

## Size distribution of the hard remains of prey in the digestive tract of northern fur seal (*Callorhinus ursinus*) and related biases in diet estimation by scat analysis

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**Abstract.** Distributions of fish otoliths and squid beaks in the stomach, small intestine, and large intestine of northern fur seal (*Callorhinus ursinus*) were examined to assess their relevance to biases in diet estimation by scat analysis. The contents of the digestive tracts of 51 seals collected in the western North Pacific off northern Japan were inspected. The large intestines contained more fish otoliths and squid beaks than either the stomachs or the small intestines. The prey composition estimated from hard parts in the small intestines was similar to the large intestines, but there was a greater dominance of squid in the stomachs. Squid beaks found in the digestive tracts ranged from 2.26–22.20 mm in wing length, although large beaks ( $\geq 10$  mm) were found only in the stomachs. In addition, there were significant differences in the sizes of fish otoliths found in the stomachs and the large intestines. The difference of the prey composition and the size may have resulted from the limited passage of large particles at the pyloric end of the stomach. In order to improve the accuracy of scat analysis, we must investigate to restrict passing the large particles.

**Key words:** beak size, northern fur seal, otolith size, passage restriction, scat analysis.

Marine mammals are major predators in marine ecosystems; therefore knowledge of their diets is essential for understanding marine food webs. Early diet studies were generally based on stomach content analysis (Pierce and Boyle 1991). This method allows prey species to be identified, and the quantity and size of prey to be estimated, using undigested hard remains, however, fresh prey remains must be collected from the stomach within a few hours of being eaten, before they pass further along the digestive tract (Pierce and Boyle 1991). Furthermore, animals must be sacrificed in order to collect stomach samples.

Since pinnipeds sometimes haul out on land or ice, their diet can also be studied from faeces collected at landing sites. Scat analysis is a non-lethal method for estimating diet composition using the undigested hard parts of prey. This method has been applied to a wide range of pinniped species (e.g. Daneri and Carlini 1999;

Dellinger and Trillmich 1999; Bowen 2000; Browne et al. 2002), including northern fur seals (*Callorhinus ursinus*) (e.g. Antonelis 1996; Sinclair et al. 1996; Antonelis et al. 1997; Kiyota et al. 1999). Scat analysis, however, leads to predictable bias in the quantitative estimation of diet composition (Jobling and Breiby 1986; Jobling 1987). These authors have attributed these biases to differing rates of erosion of hard prey parts related to variation in their shape and size. Feeding experiments with captive animals on controlled diets have highlighted errors in the results of scat analysis when applied to harbour seals (*Phoca vitulina*) (Tollit et al. 1997), California sea lions (*Zalophus californianus*) (Orr and Harvey 2001) and Antarctic fur seals (*Arctocephalus gazella*) (Staniland 2002). These experiments have indicated that the rate of recovery of hard prey remains in faeces is influenced by rates of erosion and passage through the digestive tract. As a consequence, the distribution of hard remains may

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vary in different regions of the digestive tract. Although Pitcher (1980) compared evidence of prey occurrence in the stomach contents and faeces of harbour seal, differences among digestive organs have not been directly investigated in pinnipeds.

In the present study, the distributions of fish otoliths and squid beaks were used as major prey identification keys in the stomach, small and large intestines of the northern fur seal. Differences in prey composition were analyzed in order to determine their relevance to diet estimation.

## Materials and methods

The 51 northern fur seals (9 juvenile males, 14 juvenile females, and 28 adult females) used for this study were collected during daylight, off the Pacific coast of northern Japan (36.9–38.2°N; 141.4–143.3°E), as part of ongoing research monitoring reproductive condition in this species. Twenty one animals were collected during the period 23–28 April 1997 and a further 30 were collected during the period 16–22 April 1998.

The fur seals were dissected onboard the research vessels, and their stomachs, small, and large intestines were removed and frozen at  $-20^{\circ}\text{C}$ . These organs were later thawed in the laboratory, and weighed. Each organ was incised, and its contents were flushed out using running water. The wet weights of the contents were calculated by subtracting the weight of each emptied organ from its original weight. The contents of the digestive organs were gently washed through a series of three sieves (2.0 mm, 1.0 mm, and 0.5 mm). All particles remaining on the sieves were collected and preserved in 70% ethanol. Fish otoliths and squid beaks were removed and counted under a binocular microscope. Partly-digested and undigested prey were collected separately, and their hard body parts were removed and counted.

