Intra-population variation in isotopic niche in herring-eating killer whales off Iceland

Filipa I. P. Samarra1,*, Morgana Vighi2, Alex Aguilar2, Gísli A. Vikingsson1

1Marine and Freshwater Research Institute, Skúlagata 4, 101 Reykjavík, Iceland
2Biodiversity Research Institute (IRBio) and Department of Evolutionary Biology, Ecology and Environmental Sciences, Faculty of Biology, University of Barcelona, Av. Diagonal 643, 08028 Barcelona, Spain

ABSTRACT: Among-individual variation in dietary preferences can impact community dynamics and be a driving force for evolutionary divergence, although it can be difficult to assess in free-ranging marine mammal populations. In this study, we investigate the existence of variation in isotopic niche within a population of putative herring-specialist killer whales. Isotopic ratios of carbon and nitrogen were measured in 67 skin biopsy samples from 56 individual killer whales, sampled in herring overwintering (winter) grounds and spawning (summer) grounds in Iceland when the whales were presumably feeding on herring. Whales that appeared to follow herring year round (n = 31) had lower $\delta^{15}N$ values, consistent with a diet predominantly composed of herring. This supports the existence of herring specialists in the population. In contrast, whales that were only photo-identified either in winter or in summer (n = 25) had larger variation in $\delta^{15}N$ values. A discriminant function analysis clearly distinguished between putative herring specialists and whales seasonally travelling to Scotland in summer (n = 3), which exhibited distinctly larger $\delta^{15}N$ values indicative of a diet including higher trophic level prey. This study shows that herring-eating killer whales in Iceland exhibit intra-population ecological variation, whereby individuals or groups differ in the proportional contribution of different prey items to their diet. This variation occurs in the absence of social and, potentially, reproductive isolation. Although further information will be required to assess the degree of structuring within the population, such heterogeneity should be taken into account in future conservation and management plans.

KEY WORDS: Niche width · Prey specialisation · Stable isotopes · Foraging · Orca

INTRODUCTION

Ecological variation, in the form of diversity in resource use, has long been recognised as a potential driving force for evolutionary divergence potentially leading to sympatric speciation [e.g. Mayr 1947, Smith & Skúlason 1996, Rundle & Nosil 2005]. However, traditional studies of resource use have often treated individuals within a population as equivalent. The niche variation hypothesis proposed by Van Valen (1965) suggests that generalist populations are more variable than those that specialise in a resource and that such variation may be achieved by higher among-individual heterogeneity. This hypothesis has gained recent support as apparently generalist populations have been shown to be ecologically heterogeneous, where individuals differ in their use of a common resource pool (Bolnick et al. 2003, 2007, Araújo et al. 2011), which could have significant effects on population and community dynamics (Bolnick et al. 2011). Highly mobile apex predator populations composed of individual specialists can affect ecosystem dynamics by linking separate food chains (Araújo et al. 2011). Long-lived top predators such as marine...
mammals are of particular interest since possible cultural transmission of their foraging behaviours could be an important mechanism underlying among-individual variation in resource use and its maintenance over generations (Estes et al. 2003, Sargeant et al. 2005, Torres & Read 2009). However, obtaining the necessary long-term data to assess the existence of individual preferences and possible genetic isolation in free-ranging marine mammal populations is difficult.

Measuring tissue chemical tracers in a predator acquired through prey offers an integrated measure of all prey assimilated over a period of days to years, thus providing a long-term overview of prey preferences (Michener & Schell 1994). The tracers most commonly used for dietary studies are stable isotope ratios, especially those of nitrogen (\(^{15}\text{N}/^{14}\text{N}\), denoted as \(\delta^{15}\text{N}\)) and carbon (\(^{13}\text{C}/^{12}\text{C}\), denoted as \(\delta^{13}\text{C}\)). Both are enriched in a predictable manner in consumers relative to their prey, although the enrichment of \(\delta^{15}\text{N}\) is more pronounced than that of \(\delta^{13}\text{C}\), providing a good estimate of a species’ trophic position (DeNiro & Epstein 1981, Hobson & Welch 1992). \(\delta^{13}\text{C}\) can provide information on predator foraging areas as it generally varies with latitude and, in the marine environment, presents clear differences between offshore vs. inshore or benthic vs. pelagic food sources (e.g. Cherel & Hobson 2007). As a result of these well-defined patterns of variation, variance in isotopic ratios can be used to infer trophic niche width (Bearhop et al. 2004) as well as to reliably identify individual-level variation in foraging preferences (Newsome et al. 2009a).

