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RESEARCH ARTICLE

Quantitative fatty acid signature analysis reveals a high level of dietary specialization in killer whales across the North Atlantic

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Abstract

1. Quantifying the diet composition of apex marine predators such as killer whales (*Orcinus orca)* is critical to assessing their food web impacts. Yet, with few exceptions, the feeding ecology of these apex predators remains poorly understood.
2. Here, we use our newly validated quantitative fatty acid signature analysis (QFASA) approach on nearly 200 killer whales and over 900 potential prey to model their diets across the 5000 km span of the North Atlantic.
3. Diet estimates show that killer whales mainly consume other whales in the western North Atlantic (Canadian Arctic, Eastern Canada), seals in the mid-North Atlantic (Greenland), and fish in the eastern North Atlantic (Iceland, Faroe Islands, Norway). Nonetheless, diet estimates also varied widely among individuals within most regions. This level of inter-individual feeding variation should be considered for future ecological studies focusing on killer whales in the North Atlantic and other oceans.
4. These estimates reveal remarkable population- and individual-level variation in the trophic ecology of these killer whales, which can help to assess how their predation impacts community and ecosystem dynamics in changing North Atlantic marine ecosystems.
5. This new approach provides researchers with an invaluable tool to study the feeding ecology of oceanic top predators.

KEYWORDS
blubber, cetacean, diets, feeding ecology, individual specialization, *Orcinus orca*, predation, QFASA

1 | INTRODUCTION

Elucidating the trophic interactions of marine predators is critical for understanding their ecological impacts on communities (Estes et al., 2016). It is also important to monitor the impacts of environmental changes like climate change on community dynamics (Grose et al., 2020; Sadykova et al., 2020). As the oceans warm, community dynamics are impacted, especially in the higher latitudes (Kortsch et al., 2015; Pecuchet et al., 2020; Post et al., 2019). Indeed, climate change has already led to increases in the presence of predators like killer whales (*Orcinus orca*) in the Arctic and is expected to modify their feeding habits (Ferguson et al., 2010). Yet, the feeding ecology of killer whales across many ocean regions remains uncertain, despite decades of research on different populations.

Multiple recent studies have called for an ocean-wide comparison of the diets of North Atlantic (NA) killer whales (Dietz et al., 2020; Foote, 2022; Jourdain et al., 2019). Initial studies provided some insight into the trophic interactions of NA killer whales, although they were primarily based on behavioural observations. From these, Norwegian and Icelandic killer whales are thought to mostly forage on fish like Atlantic herring (*Clupea harengus*) and occasionally on marine mammals (Samarra & Foote, 2015; Sigurjónsson, 1988; Simila & Ugarte, 1993; Vongraven & Bisther, 2014). Conversely, killer whales possibly target marine mammals off Greenland and along the east coast of Canada (Ferguson et al., 2010; Ferguson, Higdon, et al., 2012; Higdon et al., 2012). Foote et al. (2009) suggested the existence of two NA killer whale ecotypes based on morphological and genetic data: Type 1 being a generalist that relies mostly on Atlantic herring but also on some pinnipeds and cetaceans and Type 2 being a specialist that feeds predominantly on marine mammals (Foote et al., 2009). However, Foote recently published a letter calling to drop the type 1/type 2 classification for NA killer whales and focus on collecting more samples, specifically in remote areas, to understand the feeding ecology of these predators across the NA ocean (Foote, 2022). Understanding the feeding ecology of elusive and wide-ranging marine predators such as killer whales is challenging and requires the use of time-integrated dietary tracers such as stable isotopes or fatty acid signature analysis that represent the long-term diet of individuals, particularly when observational evidence is limited or when stomach contents are unavailable (Kiszka et al., 2021; Trites & Spitz, 2018).

To date, few studies have used chemical tracers to investigate the feeding ecology of NA killer whales. Studies of stable carbon and nitrogen isotope analysis and organic contaminants were consistent with observations suggesting that Icelandic and Norwegian killer whales seem to rely mostly on fish but also reported some degree of individual specialization on marine mammals like seals or porpoises (Andvik et al., 2020; Foote, 2012; Remili et al., 2021; Samarra, Vighi, et al., 2017; Wolkers et al., 2007). Greenlandic and Canadian whales seem to rely to some extent on marine mammals based on chemical tracers (Bourque et al., 2018; Matthews et al., 2021; Matthews & Ferguson, 2014; Pedro et al., 2017). Although stable isotopes provide information on the carbon source and relative trophic position, stable isotope mixing models result in large confidence intervals for prey proportions, whereas fatty acid signatures may provide more precise estimates. Fatty acids are the main constituent of most lipids and are released from ingested lipid molecules (e.g., triacylglycerols) during digestion (Budge et al., 2006). Fatty acids of carbon chain-length 14 or greater pass into an animal's circulation and are deposited into their lipid storage tissues, such as blubber, with little modification or in a predictable pattern, thus providing a time-integrated record of dietary intake (Iverson et al., 2004). In eastern North Pacific killer whales, fatty acid profiles were sufficiently distinct among the three reported ecotypes (resident, transient and offshore) to enable individual animals to be classified according to ecotype based on their fatty acid signature alone (Herman et al., 2005). Therefore, comparing fatty acid profiles, that is, qualitative fatty acid analysis, among killer whale populations and individuals may allow for identifying foraging specialization across the NA (Budge et al., 2006). However, qualitative fatty acid analysis provides no information on the relative contribution of each prey species to a predator's diet.

A greater understanding of diets may be generated using quantitative fatty acid analysis (QFASA). QFASA was developed to estimate the combination of prey FA signatures that comes closest to matching that observed in the predator after accounting for predator metabolism and de novo synthesis (Iverson et al., 2004). The method requires information on the fatty acid composition (from a subset of fatty acids that is known to reflect dietary sources) of all major potential prey species and of the predator. The method also requires species-specific calibration coefficients (CCs) that account for predator metabolism and a statistical model to minimize the statistical distance between the predator and the weighted mixture of prey species representing the diet (Iverson et al., 2004). The analysis results in diet estimates that represent the relative contribution of multiple prey sources for each sampled individual predator. We recently developed and validated QFASA for killer whales, including the determination of killer whale-specific CCs, allowing us to use this technique to explore inter- and intra-population variation in QFASA diet estimates for the first time in this species (Remili et al., 2022).

There is a need to use higher-resolution chemical tracers, like fatty acids, in samples collected within similar time frames and across regions to improve our understanding of killer whale feeding in the NA Ocean (Foote, 2022; Jourdain et al., 2019; Remili et al., 2022). Inter-population and inter-individual differences in feeding ecology may result in, for example, differential risks related to changes in prey availability due to climate change and related to exposures to

environmental contaminants for killer whales (Andvik et al., 2020; Pedro et al., 2017; Remili et al., 2021). In addition, understanding the ecological impacts of killer whales on prey populations entails renewed efforts to resolve the question of the feeding ecology of NA killer whales. In this study, we present a new approach to estimate the diets of killer whales, which may, in turn, inform on their predation pressure in a changing environment. We assess for the first time both inter- and intra-population variation in the diets of NA killer whales, using both qualitative and newly developed QFASA estimation approaches based on nearly 200 killer whales sampled from west to east across the entire NA Ocean, as well as over 900 specimens of their potential prey species.

