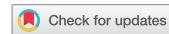


SHORT COMMUNICATION



## Seashell and debris ingestion by African penguins

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### ABSTRACT

African penguins (*Spheniscus demersus*) are pursuit-diving seabirds endemic to the coast of southern Africa. In this study, we investigate the presence of seashells and anthropogenic debris (e.g. plastic, glass, nylon) in the stomach contents of adult African penguins, as determined from sampling of live penguins through the water off-loading technique ( $n = 4,793$ ) and from post-mortem examination of penguin carcasses ( $n = 159$ ). Seashells were present in stomach contents sampled from 106 live (2.2%) and three dead (1.9%) penguins. Seashells originated from a variety of intertidal and subtidal organisms including molluscs, barnacles and bryozoans, and the eroded condition of the shell fragments suggests that they were picked up from shell deposits on the beach or in the surf zone. Seashell ingestion appears to be more frequent in the months of peak egg laying. A subset of stomach samples from known-sex individuals revealed that seashells were only present in the stomachs of adult females. In post-mortem examination, the presence of seashells in the stomach was accompanied by anatomical evidence that egg laying was imminent or had recently occurred. Anthropogenic debris was found in one (0.6%) and eight (0.2%) stomach content samples obtained from dead and live penguins, respectively. In some cases, the ingestion of anthropogenic debris co-occurred with that of seashells, and their size and shape were similar. Our findings demonstrate that adult female African penguins occasionally ingest seashells, possibly as a calcium supplementation strategy, and raise concern that in doing so they may also accidentally ingest anthropogenic debris.

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## Introduction

The African penguin (*Spheniscus demersus*) is a seabird endemic to the coasts of Namibia and South Africa. Its population has declined by ~95% in the 20th century, and the species is currently classified as 'endangered' with a remaining population of approximately 25,000 breeding pairs (BirdLife International 2016; Nel *et al.* 2003; Crawford *et al.* 2011). Dietary studies on African penguins have shown that the species relies primarily on small pelagic schooling fishes, such as anchovies (*Engraulis capensis*) and sardines (*Sardinops sagax*), with squid and crustaceans being consumed to a lesser extent (Wilson 1985; Randall and Randall 1986; Connan *et al.* 2016). There is only one brief mention of the consumption of bivalve shells by this species (Randall and Davidson 1981).

The consumption of calcium-rich materials (egg shells, bones, mussel shells, limestone, etc.) is a common strategy of birds to cope with the increased calcium demands

associated with egg laying (Graveland and Gijzen 1994; Johnson and Barclay 1996). It is generally assumed that fish-eating birds do not require ingestion of seashells because their diet is sufficiently calcium-rich (Brenninkmeijer *et al.* 1997; Boersma *et al.* 2004). However, several exceptions have been documented, including the sporadic ingestion of mollusc shells by penguins (Paulin 1975; Randall and Davidson 1981; Boersma *et al.* 2004; Massaro and Davis 2005). In Magellanic penguins (*Spheniscus magellanicus*), this behaviour is more prevalent in females during the egg laying period, and has thus been interpreted as a calcium supplementation strategy (Boersma *et al.* 2004).

Following opportunistic observations of seashell fragments in the stomachs of African penguins during post-mortem examinations and radiographic exams (Figure 1), we decided to investigate the prevalence of shell fragments and anthropogenic debris in a large sample of stomach contents from live and dead African penguins.



**Figure 1.** Radiographs of an adult female African penguin admitted for rehabilitation at SANCCOB, showing an egg in the oviduct and the presence of seashell fragments in the stomach (arrows). Legend: (a) dorso-ventral exposure, (b) latero-lateral exposure, (c) seashell fragments recovered from the stomach using the water off-loading technique.

## Methods

### *Stomach contents from live penguins*

As part of long-term monitoring studies, the stomach contents of 4,793 adult African penguins were sampled between 1980 and 2017 at ten islands in Namibia (Mercury, Ichaboe, Halifax and Possession Islands), South Africa's Western Cape (Malgas, Dassen, Robben and Dyer Islands), and Eastern Cape (St. Croix and Bird Islands) (Figure S1). Penguins were caught as they came out of the water after feeding (as determined by the distension of the lower abdomen), and their stomach contents were obtained through the water off-loading technique (Wilson 1984). No individuals were knowingly resampled; however, flipper banding of African penguins has been discontinued since 2005 and it is plausible that some individuals were resampled in different years. Food items were sieved and the presence of seashells and anthropogenic debris (plastic, glass, nylon, etc.) was

recorded. For individuals sampled at St Croix and Bird Islands since 2012, sex was determined based on beak measurements (Pichegru *et al.* 2013).