The killer whale *Orcinus orca* is a marine top predator with a cosmopolitan distribution (Forney & Wade 2006) that is as a species considered to be a generalist (Hoyt 1984, Jefferson et al. 1991). However, some populations appear to specialise in specific prey types, such as fish or marine mammals (reviewed in de Bruyn et al. 2013), or even particular prey species, such as Chinook salmon *Oncorhynchus tshawytscha* (Ford et al. 1998, 2016, Ford & Ellis 2006, Hanson et al. 2010). Ecotypes differing in diet, morphology, genetics and behaviour have been recognised in the North Pacific and Antarctica (e.g. Ford et al. 1998, 2011, Barrett-Lennard 2000, Pitman & Ensor 2003, Pitman et al. 2007, 2011, LeDuc et al. 2008, Pitman & Durban 2010, 2012). Dietary differences between these ecotypes and prey specialisation have been supported by visual observation of feeding events (e.g. Ford et al. 1998, 2011, Saulitis et al. 2000, Pitman & Ensor 2003, Burdin et al. 2004, Ford & Ellis 2006, Dahlheim et al. 2008, Pitman & Durban 2010, 2012), analysis of stomach contents or faecal material (Ford et al. 1998, 2016) and stable isotope analysis (Herman et al. 2005, Krahnn et al. 2007a,b, Newsome et al. 2009b, Durban et al. 2017). Specialised resource use appears to be culturally transmitted through generations within matrilineal social units (Riesch et al. 2012, Ford & Ellis 2014). The existence of ecological divergence and the transmission of resource use patterns through generations may be a mechanism that maintains social and reproductive isolation and, over long time scales, leads to the formation of separate ecotypes in sympatry (Moura et al. 2014, Foote et al. 2016).

The degree of dietary specialisation in killer whales has been proposed to relate to habitat productivity and availability of high-quality prey (Baird et al. 2006). Observations in tropical regions support a broad dietary range (Baird et al. 2006, Weir et al. 2010, Bolaños-Jiménez et al. 2014). Nevertheless, killer whales with an apparently mixed diet, i.e. including fish and marine mammals, have also been reported in high-latitude, productive regions both in the southern and northern hemispheres (Argentina: Íñiguez et al. 2002; Chilean Patagonia: Capella et al. 2014; Crozet Islands: Guinet 1992, Tixier et al. 2015; Marion Island: Reisinger et al. 2016; Norway: Vongraven & Bisther 2014; and South Africa: Best et al. 2010). Although prey specialisation is extensively documented in some well-studied populations and this has allowed clear ecotype assignment, the lack of long-term ecological data in other areas has impeded clarification of whether prey specialisation is a universal trait of the species (de Bruyn et al. 2013). Dietary studies providing long-term ecological data are, thus, crucial to understand the extent of ecological diversity within and between extant populations and to assess the role such variation may play in eventual ecotype formation in these long-lived top predators.

In Iceland and Norway, the occurrence of large aggregations of killer whales feeding on herring *Clupea harengus* has prompted suggestions of the existence of coastal communities or populations specialising on this prey (Sigurjónsson et al. 1988, Similä et al. 1996). Stable isotope analyses of the skin of individuals sampled in herring overwintering grounds in Norway supported at least seasonal herring specialisation in this location (Foote et al. 2012). These whales share the same complex feeding strategy (Similä & Ugarte 1993, Simon et al. 2007, Samarra & Miller 2015), suggesting similar ecology. However, observations of small subsets of killer whales switching prey in Norway (Vongraven & Bisther 2014) and
seasonally travelling away from the known herring stock distribution areas in Iceland (Samarra & Foote 2015) suggest the existence of ecological variation within herring-eating killer whales, whereby individuals may differ in the proportional contribution of different prey types to their diets, as proposed for Type 1 killer whales in the North Atlantic (Foote et al. 2009). Yet, the lack of foraging observations combined with isotopic analyses from known individuals has limited our understanding of the extent of prey specialisation in putative herring-specialist killer whales.