2 | MATERIALS AND METHODS

2.1 | Sample collection

For killer whales, we collected 191 blubber samples from biopsied, stranded, or subsistence-harvested individuals, including 58 individuals from the Eastern Canadian Arctic (Pond Inlet and Pangnirtung, Nunavut from 2009 to 2020), five individuals from Eastern Canada (Saint-Pierre & Miquelon, from 2019 to 2021), one individual from West Greenland (Nuuk, 2021), 18 individuals from East Greenland (Tasiilaq and Scoresby Sund, from 2012 to 2021), 48 individuals from Iceland (Grundarfjörður and Vestmannaeyjar, from 2014 to 2016), two individuals from the Faroe Islands (2008), and 59 individuals from Norway (Skjervøy area, from 2017 to 2019).

Details of the samples collected from 2008 to 2021 are available in Table S1. For Greenlandic killer whales, full blubber samples (and attached skin for proper orientation) were opportunistically collected from subsistence harvest events and cut into ten equal layers, with layer 1 being closest to the muscle and layer 10 being closer to the skin of the animal. More details of the sampling can be found in Pedro et al., 2017. Faroese samples were collected from stranding events. The blubber was not oxidized, and the samples' surfaces were shaved to access the freshest tissue. Samples were then processed in a similar way to the Greenlandic samples, as described in an earlier study (Bourque et al., 2018). Only the outer blubber from these samples, representing the length of a biopsy, was used in this study (Remili et al., 2022). The remaining samples consisting of skin and blubber biopsies were collected from live free-ranging killer whales using an ARTS pneumatic darting system (LKARTS-Norway, Norway) or a crossbow and stainless-steel biopsy tips (CetaDart, Denmark) ranging from 25 × 7 mm to 40 × 5 mm, depending on the location. Samples in the Canadian Arctic were collected with Fisheries and Oceans Canada Licence to Fish #s S-09/10-1009-NU, S-13/14-1024-NU, S-18/19-1029-NU, S-19/20-1004-NU, and S-20/21-1018-NU (Animal Use Protocols: FWI-ACC-2009-008, FWI-ACC-2013-022, FWI-ACC-2018-008, FWI-ACC-2019-11, and FWI-ACC-2020-19 approved by Fisheries and Oceans Canada's Freshwater Institute's Animal Care Committee). Samples from Saint Pierre et Miquelon (France) were collected under permit #431 (July 17th, 2019) granted by Préfecture de Saint Pierre et Miquelon. Samples in Iceland were collected under the institutional permit of the Marine and Freshwater Research Institute. Sampling of Norwegian killer whales was conducted in accordance with FOTS permits #8165 and 24075. Biopsy tips were sterilized before use and stored in clean plastic bags. All samples were generally collected from the body's mid-lateral region, below the dorsal fin, and stored frozen in the field at −20°C in aluminium foil. Once back at the lab, samples were stored at −80°C until analysis. The full list of more than 900 prey samples collected (as well as their locations and the tissue type) includes Atlantic herring, Atlantic mackerel (*Scomber scombrus*), bearded seal (*Erignathus barbatus*), beluga whale (*Delphinapterus leucas*), bowhead whale (*Balaena mysticetus*), fin whale (*Balaenoptera physalus*), Greenland shark (*Somniosus microcephalus*), harbor porpoise (*Phocoena phocoena*), harbor seal (*Phoca vitulina*), harp seal (*Pagophilus groenlandicus*), hooded seal (*Cystophora cristata*), humpback whale (*Megaptera novaeangliae*), lumpfish (*Cyclopterus lumpus*), minke whale (*Balaenoptera acutorostrata*), narwhal (*Monodon monoceros*), and ringed seal (*Pusa hispida*) (Table S1). All details on fatty acid extractions and fatty acid QA/QC can be found in the Supplemental text in the Supporting Information.

2.2 | Statistical analyses

All fatty acid datasets containing the same number of fatty acids ($n = 68$) were renormalized to sum to 100% prior to subsequent data analysis. Only the fatty acids identified as mainly originating from the diet were included (Iverson et al., 2004; Remili et al., 2022). Of those, only dietary fatty acids above 0.1% of the total FA signature ($n = 15$) were included to minimize analytical variation associated with small peaks on the GC-FID (Table S2). First, we performed a principal component analysis (PCA) on arcsine-transformed FA signatures across the NA to visually assess the FA niche widths and overlaps across the ocean basin (using the FACTOMINER package).

Following the PCA, we applied the newly validated QFASA model (Remili et al., 2022) to the 191 killer whales using the QFASAR package in R (version 3.6.1). QFASA produces diet estimates representing the estimated percentage of each prey species from the prey library in the diet of each predator (Remili et al., 2022). The means and standard error (SEs) of the diet estimates were obtained using bootstrap sampling ($n = 100$). The estimates were then corrected to account for differences among prey species in lipid content (Table S1). QFASA is very sensitive to the choice of prey species included in the prey library, which prompted us to select different prey in different geographical regions based on available literature regarding the known diet items of each killer whale regional group. For instance, we omitted beluga and narwhal in the Icelandic prey library because these prey species are not encountered in Iceland and were never reported to belong to Icelandic killer whales' diets. The list of prey species included in each prey library can be found in Table S1, and the justifications for the choice of prey can be found in the Supplementary text.

QFASA relies on the principle that predator FA signatures can be modelled as a linear mixture of the prey FA signatures (Iverson et al., 2004). Thus, we expect the predator FA signature to be within the prey FA range. Not meeting this criterion indicates poor CCs or an incomplete prey library (Bromaghin, 2017). We tested our data using the function *pred_beyond_prey* in QFASAR to find the proportion of predator FA values outside the range of the prey values. A second QFASA assumption is limited overlap in the FA signatures among prey species (i.e., that the FA signature of each prey species is distinct; Iverson et al., 2004). To test this assumption, we used the *leave_one_prey_out* (LOPO) function, which removes one prey signature from the library at a time and recomputes the mean prey-type, and then estimates the diet of the removed prey signature. The analysis performs this computation on each prey signature, one at a time. The final output indicates the proportion of samples attributed to the correct species.

Following the QFASA analyses, we extracted the individual diet proportions and calculated the population-wide individual specialization (IS), which is the average individual proportional similarity (PS_i), with PS_i defined as the diet overlap between an individual *i* and the population:

$$PS_i = 1 - 0.5 \sum_j \left| p_{ij} - q_j \right|,$$

where p_{ij} is the proportion of species *j* in the diet of individual *i*, and q_j is the average proportion of species *j* in the population's diet (Bolnick et al., 2002). The closer IS is to 100%, the more an individual's diet aligns with that of the whole population. Conversely, a lower IS percentage shows that an individual's diet differs from the population-wide diet.

