In vitro digestibility of some prey species of dolphins

Keiko Sekiguchi*
Peter B. Best
Mammal Research Institute
University of Pretoria, Pretoria 0002, South Africa
Mailing address: Division of Natural Science, International Christian University
3-10-2 Osawa, Mitaka-City, Tokyo 181, Japan
*E-mail address: keikos@icu.ac.jp

Studies of dolphin (Cetacea, Odontoceti) food habits are conducted by examining stomach contents because it is difficult to observe feeding behavior directly. It is rare, however, to find prey items intact in stomachs; often only fragments of muscle and some hard parts remain. Identification of prey species and estimation of their original size are usually carried out with trace remains, such as cephalopod beaks (Clarke, 1980) and fish otoliths (Fitch and Brownell, 1968), because of their species-specific shapes and allometric relationships with body size (Clarke, 1962; Jobling and Breiby, 1986).

There are several problems with using cephalopod beaks and fish otoliths in dietary studies. Otoliths are composed of calcium carbonate and can be eroded by stomach acids (McMahon and Tash, 1979; da Silva and Neilson, 1985; Murie and Lavigne, 1985, 1986; Jobling and Breiby, 1986; Harvey, 1989). Reduction in otolith size depends on the length of time they are exposed to stomach acids. Because otoliths are located inside the skull, the length of time they are exposed to acids may differ depending on the overall digestibility of the fish species concerned. Some species are identifiable even after their otoliths have been eroded and reduced in size. For such species, it may be difficult to tell if the otolith is of a reduced or original size (McMahon and Tash, 1979). Because estimation of fish prey size is usually based on a regression between otolith size and the weight or length of the prey, any reduction in otolith size that is not detected may cause prey size to be underestimated.

The use of cephalopod beaks may create different problems. Although Bigg and Fawcett (1985) reported that soft-bodied squids (*Loligo opalescens*) decreased in weight faster than herring (*Clupea harengus pallasi*) in an artificial digestion solution, cephalopod beaks were not dissolved by gastric acids. Cephalopod beaks may, therefore, accumulate in cetacean stomachs. It has been observed that some marine mammals occasionally regurgitate squid beaks (Clarke, 1980; Pitcher, 1980). Cephalopod beaks present in a stomach may, consequently, represent the remains of more than one meal and thus may result in overestimations of the proportion of squid to fish in the predator's diet.

Bigg and Perez (1985) introduced the "modified volume" method to avoid the problem of the accumulation of cephalopod beaks. This method uses the frequency of occurrence of nontrace remains to calculate the ratio between cephalopods and fish in a meal. However, if all prey remnants come from the same meal, any difference in digestibility between prey items will affect the relative frequency of occurrence of nontrace remains when the stomach is examined. As an extreme case, prey items that are digested very rapidly would not be represented by "nontrace remains" in the stomach soon after feeding.

Differentials in digestion rates between Loligo squid and herring in an artificial digestion solution, as demonstrated by Bigg and Fawcett (1985), may apply to other prey species. For example, Jackson et al. (1987) could not detect differences in the rates that fish and squid were completely digested in vitro but noted that exoskeletons of intact crustaceans resisted digestion. Thus, it is possible that digestion rates for each prey species, or prey type, could be used as "correction factors" in dietary analysis.

The present study investigates the differences in digestion rates of major prey species of dolphins in artificial digestion solutions. In addition, digestion rates of different sizes of the same prey species are considered. Digestion rates are then calculated to establish the basis for a revised method of dietary analysis.

Materials and methods

The following fish and squid species were used in a set of six experiments: 1) 5 lanternfishes (Myctophidae), 5 large and 5 small Cape anchovies (*Engraulis capensis*, Engraulidae); 2) 5 large and 5 small round herrings (*Etrumeus whiteheadi*, Clupeidae); 3) 5 large and 5 small pilchards (*Sardinops sagax*, Clupeidae); 4) 5 hakes (*Merluccius* sp., Merlucciidae) and 5 chokka squids (*Loligo vulgaris reynaudii*, Loliginidae); 5) 5 maasbankers (horse mackerel) (*Trachurus trachurus capensis*, Carangidae) and

