Marine Mammal Science

MARINE MAMMAL SCIENCE, **(*): ***–*** (*** 2012)
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DOI: 10.1111/j.1748-7692.2012.00563.x

Dietary variation within and between populations of northeast Atlantic killer whales, *Orcinus orca*, inferred from δ^{13}C and δ^{15}N analyses

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ABSTRACT

Epidermal skin samples from eastern North Atlantic killer whales, *Orcinus orca*, were analyzed for carbon and nitrogen stable isotope ratios. From those, comparisons within a data set of 17 samples collected from Tysfjord, Norway, in November suggested that diet is relatively specialized during this time period at this location. There were significant differences between a small set of samples from Iceland and those collected from Norway, which had all been assigned to the same population by a previous population genetics study. The results would be consistent with matrilines feeding on either the Norwegian or Icelandic stocks of Atlantic herring (*Clupea harengus*). There was no significant difference within Icelandic samples between those assigned to the population known to feed upon herring and those assigned to a population hypothesized to follow Atlantic mackerel (*Scomber scombrus*). The greatest differences were between the epidermal samples analyzed in this study and tooth and bone collagen samples from the North Sea that were analyzed previously, which also showed significantly more variation in isotopic ratios than found for skin samples. These differences could reflect differences in turnover rate, differences in diet-tissue fractionation and discrimination due to the amino acid composition of the different tissues, and/or greater competition promoting dietary variation between groups in the North Sea.

Key words: killer whale, *Orcinus orca*, carbon, nitrogen, stable isotope, foraging ecology.

The analysis of naturally occurring variation in the ratio of isotopes of elements within the tissues of an organism has become a powerful tool for ecologists and has been used to address a range of questions including studying differences in the diet between individuals, sex/age classes, and populations; studying migration patterns and geographic range; and studying life history strategies (Hobson 1999, Hobson

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and Wassenaar 1999, Newsome *et al*. 2010, Newton 2010). Stable isotope studies comparing the ratios of nitrogen ($^{15}N/^{14}N$) and carbon ($^{13}C/^{12}C$) can be particularly informative for comparing differences in diet, trophic position and geographic range (Hobson and Wassenaar 1999, Newsome *et al*. 2010, Newton 2010). This is because of a stepwise enrichment of ^{15}N with each increase in trophic level, which reflects differences in fractionation between synthesis and excretion. Nitrogen isotopes, ^{15}N and ^{14}N, in animal protein are assimilated from dietary protein, and excreted in urine and other nitrogenous excretions; however, these are enriched in ^{14}N relative to the body nitrogen pools (Ponsard and Averbuch 1999). Enrichment of ^{13}C can also occur, in smaller stepwise increments, with increases in trophic level, however, $\delta^{13}C$ is mainly determined by the source of primary production. For example, in marine ecosystems $\delta^{13}C$ varies among plant types, *e.g*., phytoplankton, macroalgae, and sea grass, leading to a difference between nearshore and pelagic ecosystems (Newsome *et al*. 2010). In the northeastern Pacific, studies using stable isotope analyses have provided additional evidence for dietary differences among killer whale (*Orcinus orca*) ecotypes (Herman *et al*. 2005, Krahn *et al*. 2007*b*, Newsome *et al*. 2009). The results have shown that the mammal-eating "transient" ecotype is significantly enriched in ^{15}N relative to the fish-eating "resident" ecotype, while the "offshore" ecotype was intermediate between the other two (Herman *et al*. 2005). There were also differences between populations within each ecotype in $\delta^{13}C$ that probably reflect geographic variation in the primary production.

Here, we present the results of ^{15}N and ^{13}C isotope analyses of epidermal skin samples collected from eastern North Atlantic killer whales to investigate intra- and interpopulation differences in diet and ecology. Two previously published studies have conducted stable isotope analyses on free-ranging northeastern Atlantic killer whales. The first combined isotopic analyses with organochlorine and persistent organic pollutant analyses of blubber samples from a small data set of five individuals that had stranded around the British Isles and Ireland (McHugh *et al*. 2007). One of the five individuals had elevated contaminant levels and enriched isotopic ratios relative to the others suggesting a more mammal-based diet (McHugh *et al*. 2007). A second study used tooth and bone samples (Foote *et al*. 2009), which would be expected to give a longer-term (multiyear) dietary signature than skin samples (Newsome *et al*. 2010). The isotopic ratios of 40 individuals from the North Sea and neighboring northeastern Atlantic were measured and combined with genetic analysis to sequence the hypervariable region of the mitochondrial DNA (mtDNA) control region, and with measurements of apical tooth wear. One maternal lineage (mtDNA haplotype: Atl_2_28) had invariant isotope ratios consistent with a specialized diet, and stomach contents from a single individual contained minke whale (*Balaenoptera acutorostrata*) baleen (Eschricht 1866). Specimens with this haplotype had almost no apical tooth wear. In contrast, most adult specimens belonging to several closely related maternal lineages (mtDNA haplotypes: Atl_1_29, Atl_1_33, Atl_1_35) had severe apical tooth wear, with teeth often being worn to the gums. The lineages were the same as those biopsy sampled while foraging on herring or mackerel in this region (Foote *et al*. 2011). While the tooth wear would suggest that all the individuals that belong to these lineages shared a component of the diet or foraging method, isotopic ratios indicated each lineage had a broad niche width due to long-term differences between individuals in the proportion of prey items consumed (Foote *et al*. 2009).