Finally, as a check of the robustness of the QFASA approach, we tested for correlations between the percentage of marine mammals estimated in the diets (Arcsine-transformed) and nitrogen isotope ($\delta^{15}N$) values and between marine mammal consumption and the sum concentrations of a diet-derived contaminant group, polychlorinated biphenyls (∑PCBs, log-transformed to achieve normality). These correlations were run for Icelandic male killer whales for which we had previously published both isotope and PCB data (Remili et al., 2021; Samarra, Vighi, et al., 2017). We chose males because, unlike females, they do not transfer some of their contaminant load to their offspring, and thus their PCB concentrations are not impacted by pregnancies and lactation (Borrell et al., 1995; Wells et al., 2005).

3 | RESULTS

The QFASA modeling approach provided the first detailed species-specific diet estimates for NA killer whales, revealing a remarkable range of diet compositions among and within populations. Diet estimates ranged from cetacean-dominated in the western NA (Canadian Arctic, Eastern Canada) to pinniped-dominated in the mid-NA (Greenland) to fish-dominated in the eastern NA (Iceland, Faroe Islands, Norway; Figure 1, Table S2 and S3).

QFASA estimates showed that killer whales from the western and mid-NA regions had high contributions of marine mammals in their diets, but with important differences among locations. Canadian Arctic and Eastern Canada killer whales mostly consumed cetaceans (53% ±2 and 82% ±14, respectively). Belugas and narwhals were the primary prey for Canadian Arctic killer whales, while baleen whales (fin, humpback, and minke whales) and harbor porpoises were the main prey identified for Eastern Canada killer whales. Additionally, in Canada, sampled killer whales exhibited significant spatial variation in their dietary preferences. In the Eastern Canadian Arctic, more than half of the killer whales ($n = 33$) had beluga and narwhal diet contributions above 50%, while a quarter of the whales ($n = 14$) had ringed seal

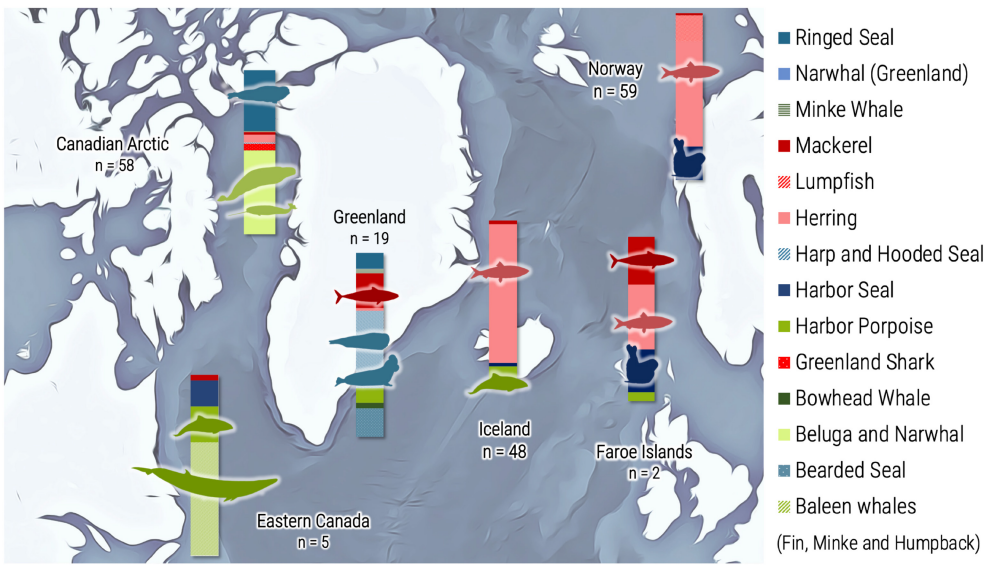

FIGURE 1 Mean proportional contributions of prey species to the diets of North Atlantic killer whales by region sampled from 2008 to 2021 based on quantitative fatty acid signature analysis. Additional information can be found in Table S2.

diet contributions above 50%, and seven whales had herring diet contributions above 20% (Table S3). In Eastern Canada, four of the five killer whales mainly fed on baleen whales (above 60%), while one individual mostly consumed harbor porpoises. In the mid-NA, Greenland killer whale diets included mainly seals (total seal: 66% ±5) and a lower contribution of cetaceans (total cetacean: 13% ±2) and fish (20% ±3). For Greenland killer whales, mackerel was the most significant source of fish, and half of the sampled individuals had a contribution of mackerel above ~20% (Table S3).

The eastern NA killer whales showed high proportions of herring in their diets: 62% ±4 for Norway, 39% ±39 for the Faroe Islands, and 82% ±4 for Iceland, with minor contributions of lumpfish, mackerel, and marine mammals. One third of the individual ($n=18$) Norwegian killer whales had lumpfish contributing more than 20% to their diet. In Iceland, ten individuals had marine mammal estimates above 30%, and in Norway, twelve individuals had harbor seal estimates over 30%.

The individual specialization (IS) index calculated for each regional group or subgroup revealed specialization differences across the NA (Figure 2a). The closer the IS index is to 1, the more the individuals' diets overlap with the population's mean diet. Thus, a lower IS estimate indicates a stronger degree of individual specialization. In the western NA, Eastern Canadian Arctic killer whales showed a moderate degree of individual specialization (IS index: 0.72 ± 0.02), with some individuals specializing on ringed seals and others on belugas and narwhals (Figure 2b). In Eastern Canada, individual specialization was also present (IS: 0.64 ± 0.08), with individuals consuming varied combinations of marine mammal species (Figure 2c, Table S3). In the mid-NA, Greenland killer whales showed a higher degree of individual specialization (IS index: 0.58 ± 0.04), with whales displaying varying feeding patterns ranging from seal-dominated diets to mixed diets with fish and marine mammals like seals or cetaceans (Figure 2d). In the Eastern NA, however, individual killer whales in Norway and Iceland showed substantial overlap with the population mean diet (IS index: 0.80 ± 0.01 for Norway; 0.80 ± 0.03 for Iceland), indicating that most of the killer whale diets are similar and in accordance with the population use of resources (Figure 2f,g). For a handful of individuals in Norway ($n=1$) and Iceland ($n=7$) previously reported to feed on marine mammals based on visual observation, there was less overlap with the population mean diet (IS index: 0.58 for the Norwegian individual; 0.51 ± 0.11 for the Icelandic individuals), indicating that these killer whales rely on different resources compared to most other individuals in the populations (Figure 2, Table S3). The IS index was low for Faroese whales (0.60) but based on only two individuals with different diets (Figure 2e).

