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STOMACH SAMPLING IN THE YELLOW-EYED PENGUIN: EROSION OF OTOLITHS AND SQUID BEAKS

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Abstract.—Experimental feeding of Yellow-eyed Penguins (*Megadyptes antipodes*) with meals containing cephalopod beak pairs and otoliths of known weight, and subsequent flushing of stomachs at 2–48 h intervals after feeding, were carried out to determine the rate of digestion of beaks and otoliths. Otoliths and beaks were placed in HCl to observe changes. All otoliths were totally digested after 24 h in penguin stomachs, disappearance rate was inversely related to size of the otolith. After 24 h squid beaks showed increasing signs of wear, the extent of which was dependent on the presence or absence of small stones in the stomach. Experiments showed that HCl only causes otolith erosion at pH 1.5; erosion rate was twice as slow as in the stomachs. Acid had no effect on squid beaks after 78 h immersion.

EROSIÓN DE OTOLITOS Y PICOS DE CALAMARES EN EL ESTÓMAGO DE PINGÜINOS (*MEGADYPTES ANTIPODES*)

Resumen.—Se llevó a cabo alimentación experimental de pingüinos (*Megadyptes antipodes*) con comida previamente pesada que contenía otolitos y picos de cefalópodos, para determinar la velocidad de digestión de picos, y de picos-y-otolitos. Para determinar la velocidad de digestión se lavó el estómago de las aves utilizando la técnica de Wilson (1984) a intervalos de 2–48 h, una vez los pingüinos eran alimentados. Otolitos y picos fueron también colocados en HCl para observar cambios en estos. Los otolitos fueron totalmente digeridos luego de pasar 24 h en el estómago de los pingüinos. La velocidad de desaparición fue inversamente proporcional al tamaño del otolito. Después de 24 h los picos de los calamares mostraban signos de desgaste; la extención del desgaste estuvo relacionado con la presencia o ausencia de gúijaros en el estómago de los pingüinos. Los experimentos *in vitro* demostraron que el HCl solo causa erosión de los otolitos a un pH de 1.5; la velocidad de erosión resultó ser dos veces más lenta que en el estómago de los pingüinos. Los picos de calamares no se afectaron después de estar sumergidos por 78 h en ácido.

The problem of erosion and differential digestion of diagnostic remains in seabird stomach samples has been acknowledged and discussed by several workers (Adams and Klages 1987, Ainley et al. 1984, Blake et al. 1985, Gaston and Noble 1985, Pascoe 1986), and some attempts have been made to quantify the extent to which the process of digestion can introduce errors into reconstructions of marine bird and mammal diets (Duffy and Laurenson 1983, Furness et al. 1984, Gales 1987, Jackson and Ryan 1986, Murie and Lavigne 1986, Wilson et al. 1985). Commonly used diagnostic remains include the sagittal otoliths of teleost fish (Adams and Klages 1987, Fitch and Brownell 1968, Montague et al. 1986, Pascoe 1986, Skira 1986, Wilson 1985), and the beaks of cephalopods (Adams and Klages 1987, Prince 1980, Randall et al. 1981). The speed at which an otolith or cephalopod beak is digested depends upon its size, shape

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and composition, as well as the degree of acidity in the predator's stomach and the exposure time (Jobling and Breiby 1986).

Differential rates of erosion may mean that the contribution made to the diet by fish with small otoliths may be under-estimated relative to fish with large otoliths (Jobling and Breiby 1986), whereas the contribution of cephalopods relative to fish may be greatly over-estimated. These errors will be magnified when several types of prey comprise the diet.

Few studies have investigated the *in vivo* effects of gastric digestion and acid exposure on fish otoliths (Jobling and Breiby 1986). The diet of the Yellow-eyed Penguin is made up of both squid and fish species with otoliths of differing size. Yellow-eyed Penguins do not regurgitate otoliths and beaks daily as do some other seabirds (Duffy and Laurenson 1983, Lalas 1983), and neither otoliths nor beaks are to be found in the feces as they are in some mammals (da Silva and Neilson 1985). Yellow-eyed Penguins forage on a daily basis, they usually leave the colony at dawn and return at dusk. I wanted to determine: (1) whether otoliths recovered from stomach contents collected from penguins represented a single day's foraging, (2) whether small otoliths were under-represented, especially if the prey were ingested early in the day, and (3) the fate of squid beaks after ingestion.

