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SHORT NOTE

Use the backbone of your samples: fish vertebrae reduces biases associated with otoliths in seabird diet studies

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Abstract A literature review showed that most recent conventional dietary studies of Procellariiformes have used otoliths alone to identify fish prey. Using data from a dietary study of Cory's Shearwaters *Calonectris diomedea*, based on 673 regurgitates from adult birds, we quantitatively compared the contribution of otoliths and vertebrae for prey identification and quantification. By using otoliths alone, the importance of the main fish prey was greatly underestimated and several species would have been considered completely absent. Therefore, we strongly recommend the combined use of vertebrae, otoliths and other fish remains in order to improve the quality of dietary studies of seabirds.

Keywords Top predator · Prey identification · Fish bones · Diet sampling · Cory's Shearwater · *Calonectris diomedea*

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Zusammenfassung

Proben mit Rückgrat: die Wirbelsäulen von Fischen helfen, verzerrte Interpretationen im Zusammenhang mit Otolithen und Ernährungsstudien bei Seevögeln zu korrigieren

Eine Auswertung der Literatur hat gezeigt, dass bei den neueren, üblichen Untersuchungen an Röhrennasen (Procellariiformes) nur Otolithen verwendet wurden, um die Beute der Vögel zu bestimmen. Anhand einer Studie über die Ernährung von Gelbschnabel-Sturmtauchern (*Calonectris diomedea*) verglichen wir in einer quantitativen Auswertung von 673 Proben von Erbrochenem adulter Vögel, welchen Beitrag einerseits die Otolithen, andererseits die Wirbelsäulen bei der Bestimmung und Quantifizierung der Beutetiere spielten. Berücksichtigte man nur die Otolithen, wurde die Bedeutung des wichtigsten Beutefisches stark unterschätzt, und einige Fischarten kamen überhaupt nicht vor. Deshalb empfehlen wir dringend, beides, Otolithen und diverse Fisch-Überreste, zu verwenden, um die Aussage-Qualität von Ernährungsstudien an Seevögeln zu verbessern.

Introduction

Traditionally, the study of seabird diet has been based on the direct observation of prey carried in the bill, analysis of pellets, regurgitates (spontaneous or induced through stomach flushing), faeces or stomachs collected from dead birds (Duffy and Jackson 1986; Barrett et al. 2007). The major advantage of these conventional diet methods is their high taxonomic resolution, when compared to more recent techniques such as stable isotope analysis (Barrett et al.

2007; Karnovsky et al. 2012). In this context, the utility of otoliths to identify fish species occurring in the diet of marine birds is indisputable, as these structures possess a species-specific morphology (Tuset et al. 2008). Additionally, their supposed resilience to corrosion in digestive tracts has also been pointed out as an advantage, in relation to other hard structures (Treacy and Crawford 1981). However, dietary studies based exclusively on otoliths can be severely biased, since their recovery rate from seabird diet samples depends on fish age/size, species (i.e., on the relative size of the otolith), number and time elapsed since ingestion (Gales 1988; Johnstone et al. 1990; Zijlstra and Van Eerden 1995; Votier et al. 2003). Several studies with non-avian marine predators have also found strong evidence that the use of otoliths can underestimate the importance and even the presence of some prey species (for review, see Bowen and Iverson 2012).

Fish vertebrae and other diagnostic bones, although much less used than otoliths in seabird diet studies, can be successfully used to identify and quantify prey, as well as to estimate prey size (e.g. Granadeiro and Silva 2000; Herling et al. 2005). Votier et al. (2003) compared the use of vertebrae and otoliths for estimation of relative abundance of prey in Great Skua *Catharacta skua* pellets and found that species with fragile otoliths are generally underestimated, except if other structures such as fish bones are used for identification. To the best of our knowledge, no previous study has examined quantitatively the performance of methods based on fish vertebrae and on otoliths in identifying diet composition and quantifying prey consumption from regurgitates (either spontaneous or induced) of Procellariiform seabirds (but see Neves et al. 2012, for comparison of a single prey). In a search of Web of Science and Google Scholar using five keywords (diet, shearwater, petrel, albatross and fulmar) in the period 2000–2012, we found 52 studies of seabird diet (only Procellariiformes) in which the method used to identify fish remains in regurgitates was clearly stated. In 59.6 % of these, otoliths (but not vertebrae) were used to identify fish prey, which confirms that most researchers still rely exclusively on otoliths to characterise the diet of Procellariiformes. The present study aims to compare the performance of the two methods to analyse the diet of a Procellariiform seabird: the most widespread, based on otoliths only and an alternative method, which considers fish vertebrae.

Methods

All the data used in this work resulted from a broader study that investigated the feeding ecology of Cory's Shearwater *Calonectris diomedea* at Selvagem Grande (30°09′N 15°52′W), an oceanic island located in the northeast Atlantic (ca 350 km off the Morocco coast).