For Icelandic killer whales specifically, diets estimated by QFASA were also compared to other available indicators of their position in the food web based on measurements realized on the same skin biopsies (Figure 3). Contaminant concentrations, that is, polychlorinated biphenyls (∑PCBs) and δ^{15}N values, were both moderately correlated with the estimated percentage of marine mammals in the whales' diets (Figure 3). The Pearson correlation coefficient between the total percentage of marine mammals (Arcsine-transformed) and log ∑PCB concentrations was $R=0.53$ ($p<0.01$) in Icelandic male killer whales, while it was $R=0.43$ ($p=0.02$) for the correlation with δ^{15}N values in the same whales. It should be noted that two killer whales that had previously been observed feeding on seals had a rather low estimated proportion of seal prey in their diet, even though their contaminant values were high (Figure S1).

The differences in QFASA estimates across the NA killer whales were further reinforced by qualitative differences, with killer whale fatty acid profiles themselves being distinctive in each region (Figure 4). Killer whales from Eastern Canada showed somewhat similar fatty acid profiles to Greenland and Eastern Canadian Arctic killer whales, but fatty acid signatures from these three regions were well separated from those of the killer whales from the Eastern NA. The Norwegian and Icelandic killer whales had highly overlapping fatty acid signatures. Nonetheless, for Norway and Iceland, several individuals identified in Figure 4 with an asterisk showed fatty acid profiles outside that of the eastern NA groups and closer in the PCA to the western and mid-NA groups. These individuals are known to have consumed marine mammals, as inferred from previous observations and/or feeding tracers (Remili et al., 2021; Samarra, Vighi, et al., 2017).

All prey species included in the QFASA prey libraries separated relatively well on the prey fatty acid PCA (Figure 5). There was some noticeable overlap of certain cetacean species. Beluga whales had the largest ellipse, which caused their FA signatures to overlap slightly with the FA signatures of narwhals, bowhead whales, and harbor porpoises. The QFASA *leave_one_prey_out* (LOPO) diagnostic revealed that beluga and narwhal FA signatures were close enough that the model was unable to distinguish between the two species (Table S4). As a result, when included in the same library, we merged the two species. Harp and hooded seals were also merged based on the QFASA diagnostics of our previous study (Remili et al., 2022). Species sampled in different regions, like herring, mackerel, and narwhals, grouped close together in the prey PCA, which suggests a limited degree of geographical dietary variation within the species; thus, the ellipses for the same species but different regions still grouped closely enough that models could accurately identify them from other species (Table S4). Nonetheless, we decided to use region-specific prey libraries to be most representative of the potential diet of killer whales in each region. For example, Greenland herring was used to estimate the diets of Greenland killer whales, while Iceland herring was used to estimate the diets of Icelandic killer whales (more details in Table S1 and Supplemental text). Fatty acid percentages for all NA killer whales, Icelandic prey (harbor seal, herring, mackerel), and Norwegian prey (herring, mackerel, and lumpfish) can be found in Tables S5 and S6.

Various checks of the QFASA models supported its utility for modeling the diets of NA killer whales. Overall, the QFASA diagnostics indicated that the choices of prey species and calibration coefficients were adequate. The *Leave_one_prey_out* analyses ranged from

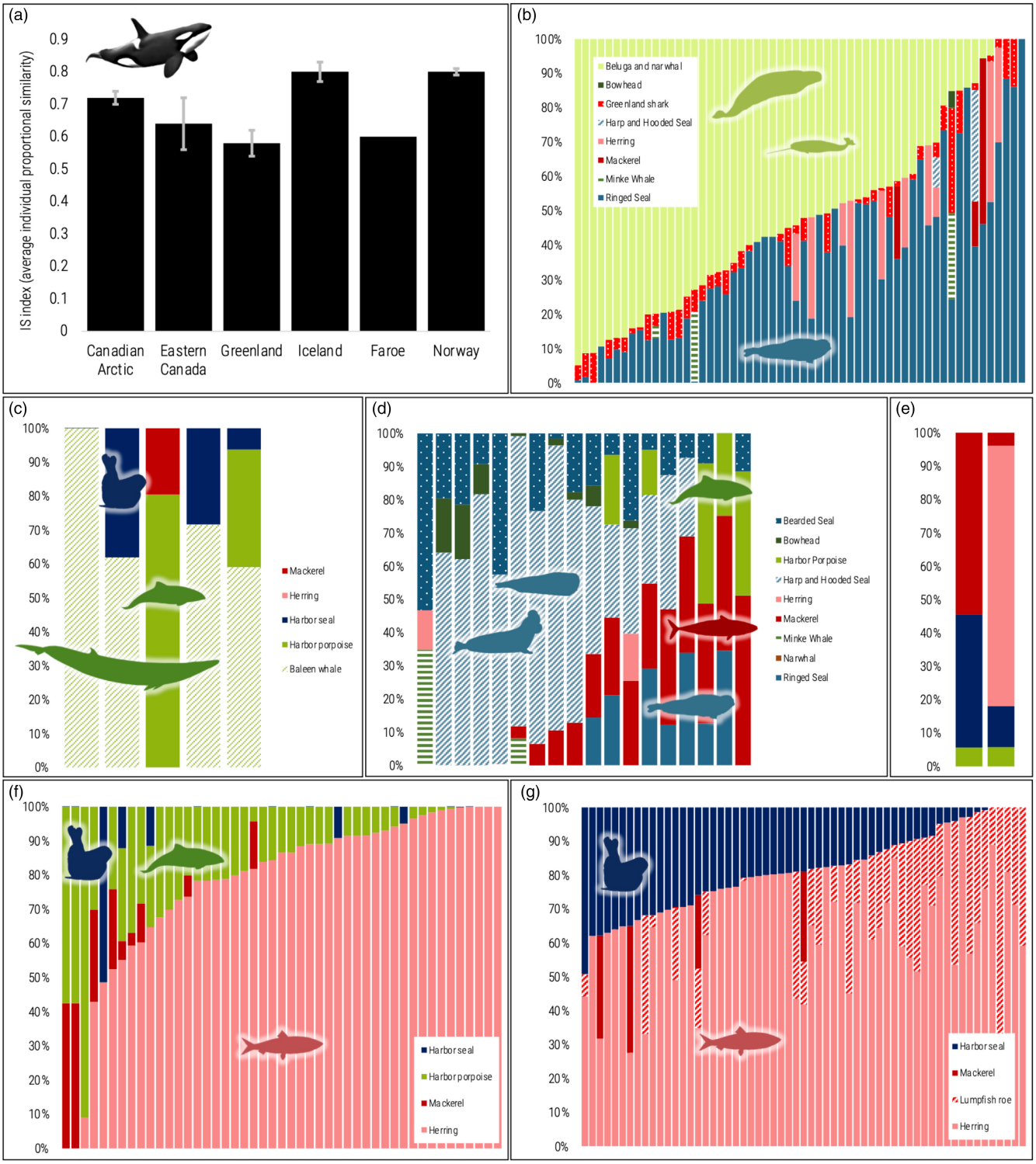

FIGURE 2 Individual dietary specialization among North Atlantic killer whales sampled from 2008 to 2021. (a) The individual specialization (IS) index across different geographical locations represents the average individual proportional similarity (PS_i), defined as the diet overlap between an individual *i* and the population mean diet; (b) Individual dietary composition of Canadian Arctic killer whales; (c) individual feeding patterns of Eastern Canada killer whales; (d) individual feeding patterns of Greenlandic killer whales; (e) Individual feeding patterns of Faroe Islands killer whales; (f) individual feeding patterns of Icelandic killer whales and (g) individual feeding patterns of Norwegian killer whales. Each bar on the *x*-axis for figures (2b–2g) represents one individual from the location. The detailed estimates for each individual can be found in Table S3.

