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Completing the picture: using vertebrae as well as otoliths in diet analysis reveals new preferred prey of great skuas

Susanna Quer[1] · Graham John Pierce[2] · Cristian N. Waggershauser[3] · Lucy Gilbert[4]

Abstract

Knowledge of the diet of marine predators such as seabirds is fundamental to understanding the ecological cascades they may influence and the impact that environmental changes may have on them. Diet analysis of seabirds frequently relies on the identification of fish otoliths in pellets. However, it is recognised that the true dietary importance of fish with small and fragile otoliths is likely underestimated, requiring an additional method. In this study, we compared the identification of otoliths with that of vertebrae in pellets to gain a more complete picture of seabird diet. We identified fish otoliths and vertebrae from 2584 great skua *Stercorarius skua* pellets collected between 2014 and 2017 from five colonies in Scotland. Diet varied markedly between colonies, comprising mostly fish in Shetland and mostly birds in St Kilda. 10% of pellets contained otoliths compared to 70% with fish vertebrae. Atlantic herring *Clupea harengus* and Atlantic mackerel *Scomber scombrus* were the most common fish species at all colonies when using vertebrae in contrast to being virtually absent when using otoliths. Conversely, the occurrence of Norway pout *Trisopterus esmarkii* and pollock *Pollachius pollachius* otoliths was six and eight times, respectively, higher than for vertebrae. Therefore, combining data from both otoliths and vertebrae provides a more complete profile of the fish component of seabird diet. This is fundamental to improving our understanding of the impacts of marine management policies on seabirds, as well as how changes in the population size of such seabird species might affect their prey species.

✉ Lucy Gilbert
lucy.gilbert@glasgow.ac.uk

Susanna Quer
squerime@gmail.com

1   Marine Laboratory, 375 Victoria Rd, Aberdeen AB11 9DB, UK

2   IIM CSIC, Rúa de Eduardo Cabello, 6, 36208 Vigo, Pontevedra, Spain

3   Institute for Biodiversity and Freshwater Conservation, University of the Highland and Islands, 12B Ness Walk, Inverness IV3 5SQ, UK

4   School of Biodiversity, One Health and Veterinary Medicine, University of Glasgow, Glasgow G12 8QQ, UK

Introduction

Knowledge of the diet of species is fundamental to understanding their ecology, their cascading ecological effects, and the impact that environmental changes may have on them. Observed changes in diet can be an early sign of changes in marine ecosystems and fishing practices, both key factors driving population reductions in marine predators; for example, drastic reductions in lesser sandeel *Ammodytes marinus* availability caused low breeding success and population declines in Atlantic puffins *Fratercula arctica*, black-legged kittiwakes *Rissa tridactyla*, Arctic terns *Sterna paradisaea* and harbour porpoise *Phocoena phocoena* in the northern North Sea through the 1990s (Heubeck et al. 1999; Furness and Tasker 2000; MacLeod et al. 2007; Sharples et al. 2009) Identifying the diet of seabirds or other marine scavengers and predators is crucial for assessing their responses to such changes in resource availability as well as providing insight into foraging decisions and diet switching, and thus their resilience to environmental changes.

There are many ways of identifying prey of marine birds and mammals (Jordon 2005) and the advantages and disadvantages of each method have been thoroughly reviewed by Duffy and Jackson (1986); Barrett et al. (2007); Bowen and Iverson (2012) and see also Votier et al. (2003). For example, direct observation of prey items carried into a breeding colony by fish-carrying bird species allows large sample sizes to be collected without disturbing the birds, but is labour intensive, is useful mainly for assessing diet of chicks (Votier et al. 2003), and prey identification can be difficult at a distance. Stomach contents can be collected when seabirds regurgitate food when captured or by stomach lavage/flushing or the use of emetics. Diet is linked to individual birds, but is labour intensive, usually requires a licence and training, necessarily causes distress to the birds and sample sizes are limited to how many birds can be captured (Barret et al. 2007). Biochemical and molecular analyses of tissue, stomach contents or faeces, such as the use of stable isotopes, gel electrophoresis, fatty acids and DNA have also been used (Pierce et al. 1990; Bearhop et al. 2001; Barret et al. 2007). In particular, stable isotopes of Carbon and Nitrogen are particularly valuable for tracing long-distance movements and identifying trophic levels (rather than species) (Bearhop et al. 2001; Barret et al. 2007). However, tissue or feather samples are required, as well as baseline samples from foraging areas. Collection of faeces is non-invasive and can reveal identifiable prey remains such as bones in some marine species, especially pinnipeds (Pierce et al. 1991) but, for seabirds, only small bones or fragments are generally excreted in faeces. Instead, many seabird species regurgitate discrete pellets containing large, identifiable prey remains including fur, feathers and a range of bones including otoliths (fish ear bones) and vertebrae. For some seabird species such as gulls, terns and skuas that breed in easily accessible colonies, large quantities of pellets can be collected quickly, easily and cheaply with minimal disturbance (Duffy and Jackson 1986; Votier et al. 2003; Barrett et al. 2007). While pellets are not always traceable to individual birds, and are regurgitated with varying frequency, pellet analysis has proven to be a particularly robust and consistent method to monitor the dietary response of seabird populations to environmental changes (e.g. Johansen et al. 2001; Votier et al. 2008; Church et al. 2018).