Sagittal otoliths were identified, and left and right otoliths were distinguished wherever possible. Fish species identification was based upon otolith morphology, following Ohizumi et al. (2001). Fish numbers were estimated using the maximum count of either left or right otoliths, plus half of the total number of otoliths of undetermined orientation. Otoliths found in the cranial bones of partly-digested or undigested prey were included in the estimates. Squid species were identified, and individuals counted using the lower beaks (Clarke 1986). Beaks found in the buccal mass of partly-digested or undigested prey were included in the estimates. The

occurrence, number, and percentage of prey species were determined separately for the stomach, small, and large intestines.

Otolith length (OL) was measured as the longest distance from the anterior rostrum to the posterior edge, parallel to the sulcus. The wing lengths of both the lower (LWL) and upper beaks of squid were measured, but otoliths in the cranial bones and beaks in the buccal mass were not measured.

The diameter of the pyloric end of the stomach of the northern fur seal was 10.5 mm measured in a female with body weight of 37.8 kg. Therefore, the otoliths were classified into two groups (small OL < 5 mm, or large OL  $\geq$  5 mm) and the beaks were classified into three groups (small LWL < 5 mm, middle  $5\text{ mm} \leq$  LWL < 10 mm, or large LWL  $\geq$  10 mm), separately for the stomach, small and large intestines. The size distributions of otoliths and beaks were compared between digestive organs and years using ANOVA and Dunnett's and Tukey's multiple comparison. All statistical analyses were performed using SPSS package (SPSS, Inc., Release 10.0 J 1999).

## Results

### *Contents of digestive tracts*

The weights of the stomach contents were not parametrically distributed, therefore medians were calculated. The median wet weights ( $\pm$  quartile deviation) of the contents were  $23.9 \pm 32.4$  g for the stomach,  $124.1 \pm 43.8$  g for the small intestine, and  $59.0 \pm 36.0$  g for the large intestine. Eleven otoliths in cranial bones and four beaks in buccal mass were found in stomachs in 1998. Relatively few otoliths and beaks ( $n = 58$  in 1997;  $n = 828$  in 1998), were discovered in the small intestine although it was the heaviest organ. Despite its relatively light weight, the large intestine contained by far the highest number of otoliths and beaks ( $n = 4,126$  in 1997;  $n = 4,267$  in 1998).

### *Prey composition*

Fifteen species of fish were identified from the digestive tract contents, nine of which were present in the stomach, eight in the small intestine, and 14 in the large intestine. Eight squid species were identified, all eight of which were present in the stomach, two were identified in the small intestine, and five in the large intestine. Remains of the rough lanternfish (*Myctophum asperum*) and of a Chiroteuthidae sp. squid were identified in each

**Table 1.** The relative frequencies of prey species identified from hard part remains in the stomachs, small intestines, and large intestines of northern fur seals collected in 1997 and 1998.

