the recent downlisting of many humpback whale management units under the Endangered Species Act in the USA (Federal Register, 2016). As such, the accurate identification of demographically discrete breeding stocks is paramount to the effective management of this species. Though some of these stocks and substocks are not necessarily threatened with extinction (Bettridge et al., 2015), they are still undergoing recovery from depletion by commercial whaling, including substocks within BSB and BSC (IWC, 2011).

Our analyses provide new habitat-based evidence supporting findings of previous genetic studies that BSB1 and BSC3 are

demographically independent and should, therefore, continue to be managed as separate substocks (e.g. IWC, 2011; Kershaw et al., 2017; Rosenbaum et al., 2009). The observation of potential dietary overlap between Mayotte (BSC2) and Madagascar (BSC3) also supports previous findings that the boundary between these substocks is porous and that they may, in fact, represent a single 'mixed stock' (Best et al., 1998; Cerchio et al., 2016; Ersts et al., 2011; Fossette et al., 2014; Kershaw et al., 2017). This additional evidence that there may be greater connectivity between at least some of the substocks within BSC than is currently accounted for in management designations has implications for estimates of abundance and recovery levels for BSC. Although we are unable to draw definitive conclusions regarding feeding area location and its relation to demographic independence from our results, the potential role that selection of feeding habitat may play in shaping population structure underscores the need for substock differentiation and relationships to undergo further evaluation, particularly when new evidence becomes available.

On a broader level, our study further demonstrates the potential for baleen whales to act as sentinels for environmental change in polar regions (Bengtson Nash et al., 2018; Moore, 2008; Seyboth et al., 2018). Inference of the diet of baleen whales through profiling stable isotope ratios allows a unique insight into environmental changes occurring in ecosystems, such as prey base and food web structure (Moore, 2008). Using large whales as sentinels for ecosystem change in polar regions may be particularly useful, as the opportunities for ecosystem-scale field research in these remote and often inhospitable areas are generally limited. To use humpback whales as sentinels of changes to the prey base and foraging habitat in the Southern Ocean, stable isotope profiling efforts should be expanded so that sampling occurs on an annual basis and is ideally implemented for all breeding stocks in the Southern Hemisphere.

5 | CONCLUSIONS

This study illustrates the importance and benefits of non-lethal sampling for determining whale ecology and prey consumption, and how these results can be interpreted in the context of other population-level data (e.g. genetics, telemetry, acoustics). For large baleen whales, skin biopsy samples can help answer questions about the location of migration routes (Carroll et al., 2015) and feeding areas. Stable isotopes reveal that sampled humpback breeding stocks and substocks are separated spatially and/or temporally during summer feeding. In addition, the results may suggest that whales sampled off Gabon (BSB1) are either switching feeding areas in different years or different groups of whales sampled at this location are utilizing different feeding areas. Stable isotope analysis, therefore, provides a corollary method of breeding stock delineation that can provide insights into whale behaviour approaching or on Southern Ocean feeding areas, where opportunities for observation and sampling are extremely limited. In the future, any study of the isotopic ecology of South Atlantic and south-western Indian Ocean humpback whale populations will benefit from additional sampling of whale skins and sampling of potential prey species from feeding grounds, as well as combination with other emerging techniques, such as radiocarbon measurements (Eisenmann et al., 2017) and measurements of persistent organic pollutants (Das et al., 2017). Quantitatively determining ecological characteristics is a method that can also be widely applied to other humpback whale breeding stocks in the Southern Hemisphere to shed light on the complicated population dynamics of these animals that are an integral part of the marine ecosystem and are still recovering from past commercial whaling. This research adds to a growing body of literature demonstrating marine mammal trophic interactions, augmenting understanding of how these species can act as sentinels of environmental change occurring in oceans worldwide (Bengtson Nash et al., 2018; Moore, 2008; Seyboth et al., 2018).

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AUTHOR CONTRIBUTIONS

SM and HCR conceived this idea and designed the study; SM sampled the existing biopsies, arranged for stable isotope analysis, analysed the data, and drafted the manuscript; HCR gave access to biopsies, interpreted the data, and drafted the manuscript; FK interpreted the data and drafted the manuscript. All authors gave final approval for publication.

ETHICS STATEMENT

Research was conducted under permits obtained from the relevant governing bodies. Original sampling was conducted under approval from relevant institutional sampling committees. For Gabon, research was conducted in partnership with the Ministry of Water and Forests. In Madagascar, research proposals were approved by the Ministère des Eaux et Forêts de Madagascar, Direction de la Pêche et des Ressources Halieutiques, Département de la Biologie Animale de l'Etablissement d'Enseignement Supérieur des Sciences of the University of Antananarivo, and Institut Halieutiques et des Ressources Marines of the University of Toliara. In Mayotte, research was conducted under the Collectivité Départmentale de Mayotte and the Direction de l'Agriculture et de la Forêt de Mayotte.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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