METHODS

Penguins were captured as they returned from foraging at dusk. Their stomachs were flushed twice to remove all otoliths and beaks using the water off-loading technique of Wilson (1984) and they were penned and left overnight to ensure empty stomachs. On the following morning penguins were fed with meals consisting of 400 g of arrow squid (*Nototodarus sloanii*), their mantles containing pairs of otoliths from three species of fish commonly found in the diet of Yellow-eyed Penguins (van Heezik 1988), as well as pairs of squid beaks of varying size. Penguins were not fed whole opalfish, cod, or sprat as they were unavailable. Mean weights $\pm$ SD of otoliths were as follows: red cod (*Pseudophycis bachus*) 33.64 $\pm$ 4.70 mg ($n = 27$), opalfish (*Hemerocoetes monopterygius*) 7.62 $\pm$ 1.95 mg ($n = 27$), and sprat (*Sprattus antipodum*) 2.56 $\pm$ 0.35 mg ($n = 18$) (see Fig. 1). Size ranges were typical of prey normally ingested by Yellow-eyed Penguins.

Using the water off-loading technique, stomach contents from two penguins were recovered at 2, 4, 6, 8, 12, 16, 24, 36, and 48 h after feeding. Penguins were flushed until only clear water was regurgitated (2–3 times). Otoliths were again counted and weighed. Beaks were examined for signs of erosion or breakage.

Stomach samples (10 ml) were extracted by using a syringe attached to a gastric tube inserted into the stomach. Samples were extracted from 12 penguins before stomach flushing. These were described and tested for pH. Pairs of pre-weighed otoliths from four species of fish, and pairs of squid beaks were immersed in two solutions of hydrochloric acid (pH 1.5 and 3.5), and agitated to simulate conditions in penguins' stomachs. Otoliths were removed at regular intervals, blotted, dried for 5 min in an

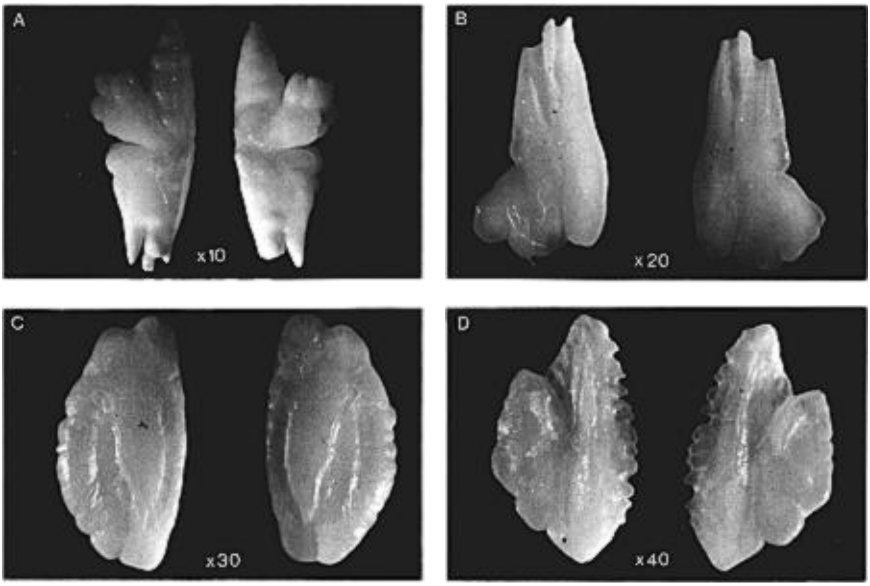

FIGURE 1. Representative otoliths from red cod (A), ahuru (B), opalfish (C), and sprat (D).

oven and weighed. Any effect of the acid on the beaks was ascertained by eye. The progress of beaks was followed by eye.

RESULTS

Feed-pumpback experiment.—The weights of recovered otoliths, expressed as a percentage of the original mean weight, declined with increased time in the stomach (Figs. 2 and 3). Otolith pairs weighing <3 mg were not recovered after 6 h, and recovery was poor after 4 h (Table 1). Otolith pairs weighing 3–10 mg were absent after 16 h and recovery was poor after 6 h. Those pairs weighing 20–40 mg were recovered up to 24 h after ingestion. Signs of erosion were evident within 2 h.