Binary logistic regression analysis was used to evaluate whether the presence of seashells in the stomach samples obtained from live penguins (event) was determined by the month and island where the stomach sample had been obtained (predictors). The months of the primary and secondary incubation peaks in each region were derived from previous studies (Randall 1983; Crawford *et al.* 1999; Wolfaardt and Nel 2003; Kemper 2006): October–December (primary) and June–July (secondary) in Namibia, February–March (primary) and October–January (secondary) in the Western Cape, and March–April (primary) and January–February and May–June (secondary) in the Eastern Cape. Fisher exact tests were used to determine whether the presence of seashells was associated with incubation peaks, sex or with the presence of anthropogenic debris.

## Stomach contents from penguin carcasses

The stomachs of 159 penguin carcasses collected during a one-year period (1 June 2017 to 31 May 2018) were dissected and the presence of seashells and anthropogenic debris was recorded. These carcasses had been collected by park rangers and local authorities along the coast of the Western Cape (including Dassen, Robben and Dyer islands and the mainland colonies of Boulders and Stony Point; Figure S1) and were submitted for necropsy at the Southern African Foundation for the Conservation of Coastal Birds (SANCCOB) as part of a disease surveillance programme. Post-mortem examination was conducted following standardised protocols (Hocken 2002). The body condition of penguins was categorised according to a five-level scoring method (Clements and Sanchez 2015) and sex was determined through the dissection of the gonads. Fisher exact tests were used to determine whether the presence of seashells was associated with sex or with the presence of anthropogenic debris.

## Identification of hard items

Seashell remains in the stomachs of seven penguins were examined in order to identify which species they represented. These samples comprised: (a) photographs of seashells recovered from two live penguins sampled at St Croix Island in May 2012 (cases 1 and 2); (b) frozen stomach samples recovered from three penguin carcasses necropsied at SANCCOB during the 2017–2018 study period (cases 3 to 5); (c) photographs of seashells recovered from a penguin carcase necropsied at SANCCOB in June 2016 (case 6); (d) the frozen stomach sample recovered from a penguin carcase necropsied at SANCCOB in December 2016 (case 7). It should be noted that the samples in cases 1, 2, 6, and 7 were obtained before the systematic carcass sampling period. Shells were identified to species level by visual matching with a local identification guide (Branch *et al.* 2008). Eroded and highly

fragmented specimens were assigned to the lowest taxonomic level of confident identification.

## Results

### Seashell fragments

One hundred and six (2.2%) of the 4,793 stomach samples obtained from live adult penguins contained seashell fragments (Table 1). Using binary logistic regression analysis, it was clear that both island ( $P = 0.008$ ) and month of sample collection ( $P < 0.001$ ) were significant predictors of the presence of seashells ( $R^2 = 0.086$ ,  $P < 0.001$ ). Figure S2 represents the seasonal distribution of the presence of seashells across study sites. At the Western Cape, the frequency of seashells was higher in stomach samples collected during the peak incubation months (3.0%) than in the remainder of the year (1.5%) ( $n = 3,572$ ;  $P < 0.001$ ), however this pattern could not be demonstrated for Namibia (2.1% vs. 0.7%;  $n = 832$ ;  $P = 0.125$ ) nor the Eastern Cape (4.7% vs. 4.2%;  $n = 389$ ;  $P > 0.9$ ).

Seashell mass was recorded for 83 samples, with a median 1.5 g per sample (minimum = 0.1 g, first quartile = 0.7 g, third quartile = 4.1 g, maximum = 28 g). For individuals sampled at St Croix and Bird Islands, and whose sex was determined, seashells were found in the stomach of females (10 of 88 individuals) but not in the stomach of males (58 individuals); the relationship between sex and the presence of seashells was significant for known-sex live penguins ( $P = 0.006$ ).