Here, we combine measurements of variation in δ¹³C and δ¹⁵N values in skin samples of free-ranging killer whales in Iceland with individual information, including sex, sighting frequency and movement patterns, to test the hypothesis that this population exhibits diet specialisation on herring. To do this, we sampled killer whales while they were observed presumably feeding on herring in the overwintering and spawning grounds of the Icelandic summer-spawning (ISS) herring during winter and summer, respectively. We hypothesised that if killer whales were herring specialists, following the herring year round between overwintering, feeding and spawning grounds, their trophic niche would be narrow and variance in their stable isotope ratio values would be low. This study contributes towards our understanding of the foraging ecology of Northeast Atlantic herring-eating killer whales and to what extent prey specialisation may be a generalised trait of this species.

MATERIALS AND METHODS

Sample and data collection

Killer whale biopsy samples were collected in winter and summer from ISS herring overwintering and spawning grounds, respectively, where killer whales are frequently seen feeding on herring. Sample collection in winter took place in February and March in 2013 and 2014 in Grundarfjörður and Kolgrafafljörður (West Iceland), 2 fjords that at the time formed part of the overwintering grounds of ISS herring (Fig. 1A, ICES 2014). Effort varied primarily due to weather and research priorities. In 2013, 2 out of 26 d with killer whale encounters were dedicated to biopsy sampling, while in 2014 there were 23 d with killer whale encounters and attempted biopsy collection. Sample collection in summer occurred in July 2014 in Vestmannaeyjar (South Iceland, 15 d with killer whale encounters), a spawning ground of ISS herring (Fig. 1B, Jakobsson & Stefánsson 1999). In both locations, killer whales aggregated seasonally coinciding with the migration of herring into the area.

Biopsy samples of skin and blubber were collected using a pneumatic rifle with 35 or 40 mm biopsy tips in 2013 and an ARTS pneumatic darting system (Kvadsheim et al. 2009) and stainless steel 25 mm biopsy tips in 2014. Biopsy tips were sterilised before use and stored in clean plastic bags. Samples were generally collected from the mid-lateral region of the body, below the dorsal fin. The whole layer of skin was used in subsequent analyses. Skin biopsy samples collected in 2013 were stored in ethanol (n = 8), while skin samples collected in 2014 were stored frozen (n = 51, Table S1 in the Supplement). While freezing is not considered to cause changes in the stable isotope values of tissues, in cetacean skin samples, ethanol preservation slightly depletes δ¹³C values but has no significant effect on δ¹⁵N values (Kiszka et al. 2014). We tested for potential differences between the 2 preservation methods and found no difference in δ¹⁵N (Wilcoxon rank sum test: W = 82, p = 0.77) or δ¹³C values (t-test: t-value = 1.91, df = 12.48, p = 0.08). Given these results, we combined the 2 sets of data. The turnover time of skin, that is, the time lapse during which the stable isotopic signal of a given type of diet remains in skin, is a few weeks in odontocete cetaceans (Browning et al. 2014, Giménez et al. 2016) unlike other tissues, such as teeth, which can
provide a lifelong sequential record of dietary preferences (Foote et al. 2009, Newsome et al. 2009b, Matthews & Ferguson 2014).