Most studies using pellets have used fish otoliths (e.g., Johnstone et al. 1990; Bearhop et al. 2001; Barrett et al. 2007; Church et al. 2018; Jakubas et al. 2018; Votier et al. 2003, 2008) because they are readily identifiable due to their well catalogued species-specific shapes and patterns (Härkönen 1986). The identification of otoliths from pellets has been powerful enough to determine links between the diets of several seabird species and commercial fisheries activities (Bearhop et al. 2001; Votier et al. 2008; Barrett et al. 2007; Church et al. 2018). Otoliths can be used to estimate the size of the prey, but they are easily damaged during digestion, which can limit their use (Granadeiro and Silva 2000). Due to this issue, a key challenge with the use of otoliths for assessing diet of marine predators is that some fish species are known to be under-represented or even absent from pellets, faeces and stomach contents because they have small or fragile otoliths (Pierce et al. 1991; Brown and Pierce 1998; Votier et al. 2003; Alonso et al. 2013; Gosch et al. 2014; reviewed by Bowen and Iverson 2012) and some fish such as *Rajidae* have no otoliths at all. Importantly, otoliths from the abundant pelagic sandeels *Ammodytidae* spp., European sprat *Sprattus sprattus*, Atlantic herring *Clupea harengus* and Atlantic mackerel *Scomber scombrus*, are rarely found in pellets despite being major components of the diet of many northeast Atlantic seabird species (Bearhop et al. 2001; Votier et al. 2001, 2003).

Therefore, in addition to otoliths, the identification of other fish skeletal elements, such as vertebrae, could prove valuable in providing a more comprehensive quantification of the diet of seabirds, as recommended by Alonso et al. (2013), which would therefore allow for more accurate monitoring of diet responses to changes in the environment and fisheries management. Like otoliths, fish vertebrae have species-specific shapes and patterns, allowing species identification (Watt et al. 1997; Granadeiro and Silva 2000) and vertebrae size can be used to estimate fish size (Granadeiro and Silva 2000) just as can otoliths (Härkönen 1986; Granadeiro and Silva 2000). However, far fewer studies of seabird diet have used vertebrae than have used otoliths, perhaps because species-specific vertebrae identification is less well known, or there may be a perception that identifying fish from otoliths is easier than it is from vertebrae (Bearhop et al. 2001). It could also be that the use of otoliths allows for direct comparison with previous studies that have used otoliths, for which there is critical mass in the published literature. Some of the seabird diet studies that used vertebrae include Neves et al. (2012) who used a combination of flesh and bones, including otoliths and vertebrae, to identify prey of Cory's shearwaters *Calonectris diomedea* in the Azores using stomach lavage of 959 birds. Votier et al. (2003) compared different methods for analysing the diet of great skuas: observations of prey items brought to the territory, regurgitates during handling, stomach lavage, prey remains left in the territory and pellets. Otoliths, other bones and scales were used to identify prey from pellets. Although a comparison of vertebrae with otoliths was not the aim, they found that otoliths grossly under-represented Atlantic mackerel and herring. The only study to our knowledge that has specifically aimed to compare the use of otoliths with vertebrae for identifying seabird prey is Alonso et al. (2013), who analysed 673 samples from stomach lavage of Cory's shearwaters in the Azores. They found higher occurrences of vertebrae than otoliths for all fish species, and 12 of the

rarer fish species were represented by vertebrae only, partially explained by fish containing many vertebrae but only two otoliths. Alonso et al. also found that the proportional occurrence of vertebrae versus otoliths differed between fish species, e.g. for flying fish (Exocoetidae) otoliths occurred slightly less than vertebrae, whereas otoliths greatly underrepresented sardines *Sardina pilchardus*. To our knowledge, no study to date has specifically aimed to quantify diet outcomes from otoliths compared with vertebrae using pellets, even though pellets are the most frequently used mode of assessing diet of many seabirds, especially cormorants, gulls, terns and skuas (Barrett et al. 2007).

In this study, we aimed to quantify the impact on diet analysis outcomes of using fish vertebrae in comparison to otoliths for identifying fish in seabird pellets. To achieve this aim, we collected great skua *Stercorarius skua* pellets from five Scottish colonies collected over four years. We analysed the diet by recording the presence of fish species per pellet by identifying otoliths in comparison to identifying vertebrae. *Great skuas* are generalist scavengers, predators and kleptoparasites and are known to follow fishing vessels, with some populations reported to have relied heavily on discarded fish (Votier et al. 2004b). Given previous observations that otoliths from Atlantic mackerel and herring are rarely found in pellets (e.g. Votier et al. 2003), we specifically hypothesised that the use of vertebrae will fill this gap and, together with otoliths, provide a more complete and balanced quantification of seabird diet, including for other fish species. Furthermore, we aimed to assess the relative importance of using vertebrae across the different colonies and regions included in this study.