Population structure of northeast Atlantic killer whales was further investigated using a data set of epidermal samples utilizing both maternally inherited mtDNA and biparentally inherited nuclear DNA (microsatellites) (Foote *et al*. 2011). The

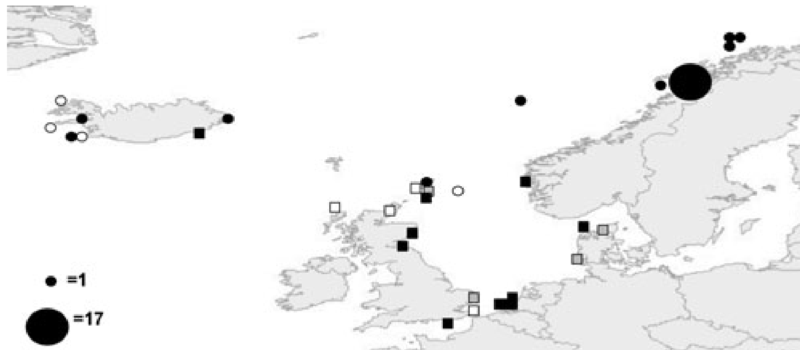

Figure 1. Sampling location for skin samples (circles) and tooth and bone samples (squares). Closed circled indicate samples assigned to population-A, open circled indicate samples assigned to population-B. Squares are colored based on mtDNA haplotype: black = Atl_1_33, gray = Atl_1_35, white = Atl_1_29.

tooth and bone samples discussed above were not included in the microsatellite analysis due to the problems associated with using degraded samples for microsatellite analysis, *e.g.*, allelic dropout (Morin *et al.* 2010). That study found that killer whales sampled on both the Norwegian and Icelandic herring grounds were classified as one population (population-A) using microsatellites, but there was differentiation between Norway and Iceland based on mtDNA haplotype frequency (Foote *et al.* 2011). There were also no photo-identification matches between Norwegian and Icelandic herring grounds suggesting no or low levels of migration between them (Foote *et al.* 2010). However, there were photo-identification matches between Iceland and the Shetland Isles, which are close to Norway (Foote *et al.* 2010, Beck *et al.* 2011). It therefore appears that population-A is comprised of two subpopulations, one following the Icelandic summer-spawning herring stock of Atlantic herring (*Clupea harengus*), the other following the Norwegian spring-spawning stock (Similä *et al.* 1996; Simon *et al.* 2007; Foote *et al.* 2010, 2011). A second population (population-B) was only defined from a small number of samples, most of them from stranded animals, however, one individual was biopsy sampled from a pelagic trawler while feeding on mackerel (*Scomber scombrus*) that were escaping the nets. The samples assigned to this population were distributed along the migration route of the northeast Atlantic mackerel stock (Foote *et al.* 2011). It was therefore hypothesized that fidelity to different fish stocks may have led to sufficient temporal and spatial separation to reduce gene flow and cause population structuring (Foote *et al.* 2011). In this study, we investigate this hypothesis further by comparing $\delta^{13}C$ and $\delta^{15}N$ stable isotope ratios between and within populations from this data set.

METHODS

Collection of Samples

Samples were collected from live free-ranging killer whales between Iceland and Norway (see Fig. 1) by remote biopsy sampling using a Barnett Panzer V 150 lb pull-strength crossbow, or modified .22 caliber rifle, or the pneumatic LK-ARTS

projector and specially designed darts produced by Ceta-Dart (see Palsbøll *et al.* 1991), Paxarms (see Krützen *et al.* 2002) and ARTdart by LKARTS-Norway. Six skin samples used in this study were also collected from stranded animals by the Marine Research Institute, Reykjavík, Iceland, and one was collected by the Wildlife Unit of the Scottish Agricultural College Veterinary Services, Inverness, Scotland.