All sampled individuals were photographically identified (Bigg 1982) to try to avoid within-season repeated sampling of the same individual. Differences between repeated within-season samples from the same whale yielded δ13C and δ15N values within limits of analytical error and therefore were averaged. Sex was assigned based on genetic analysis. No calves or young juveniles were sampled, but each sex class may include subadult or adult individuals. Each individual’s encounter history was based on a photographic database collected between 2008–2010 and 2013–2015 in Vestmannaeyjar and between 2013 and 2015 in Grundarfjörður and Kolgrafafjörður (F. I. P. Samarra unpubl. data). This included the individual’s sighting frequency, corresponding to the total number of days with sightings in the database, and a movement pattern, classified as following herring year round (Group A) if photographed in both herring overwintering and spawning grounds or seen only in summer or winter (Group B). Of those individuals sighted only in summer or winter, seasonal movement patterns are only known for a few individuals that have been seen to move between Iceland in winter and Scotland in summer (Group C; Samarra & Foote 2015), away from the known distribution of ISS herring. All other individuals were only seen either in the summer or in the winter, and their year-round movements are unknown. There are more ISS herring overwintering and spawning grounds than those sampled in this study (Jakobsson & Stefánsson 1999, ICES 2014). Consequently, Group B may include individuals that do follow herring year round but to unknown grounds or individuals that seasonally move to other unknown areas.

**Stable isotope analysis**

All samples were dried at 60°C for 48 h and then powdered with a mortar and pestle. Lipid extraction was performed using a 2:1 solution of chloroform and methanol, and samples were then dried again at 60°C for 48 h to remove any remaining solution. Approximately 0.3 mg of powdered samples was weighed into tin capsules and then automatically combusted at 1000°C in a continuous-flow isotope ratio mass spectrometer (Flash 1112 IRMS Delta C Series EA; Thermo-Finnigan). All analyses were undertaken at the Centres Científics i Tecnològics of the University of Barcelona (CCiT-UB). Standards for 13C and 15N were Vienna Pee Dee Belemnite (V-PDB) and atmospheric nitrogen, respectively. International isotope secondary standards of known 13C/12C and 15N/14N ratios in relation to V-PDB and air, respectively, were used for calibration of δ13C and δ15N, including polyethylene (IAEA-CH-7, δ13C = −31.8‰, sucrose (IAEA-CH-6, δ13C = −10.4‰), ammonium sulfate (IAEA-N-1, δ15N = +0.4‰; IAEA-N-2, δ15N = +20.3‰), potassium nitrate (IAEA-NO-3, δ15N = +4.7‰) and L-glutamic acid (USGS40, δ13C = −26.2‰ and δ15N = −4.6‰). All reference materials used are distributed by the International Atomic Energy Agency (IAEA). Standards were included after every 10 samples. Results were expressed as per mille (‰) following the delta (δ) notation. Replicate measurements of internal laboratory standards indicate measurement errors of 0.28 ± 0.15‰ for δ13C and 0.34 ± 0.16‰ for δ15N.

**Statistical analysis**

We tested the effects of sex (Male vs. Female), season (Winter vs. Summer), sighting frequency and movement pattern (Group A vs. Group B) on the δ13C and δ15N values of killer whale skin samples using either a generalised linear model or a combination of Wilcoxon rank sum tests and Spearman’s rank correlation, depending on whether the data followed a normal distribution or not, respectively. A Bonferroni correction was used when applicable to adjust the significance level to account for multiple comparisons. All analyses were conducted in R 3.2.2 for Mac OS X (R Core Team 2015).

Isotopic niche width of individuals that moved between herring grounds year round (Group A) or were seen in only 1 season (Group B) was estimated in a Bayesian framework based on multivariate ellipse-based metrics, which allowed for sampling error to propagate to generated estimates, providing robust statistical comparisons between samples (Jackson et al. 2011). Standard ellipses corrected for sample size (SEA_{C}), which are less influenced by extreme values and are equivalent to standard deviation in univariate cases, were generated using the Stable Isotope Bayesian Ellipses in R (SIBER) package (Jackson et al. 2011). The area of a SEA_{C} contains approximately 40% of the data, regardless of sample size. Differences in SEA_{C} between groups were statistically tested by comparing the probability distributions of standard ellipse areas for both groups generated as the outcome of 10⁶ resampling runs (Jackson et al. 2011).
To test for fine-scale differences in stable isotopic signatures of whales with different movement patterns (Group A, B or C), we also input the stable isotope measurements into a multivariate discriminant function analysis (DFA), where movement pattern was used as the grouping variable and the cross-validation performed through a jackknife technique implemented in the \textit{lda} function of the MASS package version 7.3-16 (Venables & Ripley 2002) in R. The overall proportion of correct classifications and the proportion of correct classifications by location were calculated and compared to the proportion of by-chance accuracy, which was assumed to be equal (~33%) for all movement patterns.