Fieldwork was conducted during the chick-rearing periods (August and September) of 2008, 2009 and 2010. Diet samples were obtained from breeding adults returning to the nest to feed their chick, using the ''water offloading'' technique (Wilson 1984), by flushing out the stomach contents with salt water. The excess of salt water was drained from the food samples through a sieve and all fresh prey were identified to the lowest possible taxonomic level using available guides (Whitehead et al. 1986). The remaining items were stored in ethanol (70 %) until further analysis. In the laboratory, digested fish were (separately) identified from vertebrae and otoliths, using our own reference collection and published information (Granadeiro and Silva 2000; Tuset et al. 2008). The number of fish was estimated either by the number of paired otoliths of similar size or from the number of highly distinctive vertebrae of each species (e.g. first caudal vertebrae; see Granadeiro and Silva 2000). Furthermore, all fish vertebrae from each species were counted and the total number of specific abdominal or caudal vertebrae was used to estimate the number of each fish species in the sample, whenever necessary (e.g. in the absence of the first caudal vertebrae). Cephalopods were quantified from the number of mantles and other fresh remains such as beaks. Eroded beaks were not included in the analysis, since they can be retained in the stomach for long periods (Barrett et al. 2007).

We calculated frequencies of occurrence (FO), as the percentage of samples with a given prey type, and numeric frequencies (NF) as the number of individuals of each prey type in relation to the total number of individuals (considering two possibilities: including cephalopods and excluding cephalopods). Chi-square tests were performed to assess differences in FO and NF of each prey, using either otoliths or vertebrae for prey identification and quantification. All prey that presented a FO lower than 3.0 % (in both methods) were pooled.

Results

Using only vertebrae for identification, Chub Mackerel *Scomber colias* stood out as the main fish prey in the diet of Cory's Shearwaters, occurring in 42.6 % of the diet samples ($n = 673$). Pilot-Fish *Naucrates ductor*, Sardine *Sardina pilchardus* and Flying-Fish (Exocoetidae) also occurred frequently in their diet (Table 1). The analysis based on otoliths alone showed a relatively similar ranking in the importance of these prey, but their occurrence in the diet was severely underestimated (Table 1; Fig. 1), with Chub Mackerel occurring in only 16.2 % of the diet samples. The NF of prey also differed profoundly when

Table 1 Estimated frequency of occurrence (%) and numeric frequency (%) of the main fish species present in the diet of Cory's shearwater *Calonectris diomedea* ($n = 673$ regurgitates), calculated using exclusively otoliths or vertebrae

Frequency of occurrence					Numeric frequency			
					Without cephalopods		With cephalopods	
Prey	Otoliths %	Vertebrae %	χ	p	Otoliths (%, $n = 725$)	Vertebrae (%, $n = 1{,}776$)	Otoliths (%, $n = 1{,}187$)	Vertebrae (%, $n = 2{,}238$)
Chub Mackerel	16.2	42.6	113.4	***	53.2	40.8	19.3	30.2
Pilot-fish	4.6	15.2	42.1	***	17.5	17.6	6.3	13.0
Sardine	0.6	11.6	71.1	***	1.5	9.2	0.6	6.8
Flying-fish	5.1	8.2	5.3	*	15.2	5.7	5.5	4.2
Horse Mackerel	1.0	3.7	10.4	**	2.7	3.2	1.0	2.4
Trichiuridae	0.1	3.4	20.5	***	0.4	1.8	0.1	1.4
Other fish species	2.8	15.5	64.6	***	8.7	12.9	3.5	16.1

Numeric frequencies of prey were estimated either considering or not the number of cephalopods ($n = 462$) present in the samples. χ^2 tests were used to assess differences between prey occurrences estimated by the two methods

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

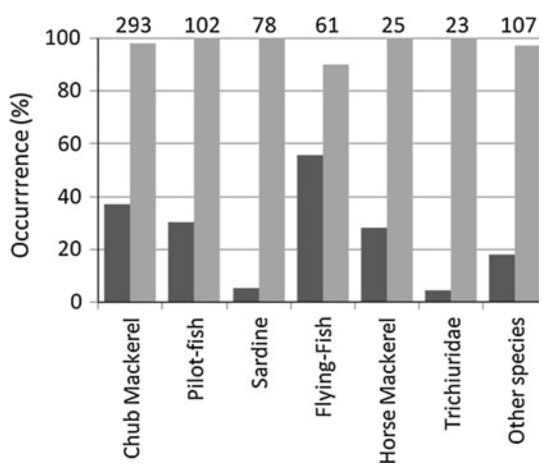

Fig. 1 Percentage of samples with prey identified by otoliths (*dark grey*) and vertebrae (*light grey*) in relation to all samples where each prey was detected (the number of those samples is presented *above bars*). Few individuals [Chub Mackerel ($n = 6$) and Flying-Fishes ($n = 5$)] were detected using exclusively other body parts, as mouth pieces or pectoral fins