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5 red squids (*Todaropsis eblanae*, Ommastrephidae); and 6) 5 pelagic gobies (*Sufflogobius bibarbatus*, Gobiidae) and 5 lanternfishes. These taxa are commonly found in stomachs of dolphins (including common dolphins, *Delphinus delphis*, dusky dolphins, *Lagenorhynchus obscurus*, and Heaviside's dolphins, *Cephalorhynchus heavisidii*) along the west coast of southern Africa (Sekiguchi et al., 1992). Table 1 shows the sizes of sample species used: all were collected in trawls by the RV *Africana*, November 1987 or January 1988, and frozen at –20°C.

For the first experiment, the procedure followed that of Jackson et al. (1987). Four liters of a digestion solution of 0.15% HCl, 0.05% Na_2CO_3 (buffer) and 1.0% pepsin (pepsin A powder, BDH Chemicals Ltd.) were adjusted to an initial pH of 2.30, near the midpoint of the range of that recorded for cetacean stomachs (pH=1.4 to 3.0, Ishihara, 1960; pH=1.8 to 3.0, Smith, 1972; pH=1.5 to 3.5, Jobling and Breiby, 1986). A Beckman expanded scale pH meter was used to monitor pH. The solution was then divided into 240-mL portions in each of seven 600-mL beakers, and 1,150 mL portions in each of two 5-L beakers.

The beakers were placed in two water baths continuously agitated (rocked) 20–30 times per minute at 38°C. Each fish was put in a small fiber glass bag (mesh size 0.5 × 0.5 mm) and then suspended in the solution. Four samples were placed in each of the 5-L beakers and a single sample in each of the 600-mL beakers. The pH for each beaker was maintained between 1.90 and 3.37; pH increased with time and was adjusted by adding HCl.

Owing to the effort required to maintain pH in individual beakers, one large PVC container (40 × 28 × 20 cm) made specifically to fit in the water bath was used in subsequent experiments. Ten liters of digestion solution, consisting of 0.50–0.56% HCl, 0.27–0.29% Na_2CO_3, and 1.0% pepsin, were maintained at 36.0 to 39.1°C in the PVC container. A Beckman expanded-scale pH meter was placed in the corner of the container to monitor pH constantly. The pH was maintained between 2.25 and 2.51 by occasional

Table 1

The species used in the artificial digestion experiments; their total length (TL, cm) or dorsal mantle length (DML, cm) and corresponding weight (WT, g). (L=large size and S=small size groups.)

Sample species		Length (cm) and weight (g)				
Cape anchovy (L)	TL	13.6	12.7	12.7	12.8	11.7
	WT	19.48	17.28	21.25	18.09	16.45
Cape anchovy (S)	TL	9.9	9.6	9.6	9.4	9.6
	WT	8.25	8.05	8.01	7.81	7.56
Round herring (L)	TL	19.5	18.7	19.5	21.0	19.8
	WT	68.95	63.49	79.03	96.27	79.47
Round herring (S)	TL	14.4	14.8	14.8	14.8	15.2
	WT	28.28	33.47	34.88	36.77	34.69
Pilchard (L)	TL	20.8	20.0	20.8	20.0	20.3
	WT	108.70	104.55	105.27	103.11	103.72
Pilchard (S)	TL	13.7	13.0	14.0	14.1	13.5
	WT	31.0	27.88	30.59	30.89	30.65
Hake	TL	17.0	17.4	17.3	17.5	16.5
	WT	46.1	51.2	48.4	52.2	41.5
Maasbanker	TL	18.8	19.4	18.9	19.1	16.5
	WT	79.5	83.3	80.2	80.4	53.6
Goby	TL	8.7	7.8	8.2	8.5	8.6
	WT	10.2	7.1	8.6	8.9	10.0
Lanternfish	TL	5.3	5.1	3.5	3.9	4.5
		4.4	4.4	4.2	4.1	4.6
	WT	1.85	1.64	0.61	0.93	1.16
		1.1	1.1	0.8	1.0	1.0
Chokka squid	DML	16.0	18.5	16.5	15.8	15.8
	WT	118.3	152.9	120.7	104.4	98.8
Red squid	DML	10.4	9.6	10.7	9.9	9.2
	WT	60.6	55.8	61.2	49.1	40.8

addition of 10% HCl (45 to 655 mL per experiment in total). The water bath rocked the container about 40 times per minute. As in the first experiment, each sample item was placed in a small mesh bag and suspended in the digestion solution.