Great skuas are particularly interesting and useful for quantifying the impact of using vertebrae on our knowledge of their diet, because many previous studies have characterized great skua diet, mainly using otoliths in pellets (Bearhop et al. 2001; Votier et al. 2003, 2008; Church et al. 2018), but also using stable isotopes (Bearhop et al. 2001) and a range of other methods (Votier et al. 2003). Furthermore, it is particularly important to have a complete picture of great skua diet, both because great skuas are apex predators, contributing to local declines in some seabird populations (Furness 1978; Heubeck et al. 1999), and knowledge of their diet is useful for reflecting temporal trends in fisheries (Church et al. 2018), as well as making predictions of the impacts of changes in fisheries management policy (Votier et al. 2004b). Of particular, timely importance is the global epizootic of highly pathogenic avian influenza virus that has caused mass mortality in many seabird species, including a 77% collapse in UK great skua populations in just two years 2021 and 2022; (Tremlett et al. 2024). It is now especially useful to have up-to-date baseline diet data as well as robust and non-invasive methods of monitoring the complete diet of great skuas, to enable comparative research on the impacts

of the altered seabird landscape (e.g. low intra-specific competition, changed seabird prey availability) on great skua diet.

Materials and methods

Diet sample collection

Great skua pellets were collected from "club sites" (areas within breeding colonies where non-breeding birds and off-duty breeding birds interact; Church et al. 2018) across five large great skua colonies in Scotland, UK. We used club sites because a large number of pellets can be collected quickly with minimal disturbance of nest sites. This was ideal for our purposes of testing the occurrence of otoliths versus vertebrae. Four of the colonies were in the Shetland Islands: Hermaness National Nature Reserve (3 club sites), Isle of Noss National Nature Reserve (3 club sites), Foula (3 club sites) and Fetlar (1 club site), and one colony was on Hirta in the St Kilda archipelago (1 club site). Pellets were carefully collected during the breeding season (June to August) between 2014 and 2017 and all pellets found were removed from each club site during each visit and kept frozen until later dissection. Sometimes, older pellets containing fish bones were no longer held tightly together but, instead, had disassembled into discrete piles of bones. In these cases, we picked up as many of the bones as possible, but sometimes a minor proportion of very small bones would fall deep into the moss, preventing easy collection. Each pellet, including disassembled discrete piles of bones, was collected into a separate sealed sample bag to prevent cross-contamination between samples.

Pellets were dissected in the laboratory by gently teasing them apart in a tray using tweezers. A magnifying glass was used to ensure the detection of small vertebrae and otoliths (less than 2 mm). Fish remains from pellets were identified to the lowest taxon possible (usually species) from otoliths using Härkönen (1986) and from vertebrae using Watt et al. (1997). Otoliths and vertebrae that could not be identified to species level were reported at genus or family levels (Pierce et al. 1991), and all otoliths, including broken ones, were counted and recorded per pellet. From this we calculated the proportion of otoliths that were successfully identified to species level. We did not record the number of every individual vertebra due to their large quantity and the fact that sometimes small vertebrae were not recoverable during the collection process at club sites, but we did record instances where we could not identify the fish species from the group of vertebrae within a pellet. We could not determine how many individual fish (of a particular species) were associated with a group of vertebrae (or otoliths) from a pellet. Therefore, for the purposes of assessing success rate of identifying

fish species per pellet, we allocated an index value of one (of a particular species) identified per pellet, even though the number of vertebrae could vary from one to at least 50. Identification success rate was therefore estimated at the pellet level as the number of species identified per pellet divided by the cumulative number of species per pellet (which is equivalent to the average number of species identified successfully (using vertebrae) per pellet multiplied by the total number of pellets containing vertebrae).

Calculating the occurrence of each fish species in great skua pellets

We created a database that listed the presence/absence and numbers of otoliths, vertebrae or other bones for each fish species (or family) identified in each pellet. A frequency of occurrence (FO) metric was produced to enable comparison between the use of otoliths and vertebrae. The FO was the percentage of pellets (out of the total number of pellets collected, n = 1742) containing at least one otolith or vertebra of a fish species. A further metric that is sometimes used is numerical frequency, which in this case would be the numbers of individual otoliths and vertebrae counted of each fish species. We did not use this metric because (i) each fish has only two otoliths but many vertebrae and the number of vertebrae vary with species (Ford 1933; MacKay and Garside 1969), creating inherent bias and making meaningful comparison between otoliths and vertebrae difficult; and (ii) we did not count every single vertebra per pellet due to the large number of vertebrae and the this would be inaccurate due to loss of some small vertebrae during collection at club sites (as previously explained). Because FO was based on the presence of a species using otoliths or vertebrae in each pellet it is not biased by how many individual otoliths or vertebrae were found in each pellet.