Prey species		Percentage of number						Percentage of occurrence					
		Stomach		Small intestine		Large intestine		Stomach		Small intestine		Large intestine	
Scientific name	Common name	1997	1998	1997	1998	1997	1998	1997	1998	1997	1998	1997	1998
<b>Fish</b>													
<i>Bathylagus ochotensis</i>	Popeye blacksmelt	—	0.6	—	1.0	0.2	3.0	—	13.3	—	10.0	4.8	40.0
<i>Engraulis japonicus</i>	Japanese anchovy	—	0.2	—	0.2	—	0.4	—	10.0	—	3.3	—	16.7
<i>Scopelosaurus harryi</i>	Scaly paperbone	—	—	—	—	—	0.04	—	—	—	—	—	3.3
<i>Myctophum asperum</i>	Rough lanternfish	—	—	—	—	0.2	—	—	—	—	—	4.8	—
<i>Symbolophorus californiensis</i>	Bigfin lanternfish	3.3	0.2	—	0.4	—	0.9	4.8	10.0	—	6.7	—	23.3
<i>Tarletonbeania taylori</i>	Taylor's lanternfish	—	—	—	—	0.5	0.9	—	—	—	—	14.3	26.7
<i>Ceratoscopelus warmingi</i>	Dogtooth lampfish	—	2.3	2.5	1.3	0.2	1.5	—	13.3	4.8	13.3	14.3	30.0
<i>Stenobranchius leucopsarus</i>	Northern lampfish	—	—	—	0.2	0.1	—	—	—	—	3.3	9.5	—
<i>Lampanyctus regalis</i>	Pinpoint lampfish	—	—	—	—	—	0.1	—	—	—	—	—	6.7
<i>Diaphus theta</i>	California headlanternfish	3.3	3.2	19.8	25.6	32.8	27.2	14.3	6.7	9.5	16.7	85.7	73.3
<i>D. gigas</i>	Brightnose headlightfish	—	0.4	4.9	0.4	0.2	—	—	3.3	4.8	6.7	4.8	—
<i>D. watasei</i>	Darkblue headlightfish	—	0.2	—	—	—	—	—	3.3	—	—	—	—
<i>Notoscopelus japonicus</i>	Patchwork lampfish	15.5	9.9	17.3	16.6	9.2	11.8	9.5	30.0	23.8	43.3	61.9	63.3
<i>Paralepis</i> sp.		—	—	—	—	—	0.1	—	—	—	—	—	6.7
<i>Lestidium</i> sp.		—	0.1	—	—	0.1	0.1	—	3.3	—	—	9.5	6.7
unidentified fish		32.2	11.6	53.1	23.3	47.5	32.4	9.5	33.3	38.1	50.0	95.2	100.0
<b>Squid</b>													
<i>Loligo bleekeri</i>	Spenar loliginid squid	—	0.1	—	—	—	—	—	3.3	—	—	—	—
<i>Watasenia scintillans</i>	Firefly squid	30.2	67.5	2.5	30.4	8.6	21.0	23.8	43.3	4.8	40.0	71.4	76.7
<i>Onychoteuthis borealijaponica</i>	Boreal clubhook squid	0.8	0.1	—	—	—	—	4.8	6.7	—	—	—	—
<i>Gonatus pyros</i>	Photogenic gonate squid	—	—	—	—	—	0.1	—	—	—	—	—	10.0
<i>G. berryi</i>	Berry armhook squid	0.8	0.1	—	—	—	0.1	4.8	3.3	—	—	—	3.3
<i>Gonatopsis borealis</i>	Boreopacific gonate squid	11.4	3.1	—	0.2	0.3	0.1	28.6	36.7	—	3.3	14.3	3.3
<i>Todarodes pacificus</i>	Japanese common squid	0.8	0.2	—	—	0.1	0.1	4.8	10.0	—	—	4.8	3.3
Chiroteuthidae sp.		1.6	—	—	—	—	—	9.5	—	—	—	—	—
unidentified squid		—	0.1	—	0.2	—	0.2	—	3.3	—	3.3	—	3.3
Total number of prey		122.5	1347.5	40.5	450.0	2302.5	2374.5						
Total number of fish		66.5	386.5	39.5	311.0	2095.5	1865.5						
Total number of squid		56.0	961.0	1.0	139.0	207.0	509.0						

of part of the digestive tract, but only in 1997. In contrast, Japanese anchovy (*Engraulis japonicus*), scaly paperbone (*Scopelosaurus harryi*), pinpoint lampfish (*Lampanyctus regalis*), darkblue headlightfish (*Diaphus watasei*), *Paralepis* sp., spenar loliginid squid (*Loligo bleekeri*) and photogenic gonate squid (*Gonatus pyros*) were found only in 1998. The dominant prey species in all sections of the digestive tract in both 1997 and 1998, were California headlanternfish (*D. theta*), patchwork lampfish (*Notoscopelus japonicus*), and firefly squid (*Watasenia scintillans*) (Table 1).

The boreopacific gonate squid (*Gonatopsis borealis*) was present in large numbers in the stomachs in both 1997 and 1998 (Table 1). Of the two dominant fish spe-

cies, the patchwork lampfish was found in greater numbers in the stomach, while the California headlanternfish was more common in the small and large intestines. The prey composition of the stomach was characterized by the dominance of squids, while the small and large intestines contained higher numbers of fish remains (Fig. 1).

#### Otolith and beak sizes

All otoliths measured were 8.37 mm or less in length. The size distributions differed only slightly among the sections of the digestive tract. However, the stomach contained a slightly higher proportion of large otoliths than that of small otoliths (Fig. 2). Moreover, the otoliths found in the stomach were significantly larger

