Vomit or Flush? Diet analysis using samples from spontaneous regurgitates or the water-off-load technique

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Abstract

Several methods, each with specific advantages and disadvantages, are frequently used to obtain diet samples from seabirds. The collection of regurgitates (REG) as well as samples from the stomach water-off-load (WOL) or flushing technique are some of the most commonly used approaches. During the Austral breeding season of 2005/06 diet samples from Cape Petrel *Daption capense* and Snow Petrel *Pagodroma nivea* were collected at Signy Island, South Orkney Islands, Antarctica. Primarily, diet samples were obtained by stomach flushing but occasionally (8 Snow Petrels and 12 Cape Petrels) birds spontaneously regurgitated their stomach contents before flushing. These regurgitates were sampled completely and stored separately. Afterwards the remainder of the bird's stomach contents was flushed. By doing so, a comparison could then be made between the REG samples and the total stomach contents (REG+WOL). In the REG samples of both species the fraction of fish was underestimated and the fraction of crustaceans overestimated compared to the REG+WOL samples. This study shows that methodology-specific outcomes are potentially to be expected when doing dietary research. Using REG samples is shown not to be suitable for quantitative assessments of the diets of petrels. The WOL technique, which collects the entire stomach content, suits this purpose better.

Introduction

The fulmarine petrels (*Fulmarinae*) are a group of seabirds within the order of procellariids. Their diets and those of other seabirds have been extensively studied (e.g. Bierman & Voous 1950; Ainley *et al*. 1992; Soave *et al*. 1996; Hodum & Hobson 2000; van Franeker *et al*. 2001; Cherel *et al*. 2002) and with a variety of different diet sampling techniques (reviewed by Barrett *et al*. 2007). Two of the most widely used methods are the collection of regurgitates (REG) and stomach flushing or water-off-load technique (WOL; Wilson 1984) and both techniques have specific advantages and disadvantages (Votier *et al*. 2003). Although collecting regurgitates is less stressful for birds, the use of regurgitates to sample diets also has disadvantages. For example, meal size cannot be determined from regurgitates due to the likelihood of incomplete regurgitation (the entire stomach contents are not regurgitated), variation in the proportion of ingested food items, and the fact that some food types are easier to regurgitate than others (e.g. Votier *et al*. 2003; Barrett *et al*. 2007). The water-off-load technique may provide a more accurate picture of meal size and diet, but is likely to be more stressful for birds. Some bird species, such as auks, do not easily regurgitate and might be less suitable for this technique although it has been used successfully in this group (Wilson *et al*. 2004). As a quantitative approach is critical if we are to understand nutrient cycling and interactions between predators and their prey, choosing an adequate method based on knowledge of the limitations of the different methods is of utmost importance.

Van Franeker *et al*. (2001) compared different diet study methods in Antarctic fulmarine petrels and suggested a bias towards crustacean prey in regurgitated samples. However, these conclusions were based on REG and WOL samples from different individual birds. They proposed to resolve the question of such potential bias by future WOL studies that could separately store and analyse voluntary regurgitates of birds captured for WOL sampling. Such an approach became possible when we captured Cape Petrels *Daption capense* and Snow Petrels *Pagodroma nivea* to sample diets using the WOL technique, and some birds regurgitated part of their stomach contents on capture. These regurgitates were collected and analysed separately from subsequent WOL samples. By doing so we were able to study potential differences between REG and WOL samples of the stomach content within individual birds of two species of petrels.

Methods

The feeding ecology of adult Cape and Snow Petrels was studied at Signy Island, South Orkney Islands (60°42'S 45°35'W) from 14 December 2005 to 21 February 2006 (Figure 1). We studied petrels at several colonies around the island (Factory Cove, Pinder Gully, Gourlay Peninsula, Observation Corner, and North Point). Chick-feeding petrels were captured with a noose pole on return to the colony after a foraging trip. We used the WOL technique (Wilson 1984) but voluntary regurgitates upon capture were collected as well to analyse diet composition in REG and WOL+REG samples.