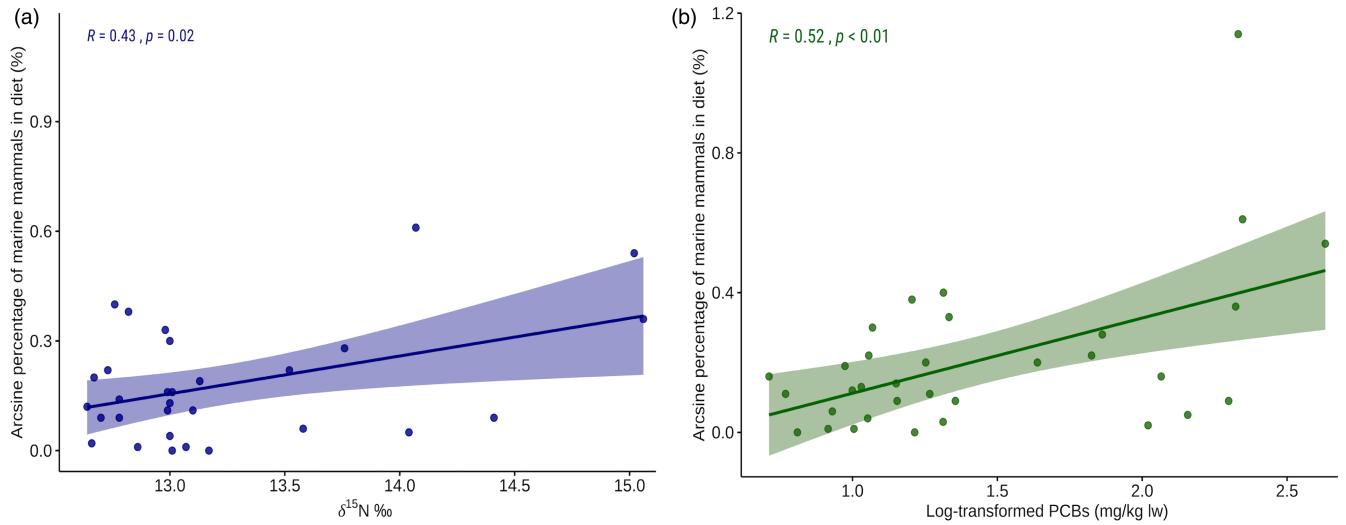

FIGURE 3 Relationship for Icelandic male killer whales ($n = 33$) between quantitative fatty acid signature analysis (QFASA) based estimates of marine mammal consumption and (a) polychlorinated biphenyl (∑PCBs) concentrations (Remili et al., 2021) and (b) δ^{15}N values (Samarra, Vighi, et al., 2017). PCB concentrations, δ^{15}N values, and fatty acid signatures were determined on the same skin and blubber biopsies, allowing a meaningful comparison of the three measurements. The Pearson correlation was calculated for the total marine mammal estimate (harbor seal + harbor porpoise) versus log ∑PCBs or δ^{15}N.

77.3% (Canada) to 89.1% (Norway) mean correct species attribution rates. The *Pred_beyond_prey* diagnostic, which represents the proportion of the predator fatty acid profiles outside the range of the prey FA profiles, ranged from 27.1% (in Greenland) to 53.5% (in the Faroe Islands; Table S4).

4 | DISCUSSION

The QFASA diet estimates obtained for each region identify new prey species and, for the first time, provide species-level diet estimates for killer whales across the NA Ocean. Killer whales' diet estimates showed that populations seem to feed on a mix of cetaceans and pinnipeds in the western NA, a mix of pinnipeds and fish in the mid-NA, and a majority of fish with some marine mammals in the eastern NA. Yet, within most locations, individual feeding preferences were also observed. These estimates are considered robust for these killer whales based on model diagnostics and consistency with other, more limited evidence from observation and measurements of other chemical tracers.

Estimates of predation on beluga and narwhal in the Canadian Arctic are consistent with local observations and coincide with a recent Arctic invasion by killer whales (Ferguson et al., 2010). In this region, the reduction of sea ice and northward range-shifting prey has led to an increased occurrence of killer whales and increased predation pressure on Arctic cetaceans, particularly narwhal and beluga whales (Ferguson et al., 2010; Ferguson, Higdon, et al., 2012; Ferguson, Kingsley, et al., 2012; Higdon et al., 2012; Matthews et al., 2019; Westdal et al., 2013). These reports have also suggested possible killer whale predation on ringed seals, the most abundant marine mammal in the Arctic (Ferguson, Higdon, et al., 2012). Our QFASA estimates quantify this predation, with ringed seals estimated as the dominant prey in a quarter of the killer whales sampled in the Canadian Arctic. These findings are important in the context of changing predator–prey dynamics in the Arctic and support the need to further investigate the top-down impacts of increasing predation pressure of killer whales on Arctic marine mammals.

In Greenland, the high relative importance of harp and hooded seals was consistent with stomach contents recovered for some of the same individuals (Remili et al., 2022). A moderate contribution of mackerel was identified by QFASA and could be explained by the possible northward distribution shifts of mackerel stocks in the NA (Berge et al., 2015; Jansen et al., 2016), and possibly by killer whales following such fish prey (Nøttestad et al., 2014; Remili et al., 2022). Predation on bearded seals has not been reported to the best of our knowledge, but this abundant prey species was consistently estimated in the diet of killer whales, particularly off Tasiilaq, Greenland, where the whales were harvested (Mattmüller et al., 2022).