Beaks were recovered intact and with no obvious signs of erosion 16 h after ingestion (Table 2). After 16 h beaks became fractured with frayed and torn wings ("intermediate" following Jackson and Ryan's (1986) description), but the upper rostral length (see Clarke 1980) was still measurable at 48 h in the majority of cases. The degree of beak fragmentation may have been related to the quantity of stones in the stomach. The 2 stomachs containing shattered beaks also contained 23 g and 17 g of small pebbles.

Mean stomach pH was 3.0 (n = 12, SD = 2.0, range = 1.29–7.01). Stomach pH became less acidic as a function of food consistency or concentration: watery samples (pH 1.29–1.85) were also green in color, indicating the presence of bile (Sladen 1958). Stomachs containing little food had pH's ranging from 2.40 to 3.15, and those with food of a thick soupy consistency had pH's from 4.76 to 7.01.

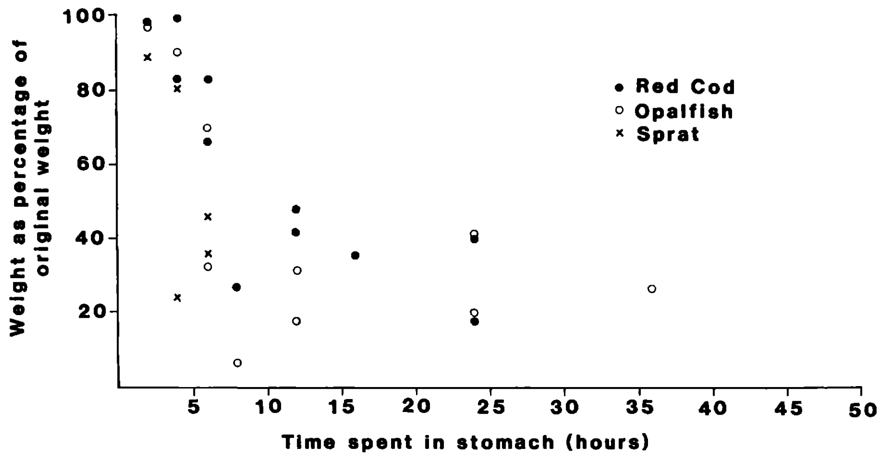

FIGURE 2. Otolith weight, expressed as percent of the mean original weight, in relation to time spent in the stomach. No point means no otoliths were recovered.

Laboratory experiment.—At pH 3.5 no erosion of otoliths or beaks was evident over an 80 h period. In HCl (pH 1.5), erosion rate differed among fish species (Fig. 4). Erosion was fastest for sprat otoliths, which are thin and delicate in shape, and was slowest for those of red cod, which are robust. Beaks remained uneroded.

DISCUSSION

Most of the otoliths used in the *in vivo* experiments were completely digested within a 24 h period, and small otoliths disappeared more rapidly than large otoliths. Therefore, stomach contents collected at the end of a day of foraging would contain mostly otoliths from that day only.

Simulation experiments indicated that the digestive process is not merely due to the stomach acidity, particularly where squid beaks are concerned. Even though substantial erosion did take place at pH 1.5, the initial rapid drop in otolith weight took more than twice as long for otoliths in the laboratory as for those in penguin stomachs. After about

TABLE 1. Percent recovery of whole otoliths after time intervals in the stomach.

	Hours after ingestion								
	2	4	6	8	12	16	24	36	48
Red Cod	100%	75%	83%	83%	100%	67%	83%	0	0
n	12/12	9/12	10/12	5/6	12/12	4/6	20/24	0/24	0/24
Opalfish	100%	42%	58%	17%	33%	0	4%	4%	0
n	12/12	5/12	7/12	1/6	4/12	0/4	1/24	1/24	0/24
Sprat	75%	63%	20%	0	0	0	0	0	0
n	3/4	5/8	2/10	0/4	0/10	0/4	0/24	0/24	0/24