Seashells were recorded in the stomachs of three (1.9%) of the 159 carcases examined at the Western Cape province of South Africa in 2017–2018. All three were adult females, representing 8.8% of the 34 adult females necropsied during the period. None of the 85 immature penguins, 37 adult males and three adults of unknown sex necropsied during the study period contained seashells in their stomachs. The small sample size precluded the detection of a significant association

**Table 1.** Distribution of the stomach samples from live adult African penguins examined in relation to the island and month in which the samples were collected. Numbers within brackets represent the number of stomach samples containing seashell fragments.

Region	Island	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
Namibia	Mercury	7	50		10 (1)						1	137 (1)	90 (6)	295 (8)
	Ichaboe	144	134							3	1	86	23	391
	Halifax											18	3 (1)	21 (1)
	Possession	44	10	30						15 (2)			26	125 (2)
	Malgas											1	5	6
Western Cape	Dassen	44	101 (3)	196 (1)	188 (4)	158 (4)	145	17	21	80 (4)	178 (10)	17	50 (3)	1,195 (29)
	Robben	90 (8)	214 (13)	304 (4)	161 (5)	340 (1)	122 (2)	148	267 (6)	266 (4)	132 (1)	89	18 (1)	2,151 (45)
	Dyer			38 (3)	22	10		7	58		85			220 (3)
	St Croix		5	34 (3)	37 (1)	37 (3)	22 (1)	15 (2)	5					155 (10)
	Bird		9	77 (4)	56 (3)	30 (1)	34	16	12					234 (8)
Total		329 (8)	523 (16)	679 (15)	474 (14)	575 (9)	323 (3)	203 (2)	366 (6)	362 (10)	396 (11)	348 (1)	215 (11)	4,793 (106)

between sex and the presence of seashells among known-sex adult penguin carcasses ( $P = 0.105$ ).

Photographs and stomach content samples from seven penguins that had ingested seashells were further analysed; all were adult-plumaged females and were in good body condition (score 4). The dissection of the gonads of five carcasses (cases 3 to 7) revealed fully developed and active left ovaries (distended blood vessels, presence of follicles  $> 5$  mm) and oviducts (enlarged lumen and thickened walls), indicating egg production was imminent or had recently occurred (Figure S3). The cause of death was determined as follows: clostridial infection (case 3), predation (cases 4, 6 and 7), and hit by car (case 5).

The stomach contents of these seven penguins showed a variety of fragments of benthic fauna from the intertidal and subtidal zones, including Bivalvia (*Atactodea striata*, *Aulacomya atra*, *Limaria tuberculata* and *Venus verrucosa*), Gastropoda (*Turbo sarmaticus*), Polyplacophora (*Dinoplax gigas*), along with unidentified limpets, periwinkles and whelks (Gastropoda), unidentified barnacles (Crustacea: Thoracica: *Sessilia*) and bryozoans (Bryozoa) (Table S1, Figure S4). The shells were often severely fragmented and eroded, hence the majority of the fragments could not be identified to species level and these were instead assigned a higher taxonomic grouping.

### **Anthropogenic debris**

The presence of anthropogenic debris was recorded in the stomach samples of eight live penguins (0.2%). These were only recorded in samples from the Western Cape: Dassen (4 samples), Robben (3) and Dyer islands (1). Two of these samples contained both seashells and anthropogenic debris; there was no significant relationship between the presence of seashells and debris in live penguins ( $P = 0.642$ ). Debris mass was recorded for the eight samples, with a median 0.35 g per sample (minimum = 0.1 g, maximum = 2.1 g). Debris items were identified as: hard plastic fragment (5 samples), glass fragment (1), paint chip (1) and nylon filaments (1).

For the stomach samples of penguin carcasses examined in the Western Cape in 2017–2018, only one sample (0.6%) contained anthropogenic debris. This sample contained a hard plastic fragment and also contained seashells (case 4 in Table S1), implying a significant relationship between the presence of seashells and debris in penguin carcasses ( $P = 0.019$ ). Additionally, one of another two stomach samples opportunistically obtained from a penguin carcase necropsied in 2016 (case 6 in Table S1) also had a hard plastic fragment and a sharp-edged glass fragment (Figure S5).

### **Discussion**

In this study we document the ingestion of seashells and anthropogenic debris by African penguins, using data from a large sample size (c. 4,800 samples) collected over four decades covering the entire species' geographic distribution.