Every hour, each bag was lifted from the container, all excess liquid was wiped off with a paper towel, and the bag with sample weighed to the nearest 0.1 g. The physical appearance of each sample was also recorded. Weighings were made at 1-h intervals until the sample mass (i.e. measured weight minus weight of the empty mesh bag) had decreased to 5–10% of its original mass.

To compare digestion rates between each species, the mean time to reach 20% of original weight (T_{20}) was calculated for each sample species. This percentage was chosen because the rate of decline in mass decreased when the sample reached this point. This decrease probably resulted from inaccuracies in weighing smaller masses as well as from the accumulation of less digestible remains. The T_{20} values for different size groups of the same prey species were compared first with a *t*-test. Then, one-way ANOVA and the Newman-Keuls test were applied to compare all sample species (Zar, 1974). A digestion rate ratio was calculated from the T_{20} values for each species, expressed as a proportion of that for lanternfish.

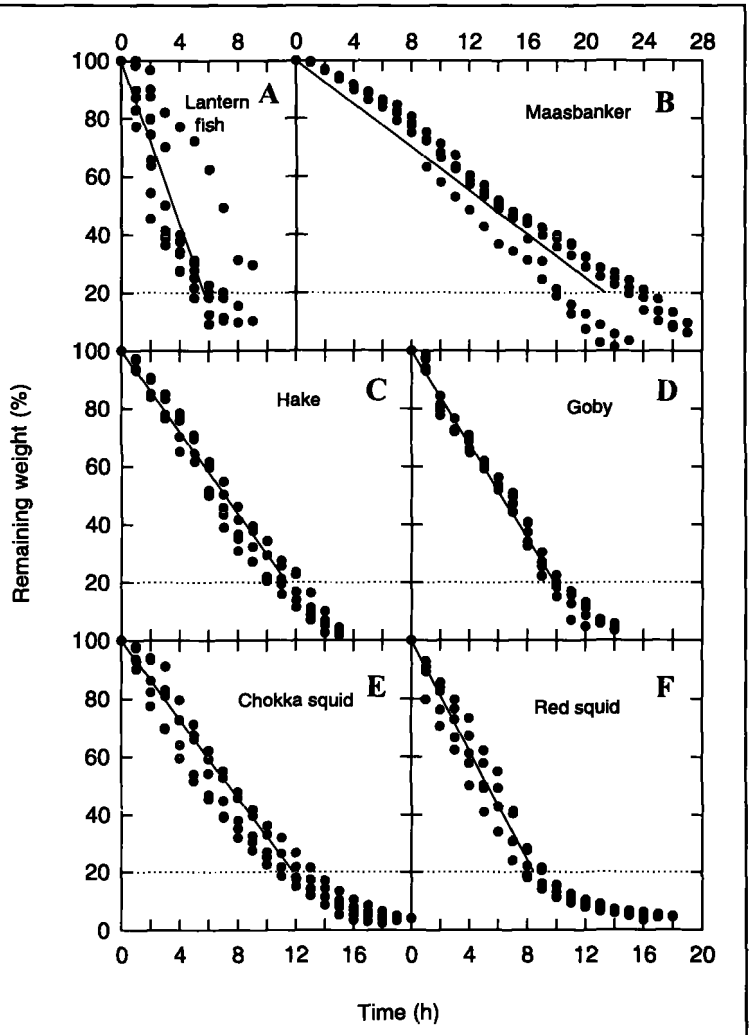

Figure 1

The rate of digestion of fish and squid species in an artificial digestion solution, expressed as the percentage of their original weight remaining at hourly intervals. The slope shown is the digestion rate to the mean time to 20% of the initial weights (T_{20}). (**A**) 10 lanternfish (Myctophidae sp.), (**B**) 5 maasbanker (*Trachurus t. capensis*), (**C**) 5 hake (*Merluccius* sp.), (**D**) 5 pelagic goby (*Sufflogobius bibarbatus*), (**E**) 5 chokka squids (*Loligo v. reynaudii*), and (**F**) 5 red squids (*Todaropsis eblanae*).