Of all NA killer whales included in this study, Iceland and Norway individuals showed the highest contribution of herring in their diets, consistent with previous reports suggesting that herring is the main prey for both populations (Jourdain et al., 2019; Samarra, Tavares, et al., 2017; Samarra, Vighi, et al., 2017; Simila et al., 1996; Simila & Ugarte, 1993; Vogel et al., 2021). QFASA estimates also indicated harbor porpoises and pinnipeds in the diets of some individuals from Iceland and Norway. These specific individuals diverged from the most common, herring-dominated diet by having one-third to more than half of their diets comprised of marine mammal species. Feeding specialization among individuals in these populations is in line with distinct behavioural observations, stable carbon and nitrogen isotope values, and pollutant concentrations within individuals of the two populations (Andvik et al., 2020; Jourdain et al., 2017,

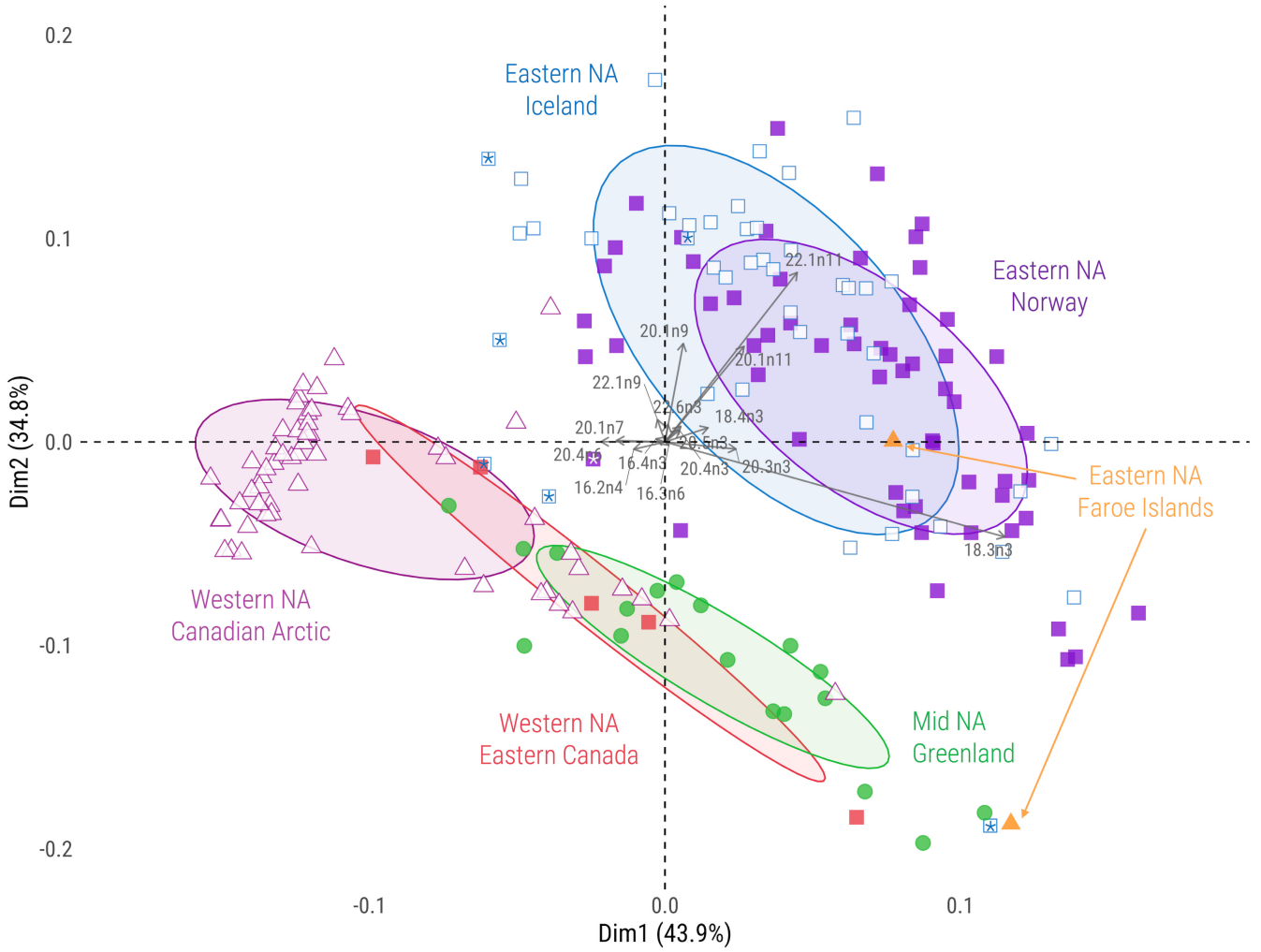

FIGURE 4 Principal component analysis of blubber fatty acid signatures of North Atlantic killer whales ($n = 191$) sampled from 2008 to 2021, grouped by region. The presence of an asterisk identifies individuals in Iceland and Norway known to have marine mammals in their diets based on field observations and/or published studies featuring other chemical tracers.

2020; Remili et al., 2021; Samarra et al., 2018; Samarra, Tavares, et al., 2017; Samarra, Vighi, et al., 2017).

We measured a substantial amount of dietary variation in each regional group, reflecting the complex feeding ecology of killer whales in the NA, supporting the recent suggestion to retire the terms "Ecotypes 1 and 2" from further use (Foote, 2022). Indeed, while Arctic and Eastern Canadian killer whales seem to predominantly prey on marine mammals according to their diet estimates, relative proportions for the different prey species consumed varied substantially among individuals. In the Arctic, about a quarter of the killer whales showed diet estimates above 50% for ringed seals, while the remaining individuals showed high diet estimates for belugas and narwhals. Only three individuals in the Canadian Arctic had baleen whales in their diet estimates, which suggests minimal predation on baleen whales in this area or for these individuals. This finding is surprising, as previous research has suggested the possibility of the importance of bowhead whale predation in the Hudson Bay region of the Canadian Arctic (Galicia et al., 2016), a region not sampled in our study. Baleen whale predation may be lower than previously suggested, or Arctic individuals targeting bowhead were not captured in our study despite a reasonably large sample size. Nearly all killer whales in Eastern Canada, however, fed on baleen whales. In Greenland, we also measured strong individual dietary variation, with half of the individuals showing a preference for seals and the other half consuming both mackerel and seals. Distinct feeding preferences among individuals were also observed in Norway and Iceland, this time with most of the killer whales feeding predominantly on herring, while a small number of individuals showed a mixed diet of fish and more than half marine mammals, including either harbor seals, harbor porpoises or both. Previous research suggested that killer whales in Norway may have to supplement their herring-dominated diet with seals because they provide better nutritional value (Bories et al., 2021). These findings thus deserve further attention in the context of rapidly changing ecosystems and geographical shifts in prey availability as a result of climate change (Fossheim et al., 2015), as well as the threats posed by bioaccumulating organic contaminants (Andvik et al., 2020; Remili et al., 2021).