Sex was determined using molecular methods (see Morin *et al.* 2005) and in some cases confirmed by the presence of male secondary sexual characteristics or observation of the sex-specific pigmentation in the genital area. In all cases there was complete agreement between the molecular and observational determinations. The age class of males was estimated based on the relative size of the dorsal fin and males were classified as juveniles if the dorsal fin had not started to "sprout," subadults if the dorsal fin had not reached its full height, *e.g.*, when the height/width ratio is approximately 1.7, this occurs at approximately 21 yr of age (Olesiuk *et al.* 1990). Females were classified as juveniles if they were estimated to not have reached adult size (approximately $\geq$5 m).

Storage and Preparation of the Samples

Most samples were wrapped in foil and frozen at -20°C; however, as the primary purpose for sampling was for DNA analyses some samples had been stored in 20% dimethyl sulphoxide (DMSO) solution saturated with salt (Amos and Hoelzel 1991). To ascertain if this preservative would affect the isotopic ratios of the sample, two of the unpreserved samples were split, and part was stored in 20% DMSO at 4°C for one year, the remainder of each sample was kept frozen at -20°C. To extract the lipids we washed each skin sample in successive rinses of distilled water, and several rinses of a 2:1 mixture of chloroform and methanol over a 24 h period, following previous studies on marine mammals (*e.g.*, Das *et al.* 2003, Marcoux *et al.* 2007). Samples were then dried at 50°C for 48 h and then ground up.

Isotopic analyses were conducted at the National Environment Research Council's Life Sciences Mass Spectrometry Facility in East Kilbride, Scotland. Approximately 0.8 mg of sample were placed in tin capsules and nitrogen and carbon isotope analyses performed simultaneously using continuous-flow isotope ratio mass spectrometry. All stable isotope ratios are expressed in per mil (‰) deviations from primary international standards, using the delta (δ) notation. Replicate measurements of internal laboratory standards (gelatine, two isotopically disparate alanines, and tryptophan) indicate a precision of $\leq$0.1‰ for δ^{13}C and $\leq$0.2‰ for δ^{15}N.

Statistical Analyses

Genetic analyses had previously been conducted to sequence the maternally inherited mitochondrial DNA (mtDNA) control region and nuclear DNA was amplified in the form of 17 polymorphic microsatellite loci, and the allele frequencies were used to assign each sample to a population. This genetic component of this work has been previously published (see Foote *et al.* 2009, 2011). The 17 samples collected from Tysfjord, Norway, were then grouped based on sex/age class and mtDNA haplotype for statistical comparison to investigate demographic and matrilineal effects on stable isotope values. All samples were grouped based on population assignment and

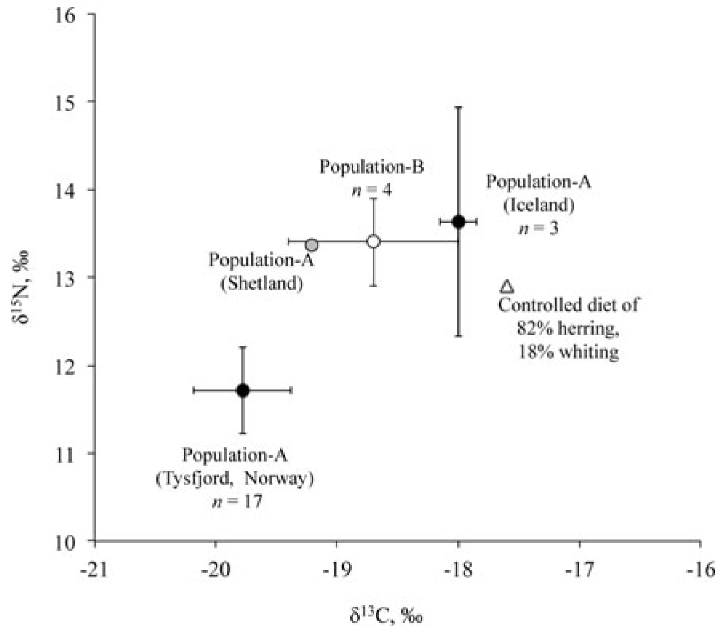

Figure 2. Nitrogen and carbon stable isotope ratios of epidermal collagen samples, for northeastern Atlantic killer populations. The triangle indicates the published isotope ratio based on blood collagen of a captive Icelandic killer whale fed on a diet of 82% Atlantic herring and 18% whiting for 308 d (Caut *et al*. 2011).

geographical origin for statistical comparison to investigate population-level differences on stable isotope values. The statistical significance of the killer whale grouping differences were calculated using the factorial ANOVA with Helmert contrasts and Type III sum of squares to control for the inequality of sample sizes.