**Diet composition**

All killer whale skin samples used were collected in known herring grounds, where the whales were observed feeding on herring. Although killer whales in Iceland have also been observed preying on seabirds, seals and minke whales (Víkingsson 2004), such observations have generally not been accompanied by identifications of the whales. Therefore, separate ecotype assignment of these latter whales could not be made. Throughout several field seasons in the herring overwintering and spawning grounds, our observations have only identified herring as a major prey item. The only 2 interactions with other prey observed were of a whale with a lumpfish \textit{Cyclopterus lumpus} in its mouth and whales throwing a salmon \textit{Salmo salar} in the air.

When killer whales were observed feeding, herring stunned by killer whale underwater tail slaps could be seen at the surface and were often caught by seabirds flying above feeding whales. In many cases, the research vessel was not close enough to collect the herring, but when possible, stunned fish were opportunistically collected and stored frozen. Samples of approximately 1 × 3 cm of herring muscle (including skin) were taken from each fish. Herring samples were processed following the same procedures for stable isotope analysis as detailed for killer whale skin samples (see Materials and methods: Stable isotope analysis).

Skin isotopic enrichment values per trophic level for nitrogen and carbon were +1.3 and +1.35, respectively, derived from a controlled diet experiment (García-Tiscar 2009). We used these values following the methodology of García-Tiscar (2009); however, the author pointed out that these values varied considerably between and within individuals examined (García-Tiscar 2009). Although the enrichment factor for nitrogen is below the 2 to 5‰ range found in other marine mammals (Newsome et al. 2010, Borrell et al. 2012), this value is consistent with the lower discrimination factors for killer whale blood and plasma measured from controlled diet experiments (García-Tiscar 2009, Caut et al. 2011) and agrees with the diet-predator enrichment expected from skin biopsies of free-ranging whales feeding on herring in Norway (Foote et al. 2012). The expected values were then directly compared to the measured mean δ^{15}N and δ^{13}C results of each group.

**RESULTS**

We collected 67 skin samples from 56 individual killer whales; 15 of them were repeated samples of the same individual killer whales within the same season and, thus, were averaged; 3 were repeated samples across different seasons and were kept as separate samples (Table S1 in the Supplement). This resulted in 59 measurements in total, 17 females and 42 males; 32 were collected in summer and 27 in winter (Table S1). Of the 59 measurements, 34 were whales that presumably followed herring year round (Group A), while the remaining 25 were whales only seen in 1 season (Group B), 3 of which were whales seen seasonally travelling to Scotland (Group C, Table S1). Killer whale skin sample δ^{15}N values ranged between 12.5 and 15.1‰ (mean ± SD: 13.2 ± 0.6‰, n = 59), and δ^{13}C values ranged between −18.5 and −16.9‰ (mean ± SD: −17.8 ± 0.3‰, n = 59, Table S1, Fig. 2).