Although Randall and Davidson (1981) mentioned having recovered bivalve shells from the stomachs of African penguins, they did not speculate on the reasons for the ingestion of these items. Boersma *et al.* (2004) suggested that seashell ingestion by Magellanic penguins might be related to the high calcium demands due to the thick egg shells in these birds. Our results are consistent with this hypothesis, considering that (a) among known-sex individuals, the ingestion was only recorded in adult females, and (b) in post-mortem examination, the presence of seashells in the stomach was accompanied by anatomical evidence that egg laying was imminent or had recently occurred.

Interestingly, the frequency of seashells in the stomachs of African penguins was markedly lower than that reported by Boersma *et al.* (2004) for Magellanic penguins. This might be related to the fact that Magellanic penguins are more synchronous breeders than African penguins (Boersma *et al.* 2013; Crawford *et al.* 2013), which would imply that a sampling strategy during the peak egg laying season where females are captured without prior knowledge of the reproductive status would be more likely to sample females close to the egg laying date in Magellanic penguins than in African penguins. This could also explain the apparently more marked seasonality in seashell ingestion in Magellanic penguins than in African penguins. African penguins can lay eggs year-round, and the peaks in egg laying and incubation can differ markedly among breeding colonies (Kemper 2006; Kemper *et al.* 2007; Crawford *et al.* 2013). Because our sampling effort was unevenly distributed across different islands and months, it is difficult to make generalisations regarding the seasonality of the presence of seashells in the stomach contents of this species. However, a comparison at the regional level does suggest that in the Western Cape (where we had the largest sample size) the ingestion of seashells tends to be most frequent in the months of peak egg laying.

Boersma *et al.* (2004) roughly estimated that female Magellanic penguins need 10.5 g of calcium in order to produce two eggs and can mobilise only 6.7–8.0 g of skeletal calcium, hence they would need to obtain an additional 2.5–3.8 g through their diet. Female African penguins are 21% lighter than female Magellanic penguins (average body mass = 3.0 and 3.8 kg, respectively),

their eggs are 15% lighter (average combined egg mass = 211.6 and 249.6 g) (Boersma *et al.* 2013; Crawford *et al.* 2013), and their eggshells are 26% thinner (average eggshell thickness = 0.60 and 0.81 mm) (Boersma *et al.* 2004; Bouwman *et al.* 2015). The dietary calcium requirements associated with egg production in African penguins are therefore likely to be smaller (in absolute terms) than those of Magellanic penguins. The mass of seashell fragments recovered from the stomach of African penguins was relatively low (median 1.5 g). However, the mass of seashells recovered may have been affected by varying percentage of seashell mass recovery using the water off-loading technique and varying digestion/absorption of seashells depending on the time since their ingestion (see Gales 1987; Neves *et al.* 2006). Furthermore, the proportion of calcium that can be absorbed is likely to vary considerably among seashell taxa and erosion condition. For these reasons, our results do not allow for an accurate assessment of the quantity of calcium obtained by African penguins through seashell ingestion.

Although some of the seashells could have been obtained from reef substrate while diving (see McInnes *et al.* 2017), their diverse taxonomic composition, the representation of different intertidal and subtidal organisms, and the eroded condition of the shell fragments strongly suggest that they were picked up from shell deposits on the beach or in the surf zone. The consumption of shells from a variety of organisms that are relatively common in the region suggests that the penguins select the seashells primarily based on size and shape rather than on species.

Anthropogenic debris was found in the stomach contents of a small number of African penguins evaluated in this study. Although there are numerous records of penguins entangled with marine debris, litter ingestion is relatively uncommon in these birds (Ryan 1987; Battisti *et al.* 2019). Juvenile Magellanic penguins appear to be an exception in this regard, with 22–89% of the individuals found along the Brazilian coast having debris in their stomachs (Tourinho *et al.* 2010; Brandão *et al.* 2011; Di Benedetto and Siciliano 2017). However, in that species the ingestion of marine debris is thought to be related to inexperience and an attempt to stave off hunger (Brandão *et al.* 2011; Di Benedetto and Siciliano 2017), which was clearly not the case in this study as the debris was found in adult penguins that were in good body condition. Instead, because some of the plastic and glass fragments found in this study frequently had a size and shape resembling that of the seashells (Figures S4 and S5), we suspect their ingestion may have been accidental while attempting to collect seashells from beach deposits.