A binocular microscope was used to sort all recognizable items of the main prey groups (fish, crustacean, squid, or others). Original prey numbers, sizes, and masses were estimated using characteristic body part remains, such as fish otoliths, fish eyes, euphausid carapaces, and euphausid eyes. Fish were identified to species level using otoliths collected in the samples following identification keys (Hecht 1987; Williams & McEldowney 1990; Reid 1996). Otolith length and/or height were measured using a Zeiss Discovery Stereomicroscope and Axiovision (version 4.8.2.0). The regression equations in Williams and McEldowney (1990) and Reid (1996) were used to reconstruct the total length and mass of fish. Crustaceans were identified using Morris *et al*. (1988), Hill (1990), Reid & Measures (1998), and Shreeve (2005). All Antarctic Krill *Euphausia superba* found in the samples seemed to belong to two demographic categories, classified as either adult (eye diameter > 1.5 mm) or juvenile (eye diameter < 1.5 mm) individuals. A sub-sample of

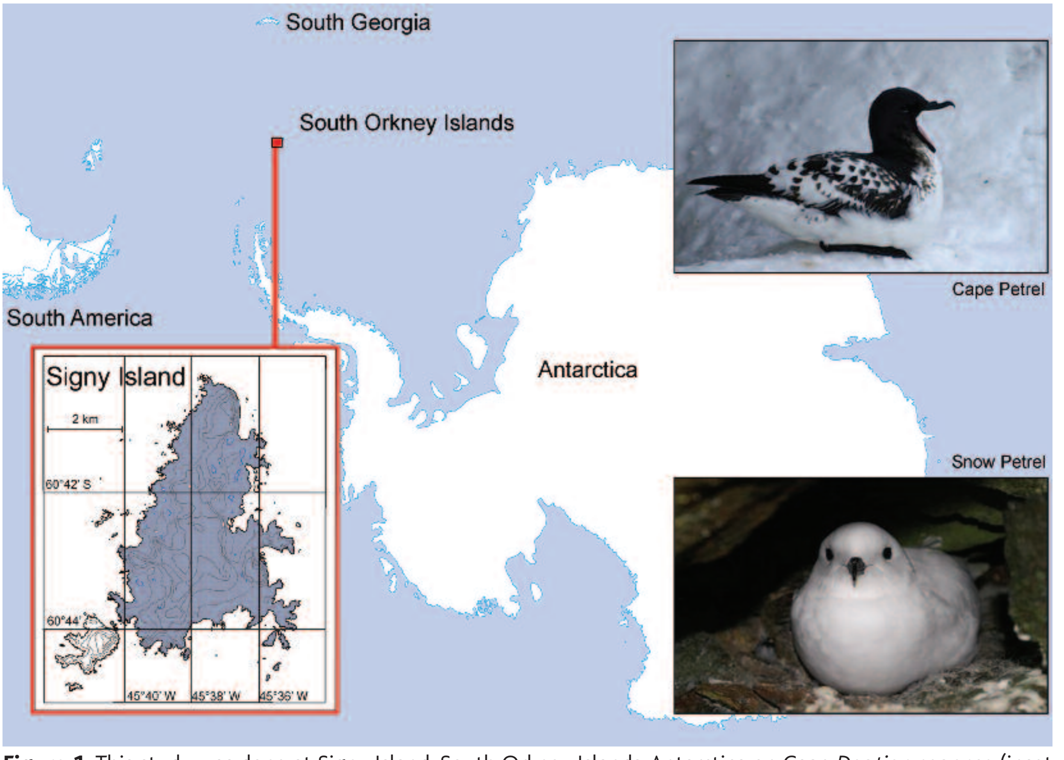

Figure 1. This study was done at Signy Island, South Orkney Islands, Antarctica on Cape *Daption capense* (inset top) and Snow Petrels *Pagodroma nivea* (inset bottom), Signy Island, December 2005. © Ruben Fijn.