Results

Samples were digested almost completely in the pepsin solution. Although digestion rates were quite different among species, the sequences of digestion of particular tissues were similar among species (Table 2). Although the head of a fish usually disintegrated when about half the body had been digested, otoliths were not always visible through the mesh bag at this stage. In the case of hake and maasbanker, the dorsal surface of the head began to be digested at an earlier stage (15% digested at 2–3 h for hake, 5–6 h for maasbanker) than that found for other fish species. Otoliths became visible (through the mesh bag) at 5–8 h for hake and at 19 h for maasbanker. Hake otoliths fell through the mesh at 9–13 h. Most otoliths were dissolved completely when the experiments with hake terminated at 20 h (except one otolith), and at 27 h (except for five otoliths) with maasbanker. Some otoliths of reduced size were recovered in experiments involving other species (i.e. 16 from anchovy, 8 from herring, and 10 from goby). All squid beaks recovered at the termination of the experiments showed no obvious signs of having been digested.

All samples decreased in weight over time (h), each species having different rates of digestion (Figs. 1 and 2). Lanternfish were digested very quickly, and were almost completely gone within 9 hours. Hake

Table 2

The generalized sequence of digestion for fish and squid in the artificial digestion experiments.

Weight remaining (%)	Squid	Fish
95–85	Begins to lose skin and viscera	Abdomen breaks up; begins to lose skin and viscera
85–60	Loses fins; muscle reduced	Most of skin and viscera are gone; loses eyes and tail; head begins to be digested
60–40	Tentacles featureless; mantle splits, exposing the pen	Head is gone; muscle reduced; releases otoliths
40–30	Flesh reduced further	Muscle disintegrates; backbone exposed
30–10	Beaks, eyes, and pen released	Muscle reduced further
<10	Beaks, eyes, part of pen, and a little flesh remain	Pieces of muscle and skin, and some vertebrae remain

and goby were also digested quickly and reduced to less than 10% of their original weight within 15 hours. Most species, however, took longer for complete digestion (about 20 h); maasbanker took as long as 27 hours to be reduced to less than 10% of its original weight.

Table 3 lists the T_{20} values for each sample species. The T_{20} values varied between species sampled (from 5.68 h to 21.35 h), but for most species, the T_{20} value was roughly 13 h. Among the 12 species and size groups sampled, maasbanker had the slowest rate of digestion and lanternfish the highest, being digested about 3.8 times faster than maasbanker. Red squid was digested faster than most fish species except lanternfish, whereas the digestion rate of chokka squid was slower than that of large round herring, hake, goby, red squid, and lanternfish.

There appeared to be differences between digestion rates of different sizes of the same species (Table 3). Smaller anchovy and pilchard were digested about 1.2 times faster than larger ones, but round herrings showed the opposite pattern. However, for anchovy and round herring, the T_{20} values for large and small fish were not significantly different (t=1.65, df=8, P=0.1381, for large fish; t=1.05, df=8, P=0.3256, for small fish). The two size groups of pilchard had significantly different T_{20} values