We calculated FO using otoliths and using vertebrae for each of the eight most commonly occurring fish species. Fish species that occurred only rarely, i.e. found in fewer than 2% of pellets, were pooled into a category called "other".

We also assessed the relative importance of otoliths *versus* vertebrae in identifying each fish species. This was done by calculating, for pellets containing each fish species separately, the proportion that were identified from otoliths and from vertebrae. For example, if there were 55 pellets containing haddock *Melanogrammus aeglefinus*, 41 of which contained haddock otoliths and 23 of which contained haddock vertebrae, the relative contribution of otoliths to identifying haddock was 41/55 = 75% while the relative contribution of vertebrae was 42%.

Statistical analysis

To compare the occurrence of each prey species between otoliths and vertebrae we used contingency tables and Chi-squared (χ^2) tests, except when sample size was low (n < 10) where we used two-tailed Fisher's Exact test (McDonald 2014), as used by previous studies of seabird diet analysis (e.g. Votier et al. 2003; Church et al. 2018). *Bonferroni* correction was applied to the *p*-value to account for multiple comparisons. Statistical tests were conducted for FO using occurrence of each species in each pellet as the unit of analysis.

Results

A total of 2584 pellets were dissected: from the Shetland Islands there were 884 from Hermaness on Unst, 833 from the Isle of Noss, 277 from Foula, 264 from Fetlar; and from the St Kilda archipelago there were 326 pellets from Hirta. Fish remains (vertebrae, otoliths or fish bones) were found in 75% of all great skua pellets across the five Scottish colonies (Table 1). The importance of fish in the diet varied between colonies: more than 70% of pellets contained fish in all the Shetland colonies, compared to 22% of pellets from St Kilda (where 93% contained the remains of other seabirds) (Fig. 1).

Fish vertebrae occurred in pellets much more frequently than did otoliths: approximately seven times more pellets contained fish vertebrae (70%) than otoliths (10%) (Table 1).

The success rate of identifying fish to species (at the pellet level) was also greater when using vertebrae (93.5%) than otoliths (78.6%) (Table 2). Failure to identify the fish (even to family) in a pellet was rare, but occurred more frequently when using vertebrae (1.9%) than otoliths (0.9%) (Table 2). Failure to identify fish to species from otoliths was usually due to otoliths being broken or worn: 64 (65%) of the 99

Table 1 Proportions and total numbers of great skua pellets (n = 2584 total pellets collected) containing fish otoliths, fish vertebrae and other fish bones

% and no. pellets containing:	% pellets	no. pellets
Any fish bones (otoliths, vertebrae or other bones)	74.6	1928
Otoliths	10.1	260
Vertebrae	69.5	1804
Otoliths or vertebrae	70.9	1832
Other fish bones	63.8	1649
Otoliths identified to species	7.78	201
Vertebrae identified to species	65.8	1699
Otoliths or vertebrae identified to species	67.4	1742

Note that not all pellets contained fish remains

Fig. 1 Percentage of pellets containing remains of fish, bird, other marine organisms such as cephalopods and goose barnacles, and terrestrial mammals such as rabbits, from five great skua *Stercorarius skua* colonies (Fetlar, Foula, Noss and Unst are in Shetland, north of mainland Scotland; St Kilda is west of mainland Scotland). Standard error bars are shown
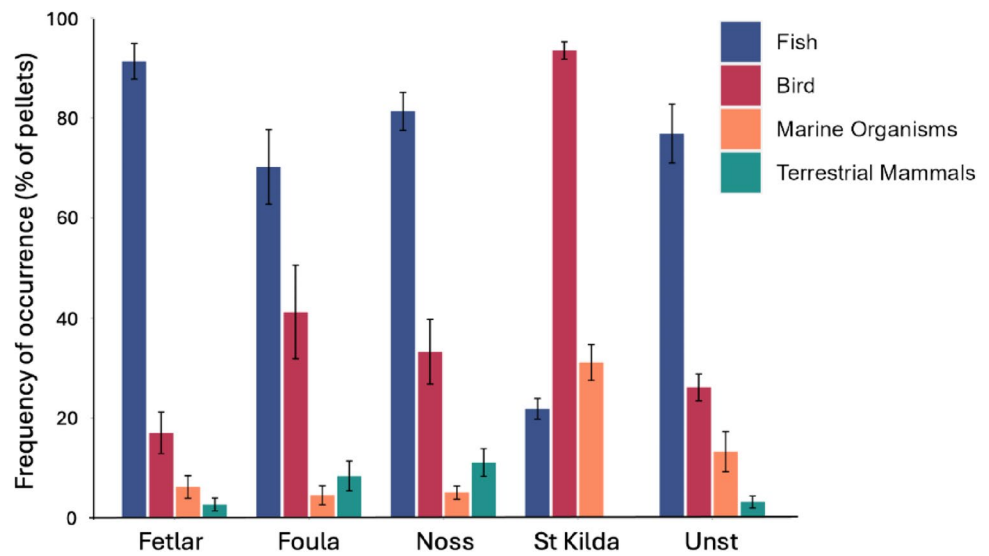