Interestingly, the ingestion of anthropogenic debris was only recorded in penguins sampled in the Western Cape province of South Africa. While this may in part be related to the greater sampling effort in this region, it could also be related to the fact that coastal areas near Cape Town are known to have a high density of anthropogenic debris from local sources (Ryan *et al.* 2018). The ingestion of marine debris can be life threatening to seabirds in the case of hard materials with sharp edges, similar to that documented in samples during this study (Figure S5), as it can lead to serious gastrointestinal injury (Ryan 1987; Brandão *et al.* 2011). Furthermore, plastics may be a source of toxic chemicals that disrupt endocrine systems, are teratogenic or interfere with egg shell formation (Fry 1995; Rochman *et al.* 2013; Bouwman *et al.* 2015). Further research and continued monitoring of the frequency and impacts of anthropogenic debris on African penguins are therefore warranted.

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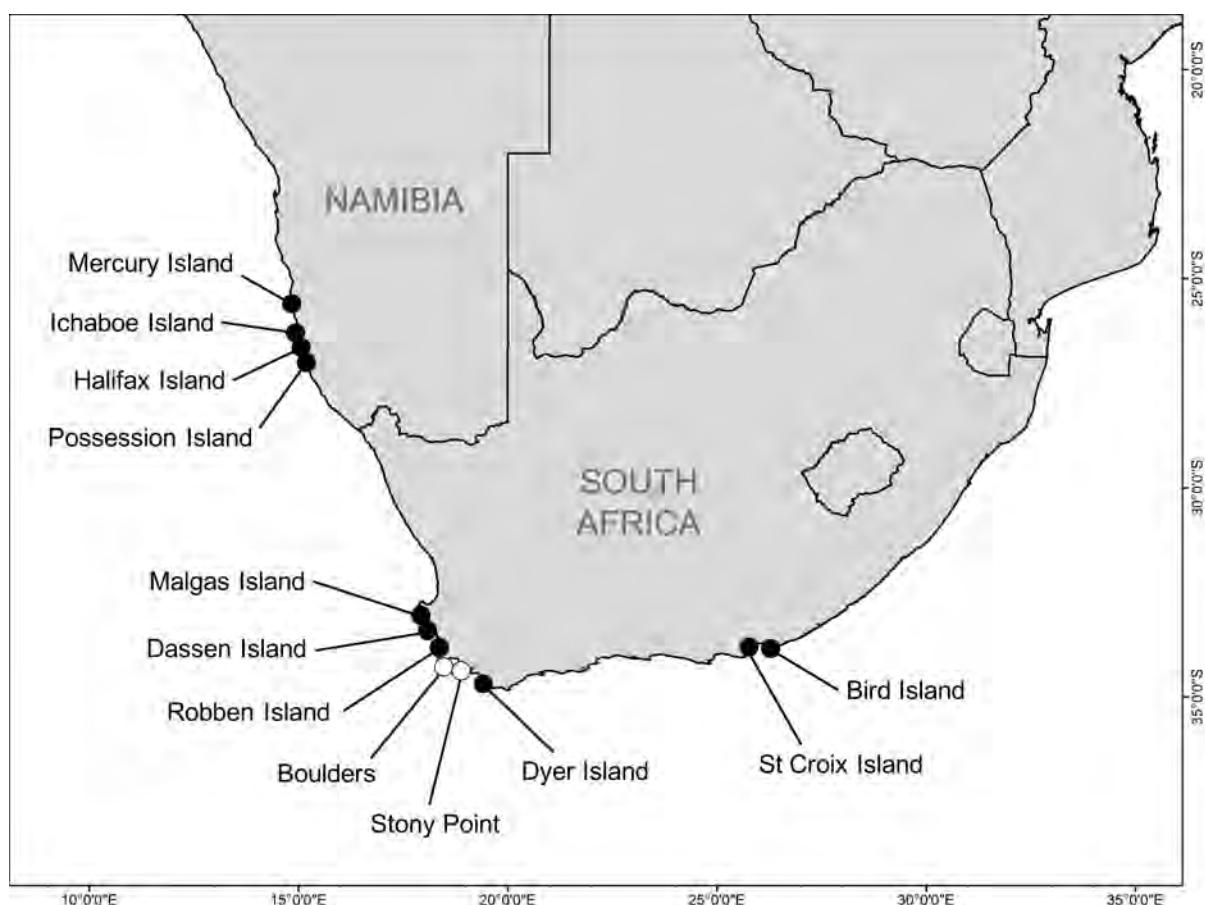
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## Supplementary materials