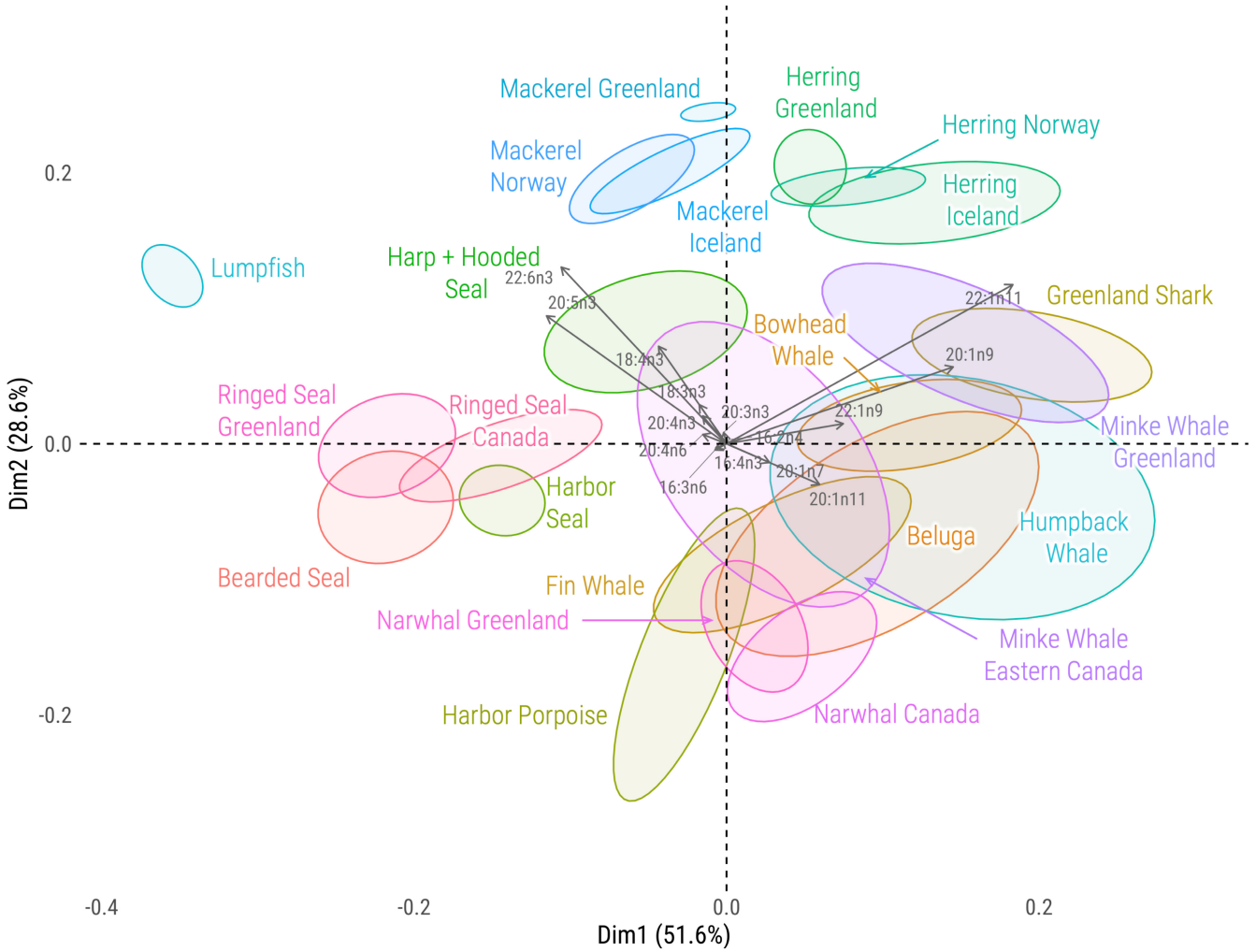

FIGURE 5 Principal component analysis of ($n = 967$) the fatty acid signatures of potential prey species of killer whales in the North Atlantic Ocean, representing the total prey input in all the quantitative fatty acid signature analysis (QFASA) models. The QFASA model for each killer whale group was run with a subset of region-specific prey. The full prey sample list is available in Table S1.

Qualitative comparisons of killer whale fatty acids revealed a gradient of FA profiles for killer whales across the NA. The FAs included in the analysis consisted of those fatty acids known to arise largely from dietary intake, thus minimizing possible confounding influences from physiological variation (e.g., de novo synthesis, metabolism; Iverson et al., 2004). According to our prey PCA, the FA signatures of individual species across regions did not differ substantially relative to the FA signature differences among species. It implies that the west-to-east FA gradient observed in the NA killer whales' profiles seems to be driven largely by differences in the diet composition and not geographic variation in prey FA profiles, in accordance with spatial FA variation shown in other studies (Thiemann et al., 2008). The results of the PCA are thus consistent with previous knowledge of the feeding ecology of killer whales. Interestingly, some of the Iceland and Norway individuals known to prey on marine mammals to a certain extent grouped closer to the Canadian and Greenlandic whales, suggesting that qualitative FA profile analyses can at least differentiate between individuals feeding predominantly on fish vs. those feeding on marine mammals. The wide spread of these previously identified "mixed-diet" individuals in Iceland suggests strong dietary plasticity in Iceland and Norway. Despite the relatively large dataset of the present study for killer whales in the North Atlantic compared to previous studies, we were unable to assess temporal and seasonal variation within or among regions in the current study due to data limitations. This would be an important avenue for future research.

QFASA for killer whales offers an invaluable new ecological tool to quantify the feeding preferences of marine predators such as cetaceans; however, some limitations should also be highlighted, specifically regarding prey library selection. Ideally, one should include all relevant prey species based on previous research using other methods, including stomach contents, stable isotopes, or behavioural observations. Conversely, researchers should select the species to include in their prey library very carefully to avoid different types of bias. Too few prey species in the library will generate false diet estimates, as the QFASA model will simply match the most probable prey based on the shortest statistical distance to the predator. If an important prey species is missing, the model will

still gravitate towards the closest prey, which may not be present or substantial in the true diet. For example, we did not include grey seals (*Halichoerus grypus*) in our Iceland and Norway prey libraries due to a lack of samples, despite reports indicating some whales feed on this species (Jourdain et al., 2017; Samarra et al., 2018). To ensure enough prey species are included in the library, researchers should pay attention that their *pred_beyond_prey* model diagnostics are not too high (Bromaghin, 2017). Anecdotally, our previous paper developing the QFASA approach for killer whales only contained Arctic seals in the prey library (Remili et al., 2022). When used on the Faroe Islands killer whales, the QFASA method estimated a high proportion (40%) of ringed seals in one of the whales' diets, which seemed unlikely based on the high-latitude habitats of ringed seals. When replacing ringed seals with our new FA data for harbor seals (a species sampled from Iceland, closer to the Faroe Islands) in the Faroe Islands prey library for the current study, the diet estimate was instead 40% harbor seal in the same killer whale. This result illustrates the need for a carefully curated prey library with geographically relevant species. One potential caveat of this study is temporal variation in the geographic range of the predators or prey included in the models. Prey species with a large geographical range, like baleen whales, may show different FA profiles based on the season. Future research efforts should be directed towards quantifying blubber FA turnover rates in marine mammal species to better constrain the period of feeding represented by the QFASA estimates. Another potential issue with prey libraries can arise when species with very similar FA signatures are included. In this case, the model may not be able to differentiate between species, which can cause a serious bias in the diet estimates. Researchers should thus check their values for the *leave_one_prey_out* model diagnostics and merge overlapping species when necessary (e.g., here, we merged species of baleen whales or Monodontidae in some of our libraries).