**Stable isotope analysis**

The δ^{13}C data followed a normal distribution (Shapiro-Wilks test: \(W = 0.98, p = 0.43\)). A generalised linear model was fitted assuming a normal distribution with identity link and with sex (Male vs. Female), season (Winter vs. Summer), movement pattern (Group A vs. Group B) and sighting frequency as explanatory variables. There was a significant effect of sex on δ^{13}C values, with males having slightly higher values than females (Male vs. Female:
coefficient estimate = 0.22, \( t \)-value = 2.56, df = 54, p = 0.01), but no other variable was significantly different (Fig. 2). Because the \( \delta^{15}N \) data did not follow a normal distribution (Shapiro-Wilks test: W = 0.80, p < 0.001), Wilcoxon rank sum tests and a Spearman’s rank correlation test were used instead. There was no significant effect of sex on \( \delta^{15}N \) (Wilcoxon rank sum test: p > 0.1). There was no relationship between sighting frequency and \( \delta^{15}N \) (Spearman’s rank correlation: \( r_S = -0.02 \), \( S = 34 759 \), p = 0.91, Fig. 2). However, there was a significant effect of season (Wilcoxon rank sum test: \( W = 631 \), p = 0.002, Fig. 2) and movement pattern (Fig. 3). Although the range of values for summer and winter was similar, killer whales sampled in summer were more likely to have lower \( \delta^{15}N \) values. Only 9% of whales sighted in summer in comparison to 30% of whales in winter had \( \delta^{15}N \) values >13.5‰, a threshold based on the largest value of the convex hull area encompassing putative herring specialists (see Fig. 3).

Whales that followed herring year round (Group A) had a significantly lower \( \delta^{15}N \) than whales only seen in 1 season (Group B; Wilcoxon rank sum test: \( W = 220 \), p = 0.001, Fig. 3). In addition, whales that followed herring year round (Group A) had a smaller convex hull total area than whales seen only in 1 season (Group A vs. Group B: total area = 0.63 vs. 3.01‰², Fig. 3) as well as a significantly lower \( \text{SEA}_{C} \) (Group A vs. Group B: 0.19 vs. 0.92‰², p = 0, Figs. 3 & 4), indicating a narrower isotopic niche width. Three individuals that followed herring year round were sampled in both winter and summer (Table S1). The mean ± SD difference between samples from winter and summer of each individual was 0.12 ± 0.12‰ for \( \delta^{15}N \) and 0.35 ± 0.30‰ for \( \delta^{13}C \).

A multivariate DFA showed high stable isotopic variation between whales that follow herring year round (Group A) and whales that travel between Iceland and Scotland (Group C), with the first discriminant function accounting for 98% of the variability. The loadings of the first discriminant function revealed that \( \delta^{15}N \) was the main discriminating predictor (loadings: \( \delta^{15}N = -2.06, \delta^{13}C = 0.44 \)). The cross-validated classification showed an overall correct classification of 64% of samples to the correct movement pattern, compared to a by-chance proportion of 33%. The largest correct classification score was 85% for Group A, followed by 67% for Group C. None of the misclassifications for Group A were assigned to Group C, and none of the misclassifications of Group C were assigned to Group A, supporting a good separation between these groups (Fig. 3).

Whales only seen in 1 location (Group B) had a correct classification score of 32%. Misclassifications were assigned to the other 2 movement types, reflecting less discrimination between whales seen in

Fig. 2. Variation in killer whale stable isotopes with sex (left panel), season (center panel) and sighting frequency (right panel: circle diameter increases with sighting frequency)

Fig. 3. \( \delta^{13}C \) and \( \delta^{15}N \) values of skin samples from killer whales seen in herring grounds year round (Group A) and whales that are only seen seasonally (Groups B and C). Solid lines represent the standard ellipses corrected for sample size (\( \text{SEA}_{C} \)), while dashed lines represent the convex hull area. Whales seen in Iceland only in winter that travel to Scotland in summer (Group C) are represented as triangles
Diet composition

We sampled 12 herring collected in summer and winter fieldwork sites during killer whale feeding events. Herring had a mean ± SD of 11.78 ± 0.50‰ for $\delta^{15}N$ and $-19.13 ± 0.47$‰ for $\delta^{13}C$; there was no statistically significant difference between samples collected in winter and summer (Wilcoxon rank sum test: $W = 26$, $p = 0.24$ for $\delta^{15}N$ and $W = 23$, $p = 0.48$ for $\delta^{13}C$). The assumption of a diet exclusively composed of herring resulted in a $\delta^{15}N_{\text{expected}}$ of 13.08‰ and a $\delta^{13}C_{\text{expected}}$ of $-17.78$‰. The modelled herring diet isotopic ratios ($\delta^{15}N_{\text{expected}}$ and $\delta^{13}C_{\text{expected}}$) were very close to the mean and within the range of values (shown as [minimum; maximum]) for killer whales following herring year round (group A, 12.93 [12.47; 13.44]‰ for $\delta^{15}N$ and $-17.84 [-18.54; -16.92]$‰ for $\delta^{13}C$) but considerably lower than the most extreme $\delta^{15}N$ values within this group, which had larger variation than group A. In comparison to whales seen travelling between Iceland and Scotland (group C), the modelled herring diet isotopic ratios ($\delta^{15}N_{\text{expected}}$ and $\delta^{13}C_{\text{expected}}$) were lower than the mean and outside the range for $\delta^{15}N$ values (14.25 [13.71; 15.02]‰) but not for $\delta^{13}C$ values ($-17.74 [-17.81; -17.61]$‰).