Results

There was very little variation in the measured δ^{13}C and δ^{15}N values for the set of samples collected from killer whales during November 2005 in Tysfjord, northern Norway (Fig. 2, Table 1). Statistical comparisons between sexes and between mtDNA haplotypes were nonsignificant for both δ^{13}C and δ^{15}N. Mean values $\pm$ 1 SD and sample sizes (n) are shown for each comparison. Sex: males ($n = 7$) *vs*. females ($n = 10$), δ^{13}C: -19.8 ± 0.4 *vs*. -19.7 ± 0.2, ($F_{1,15} = 0.468$, $P = 0.504$); δ^{15}N: 11.5 ± 0.4 *vs*. 11.8 ± 0.5, ($F_{1,15} = 1.732$, $P = 0.208$). mtDNA haplotype: Atl_1_33 ($n = 7$) *vs*. Atl_1_35 ($n = 10$), δ^{13}C: -19.7 ± 0.3 *vs*. -19.9 ± 0.3, ($F_{1,15} = 1.830$, $P = 0.196$); δ^{15}N: 11.8 ± 0.6 *vs*. 11.6 ± 0.3, ($F_{1,15} = 0.832$, $P = 0.376$).

Although not tested statistically, the two juveniles (samples 3 and 5) and one subadult (sample 14) had δ^{13}C and δ^{15}N values within the narrow range of the adults (Table 1). Additionally, two out of the three individuals (samples 19 and 20) sampled farther north in December 2007 following a shift in the migration

Table 1. Details of the samples used in this study.

Sample no.	Sample type	mtDNA haplotype	Population	Sex	Age	Region	Collection site	Collection period	Preservative	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
1	Biopsy	Atl_1_33	A	M	Adult	Norway	Tysfjord	November 2005	None	−19.3	11.9
2	Biopsy	Atl_1_33	A	M	Adult	Norway	Tysfjord	November 2005	None	−19.4	11.7
3	Biopsy	Atl_1_33	A	M	Juvenile	Norway	Tysfjord	November 2005	None	−19.7	11.7
4	Biopsy	Atl_1_35	A	F	Adult	Norway	Tysfjord	November 2005	None	−19.5	12.0
5	Biopsy	Atl_1_33	A	F	Juvenile	Norway	Tysfjord	November 2005	None	−20.0	11.9
6	Biopsy	Atl_1_33	A	F	Adult	Norway	Tysfjord	November 2005	None	−19.8	12.7
7	Biopsy	Atl_1_33	A	F	Adult	Norway	Tysfjord	November 2005	None	−19.8	11.7
8	Biopsy	Atl_1_33	A	F	Adult	Norway	Tysfjord	November 2005	None	−19.0	11.2
9	Biopsy	Atl_1_35	A	M	Adult	Norway	Tysfjord	November 2005	None	−20.4	11.8
10	Biopsy	Atl_1_33	A	M	Adult	Norway	Tysfjord	November 2005	None	−20.3	10.9
11	Biopsy	Atl_1_35	A	F	Adult	Norway	Tysfjord	November 2005	None	−20.0	11.8
12	Biopsy	Atl_1_35	A	F	Adult	Norway	Tysfjord	November 2005	None	−19.8	11.8
13	Biopsy	Atl_1_35	A	F	Adult	Norway	Tysfjord	November 2005	None	−19.9	11.4
14	Biopsy	Atl_1_35	A	M	Subadult	Norway	Tysfjord	November 2005	None	−19.9	11.2
15	Biopsy	Atl_1_33	A	F	Adult	Norway	Tysfjord	November 2005	None	−19.4	11.7
16	Biopsy	Atl_1_33	A	F	Adult	Norway	Tysfjord	November 2005	None	−19.7	12.5
17	Biopsy	Atl_1_35	A	M	Adult	Norway	Tysfjord	November 2005	None	−19.8	11.3
18	Biopsy	Atl_1_35	A	F	Adult	Norway	North of Andenes	December 2007	None	−20.9	12.9
19	Biopsy	Atl_1_33	A	M	Adult	Norway	North of Andenes	December 2007	None	−20.0	11.5
20	Biopsy	Atl_1_33	A	M	Adult	Norway	North of Andenes	December 2007	None	−19.4	11.6
21	Biopsy	Atl_1_33	A	M	Adult	NE Atlantic	67°50′N, 12°57′E	May 2001	20% DMSO	−19.9	11.5
22	Biopsy	Atl_1_35	A	F	Adult	NE Atlantic	66°47′N, 7°14′E	July 2002	20% DMSO	−19.5	12.1
23	Necropsy	Atl_1_34	A	F	Juvenile	Scotland	Shetland Isles	May 1995	None	−19.2	13.4
24	Biopsy	Atl_1_29	B	M	Adult	North Sea	60°31′N, 1°27′E	October 2008	None	−18.8	14.1
25	Necropsy	Atl_1_34	A	M	Adult	Iceland	Núpur, Berufirði	February 1998	20% DMSO	−17.8	13.1
26	Necropsy	Atl_1_29	B	M	Juvenile	Iceland	Stigahlíð, Bolungarvík	May 1998	20% DMSO	−17.9	13.0
27	Necropsy	Atl_1_34	A	F	Adult	Iceland	Beruvík, Snæfellsnesi	April 2003	20% DMSO	−18.1	12.7
28	Necropsy	Atl_1_29	B	M	Adult	Iceland	Keilisnesi, Reykjanesi	July 2003	20% DMSO	−19.3	12.9
29	Necropsy	Atl_1_34	A	M	Adult	Iceland	Gilsfjörður, Skarðstönd	November 2003	20% DMSO	−18.0	15.2
30	Necropsy	Atl_1_29	B	M	Adult	Iceland	Breiðalæk, Barðaströnd	April 2008	20% DMSO	−18.4	13.6

