Assessment of errors in cetacean diet analysis: *in vitro* digestion of otoliths

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Research into the feeding habits of cetaceans has traditionally relied on examining samples taken from the digestive tracts of commercially caught or stranded and by-caught animals. This depends on the identification and measurement of hard parts such as fish otoliths. Otoliths are often partially digested in the stomach, making quantitative estimates of fish size from otolith size biased.

As a preliminary attempt to quantify the size reduction of otoliths found in the stomachs of small cetaceans, an *in vitro* digestion experiment was carried out. Otoliths of herring, whiting, poor cod and haddock were digested at 37°C, pH=3.6, and measured every 60 min.

Linear regressions relating otolith size to digestion time and original size generally provided an adequate description of the time course of size reduction. The parameter that changed most rapidly due to digestion was otolith thickness. Differences in digestion rates between species reflect the general robustness and shape of the otoliths.

A simulation using actual dietary data showed that the overall diet composition was not radically changed by accounting for otolith digestion, although the apparent contribution of herring increased as the assumed digestion time is increased, because herring otoliths were the most susceptible to digestion.

Research into the feeding ecology of cetaceans has traditionally relied on examining samples taken from the digestive tracts of commercially caught or stranded and by-caught animals (see Pierce & Boyle, 1991, for a review). This approach relies on the presence of remains that can be recognized, in practice mostly fish otoliths and cephalopod beaks, although inclusion of other hard remains (bones) can provide valuable information (Cottrell et al., 1996). Quantitative evaluation of the diet normally involves back-calculation of prey size from measurements made on the otoliths or beaks (Clarke, 1986; Härkönen, 1986). Since otoliths consist primarily of CaCO₃, they are susceptible to digestion in the stomach.

To estimate the effect of otolith digestion in determining seal diets, feeding studies have been conducted with captive animals (Murie 1987; Hammond et al., 1994; Cottrell et al., 1996; Tollit et al., 1997) and with *in vitro* digestion experiments (Jobling & Breiby, 1986; Pierce et al., 1993). Most current dietary studies on seals are based on faecal analysis, therefore captive feeding experiments do not need to be invasive: fish are fed to the seals and otoliths recovered from the faeces. In cetaceans stomach content analysis is still the main approach used in dietary studies, therefore the use of captive feeding experiments to quantify otolith digestion is not a plausible option.

As a preliminary attempt to quantify the reduction in size of otoliths found in the stomachs of small cetaceans, an *in vitro* digestion experiment was carried out. Our particular interest was the diet of harbour porpoises (*Phocoena phocoena* (Linnaeus, 1758)) which, in western Europe is one of the most commonly stranded small cetaceans (Smeenk, 1992). However, the only data available for stomach pH of small cetaceans is an average value of 3.6 for bottlenose dolphin (*Tursiops truncatus*, Montagu 1821) (S.H. Ridgway cited in Smith, 1972). Pierce & Boyle (1991) review previous *in vitro* studies on digestion. Madge (1975) reports that gastric pH in mammals usually ranges between 1.5

and 3.5, and pH values around 2.0 were selected by Hawes (1983) and Pierce et al. (1993) for seals. Pepsin was not used in the present experiments, since in previous studies it was reported to have no apparent digestive effect on otoliths (Pierce et al., 1993).

Otoliths were taken from fish caught during research cruise surveys by the Fisheries Research Services Marine Laboratory, Aberdeen. Fish species frequently found in small cetacean diets (M.B. Santos et al., 1995, unpublished data) were used: herring *Clupea harengus*; haddock *Melanogrammus aeglefinus*; whiting *Merlangius merlangus* and poor cod *Trisopterus minutus* (Linnaeus, 1758).