intact Antarctic Krill of both groups was taken to estimate average carapace lengths ($N_{\text{adult krill}}$ = 272 and $N_{\text{juvenile krill}}$ = 45). The regression equations in Reid & Measures (1998) were then applied to reconstruct the mass of krill in a diet sample, using the number of eye pairs to determine the number of krill specimens taken. Other crustaceans were also encountered but most were complete when found so their reconstructed mass could be determined with some certainty. Complete squid or identifiable remains such as complete squid beaks were not retrieved in the samples, but some squid parts were found. An estimate of total length was made based on the size of body parts such as arms. Original mass of the most common squid species known to occur around the South Orkney Islands (*Histioteuthis sp*.) was calculated to estimate original mass following Clarke (1986). Diet composition of all birds was based on the summed mass of all samples of birds. It is expressed in terms of mean percentage Reconstructed Weight (REW) proportions of main prey groups, including fish (F), crustacean (C), squid (S), and other (O) (summarized as F:C:S:O). Differences in diet composition between the two sampling groups were tested using non-parametric Mann-Whitney *U*-tests (Quinn & Keough 2002) using SPSS 15.0 for Windows.

Results

Twelve Cape and eight Snow Petrels regurgitated part of their stomach contents before a complete WOL procedure was performed. These REG samples had average drained weights of 20.1 ± 14.5 g (mean ± SD; range = 0.6–47.0 g) for Cape Petrels and 8.2 ± 4.8 g (mean ± SD; range = 1.4–17.1 g) for Snow Petrels. WOL samples obtained afterwards had average drained weights of 19.0 ± 11.9 g (mean ± SD; range = 3.4–39.2 g) for Cape Petrels and 14.5 ± 8.7 g (mean ± SD; range = 4.2–29.0 g) for Snow Petrels.

In REG samples of Cape Petrels, two species of fish were found: *Electrona antarctica* and *E. carlsbergi*. When WOL samples from these birds were added to the REG samples, three more species of fish were found: *Lepidonotothen larseni, Gymnoscopelus braueri* and *G. nicholsi*. In REG samples of Snow Petrels, only *E. antarctica* was found. In the WOL samples from these birds *L. larseni* was also found (Table 1). No difference in occurrence of crustacean species was found in REG and WOL samples of Cape Petrels. *Euphausia superba* was their most abundant crustacean prey followed by *Themisto gaudichaudii*, different species of Gammarid amphipods and *Calanoides acutus*. In Snow Petrels differences in crustaceans did occur between REG and WOL samples. REG samples only contained *E. superba* and Gammarid amphipods, but WOL samples also contained *T. gaudichaudii* and *Pasiphae scotiae* (Table 1). Squid remains were found in both REG and WOL samples of Cape Petrels, but identification was not possible to species level. Squid remains were only found in WOL samples of Snow Petrels.

Table 1. Frequency of occurrence (%) of fish and crustacean species in collected regurgitates (REG) or complete stomach samples (REG + WOL) from Cape *Daption capense* and Snow Petrels *Pagodroma nivea* at Signy Island in 2005/2006. For fish species, bracketed numbers specify the number of otolith pairs found.

Cape Petrel (n = 12)	**REG**	**REG+WOL**
Fish		
Electrona antarctica	42% (9)	75% (34)
E. carlsbergi	8% (1)	8% (1)
Lepidonotothen larseni	-	8% (1)
Gymnoscopelus braueri	-	8% (1)
G. nicholsi	-	8% (1)
Crustaceans		
Euphausia superba	100%	100%
Themisto gaudichaudii	25%	42%
Gammarid amphipods	8%	8%
Calanoides acutus	8%	8%
Snow Petrel (n = 8)		
Fish		
Electrona antarctica	25% (4)	100% (33)
Lepidonotothen larseni	-	13% (4)
Crustaceans		
Euphausia superba	100%	100%
Themisto gaudichaudii	-	50%
Gammarid amphipods	25%	13%
Pasiphae scotiae	-	13%

Cape Petrel diet composition based on the reconstructed weight of REG samples was 21:77:01:00 (% Fish:Crustacean:Squid:Other). Diet composition for the full stomach content of REG+WOL was 36:63:01:00. These two compositions differed significantly in the fish fraction (U = 16.00, r = -945.4, P = 0.001) and crustacean fraction (U = 21.00, r = -849.9, P = 0.002). Squid (U = 66.00, r = -0.17, P = 0.50) and Other (U = 72.00, r = 0.28, P = 1.0) fractions were not significantly different.