Table 2. Stable isotope values for two samples that were split in two and stored dry and in 20% DMSO respectively for 1 year.

Sample No.	Preservative	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
8	None	−19.0	11.2
	Stored in 20% DMSO for 1 year	−19.5	10.9
24	None	−18.8	14.1
	Stored in 20% DMSO for 1 year	−19.3	14.0

route of the Norwegian spring-spawning herring stock between 2005 and 2007, had $\delta^{13}C$ and $\delta^{15}N$ values within the narrow range of values measured for individuals sampled in Tysfjord (Table 1). One female (sample 18) sampled north of Andenes in 2007 had the highest $\delta^{15}N$ value (12.9) and the lowest $\delta^{13}C$ value (−20.9) out of all the Norwegian samples, but these values were only marginally higher than those measured for individuals sampled in Tysfjord (Table 1). Two individuals (samples 21 and 22) sampled offshore on the herring feeding grounds during the summer months also had $\delta^{13}C$ and $\delta^{15}N$ values within the narrow range of values measured for individuals sampled in Tysfjord (Table 1).

Although the Icelandic sample size was small, there were significant differences between the Norwegian and Icelandic samples previously assigned to population-A, and between samples previously assigned to population B and Norwegian samples assigned to population-A. The Icelandic population-A samples and population-B samples were found to be significantly more enriched in ^{15}N (population-A Norway *vs.* population-A Iceland: $F_{1,23} = 27.002$, $P < 0.0001$; population-A Norway *vs.* population-B Iceland/North Sea: $F_{1,24} = 40.581$, $P < 0.0001$) and significantly less depleted in ^{13}C (population-A Norway *vs.* population-A Iceland: $F_{1,23} = 56.501$, $P < 0.0001$; population-A Norway *vs.* population-B Iceland/North Sea: $F_{1,24} = 18.542$, $P < 0.0001$) relative to the Norwegian population A samples (Fig. 2). There was no significant difference in either $\delta^{15}N$ ($F_{1,5} = 0.125$, $P = 0.738$) or $\delta^{13}C$ ($F_{1,5} = 3.015$, $P = 0.143$) between the Iceland population-A samples and the population-B samples (Fig. 2).

Differences between the repeat measures for the two samples that were split and half was stored in preservative 20% DMSO at 4°C for one year, while the other half remained dry frozen at −20°C (Table 2), were less than the difference between the Icelandic and Norwegian mean $\delta^{13}C$ and $\delta^{15}N$ values, but greater than the expected level of inaccuracy of ≤0.2‰ based on the repeat measures of the internal laboratory standards. Note that only the isotopic values of the dry frozen subsamples were used in the statistical analyses and Fig. 2.

There were significant differences between the isotopic values measured from skin samples for this study when compared with isotopic values measured from bone and tooth samples from a previously published study (Foote *et al.* 2009, Fig. 2). Statistical comparisons within mtDNA haplotype and between tissue types found that bone and tooth samples were significantly ($P < 0.0001$) enriched in $\delta^{13}C$ and $\delta^{15}N$ relative to the Norwegian skin samples (Fig. 3). There was also significantly greater variance (variance ratio test and homogeneity of variance test) in the isotope measurements from bone and tooth samples than from skin samples within each mtDNA haplotype ($P < 0.0001$).