Table 2 Numbers of individual otoliths, cumulative otoliths and vertebrae per pellet found in all great skua pellets and success rate of identification

	Individual otoliths (%)	Pellet-level otoliths (%)	Pellet-level vertebrae (%)
Total	462	303	2184
Identified to species (%)	363 (78.6)	231 (76.1)	2043 (93.5)
Identified to family only (%)	93 (20.1)	49 (16.1)	98 (4.5)
Not identifiable (%)	6 (1.3)	3 (0.9)	43 (1.9)
Not identified to species (%)	99 (21.4)	72 (23.9)	141 (6.4)
Too broken to identify to species (%)	64 (13.8)	50 (16.5)	Not recorded

Identification success rate was estimated for each individual otolith, and also at the pellet level. Pellet level identification success was estimated as the number of fish species identified per pellet divided by the cumulative number of species per pellet. Cumulative species per pellet is equivalent to the average number of species identified per pellet (using otoliths or vertebrae) multiplied by the total number of pellets (containing otoliths or vertebrae)

otoliths that could not be identified to species were broken (Table 2).

On average, a pellet with vertebrae contained 1.21 different fish species as identified from vertebrae, with a maximum of 4 fish species in a single pellet. Pellets with otoliths had on average 1.78 different fish species identified from otoliths per pellet with a maximum of 17 different species identified from otoliths in a single pellet.

Using vertebrae, a total of 29 fish species were identified in the diet of great skuas compared with 18 species detected using otoliths. Atlantic cod *Gadus morhua*, brown trout *Salmo trutta* and tadpole fish *Raniceps raninus* were identified exclusively by otoliths. Among the fish species identified exclusively by vertebrae were Atlantic mackerel, greater forkbeard *Limanda limanda*, European hake *Merluccius merluccius*, garfish *Belone belone*, common dragonet *Callionymus lyra*, bib *Trisopterus luscus*, butterfish *Pholis gunnellus,* northern rockling *Ciliata septentrionalis,* turbot *Scophthalmus maximus*, plaice *Pleuronectes platessa*, European flounder *Platichthys flesus*, dab *Limanda limanda*,

megrim *Lepidorhombus whiffiagonis*, scaldfish *Arnoglossus laterna* and witch *Glyptocephalus cynoglossus* (complete list of species in Supplementary Material Table S1).

Otoliths indicated prey that had not been detected by vertebrae in only 0.5% (10/1832) of pellets. In contrast, vertebrae indicated prey that had not been detected by otoliths in 21.67% (397/1832) of pellets.

Proportion of great skua pellets containing each fish species: Frequency of Occurrence (FO)

Using data on the proportion of great skua pellets containing fish vertebrae identified to species (n = 1699) and otoliths identified to species (n = 201), FOs were significantly higher when using vertebrae than otoliths for Atlantic herring and mackerel (Table 3): 83.3% of pellets contained Atlantic herring vertebrae and 20.4% contained Atlantic mackerel vertebrae whereas Atlantic herring otoliths occurred in 0.4% of pellets and Atlantic mackerel otoliths were undetected (Table 3; Fig. 2).

Table 3 Frequency of occurrence (FO) of fish species identified in great skua pellets from five Scottish colonies

Frequency of occurrence (FO) of fish species in great skua pellets

Prey	% FO otoliths	% FO vertebrae	χ^2 or Odds ratio (95% CI)	p	% FO all
Atlantic herring	0.4	83.3	2455.90	<0.0001	83.3
Atlantic mackerel	0.0	20.4	0 (0.0–0.01)	<0.0001	20.4
Norway pout	3.7	0.6	38.78	<0.0001	3.8
Blue whiting	2.8	2.7	$1.9e^{-32}$	1	4.5
Whiting	1.2	2.7	9.37	0.99	3.2
Haddock	2.4	1.3	4.60	0.045	3.2
Pollock	0.8	0.1	14.10 (2.14–595.18)	0.001	0.9
Poor cod	0.5	0.2	2.26 (0.63–10.05)	0.27	0.5
Other	1.2	6.0	57.61	<0.0001	8.4