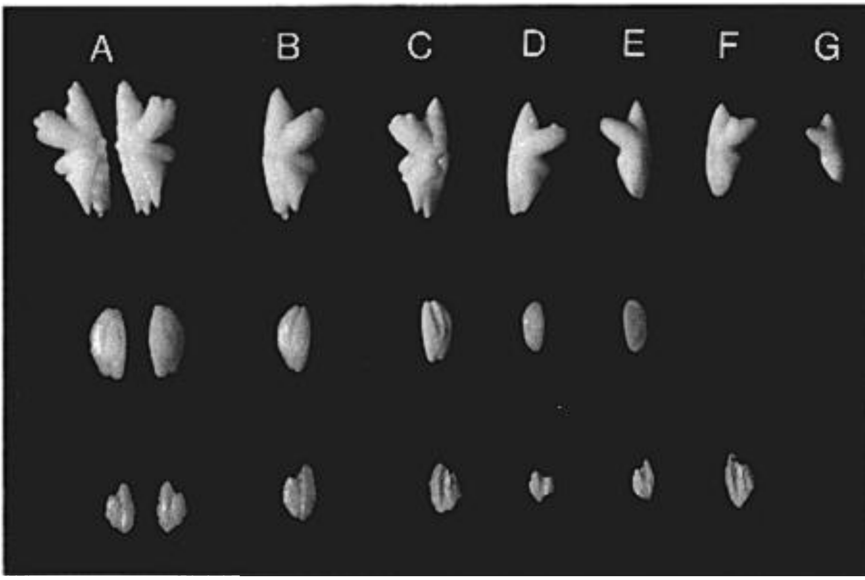

FIGURE 3. Otoliths from red cod (top), opalfish (middle) and sprat (bottom) shown after time intervals in penguins' stomachs. Intact otolith pairs are shown (A), after 2 h ingestion (B), after 6 h ingestion for red cod and opalfish and 4 h for sprat (C), after 12 h for red cod and opalfish and 4 h for sprat (D), after 16 h for red cod, 24 h for opalfish and 6 h for sprat (E), after 24 h for red cod and 6 h for sprat (F), and after 24 h for red cod (G).

22 h the erosion rate in the lab slowed down, suggesting that the otolith centrum is harder or more resistant than the exterior. The absence of any erosion of otoliths in pH 3.5 indicates that such acidity alone is not responsible for erosion.

The absence of any changes in beaks at both 1.5 and 3.5 pH suggests that, while chemical processes probably release the beaks from the flesh, some form of mechanical process must play a significant role in the breakdown of beaks. The extended retention time of beaks without loss of measurable rostral lengths could result in a considerable over-estimation of squid relative to fish in the diet of Yellow-eyed Penguins. Jackson and Ryan (1986) counted cephalopod beaks, noted their state of wear, and counted otoliths and krill eye lenses. They classified beaks into three discrete states of wear, and found a mean 87% of *Loligo* beaks recovered from 7 White-chinned Petrels *Procellaria aequinoctialis* were "fresh" ("wings" intact) after three weeks. Stomach contents of the Shy Albatross *Diomedea cauta* contained no otoliths one day after feeding, but retained beaks for 38–50 d (Furness et al. 1984). In contrast, in the present study all beaks had reached the "intermediate" stage (rostrum sharp but with broken and abraded wings) within 24 h, and the "worn" stage (all surfaces

TABLE 2. Recovery of squid beaks after time intervals in the stomach.

Time interval	No. upper beaks	No. lower beaks	Beak condition & consistency of vomit
2 h	3/5	2/5	Beaks inside buccal mass; large intact pieces of squid.
4 h	12/13	12/13	Beaks inside buccal mass; suckers off tentacles gone.
6 h	9/10	9/10	Beaks inside buccal mass; flesh smooth with no suckers; gray.
8 h[a]	4/4	4/4	Beaks loose but intact; fragments of flesh.
16 h[a]	5/5	4/5	Beaks loose but intact; no flesh, rachis and eyeballs remaining; yellow-green.
24 h	11/12	10/12	Rostral length intact on 7, but wings frayed; remaining 4 shattered and eroded.
36 h	12/12	10/12	Rostral length intact on all but wings frayed and torn; entire beak eroded.
48 h	9/12	12/12	Rostral length intact on 9 uppers, although wings frayed and entire beak eroded; remaining 3 shattered.

[a] One penguin only.

smooth and rounded) in 36 h. The relatively rapid rate of attrition of beaks in Yellow-eyed Penguin stomachs may depend on the presence of small stones or other beaks in the stomach.