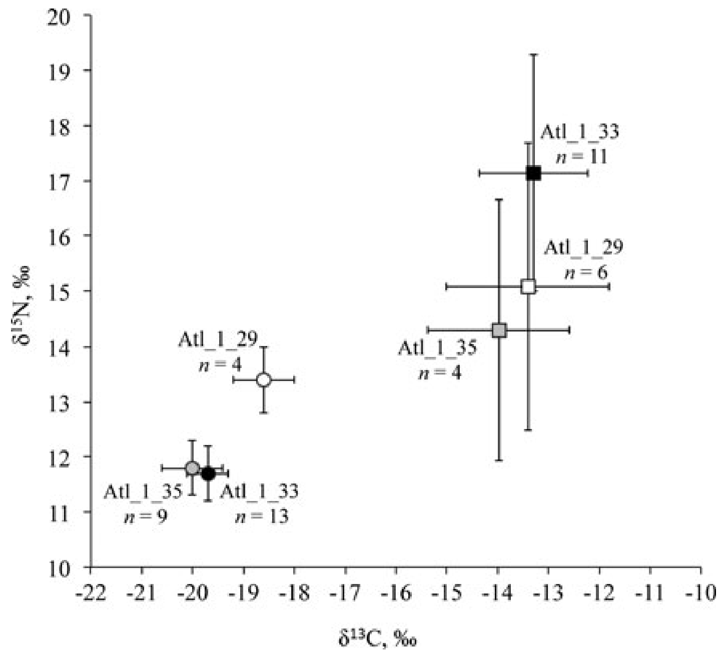

Figure 3. Nitrogen and carbon stable isotope ratios of epidermal collagen samples (circles) and bone or tooth collagen samples (squares) from Foote *et al*. (2009), for northeastern Atlantic killer lineages.

Discussion

The lack of variation in isotopic values of the samples from the Norwegian subpopulation is consistent with observations from Tysfjord in the winter months of killer whales specializing in feeding on the Norwegian spring-spawning stock of Atlantic herring (Similä and Ugarte 1993, Similä *et al*. 1996, Similä 1997). From 105 observations of confirmed predation events by killer whales in Tysfjord, 99 were of the killer whales consuming herring (Similä *et al*. 1996). The variance in stable isotope ratios of the individuals sampled in Tysfjord in November 2005 is comparable with that of the isotope ratios from skin samples collected in the inshore Pacific waters of Washington State and British Columbia from the Southern Resident killer whale population during the summer months (Herman *et al*. 2005, Krahn *et al*. 2007*a*). This population is known to feed preferentially on Chinook salmon (*Oncorhynchus tshawytscha*) in the summer months in this area (Ford *et al*. 2006, Hanson *et al*. 2010).

Differences in isotopic ratios can occur between demographic units due to periods of high growth rate or catabolism during pregnancy or lactation (Newsome *et al*. 2010), but were not evident in this sample set. Satellite tracking and comparisons of photo-identification between the wintering and spawning grounds have suggested the whales follow the seasonal migration of the Norwegian spring-spawning herring stock (Bisther and Vongraven 1995, Similä *et al*. 1996, Stenersen and Similä 2004). Although there is a large onshore-offshore movement and change in nutritional state of the herring on the summer feeding grounds compared with the wintering grounds, where they do not feed (Slotte 1999), the isotopic ratios of the two killer

whale samples from the summer feeding grounds of the herring fell within the values from samples collected on the herring's wintering grounds.

There were significant differences in isotope ratios between the Icelandic and Norwegian individuals assigned to population-A. This is in agreement with previous work, which found no evidence of movement of photo-identified individuals between Iceland and Norway (Foote *et al.* 2010) and significant differentiation based on the frequency of mtDNA haplotypes (Foote *et al.* 2011). Such differences in stable isotope ratios would be expected if the Icelandic matrilineal groups follow and mainly consume the Icelandic summer-spawning herring and the Norwegian matrilineal groups follow and mainly consume the Norwegian spring-spawning herring as previously suggested (Simon *et al.* 2007; Foote *et al.* 2010, 2011). However, there are other factors that need to be considered. Icelandic samples assigned to population-A were all stored in DMSO at 4°C, whereas the Norwegian data set consisted entirely of biopsy samples from living animals, which were dry frozen at −20°C. Previous studies using cetacean skin found that lipid extraction successfully removed the DMSO from the sample and led to comparable results to untreated skin samples (Todd *et al.* 1997, Marcoux *et al.* 2007, Lesage *et al.* 2010). The two samples that we split and kept under the two different storage methods for one year were then lipid extracted and showed a reduction of $<1.0‰$ for $\delta^{13}C$ and a reduction of $<0.3‰$ for $\delta^{15}N$ (Table 2). These differences are less than the differences between the Iceland and Norwegian samples and in the opposite direction, *e.g.*, the Icelandic samples stored in DMSO had significantly higher values of $\delta^{13}C$ and $\delta^{15}N$ than the frozen unpreserved Norwegian samples. This would therefore suggest that the differences in isotopic ratios between the Norwegian and Icelandic samples are not caused by differing methods of sample storage.