Measurements were made of otolith length, width, and thickness. The measurements were made using a binocular microscope fitted with an eyepiece graticule. The otoliths were placed in a 37°C hydrochloric acid (HCl) solution buffered with 0.05 M potassium hydrogen phthalate (COOH C₆ H₄ COOK). The pH remained within 0.1 pH-units of the set value of 3.6. At 60 min intervals otoliths were taken out of the acid solution, blotted dry and put into a pH 9.0 solution of sodium hydroxide (NaOH), to stop all further digestion. Otoliths were left in NaOH until measured. Broken otoliths were not measured, because the surface area where digestion could occur had changed.

Using only data from otoliths which survived for at least 10 h, size after digestion time *t* (St, mm) was regressed against original size (OS, mm) and digestion time (DT, min). The fitted regressions were of the form:

$$St = a + b_{OS} \times OS + b_{DT} \times DT \quad (1)$$

where *a*, *b*_{OS} and *b*_{DT} were the fitted regression coefficients. Fitted values were plotted against residuals to check goodness of fit.

Table 1. Sizes, regression constants and coefficients for otolith size (using otoliths that survived for 10+ h only) for the regression equation $St = a + b_{OS} \times OS + b_{DT} \times DT$. All constants and coefficients are significant $P < 0.001$, except those marked ns for non significant.

Species	Otolith parameter	N	average size $\pm$ SE (mm)	r^2	a	b_{OS}	b_{DT}	$b_{DT}/size$
Haddock	length	59	12.6290 $\pm$ 0.3419	0.995	0.0889 ns	1.0085	-0.0934	-0.0074
	width	60	4.6033 $\pm$ 0.1038	0.992	-0.1214	1.0245	-0.0257	-0.0056
	thickness	60	2.3230 $\pm$ 0.0749	0.757	0.7333	0.6648	-0.0203	-0.0087
Herring	length	23	4.7948 $\pm$ 0.0438	0.905	0.0472 ns	0.9956	-0.0487	-0.0102
	width	39	2.1671 $\pm$ 0.0216	0.763	0.2102	0.9042	-0.0318	-0.0147
	thickness	39	0.7202 $\pm$ 0.0125	0.879	0.0453	0.9210	-0.0165	-0.0229
Poor cod	length	40	8.5368 $\pm$ 0.0973	0.976	0.4554	0.9476	-0.0374	-0.0044
	width	46	3.9931 $\pm$ 0.0396	0.932	0.0181 ns	1.0074	-0.0130	-0.0033
	thickness	46	2.5239 $\pm$ 0.0333	0.952	0.0698 ns	0.9737	-0.0130	-0.0051
Whiting	length	41	13.5432 $\pm$ 0.3015	0.995	-0.2422	1.0200	-0.0498	-0.0037
	width	54	4.2445 $\pm$ 0.0626	0.985	0.0262 ns	0.9953	-0.0177	-0.0042
	thickness	54	1.5822 $\pm$ 0.0192	0.954	-0.0206 ns	1.0140	-0.0106	-0.0067

Figure 1. The estimated relative contributions of haddock, herring, poor cod (as *Trisopterus* sp.) and whiting to the diets of harbour porpoises, as a function of digestion time.

To quantify the effect of otolith digestion on estimated diet composition in actual samples, the regressions were applied to otolith length (or width) data from 49 harbour porpoise stomach samples collected in Scotland during 1992–1994 (M.B. Santos et al., unpublished data). The four species that were used in this experiment make up approximately 55% of the annual diet. Another 40% is made up by sandeels, whose otoliths were considered too small for this study. A full description of the diet will be presented elsewhere. The calculated regressions were rearranged to estimate original otolith size from current otolith size and the assumed digestion time. For this calculation dietary data of *Trisopterus* sp. are used for poor cod, and pooled data for haddock, pollack (*Pollachius pollachius*) and saithe (*Pollachius virens*) (Linnaeus, 1758) are used for haddock, because they cannot be identified further in prey remains (personal observations). Fish weights were then estimated from otolith size using previously established regressions (Härkönen, 1986; Coull et al., 1989; J.R.G. Hislop, unpublished data). The relative contribution of the four species to the diet was calculated for digestion times of 0, 1... 12 h.