Reported rates of erosion of otoliths vary; otoliths in pellets regurgitated by Cape Cormorants (*Phalacrocorax capensis*) were reduced by at least 25% in length after only one day (Duffy and Laurenson 1983), but Lalas (1983) reported little erosion of otoliths from pellets of Spotted (*Phalacrocorax punctatus*), Little (*Halietor melanoleucos*) and Stewart Island shags (*P. chalconotus*). Degree of erosion of otoliths in Cape Cormorants depended on several factors such as varying calcium demands, meal size, fish size and position of otolith in the fish bolus (Duffy and Laurenson 1983). Otolith digestion in seals was relatively fast, with none recovered after 12.9 h (Murie and Lavigne 1986). In the Yellow-eyed Penguin, although small otoliths such as those from sprat were absent 6–8 h after ingestion, larger otoliths such as those from red cod took longer to break down. This tendency for large robust otoliths to resist attack, whereas small fragile otoliths are readily eroded and dissolved was also noted by Jobling & Breiby (1986). At least 100% of those from red cod and 33% from opalfish, but none from sprat were present after 13–14 h, which is a long foraging trip. Therefore, otoliths weighing less than about 10 mg and ingested early in a foraging trip could possibly be digested at the trip's end. Fish species possessing otoliths of this size can sometimes comprise a significant portion of Yellow-eyed Penguin diet: up to 45% by calculated weight at some breeding areas (van Heezik 1988).

It is possible that digestion during a foraging trip is slower than in the experimental trials. Otolith digestion only begins after they are freed from the skull and this process may take longer than merely becoming free of

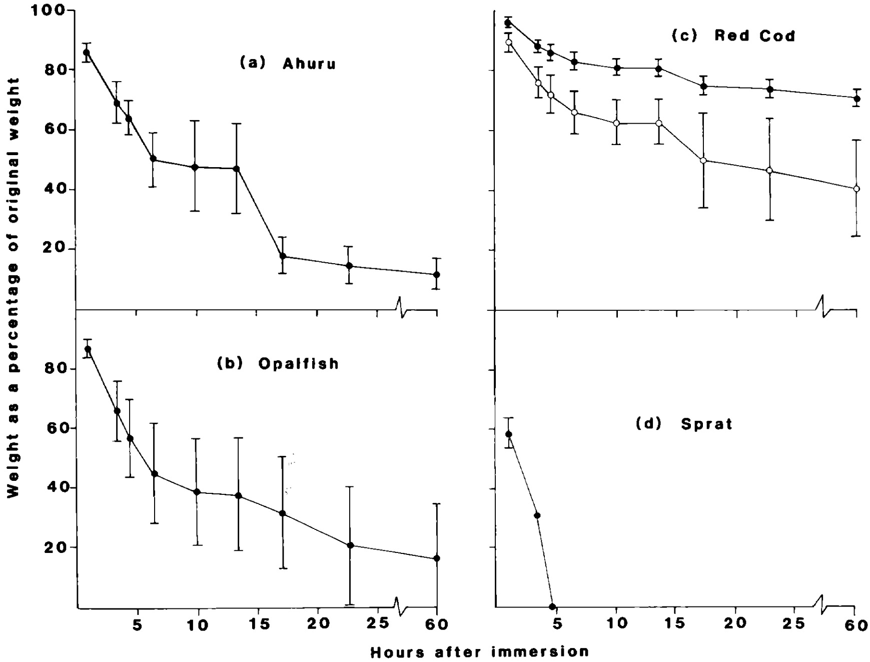

FIGURE 4. Rates of erosion of otoliths placed in HCl at pH = 1.5 from (a) ahuru, (b) opalfish, (c) red cod and (d) sprat. In (c) large otolith pairs (mean = 118.2 ± 19.0 mg/pair), are shown by closed circles and small pairs (mean = 28.2 ± 4.4) by open circles. Bars denote ± 1 SD; $n = 3$ in all cases except sprat at 3.5 h.

the squid mantles. During foraging the stomachs may be fuller than in these trials, in which case erosion of otoliths in the center of the food mass may be slowed (Gales 1987). Taking these factors into account, under-estimation of certain diet species relative to others should be considered when stomach contents comprise more than one fish species with smaller otoliths weighing less than about 10 mg per pair, if foraging trips are long, if the meal mass is not large, if fish species with smaller otoliths are ingested early in the foraging trip, and if squid are consumed as well.

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