The Icelandic samples assigned to population-A were all collected postmortem during necropsies of stranded individuals whereas the Norwegian samples were all collected by biopsy from live, free-ranging specimens, and this could also present a potential bias. If the Icelandic individuals stranded due to some chronic cause of death that led to a prolonged period of nutritional stress prior to death, this could cause enrichment of ^{15}N due to the catabolism of the body's protein supplies (Hobson *et al.* 1993, Fuller *et al.* 2005). Biopsy sampling from free-ranging Icelandic animals would be beneficial for further comparisons.

Several of the Icelandic samples were assigned with samples from around the UK and Ireland to a second population (B) by a previous population genetics study (Foote *et al.* 2011). Another sample assigned to this population was from a group feeding on mackerel in the North Sea and it was hypothesized that this population may follow the northeast Atlantic mackerel stock (Foote *et al.* 2011). There was no significant difference in mean $\delta^{15}N$ and $\delta^{13}C$ between the samples assigned to population-B and the Icelandic samples assigned to population-A (Fig. 2). This could reflect an overlap in geographic range, an overlap in diet, or both. Previously published isotopic ratios for North Atlantic mackerel and Atlantic herring suggest that there are only slight differences in $\delta^{15}N$ (Table 2) and $\delta^{13}C$ will mainly be dependent upon the sampling location. Given that most of the population-B samples were also from Iceland, stable isotope analyses may not be able to discriminate between a diet of North Atlantic mackerel and Atlantic herring for these samples. Mackerel was considered a rare vagrant into Icelandic waters until 2006, when subsequently it has been found in increasing abundance off the southeast coast of Iceland (Ástþórsson *et al.* 2009). Therefore two of our sampled individuals from population-B stranded around Iceland in years when mackerel was scarce, but herring was abundant. This leaves us unable to discriminate between the two most probable and nonmutually exclusive

Table 3. Stable isotope values for prey items from published sources.

Prey species	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Reference
Atlantic herring	-18.5 ± 0.3	11.8 ± 0.2	Kurle (2002)
Icelandic capelin	-19.8 ± 0.3	11.1 ± 0.1	Kurle (2002)
North Sea herring	-17.9 ± 1.9	13.0 ± 1.1	Das *et al*. (2003)
North Sea mackerel	-16.6 ± 0.4	16.1 ± 0.3	Das *et al*. (2003)
North Sea harbor seal	-16.2 ± 1.3	18.7 ± 0.3	Das *et al*. (2003)
North Sea harbor porpoise	-16.4 ± 1.6	16.2 ± 1.6	Das *et al*. (2003)
Atlantic herring	-18.6 ± 0.2	11.8 ± 0.2	Caut *et al*. (2011)
Atlantic mackerel (Bristol Channel and Celtic Sea)	-19.2 ± 0.3	11.9 ± 0.9	Votier *et al*. (2010)
Atlantic mackerel (western North Atlantic)	-21.28 ± 0.4	12.5 ± 0.5	Abend and Smith (1997)

causes of similar isotopic values, *e.g*., overlapping geographic range and overlapping diet for our Icelandic samples assigned to populations A and B. Combining stable isotopes with additional multiple independent chemical methods such as fatty acids and organochlorine analyses may provide better dietary resolution than a single biometric (*e.g*., Herman *et al*. 2005). Additionally, further biopsying work concurrent to observations of predation and prey sample collection would be beneficial.

The mean isotopic ratios during a controlled diet experiment on a captive Icelandic killer whale, which was fed on a diet of 82% Atlantic herring and 18% whiting for 308 d (Caut *et al*. 2011), was within the range of $\delta^{15}N$ we found for Icelandic killer whales assigned to population-A, but was slightly less depleted in $\delta^{13}C$. Two studies (García-Tiscar 2009, Caut *et al*. 2011) noted that the discrimination factor for $\Delta^{15}N$ between killer whales included in the controlled feeding experiment and the prey items was less than the normal range of 2‰–5‰ found in many previous isotope studies (Newsome *et al*. 2010). However, they found $\Delta\delta^{13}C$ was within the normal range of 0.9‰–1.9‰. Comparison with the table of isotopic ratios of prey items (Table 3) would suggest that $\Delta^{15}N$ in our study is also less than the normal discrimination factor range of 2‰–5‰, however, to confirm this requires isotopic measurements of a range of prey items from each location in our study area, which was beyond the scope of this study.