DISCUSSION

Killer whale ecotypes in the Northeast Pacific have become an important study system of ecological diversification due to their discrete diet based on either fish or mammals, which is in turn linked to behavioral differences and social and genetic segregation (Riesch et al. 2012). In contrast, this study on a population of Northeast Atlantic killer whales previously presumed to be herring specialists (Sigurjónsson et al. 1988, Simon et al. 2007) provides isotopic evidence for fine-scale within-population niche variation. The variation in $\delta^{15}N$ values spanned ~2.5‰, a value consistent with over 1 trophic level of niche width, assuming estimates of discrimination factors in killer whale skin of around ~1.3‰ (García-Tiscar 2009). When the stable isotopic values are examined in the light of individual sighting history information, variation in $\delta^{15}N$ values correlated with season and movement patterns. This indicates that while many individuals appeared to specialise on herring, not all individuals within the population were equivalent. A few individuals had stable isotope values indicative of a broader diet, which could include seasonal or opportunistic targeting of herring.

Variation in isotopic niche with movement pattern

Whales that appeared to follow herring year round had overall lower $\delta^{15}N$ than whales seen in only one season as well as a significantly narrower trophic niche width. A diet composed exclusively of herring was consistent with the stable isotopic values of these whales, and year-round specialisation on herring was supported by a very low difference in repeated samples collected from the same individuals across seasons. Among whales only seen in one season, there was increased variation in $\delta^{15}N$ values, with
some whales grouping well with whales following herring but others having higher $\delta^{15}N$ values indicative of incorporation of higher trophic level prey in their diet. This increased variation was evident in the low rate of discrimination between this and the other groups in the DFA. The individuals seen in only one season but with $\delta^{15}N$ values similar to those following herring year round also grouped well with a diet consisting mainly of herring, indicating that some whales may follow herring year round but to unknown locations.

There was particularly good discrimination in $\delta^{15}N$ values between whales following herring year round and whales travelling seasonally to Scotland. For some of the whales seen in only one season, and particularly those known to travel seasonally between Iceland and Scotland, $\delta^{15}N$ values were higher than expected based on a diet consisting exclusively of herring. While they may seasonally aggregate in herring grounds to exploit this prey, the diet of these individuals appears to also include higher trophic level prey. Indeed, two of the biopsy-sampled individuals included in this study that are seen seasonally travelling between Iceland and Scotland ($IS172$ and 997, Table S1 in the Supplement) have been confirmed attacking and consuming grey seals *Halichoerus grypus* in summer in Scotland (Fig. S1 in the Supplement). Nevertheless, the presence of individuals with presumably mixed diets was not related with sighting frequency. Indeed, the individuals sighted moving between Iceland and Scotland are sighted frequently in the herring overwintering grounds (Samarra & Foote 2015), despite their higher $\delta^{15}N$ values. Thus, although we lack sufficient information in this study to elucidate what other high trophic level prey may compose their diet in Iceland or other locations where these whales may travel to, at least some individuals within the population have a broader niche width and do not specialise exclusively on fish. Complementary analysis on other chemical tracers (e.g. Herman et al. 2005) and genetic markers should provide a better understanding of the level of dietary and demographic structuring in this population.