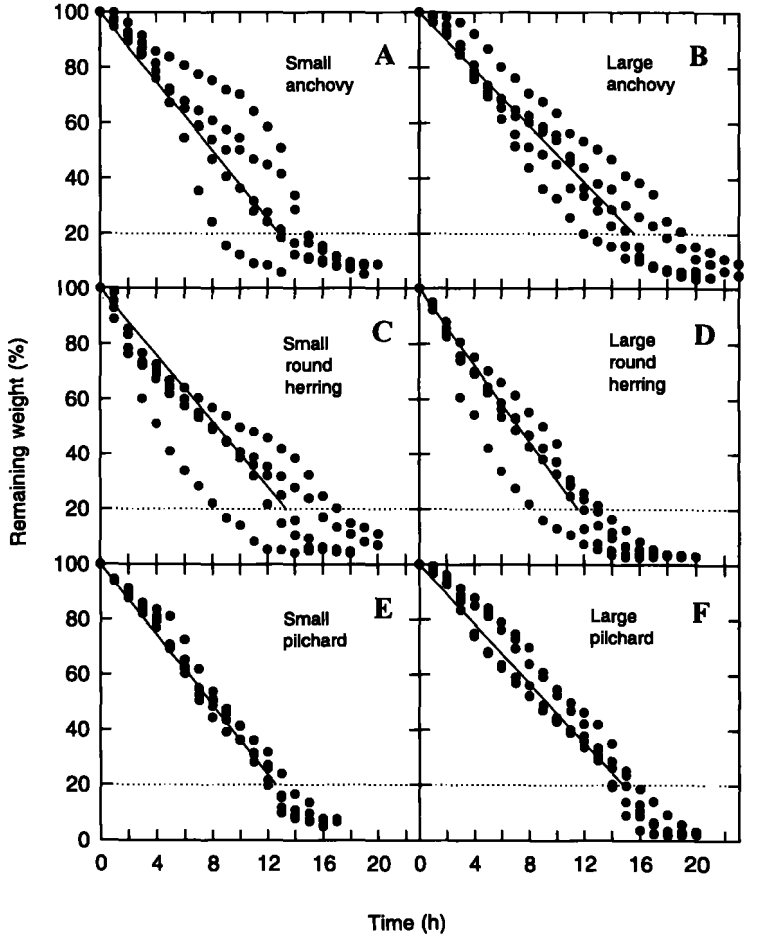

Figure 2

The rate of digestion of two different size groups of fish species in an artificial digestion solution, expressed as the percentage of their original weight remaining at hourly intervals. The slope shown is the digestion rate to the mean time to 20% of the initial weights (T_{20}). (**A**) 5 small and (**B**) 5 large specimens of anchovy (*Engraulis capensis*), (**C**) 5 small and (**D**) 5 large round herring (*Etrumeus whiteheadi*), (**E**) 5 small and (**F**) 5 large pilchard (*Sardinops sagax*).

Table 3

The list of calculated mean times and standard deviations for each species in the artificial digestion experiments when remaining weights reach 20% of the original weight (T_{20}). The digestion rate ratio shows the T_{20} value for each species in relation to that of lantern fish. (L=large size and S=small size groups—see Table 1).

Sample species		*n*	Time (h) to reach 20% of original wt. (T_{20})		Digestion rate ratio
			Mean	SD	
Maasbanker	*Trachurus t. capensis*	5	21.35	3.09	3.76
Cape anchovy (L)[1]	*Engraulis capensis*	5	15.67	2.81	2.76
Pilchard (L)	*Sardinops sagax*	5	14.82	0.80	2.61
Round herring (S)[2]	*Etrumeus whiteheadi*	5	13.35	3.33	2.35
Cape anchovy (S)[1]	*Engraulis capensis*	5	12.85	2.60	2.26
Pilchard (S)	*Sardinops sagax*	5	12.57	0.62	2.21
Chokka squid	*Loligo v. reynaudii*	5	11.82	1.04	2.08
Round herring (L)[2]	*Etrumeus whiteheadi*	5	11.54	1.96	2.03
Hake	*Merluccius* sp.	5	11.36	0.98	2.00
Goby	*Sufflogobius bibarbatus*	5	9.88	0.39	1.74
Red squid	*Todaropsis eblanae*	5	8.44	0.70	1.49
Lanternfish	Myctophidae	8	5.68	0.66	1.00

[1] T_{20} for large and small anchovy = 14.26 ± 2.95 h.
[2] T_{20} for large and small round herring = 12.45 ± 2.75 h.

(t=5.02, df=8, P=0.001). Because there was no significant difference between the two size groups of anchovy and round herring, data were combined for one-way ANOVA on all sample species.

The T_{20} values for 10 sample groups (maasbanker, large and small pilchard, anchovy, round herring, hake, goby, lanternfish, chokka squid, and red squid) showed a significant difference (one-way ANOVA, F=27.3, total df=62, P<0.0001). The Newman-Keuls test indicated maasbanker, goby, red squid, and lanternfish had different T_{20} values from other species (P<0.05).