The isotopic values from the skin samples measured in this study differed significantly to those from a previous study of tooth and bone samples (Foote *et al*. 2009). Different tissues can give different signatures for several reasons. The differences in amino acid composition among different tissues can lead to large differences in trophic discrimination (Newsome *et al*. 2010). For example, $\delta^{13}C$ in bone collagen is typically enriched by 4‰–5‰ relative to the diet, in comparison to a 0.9‰–1.9‰ enrichment typical for epidermal tissue (Newsome *et al*. 2010). This is likely a factor underlying the large differences in $\delta^{13}C$ between soft and hard tissues in this study. There are also differences in geographic location to take in to account as tooth and bone samples were mainly from the North Sea region and skin samples were mainly from Norwegian and Icelandic waters. Within our study area of the eastern North Atlantic there is large-scale spatial variation in the $\delta^{13}C$ of marine animals (Barnes *et al*. 2009). Mackerel and herring sampled by Das *et al*. (2003) from the North Sea were less depleted in $\delta^{13}C$ and more enriched in $\delta^{15}N$ than Atlantic herring or mackerel (Table 3) as were the killer whales sampled in this area.

There was also significantly higher variation in $\delta^{15}N$ and $\delta^{13}C$ values from bone and tooth collagen relative to epidermal tissue samples. This suggests that interindividual differences in the mean composition of the diet over the longer timescales reflected in tooth and bone collagen $\delta^{15}N$ and $\delta^{13}C$ values are greater than the interindividual differences in the mean diet reflected in the epidermal tissue $\delta^{15}N$ and $\delta^{13}C$ values. This could reflect differences between a long-term diverse diet and seasonal specialization, at least by some groups. Salmon bones were found in the stomach contents of a killer whale assigned to population B that stranded in Cork harbor, Ireland (McHugh *et al.* 2007). An ocean sunfish (*Mola mola*) was found in the stomach contents of another recent stranding at County Mayo, Ireland (Ryan and Holmes, in press), marine mammals were found in the stomach contents of two of the specimens from which tooth and bone samples were taken and another individual had hundreds of seabird feathers in the airways (Eschricht 1866, Kompanje 1995), all of these individuals shared mtDNA haplotype Atl_1_33 (Foote *et al.* 2009). One of these, an adult male from the Kattegat that had the remains of 14 harbor seals (*Phoca vitulina*) and 13 harbor porpoises (*Phocoena phocoena*) in its stomach (Eschricht 1866), had $\delta^{15}N$ of 16.07. This is a lower $\delta^{15}N$ than reported for harbor porpoises and harbor seals in the North Sea (Table 3) suggesting a lower trophic level component to the long-term diet of this individual. There have been a limited number of observations of photo-identified Norwegian killer whale hunting both herring and seals (Stenersen and Similä 2004). There could also be geographic variation in the degree of interindividual differences in the mean composition of the diet. The North Sea herring and mackerel stocks are smaller and spatially more heterogeneous than the Norwegian and Icelandic herring stocks or the North Atlantic mackerel stock (Payne *et al.* 2004) and this may also drive competition and lead to greater dietary diversification by killer whales in the North Sea relative to Norway or Iceland. Killer whales around the Shetland Isles have been observed consuming seals, eider ducks (*Somateria mollissima*), and herring (Smith 2006, Bolt *et al.* 2009, Deecke *et al.* 2011); however, to date no photo-identified individuals have been confirmed feeding on both fish and mammals in Shetland waters. A pattern of foraging diversification and individual or group dietary differences within a population being dependent upon food limitation has been noted for other marine mammal species (*e.g.*, the southern sea otter, *Enhydra lutris nereis*; Tinker *et al.* 2008). Further investigation of this is warranted and requires quantification of intra-individual dietary variation using time series of stable isotope data, such as has previously been achieved using growth layers of tooth dentin from killer whales (Newsome *et al.* 2009).

In conclusion, the stable isotope values measured from skin samples of northeast Atlantic killer whales support the finding of previous investigations using genetic (mtDNA) and mark-recapture analyses of differentiation between Norwegian and Icelandic herring-eating killer whales. However, stable isotope analyses were not able to confirm the hypothesis that population differentiation between Icelandic populations A and B was due to one following herring stocks and the other following mackerel.

Acknowledgments

We thank Colin Macleod, Jen Snowball and Cory Matthews for their useful discussions during this work. This paper was greatly improved by comments from two anonymous reviewers and the editor. ADF was supported by a University of Aberdeen 6th Century Scholarship and

an EU Marie Curie Intra-European Fellowship. The laboratory work conducted at the SUERC Life Sciences Mass Spectrometry Unit was supported by the National Environment Research Council (NERC). Further funding for sample collection was provided by Scottish Natural Heritage and Marine Scotland, the Scottish Government.

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Received: 10 August 2011
Accepted: 20 January 2012