FO for each fish species is the % of pellets containing at least one otolith or vertebra of that fish species. p-values indicate statistical differences between FO from otoliths and FO from vertebrae using χ^2 tests (or Fisher's Exact test where sample sizes were too small for χ^2, hence Odds ratios cited). "% FO all" represent the % FO resulting from the use of both otoliths and vertebrae to identify prey items
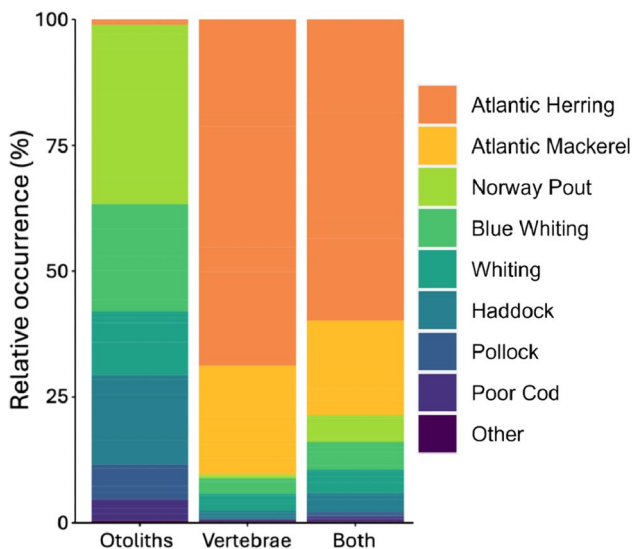
Fig. 2 Relative occurrence of fish species in great skua *Stercorarius skua* pellets calculated using otoliths only, vertebrae only and both otoliths and vertebrae combined, averaged over five Scottish colonies

Vertebrae from other species occurred much less frequently, the most frequent being blue whiting *Micromesistius poutassou* and whiting *Merlangius merlangus* vertebrae (both occurred in 2.7% of pellets; Table 3; Fig. 2).

The most frequently occurring fish species when using otoliths was Norway pout *Trisopterus esmarkii* occurring in 3.7% of pellets, followed by blue whiting otoliths in 2.8% of pellets; Table 3; Fig. 2). FO for Norway pout and pollock *Pollachius pollachius* showed significantly higher occurrence of otoliths than vertebrae (Table 3; Fig. 2). There was little evidence of statistically significant differences between the use of otoliths *versus* vertebrae in the proportion of pellets containing blue whiting, whiting, haddock and poor

cod *Trisopterus minutus* (Table 3; Fig. 2). Overall differences in the relative occurrence of the most frequent species between methods can be observed across the different colonies (Fig. 3).

Relative importance of otoliths versus vertebrae in identifying each fish species in pellets.

All pellets that contained Atlantic mackerel and almost all that contained Atlantic herring were identified from vertebrae, while no mackerel and almost no herring were identified from otoliths (Fig. 4). Of all pellets that contained whiting, more than double were identified from vertebrae than from otoliths (Fig. 4). In contrast, for pellets containing Norway pout, pollock and poor cod, at least 90% were identified thanks to otoliths, with much lower proportions of pellets identified from vertebrae (Fig. 4).

Deducing diet from a combination of methods

Combining FO data from both otoliths and vertebrae (proportion of pellets with occurrence of a fish species identified by either otoliths or vertebrae) suggests that Atlantic herring (83.3% pellets) and Atlantic mackerel (20.4%) contribute the majority of fish in the diet of great skuas, followed by blue whiting (4.5%), Norway pout (3.8%), haddock (3.2%) and whiting (3.2%) (Table 3; Fig. 2).

Discussion

Our overarching aim was to test the impact of using vertebrae, in comparison to otoliths, as an additional method for identifying fish components in the pellets of seabirds by using large sample sizes from multiple great skua colonies

Fig. 3 Relative occurrence of fish species in great skua *Stercorarius skua* pellets calculated using otoliths (left graph) or vertebrae (right graph) from each of five skua colonies (Fetlar, Foula, Noss and Unst are in Shetland, north of mainland Scotland; St Kilda is west of mainland Scotland)
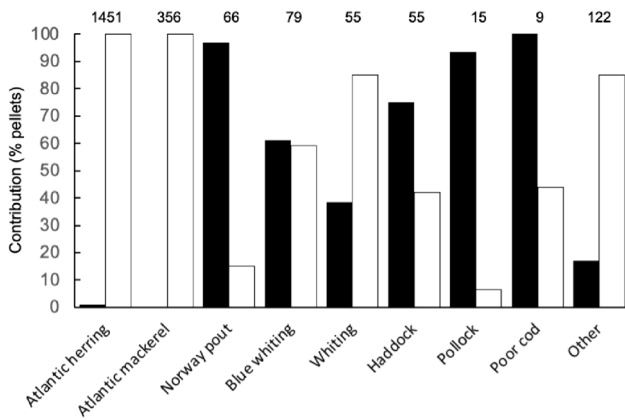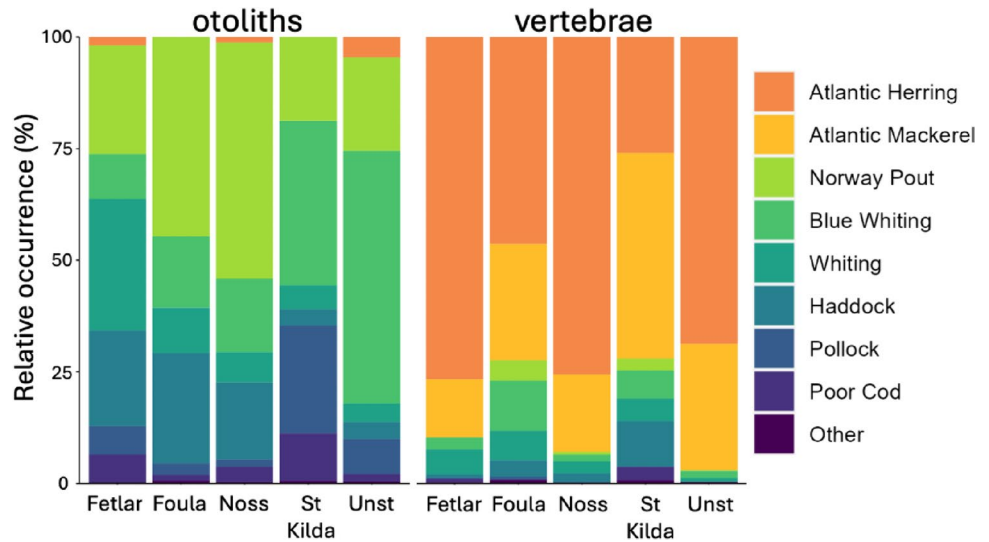