Discussion

Compared with previous digestion experiments, complete digestion of samples took longer than expected (Figs. 1 and 2). Bigg and Fawcett (1985) reported that whole herring and squid were digested within 10 h in an artificial solution of 1% HCl and 1% pepsin. Jackson et al. (1987) found that about 10–15 h were required to digest whole anchovies in vitro (pH=1.25–1.35). These time differences are probably the result of differences in acidity of the digestion solutions. In the present experiments, the solutions had a pH of ~2.3. The pH of the solution used by Bigg and Fawcett (1985) can be calculated as about 1.1. Therefore, their solution was far more acidic than ours, resulting in more rapid digestion of fish and squid tissues.

As noted, there was a general tendency for the digestion rate to decline when the remaining weight was less than 20% of the original weight. This was more pronounced for cephalopods than fish (Figs. 1 and 2). Bigg and Fawcett (1985, Fig. 16.1) reported similar trends: declines in rates of digestion can be caused by the accumulation of less digestible material, i.e. squid beaks and pens (Table 2; also Table 16.3 in Bigg and Fawcett, 1985).

Although their procedure was different from that used in the present study, the digestion experiment of Nordøy et al. (1993) for herring (*Clupea harengus*) also showed a rapid decline in digestion rate after about 70% of "dry matter disappearance" (DMD), and stated that the maximum DMD of herring is about 80%. The digestion rate decline at 80% in the present study may also be related to the digestibility of prey species of dolphins, or cetaceans in general. Undigested prey remains may be voided via gastric evacuation or, possibly, by regurgitation, as proposed for squid beaks (Clarke, 1980; Pitcher, 1980).

The validity of in vitro experiments in representing in vivo situations remains a matter of debate, but technical and other considerations make in vivo digestion experiments with dolphins impractical at this stage. Although not engaging strictly in a digestion experiment, Kastelein et al. (1993) fed captive Commerson's dolphins (*Cephalorhynchus commersonii*) on North Atlantic herring (*Clupea harengus*) and Columbia river smelt (*Thalechthys pacificus*),

into which gelatine capsules containing red dye were inserted. They found that only 40 to 155 minutes elapsed before dye appeared in feces, but it is not clear how this relates to the full digestion times of the fish. In vivo experiments with pinnipeds (another marine mammal feeding largely on cephalopods and fish) suggest somewhat faster digestion rates than those in our study. Murie and Lavigne (1985) found no fish hard parts remaining in seal stomachs 18 hours after feeding. However, stomachs could have been voided by regurgitation and gastric evacuation, whereas "hard parts" in our experiments could escape from the digestion bags only if they were reduced to less than mesh size. Thus, their results are not necessarily inconsistent with those of the present study, although mechanical break-down actions of stomachs are likely to produce faster digestion in vivo.

The in vitro digestion speeds recorded in the present study differed between species (Table 3), but there was no consistent correlation with the taxonomic position of the prey. Three fish species in the order Clupeiformes (round herring, pilchard, and anchovy) had digestion-rate ratios in the range 2.03–2.76, although large and small size groups of pilchard had significantly different T_{20} values. However, maasbanker and goby, both in the order Perciformes, showed very different digestion-rate ratios (3.76 and 1.74). While both squid species were digested faster than most fish species, chokka squid was digested more slowly than large round herring, hake, goby, and lanternfish. Bigg and Fawcett (1985) found that the squid *Loligo opalescens* was digested much faster than herring (*Clupea harengus pallasi*), both in vitro and in vivo (i.e. in a seal stomach). On the other hand, Jackson et al. (1987) found no difference in the digestion rate between fish (hake and anchovy) and squid (*Loligo*) in vitro. LeBrasseur and Stephens (1965) reported that fish (salmonids, myctophids, and hexagrammids) were digested faster than squid (gonatids) in their pepsin-hydrochloric acid solution (0.2 g pepsin/1 L, 1.5% HCl, pH 1.8). These in vitro differences quite possibly are the result of variations in the acidity of the solutions used and differences in experimental procedures.