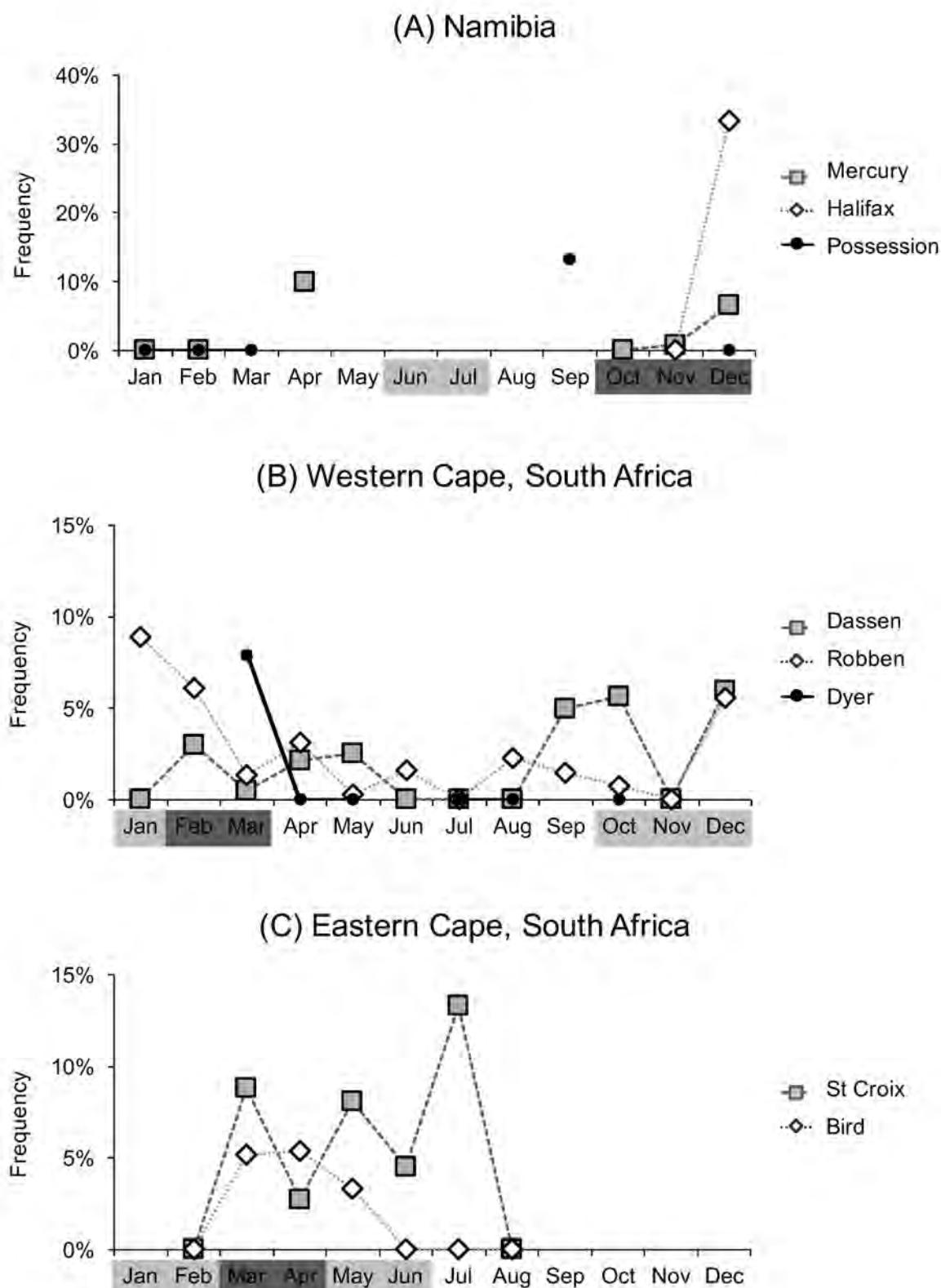
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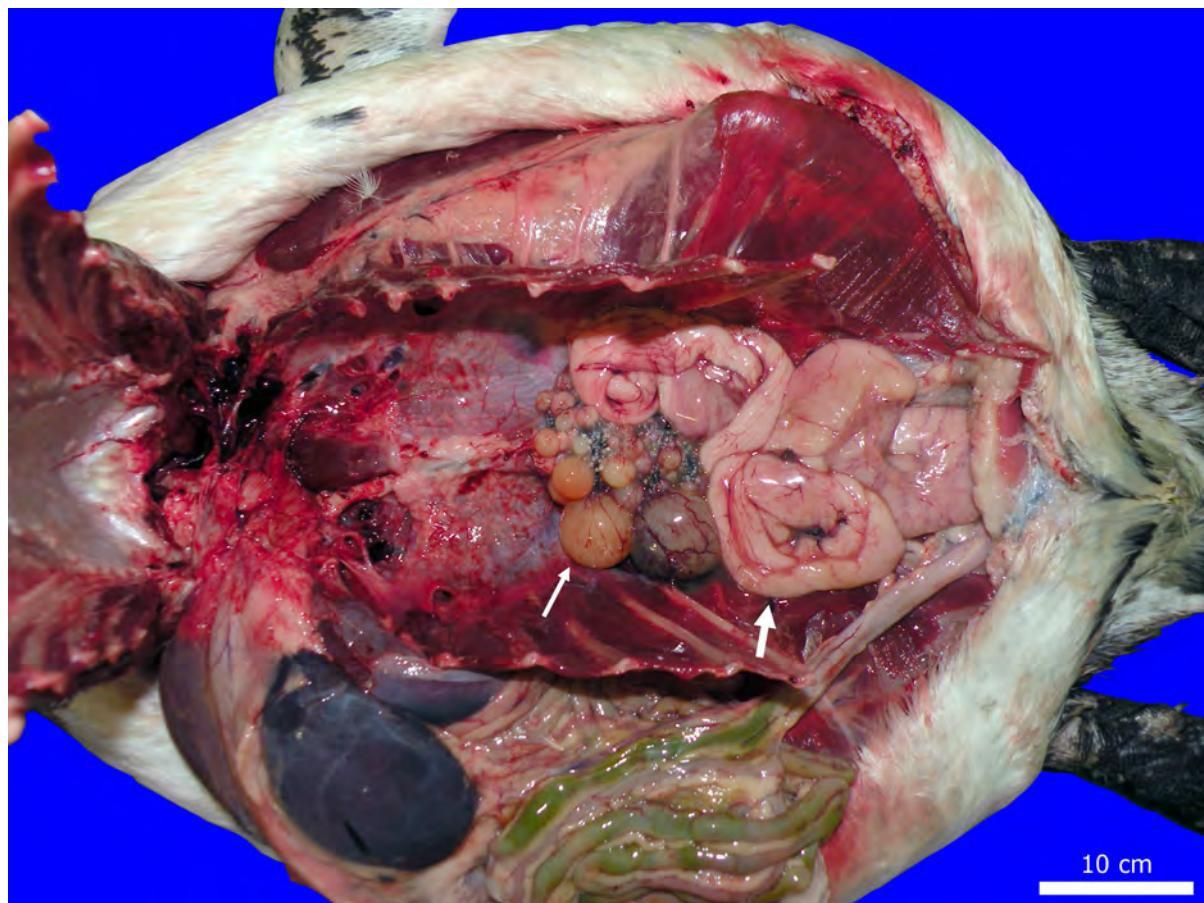
**Figure S1.** Geographic distribution of the islands (black circles) and mainland colonies (white circle) from where African penguin stomach contents were sampled.