Fig. 4 The proportional contribution of otoliths (black bars) and vertebrae (white bars) to our identification of each fish species in great skua pellets. The numbers of pellets containing either vertebrae or otoliths for each fish species, used in the calculations for each of these percentages, are shown above the bars. For example, of the 55 pellets containing haddock remains, 75% of them contained herring otoliths, while 41% of them contained herring vertebrae. The percentages from otoliths plus vertebrae add up to more than 100% for most fish species because some pellets contained both vertebrae and otoliths

with varying diets. Our results clearly show that vertebrae occur much more frequently than do otoliths in regurgitated great skua pellets, and that the predominant fish species identified in the diet of great skuas is contingent on whether vertebrae or otoliths are used for identifying fish remains. Most strikingly, as predicted, the use of vertebrae for identifying fish revealed that Atlantic herring and Atlantic mackerel were the most frequently occurring fish species of great skua pellets in all colonies. In concurrence with previous observations (e.g. Bearhop et al. 2001; Votier et al. 2003; Church et al. 2018) herring and mackerel otoliths were almost absent from pellets.

Our finding that seven times more pellets contained fish vertebrae than otoliths is not surprising because each individual fish has only two otoliths but many vertebrae. This infers an advantage of vertebrae over otoliths as they effectively increase the sampling effort, which increases the likelihood of infrequently occurring fish species to be counted in pellets: we identified 29 fish species using vertebrae but only 18 were detected using otoliths. However, fish vary greatly in the number of vertebrae they have, which is likely to bias their occurrence in pellets; for example, Atlantic mackerel have 30 vertebrae (MacKay and Garside 1969) while Atlantic herring typically 54–58 (Ford 1933). The use of a frequency of occurrence metric (presence per pellet) rather than a numerical frequency measure (counts of individual vertebrae) will greatly mitigate this bias, but there could still be a greater number of pellets with the presence of a fish with more vertebrae, and this should be taken into account when interpreting diet outcomes using vertebrae.

A further clear advantage of using vertebrae is that vertebrae are more robust, leading to a higher probability of recovery and identification than otoliths (Alonso et al. 2013), reflected by our finding that 93.5% of fish in pellets were identified to species using vertebrae compared to 78.6% using otoliths. Otolith recovery and identification rates vary according to shape, size and robustness (Tollit et al. 1997) and species with smaller or thinner otoliths (e.g. herring, mackerel, sardines) are known to have extremely low recovery rates from pellets (Pierce et al. 1990; Brown and Pierce 1998; Votier et al. 2003; Alonso et al. 2013). This emphasises the importance of exploring further methods, and the use of vertebrae suggests that Atlantic herring and mackerel comprise the majority of fish species consumed by great skuas at all the Scottish colonies we studied. Previous studies have documented the presence of herring or mackerel in the diet of Scottish

great skuas but not in such high occurrence, presumably partly because vertebrae were not used for the identification of herring or mackerel in these studies (Votier et al. 2003; 2004a, b; 2008; Bearhop et al. 2001).