The reliability of our QFASA estimates was corroborated by the moderate correlations between the total proportion of marine mammal estimates and other feeding tracers (PCBs and δ^{15}N values). However, we observed some exceptions to the relationship between the QFASA-based diet estimates and the PCBs and δ^{15}N. For instance, two of the previously identified "mixed-diet" Icelandic killer whales showed almost no marine mammal consumption based on QFASA but did show elevated blubber ∑PCB concentrations and skin δ^{15}N values. This discrepancy could be attributed to different time-integrated diet signals from the blubber fatty acid signatures compared to the blubber PCB concentrations. PCBs and other persistent organic pollutants are extremely stable chemically and not easily metabolized by cetaceans (Meyer et al., 2018). The only substantial way for cetaceans to reduce their blubber concentrations of most PCBs is via gestation, lactation, or starvation (Tanabe et al., 1982). Values of δ^{15}N reflect the trophic position of an organism and, in cetacean skin, may represent a feeding window from ~2.5 to 6 months, depending on the skin turnover rate (Wild et al., 2018). The fatty acid turnover rate in blubber is not certain but may be around a few weeks in the inner blubber, closest to the muscle for small odontocetes (Choy et al., 2019). To the best of our knowledge, estimates of the turnover rate of FAs in the outer blubber are not available but may represent the diet between several weeks and several months prior to sampling (Budge et al., 2006). As a result, blubber PCB concentrations and, possibly (although not certainly) skin δ^{15}N values may reflect dietary patterns over a longer period than outer blubber fatty acid signatures. This is an important consideration when applying QFASA to cetaceans and supports the use of multiple tracers to elucidate the feeding ecology across multiple temporal scales within a population or individual. The two Icelandic whales photographed targeting seals in Scotland were sampled in Icelandic waters among herring-feeding killer whales. The PCBs, δ^{15}N values, and FA profiles used in this study were all derived from the same biopsy, and thus the difference in feeding patterns suggested among the tracers supports seasonal variation in the dietary preferences of these two individuals (Remili et al., 2021; Samarra, Vighi, et al., 2017). Therefore, combining multiple dietary tracers may allow for the identification of seasonal feeding patterns in future research.

Early studies suggested a possible classification of NA killer whales into Type 1/Type 2 based on evidence for different feeding ecologies (Foote et al., 2009). However, a decade of further research that combined field observations of photo-identified killer whales and dietary tracers across the NA indicates more complex patterns of variations within and among killer whale groups/populations leading to the recommendation of withdrawing the simplistic dichotomy Type 1/Type 2 (Foote, 2022). Our results of QFASA modeling based on ~200 killer whales spanning from the west to the east NA Ocean provide a panoramic view of the complex feeding strategies across the NA, as well as within-population individual feeding specialization. Further research could investigate this dietary plasticity from a genetic approach to understand how population structure may arise from this dietary variation (de Bruyn et al., 2013; Tavares et al., 2018). Regardless, our findings provide new identification of prey species, and species-level diet estimates that can inform the predatory impacts of killer whales, perhaps as distinct ecological units, across the NA and other ocean basins worldwide inhabited by this ultimate apex predator.

AUTHOR CONTRIBUTIONS

Melissa A. McKinney, Anaïs Remili, Rune Dietz, and Christian Sonne designed the study with input from all co-authors. Filipa I. P. Samarra, Audun H. Rikardsen, Aqqalu Rosing-Asvid, Lisa E. Kettemer, Steven H. Ferguson, Cortney A. Watt, Cory J. D. Matthews; Rune Dietz, Christian Sonne, and Jeremy J. Kiszka provided the killer whale samples/data. Filipa I. P. Samarra, Sandra M. Granquist, Eve Jourdain, Katrine Borgå, and Anders Ruus provided the Icelandic and Norwegian prey samples. Anaïs Remili performed the fatty acid analyses and data analysis and wrote the original draft of the manuscript with input from Melissa A. McKinney. All authors reviewed and edited subsequent versions of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing interest.

DATA AVAILABILITY STATEMENT

All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. The raw data is available on the Polar Data Catalogue: https://doi.org/10.21963/13299 (Remili et al., 2023).

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SUPPORTING INFORMATION

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

Table S1. List of sample and collection details for the killer whales (*Orcinus orca*) and their prey: Atlantic herring (*Clupea harengus*), Atlantic mackerel (*Scomber scombrus*), bearded seal (*Erignathus barbatus*), beluga (*Delphinapterus leucas*), bowhead whales (*Balaena mysticetus*), fin whale (*Balenoptera physalus*), Greenland shark liver (*Somniosus microcephalus*), harbor porpoise (*Phocoena phocoena*), harbor seal (*Phoca vitulina*), harp seal (*Pagophilus groenlandicus*), hooded seal (*Cystophora cristata*), humpback whale (*Megaptera novaeangliae*), lumpfish roe (*Cyclopterus lumpus*), minke whale (*Balaenoptera acutorostrata*), narwhal (*Monodon monoceros*), and ringed seal (*Pusa hispida*).

Table S2. Mean ± SE QFASA diet estimates (in %) for all North Atlantic killer whales ($n = 150$), and individual specialization (mean percentage similarity) based on the whole population average. Please note: The IS measure of individual specialization corresponds to the average similarity between individuals' diets and the population's diet. When all individuals consume the full set of population resources, IS equals 1.0. As individuals use smaller subsets of the population diet, IS declines toward zero.

Table S3. Individual estimates for all killer whales in the NA (in %).

Table S4. QFASA modeling diagnostics: *Leave_one_prey_out* (LOPO) as the mean of correct species attribution and *Pred_beyond_prey* estimates for all regional groups of killer whales in our study.

Table S5. FA percentages (mean ± SE) in North Atlantic killer whales ($n = 150$). Only FA above 0.1% are shown. Bold fatty acids are the dietary set used in the QFASA models (16:2n4,16:3n6, 16:4n3, 18:3n3, 18:4n3, 20:1n11, 20:1n9, 20:1n7, 20:4n6, 20:3n3, 20:4n3, 20:5n3, 22:1n11, 22:1n9, 22:6n3).

Table S6. FA percentages (mean ± SE) in Icelandic prey, including harbor seals ($n = 14$), Atlantic herring ($n = 10$), and Atlantic mackerel ($n = 10$), and in Norwegian lumpfish roe ($n = 4$). Only FA above 0.1% are shown. Bold fatty acids are the dietary set used in the QFASA models.

Figure S1. Relationship between (A) contaminants (∑PCBs data from Remili et al., 2021) and diet estimates in Icelandic male killer whales ($n = 33$) and (B) $\delta^{15}N$ values and diet estimates in the same whales ($\delta^{15}N$ data from Samarra et al., 2017). PCBs, $\delta^{15}N$, and fatty acids measurements were performed on the same skin and blubber biopsies, thus allowing an accurate comparison of the three measurements. The colored bars represent the proportion of prey species in the diet, the green crosses represent the ∑PCBs in ng/g lipid weight (lw), and the blue plus signs represent the $\delta^{15}N$ values (‰).

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