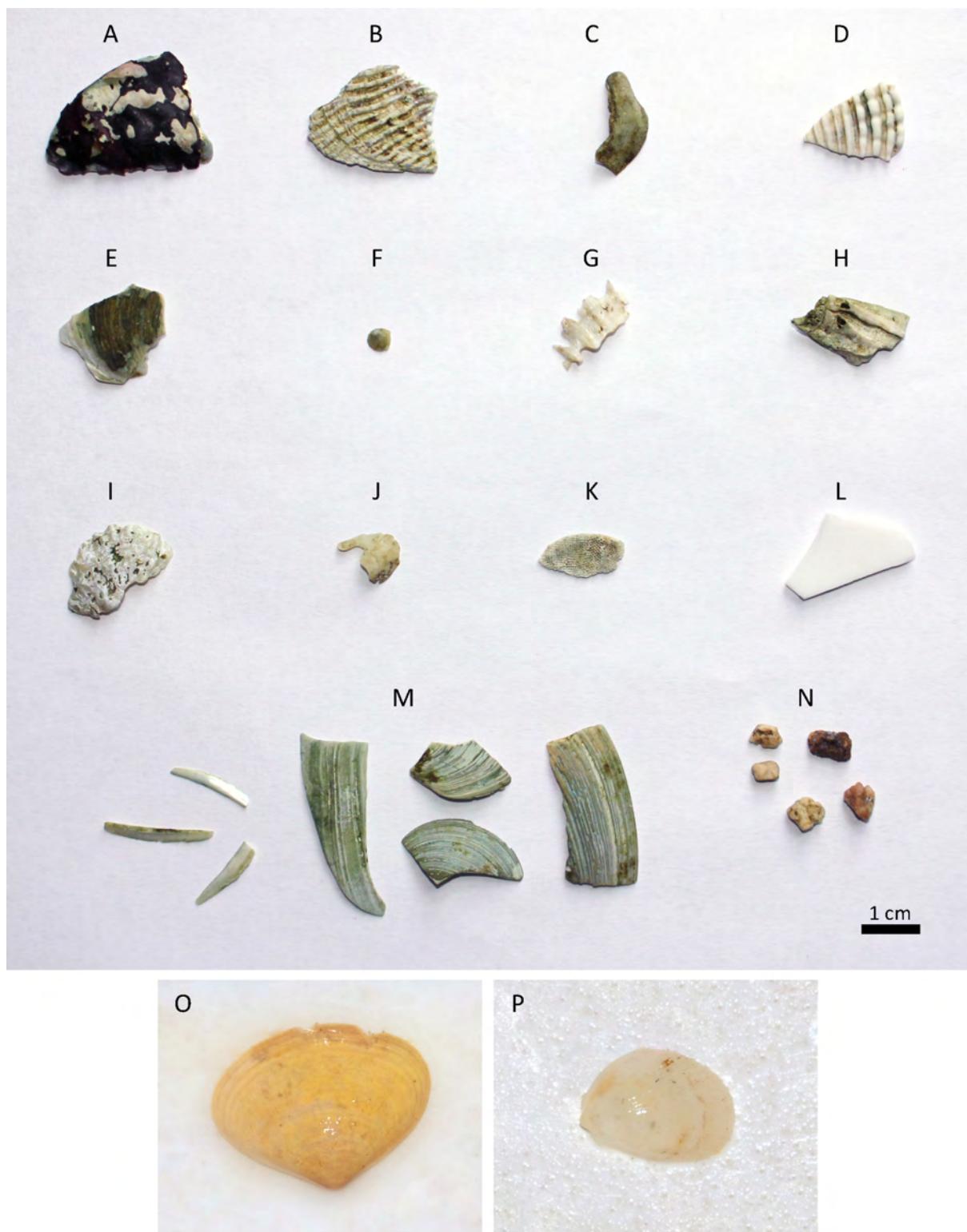
**Figure S2.** Frequency of the presence of seashells in the stomach samples from live adult African penguins in relation to the island and month in which the samples were obtained. The months of the primary (dark grey) and secondary incubation peaks (light grey) in each region are highlighted.



**Figure S3.** Reproductive tract of a female African penguin whose stomach contained seashells (case 7), showing a fully developed and active ovary (distended blood vessels, presence of follicles > 5 mm; thin arrow) and oviduct (enlarged lumen and thickened walls; thick arrow).



**Figure S4.** Hard items found in the stomach of adult female African penguins. Legend: (A) *Turbo sarmaticus*, (B) *Aulacomya atra*, (C) *Dinoplax gigas*, (D) *Venus verrucosa*, (E) unidentified mussel fragment, (F) unidentified periwinkle, (G) unidentified limpet, (H) unidentified barnacle, (I) unidentified oyster, (J) unidentified whelk, (K) unidentified bryozoan, (L) hard plastic fragment, (M) unidentified tellin/clam fragments, (N) rocks, (O) *Atactodea striata*, (P) *Limaria tuberculata*.



**Figure S5.** Stomach contents of an adult female African penguin (case 6) comprising a variety of eroded seashell fragments, a sharp glass shard (white asterisk), a plastic fragment (black asterisk), and squid beaks (black arrow).