The use of vertebrae (in addition to otoliths) as a fish identification method in pellets will therefore have the greatest impact on seabirds whose diet comprises a large proportion of herring or mackerel or other species with small, fragile otoliths such as sardines (Alonso et al. 2013). Our study showed that only 22% of great skua pellets from St Kilda contained fish, while more than 90% contained seabird remains, in contrast to Shetland colonies where more than 70% of pellets contained fish. This likely reflects the difference in commercial fishing activity (and perhaps also in seabird prey availability and individual prey preferences) between the two archipelagos and suggests that the method of fish identification (otoliths versus vertebrae) will have a greater impact on great skua diet analysis in areas where skuas feed mainly on fish (such as Shetland) than in areas with a low proportion of fish in the diet (such as St. Kilda or the Faeroe Islands; (Hammer 2017)). Neither great skuas nor other seabirds are able to dive deep enough to catch many of the demersal white fish species (such as gadoids); therefore, as great skuas scavenge from fishing vessels, the proportions of whitefish in pellets reflect fishing vessel activity and practices (Votier et al. 2004b, 2008; Church et al. 2018). The proportions of Atlantic herring and mackerel in the diet may also reflect fishing activity, but less accurately: while these species sustain a huge commercial pelagic fishing industry which likely provides plentiful scavenging opportunities for great skuas, herring and mackerel are also key prey for Northern gannets *Morus bassanus* which are frequently kleptoparasitised by great skuas. Therefore, the frequency of occurrence of vertebrae in pellets does not allow us to quantify how much of the herring and mackerel was stolen from gannets or scavenged them from fishing vessels. To a lesser extent, other seabird species, such as herring gulls *Larus argentatus* and Atlantic puffins *Fratercula arctica* may also be kleptoparasitised by great skuas in Shetland (Andersson 1976; Furness 1978), providing fish in addition to those acquired from fishing vessels.

The proportions of otoliths *versus* vertebrae found from each fish species could potentially indicate the nature of the discards: otoliths were more important than vertebrae for identifying Norway pout, haddock, pollack and poor cod, in line with previous research of great skua diet using otoliths, where otoliths identified Norway pout, blue whiting, haddock, and whiting as common components of great skua diet in Shetland (Votier et al. 2008; Church et al. 2018). These are species that must have been acquired from fishing vessels which might suggest that the heads of these species are the body part most discarded as they were most available for skuas to scavenge.

We suggest that future diet studies using pellet analysis would benefit from the use of both otoliths and vertebrae in order to gain a broader and more inclusive picture of seabird diet (as suggested by Alonso et al. 2013 for stomach contents of shearwaters). Neither the use of vertebrae nor otoliths are free from bias, however; while we know that each fish has two otoliths and many vertebrae, we do not have accurate information for how vertebrae and otoliths in pellets translate into individual fish numbers or biomass consumed. Our data show that several species of fish can occur in a single pellet and the bones from an individual fish could be spread over several pellets. Great skuas scavenge fish from fishing vessels and steal fish from other seabirds such as gannets (Andersson 1976; Furness 1978); therefore, whether vertebrae or otoliths are consumed will depend on many factors such as the species and size of the fish, the means of capture, foraging behaviour and the type of processing of the fish onboard fishing boats. For example, if fish heads are thrown overboard but the bodies are kept, then the use of vertebrae will under-represent those fish; or if the fish heads are too large to swallow, as for large Atlantic cod, the use of both otoliths and vertebrae may under-represent that species if only soft body parts can be consumed.

Notwithstanding the issues from both methods of diet analysis, if both vertebrae and otoliths are routinely used in diet analyses, sample sizes can be greatly increased which, thanks to greater sampling effort, allows for a wider variety of fish species can be identified. If the methods remain consistent, meaningful comparisons can be made between areas and time periods to better test hypotheses about the impacts of environmental changes, marine ecosystem health or fisheries practices and management on seabird diet. With new quantified information on Atlantic herring and mackerel in seabird diet, questions can be addressed about the impacts on great skua diet of commercial pelagic fisheries activities and discarding, as they have previously for white fish species (Votier et al. 2004b, 2008; Church et al. 2018), and seasonal changes in diet in relation to fisheries seasons.

While we used pellets from great skuas to test the differences in outcome between the two methods of otolith and vertebrae identification, these findings are equally applicable for analysing the diet of any other piscivorous vertebrate for which fish bone remains can be collected from pellets, stomach contents or faeces.

Concluding, otoliths have been the most commonly used method to characterise the fish component of pellets and stomach contents of many seabird species, partly due to the availability of reference collections (e.g. Härkönen 1986). Otolith studies have provided an essential understanding of the diet of seabirds as well as of their links to environmental factors and anthropogenic activities such as industrial fishing and discarding practices (Votier et al. 2008; Church et al. 2018). However, our findings show that the inclusion

of vertebrae in identifying fish remains in pellets not only greatly increases the sample size that can be included in diet analysis but, most importantly, allows the importance of species with small, delicate ototliths (such as herring and mackerel) to be realised in seabird diet. Because these pelagic species are subject to intense fishing pressure and predated by other seabird species upon which skuas kleptoparasite (e.g., Northern gannets; Bearhop et al. 2001), the use of vertebrae in pellets can now enable us to better address questions about the interactions between marine ecosystem health and pelagic fishing practices and seabirds (Cottrell et al. 1996).

Data and code availability The data and code are freely available to view by contacting L. Gilbert.

Declarations

Conflict of interest The authors have no competing interests.

Ethical approval Consenting access to colony sites for pellet collection was sought from Nature Scot and Natural Trust of Scotland. No ethical approval was required for this study.

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