Snow Petrel diet composition based on the reconstructed weight of REG samples was 19:81:00:00 (Fish:Crustacean:Squid:Other). Diet composition of REG+WOL samples was 51:48:01:00. These two compositions differed significantly in the fish fraction (U = 2.00, r = -1135.8, P = 0.001) and crustacean fraction (U = 3.00, r = -1.1, P = 0.001). Squid (U = 28.00, r = -0.35, P = 0.51) and Other (U = 28.00, r = 0.35, P = 0.51) fractions did not differ.

Discussion

Regurgitates of both petrel species underestimated the fish fraction and overestimated the crustacean fraction compared to the complete stomach contents as determined by REG and WOL samples combined. This confirms the suggestions by van Franeker *et al.* (2001) that a bias towards crustacean prey is expected in REG samples compared to WOL samples for Cape Petrels, although they were only able to conclude this from samples of different individuals, in contrast to this study. They did not find such a bias for Snow Petrels, which can be explained by regional differences in diet composition of the latter species. Snow Petrels in the study of van Franeker *et al.* (2001) at Ardery Island were almost exclusively fish eaters, thus yielding similar diet compositions for both sampling methods, whereas Snow Petrels around Signy Island have a more diverse diet with a substantial crustacean component (Fijn *et al.* 2012).

In previous studies the WOL technique was shown to be the most effective way of diet sampling (Ryan & Jackson 1986; Gales 1987) with very good recovery of different food items such as fish otoliths and fish and krill remains. However, in these studies a comparison could never be made between the two methods within one individual bird. We were able to compare the fraction in individual birds and confirm that using solely REG samples will affect the outcome of a diet study. An explanation for the difference between REG and WOL samples is that different prey items come out differently during sampling. Regurgitates are from the top layer of a bird's stomach which might cause some prey items to be present with a higher probability than others. Gales (1987) noted that krill came out first in Little Penguins *Eudyptula minor* and this might cause a crustacean bias in a 'single flush method', as was similarly proposed by Creet *et al.* (1994) and van Franeker *et al.* (2001). Being in the upper layer of the stomach could be due to the timing of ingestion of the specific prey item. Another reason could be that otoliths quickly become loose in the stomach due to a faster digestion of fish material. They might then be more likely to 'sink' in the stomach due to their small size compared to the more bulky exoskeletons of crustaceans that don't digest as rapidly as fish material. Also otoliths can become trapped in the microstructures (villi) of the stomach. Thus, most otoliths would only come out when stomach contents are collected completely, i.e. by flushing.

In previous studies to obtain diet samples via regurgitation, two different techniques have been used. The first is incidental collection of regurgitates, which are mostly small samples from the upper layers of the stomach. The second method is forced regurgitation when birds are inverted over buckets and belly massaged to obtain diet samples. The second option would be the preferred way to collect REG samples, although as Arnould & Whitehead (1991) stated, this type of REG sampling implies substantial stress for the birds. Thus, forced regurgitation and WOL probably induce a similar level of stress, in which case WOL should be the preferred choice because of the completeness of the data obtained in relation to the amount of stress for the bird. Dietary research based on regurgitates only allows qualitative comparisons but can be a preferred method of sampling, for example where researchers wish to minimise impacts on sensitive populations or species, or where comparative values (e.g. intra- or inter-annual dietary changes or comparisons between locations) are more relevant than absolute values.

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