It is possible that digestion rates are related to muscle structure. Because pepsin is an enzyme that dissolves protein, the protein composition of a body will have an effect on digestion rate. Greer-Walker and Pull (1975) found that active pelagic fish had higher proportions of red muscle than coastal or deep-sea fish species. They reported that the mean red muscle proportion was 19.8% for Clupeidae, 18.3% for Carangidae, 4.5% for Gobiidae, and 4.5% and 0.6% for the deep-sea fish families Macrouridae and Chimaeridae, respectively. The digestion rates of fish prey found in the present study (Table 3) appear to fit a pattern in which the prey species digested most slowly tend to have the highest proportions of red muscle. Red muscle, containing greater quantities of mitochondria, myoglobin, fats, and glycogen than white muscle, may have stronger resistance to pepsin in the digestion process.

Fish otoliths recovered in the present study were reduced in size, and most hake and maasbanker otoliths completely dissolved within 8–12 h after exposure. McMahon and Tash (1979) reported that otoliths in a 0.01 N HCl solution (pH=2.0–2.5) at 25°C were dissolved completely in 24 h, and a herring otolith in a pH 1.09 to 3.09 solution disappeared in 7 h (Jobling and Breiby, 1986). However, the erosion rate of otoliths of different species in acid varies (Jobling and Breiby, 1986), possibly depending on the ratio of surface area to volume (da Silva and Neilson, 1985). On the other hand, using otoliths recovered from fecal samples of captive harbor seals (*Phoca vitulina*), Harvey (1989) found no significant relation between the robustness (length/weight) of the otolith and the degree to which the resultant estimate of fish length was reduced. In seal stomachs, all otoliths were released from herring skulls within 6 h and no otoliths were found 12 h after feeding (Murie and Lavigne, 1986; Murie, 1987). In the present experiments, only fragile, somewhat eroded otoliths were recovered after about 20 h of digestion in vitro. Consequently, it would be likely that any intact otoliths that are found in dolphin stomachs are from recently ingested fish.

Walker et al. (1986) reported the recovery of anchovy (*Engraulis mordax*) otoliths from the stomach of a Pacific white-sided dolphin (*Lagenorhynchus obliquidens*) that had been held in captivity for 8 days without being fed anchovy; this finding suggested the possibility that otoliths can be retained over a period of one week. In the present experiments, a total of 16 anchovy otoliths (80%) were recovered after 20 h; these otoliths were too eroded, however, to estimate original sizes. Because the forestomach of a dolphin contains no glands, gastric juice must be refluxed from the main stomach (Harrison et al., 1970), so that the retention of otoliths for as long as 8 days should be viewed as exceptional.

The digestion sequences were similar for all experimental species (Table 2). Because otoliths are located inside a fish skull, their size reduction depends on when they are initially exposed to stomach acids. In most cases, heads of fish had disintegrated when about 40–60% of the body had been digested (Table 2), usually some 4 to 15 h after digestion began (Figs. 1 and 2), when most otoliths were probably exposed to the acids and began to erode. Harvey

(1989) found that lengths of prey estimated from the sizes of otoliths in seal feces were underestimated by an average of 27.5%. Although the erosion rate of otoliths may be different for each species (Jobling and Breiby, 1986), it should be possible to apply correction factors to avoid underestimating fish size. The stage of digestion of fish prey in a stomach, for instance, could be used as an index to suggest how much time has passed since feeding.

A significant difference in T_{20} values for different size groups of a particular prey species was only found in pilchard. Smaller anchovy were digested about 1.2 times faster than larger ones. On the other hand, larger round herring were digested about 1.2 times faster than smaller ones (Table 3). These differences were not significant, however, although there was more variation among samples for anchovy and round herring than for pilchard (Fig. 2). Larger sample sizes may be required to test for differences in digestion rates between different-size individuals of a prey species.

Although it has not been possible to calibrate these in vitro experiments with in vivo information, this paper indicates interspecific differences in relative digestion rates for several prey items taken by dolphins. It should, therefore, be possible to apply "correction factors" to estimate the original amount of particular prey consumed when prey of different digestibility occur together in a stomach. However, the wider application of such a method would require the examination of digestion rates for additional prey species.

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