**Intra-population diversity in prey specialisation**

In killer whales, the occurrence of a mixed diet, including high and low trophic level prey, has been visually observed in several locations and shown based on stable isotope analysis (Foote et al. 2009, 2013, Reisinger et al. 2016). In the North Atlantic, analysis of stranded and museum specimens showed the existence of an ecotype including individual-level variation in the proportional contribution of fish and marine mammal prey to the diet (Foote et al. 2009), a finding supported by a few observations in Norway of a group of killer whales switching between the 2 prey types (Vongraven & Bisther 2014). In Icelandic waters, sympatric killer whales appear to divide into different movement patterns that correlate with their use of resources, with apparent seasonal overlap in targeted resources. While some whales appear to specialise on herring year round, others target both herring and other higher trophic level prey and appear to maintain such preferences over time. This indicates a generalist population, but one in which isotopic niche width is primarily driven by among-individual or group variation rather than by all individuals consuming the same wide range of prey, as it has been reported to occur in other taxa (Bolnick et al. 2003, 2007, Araújo et al. 2011).

Ecological variation and divergence appears to be an important factor promoting genetic divergence in marine top predators (e.g. Louis et al. 2014), including killer whales (Foote et al. 2016). Unlike sympatric killer whale populations of different ecotypes described in other areas that are ecologically, socially and genetically isolated (e.g. Ford et al. 1998, 2011), in Iceland ecological specialisation does not appear to occur at the population level. Instead, groups within the population appear to share part of their ecological niche at least seasonally. This shared niche, together with temporary social associations (Tavares et al. 2016), may mean that there is no genetic divergence between the different groups described here and, thus, that different movement patterns and foraging traditions may be maintained without genetic divergence. This would agree with observations of the maintenance of among-individual ecological variation in North Atlantic killer whales across thousands of years without leading to sympatric speciation (Foote et al. 2013); however, genetic analyses will be required to assess whether the observed ecological diversity maps to different lineages.

Our study was composed, in most cases, of only one measurement per individual, which precluded us from evaluating within-individual variation, except for 3 individuals that showed very low variation in stable isotopic values between seasons, supporting specialisation on herring year round. Together with movement patterns that appear to be maintained over several years (Foote et al. 2010, Samarra & Foote...
2015), the analysis in this study suggests that foraging traditions may be kept in the long term. Such persistent variation in foraging behaviour is in agreement with the large variance in \( \delta^{15}N \) values, indicative of a wide ecological niche, found in long-term dietary markers (tooth and bone) for Type 1 killer whales in the North Atlantic (Foote et al. 2009). Assessing the long-term stability of dietary preferences for herring-eating killer whales, by combining repeated feeding observations and isotopic measurements of identified individuals, would be important in the future, particularly if potential changes in stable isotope baselines are concurrently monitored.

Our study strongly supports the existence of herring-specialist killer whales that target this prey year round and follow its migration. Specialisation allows individuals to develop and refine foraging techniques and makes them more efficient hunters. Indeed, herring-eating killer whales are known to employ a complex group-coordinated feeding strategy to target herring (Similä & Ugarte 1993). In highly specialised killer whale populations, low prey abundance can severely impact population demography (Ward et al. 2009, Ford et al. 2010, Esteban et al. 2016) and social connectivity (Foster et al. 2012). Ringing is a prey known to change migration routes and to be subject to severe changes in abundance (Jackson & Stefánsson 1999, Öskarsson et al. 2009), and as a consequence, whenever its abundance falls below certain levels, it is likely to impact the demography and social connectivity of herring-specialist killer whales. Consequently, assessing the degree of dependence on herring as well as determining the proportion of specialised individuals within the killer whale population and their level of foraging flexibility is of utmost relevance to investigate the effects of this top marine predator in the ecosystem and its resilience to environmental changes. Assessing the long-term variation in prey specialisation within this population will also increase our understanding of the role such variation may play in eventual ecotype formation in these long-lived top predators.

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LITERATURE CITED


Bolnick DI, Svanbäck R, Araújo MS, Persson L (2007) Comparative support for the niche variation hypothesis that more generalized populations also are more heterogeneous. Proc Natl Acad Sci USA 104:10075–10079


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