No otoliths dissolved completely after 12 h, although some broke up and could not be measured further. Linear regressions gave adequate fits to the data in most cases, although relatively low r^2 values were obtained for herring and haddock otolith

thickness (Table 1). The regression coefficients for original otolith size were generally close to 1.0; i.e. for a given digestion time, digested size was directly proportional to original size. However, the majority of regression intercepts were significantly different from zero, which probably indicates that the relationships were initially non-linear.

Dividing the regression coefficient for digestion time by the average otolith size provides a crude index of the relative rate at which the different otolith dimensions were reduced. It can then be seen that thickness decreased more rapidly than length or width in all four species, and that herring otoliths were more rapidly digested than those of the gadid species.

Results of the simulation (Figure 1) indicate that: (a) the absolute contribution by weight for the four species combined increases by more than 25% if a 12 h digestion period is assumed. [This does not imply that their importance as a proportion of the overall diet would increase, since the weight attributable to other species would presumably also increase.] (b) Regardless of the assumed digestion time, whiting is clearly more important than the other three species. However, the apparent contribution of herring increases for longer digestion times.

The assumption that otolith digestion was essentially linear is at best an approximation and may not apply to otoliths of all species. The different rates of *in vitro* otolith digestion, between measurements and between species, were consistent with expected differences in the general robustness and shape of the otoliths. Fragile, thin otoliths such as those of herring are easily digested, while bulkier otoliths of gadids are less affected. This is supported by findings from previous studies on seals (Cottrell et al., 1996; Tollit et al., 1997) and *in vitro* work (Jobling & Breiby, 1986). Thinner extremities of otoliths may digest more quickly than the bulk of the otolith. Thus the relatively large decrease in whiting otolith length reported by Tollit et al. (1997), in a feeding trial using captive seals, could be due to breaking of the fragile tip and the subsequent erosion of the break point.

The assumptions made here about the conditions inside the stomach may not be completely realistic. The solutions used might not reflect natural conditions. Apart from the paucity of good data on gastric pH in the literature, it is most unlikely that pH remains constant in the gastric fluid. The rate of digestion will also depend on stomach temperature which is likely to be lowered when fish (and seawater) are ingested and will then presumably gradually return to body temperature. The effect of

stomach rugae, that can trap hard parts for extended periods, increasing the time of digestion is also difficult to account for (Bigg & Fawcett, 1985). During the initial stages of digestion, otoliths are likely to be protected from digestion since they are inside the head of the fish, although digestion can sometimes occur in the skull (Mead & Potter, 1990).

Before correction factors for otolith degradation can be applied, the residence time of otoliths in the stomach must be known. Behavioural observations in Canada suggest one or two daily feeding periods (Read & Gaskin, 1985) which could imply that food typically takes no more than 12 h to pass beyond the stomach. However, there is no reason why all sampled animals should have died at a particular stage of the digestive cycle. Also, stranded animals, the main source of data, have often been dead for an unknown period of time. During that time the otoliths may have been subjected to digestion by acid remaining in the stomach. Although our 12 h sampling regime seems adequate to cover the normal otolith digestion period in the stomach of a harbour porpoise, it might be insufficient to cover otolith digestion in stranded animals.

There are several possible ways of quantifying the time since ingestion of a meal. Murie (1987), working on seal stomach contents, used the proportion of fish otoliths found inside skulls as an index of residence time in the stomach. However, very few complete fish-heads were seen inside the porpoise stomachs sampled (personal observations). The use of otolith digestion-classes based on surface structure (e.g. Tollit et al., 1997) carries the risk of subjectivity, resulting in differences between workers. Use of a ratio between sulcus length and otolith length might be possible, since the sulcus is relatively resistant to digestion and is usually located in the centre of the otolith.

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