estimated by the two methods, either considering only fish prey ($\chi^2_7 = 107.6$, $p < 0.001$; Table 1) or all prey together ($\chi^2_8 = 366.9$, $p < 0.001$). All fish prey, including Chub Mackerel, were underestimated in terms of NF (considering all prey), when only otoliths were used for fish identification. The exception was flying-fish. Only a minor proportion of Chub Mackerel (29.8 % of 514 individuals), Pilot-Fish (27.1 % of 203) and Flying-Fish (20.0 % of 80) were highly digested (with no flesh attached to the vertebral column or only loose vertebrae), while the major part of Sardines (69.0 % of 116), *Trachurus* spp. (59.0 % of 39) and Trichiuridae (60.9 % of 23) were highly digested. Using vertebrae, we were able to identify 28 species or genera occurring in the Cory's Shearwater diet, whereas the otoliths of only 12 species or genera were found. Among the species exclusively identified by vertebrae were the Skipjack *Katsuwonus pelamis*, Skimmer *Scomberesox saurus*, European Anchovy *Engraulis encrasicolus*, Trumpet Fish *Macrorhamphosus scolopax*, Slender Sunfish *Ranzania laevis* and Conger *Conger conger*. There were only five diet samples in which otoliths indicated prey (1 *Trachurus* spp.; 1 Exocoetidae; 3 Myctophidae) not detected by vertebrae.

Discussion

The occurrence of the main prey of Cory's Shearwater would have been dramatically underestimated if we had used only otoliths for fish identification (Table 1). Similarly, Votier et al. (2003) compared the use of vertebrae and otoliths to assess the diet of Great Skuas using pellets and found that otoliths from Atlantic Mackerel *Scomber scombrus* (representing 36 % of all fish prey) were never detected in the diet remains (Votier et al. 2003). Neves et al. (2012) also found that only one-quarter of Blue Jack Mackerel *Trachurus picturatus* in Cory's Shearwater regurgitates, detected using vertebrae, could also be identified by means of otoliths. In our study, several rarer species would not have been detected at all, because their otoliths were completely absent from diet samples. Overall, the results from these various studies indicate that the occurrence of species with small and fragile otoliths tends to be severely underestimated or missing altogether. Even for species with robust otoliths, such as Flying-Fish (personal observation) and *Trachurus* spp. (Tuset et al. 2008), the recovery rate of otoliths can be low when compared to vertebrae (Table 1; Fig. 1).

There is strong evidence that time elapsed since ingestion influences the recovery rate of otoliths (Gales 1988; Bowen and Iverson 2012). The majority of otoliths recovered (particularly from Chub Mackerel and Pilot-Fish) in our study were enclosed in fish crania and, therefore, still protected from the digestion process. For fish that were in a more advanced stage of digestion (e.g. Sardine), otolith recovery rate was very low. Like many seabird species, Cory's Shearwaters ingest their fish prey head-first and, in regurgitated samples, the anterior region of fish is often more digested than the posterior body parts. In fact, this pattern of digestion also affects the recovery of certain body structures, with a higher incidence of caudal vertebrae, in comparison to anterior vertebrae (this study) or fish heads (Granadeiro et al. 1998). Furthermore, the number of vertebrae present in a fish skeleton is much larger compared to the pair of sagitta otoliths per fish (Whitehead et al. 1986), thus the additional use of vertebrae in prey identification increases the probability of finding identifiable remains in regurgitated food samples.

Another possible source of bias in the exclusive use of otoliths in diet reconstruction is connected with the assumption that seabirds always ingest whole fishes. However, seabirds may ingest only particular fish parts, for instance when feeding on discards from fishing vessels (e.g. Votier et al. 2010). In those cases, the additional use of vertebrae and other hard remains would certainly improve diet reconstruction.

This study clearly shows that the efficiency of otoliths to identify and quantify fish prey can be low, compared to vertebrae. However, this does not imply that otoliths have no role to play in dietary studies. For many closely related fish species, vertebrae are very similar, while otoliths (if not eroded) allow a clear distinction among species (e.g. Atlantic Mackerel *S. scombrus* and Chub Mackerel *S. colias*; Tuset et al. 2008). Moreover, many discards available to seabirds are composed of only fish heads and, in that case, otoliths may be essential for prey identification. Furthermore, guides and reference collections of fish otoliths have been created in many regions (much more than vertebrae collections and guides) and therefore the use of otoliths will remain an easier option to identify prey. Nevertheless, our study indirectly underlines the urgency of gathering collections of fish vertebrae (and of producing the corresponding guides). We suggest that seabird researchers conducting dietary studies should take into account the overall fish remains present in their diet samples, rather than rely solely on otoliths for prey identification and quantification, which as shown here severely underestimates the occurrence of several key prey. This procedure would certainly improve the robustness of dietary studies and prevent severe biases associated with the recovery rates of otoliths from diet samples.

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