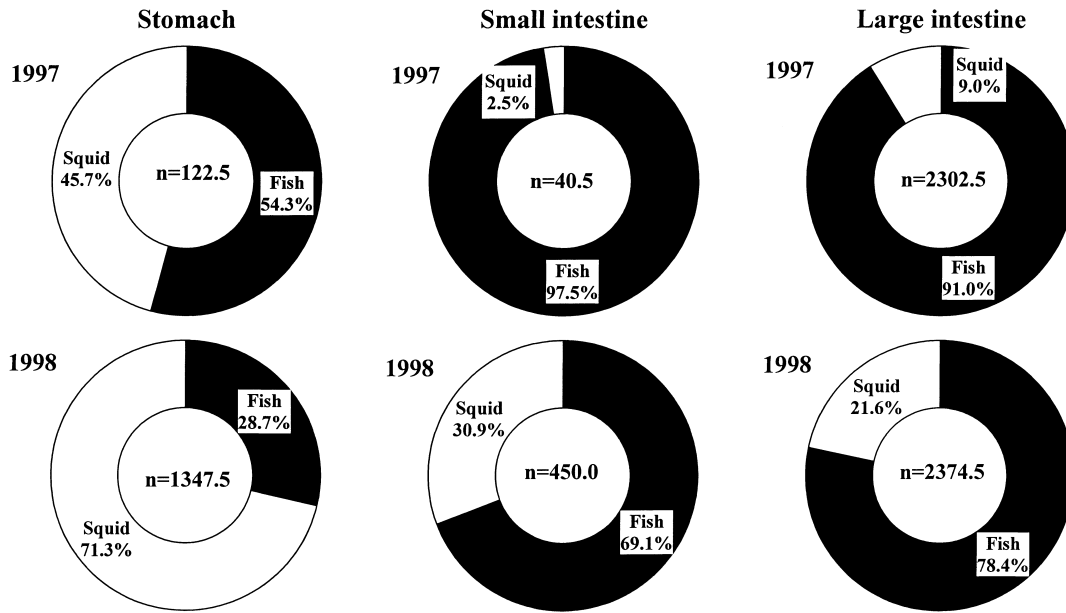


Fig. 1. The proportions of fish and squid found in the stomachs, small intestines, and large intestines of northern fur seals collected in 1997 and 1998 ( $n$  = total number of prey).

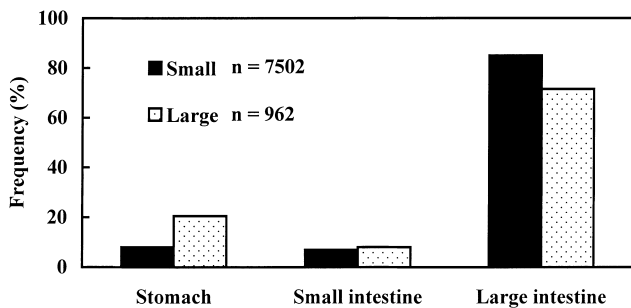


Fig. 2. The relative abundances of different sized fish otoliths (small < 5 mm; large  $\geq$  5 mm), found in the stomachs, small intestines, and large intestines of northern fur seals collected in 1997 and 1998 ( $n$  = small or large otolith number of otoliths).

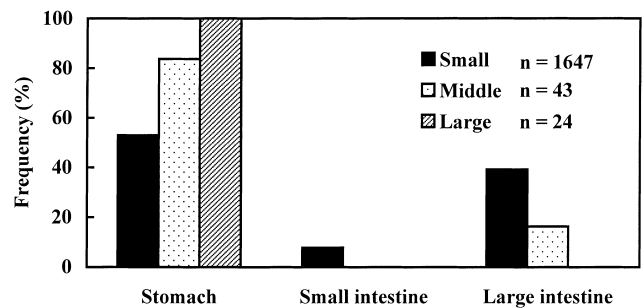


Fig. 3. The relative abundances of different sized squid lower wing length (LWL) (small < 5 mm; middle 5 mm  $\leq$  LWL < 10 mm; large  $\geq$  10 mm), found in the stomachs, small intestines, and large intestines of northern fur seals collected in 1997 and 1998 ( $n$  = small, middle or large beak number of lower beaks).

than those found in the large intestine (ANOVA, Dunnett,  $P < 0.05$ ).

Considering the dominant fish prey species separately, the otoliths of the California headlanternfish found in the stomach were significantly larger than those found in the small intestine in 1998 (ANOVA, Tukey,  $P < 0.05$ ). Otoliths of the patchwork lampfish found in the stomach were significantly larger than those found in the large intestine in both 1997 and 1998 (1997 ANOVA, Dunnett,  $P < 0.05$ ; 1998 ANOVA, Tukey,  $P < 0.05$ ).

The upper and lower beaks found in the digestive tract ranged in length from 2.26–22.20 mm. The lower beaks found in the stomach were significantly larger than those found in the large intestine (ANOVA, Dunnett,  $P < 0.05$ ). The stomach also contained almost all of the large

(100%) and middle (83.7%)-sized lower beaks (Fig. 3). A similar pattern was observed for large upper beaks. Most of the small lower beaks were from the firefly squid, whilst the large lower beaks found in the stomachs belonged mainly to the boreopacific gonate squid.

### Discussion

The contents of small intestines of the northern fur seal were found to be heavier than that of either the stomachs or the large intestines. However, the small intestine contained relatively few hard prey remains. In contrast, the large intestine contained larger numbers of hard remains. Bigg and Fawcett (1985) suggested that hard

prey parts were not affected by digestive activity in the small intestine, and passed rapidly into the large intestine. As a result, larger numbers of hard remains were observed in the large intestine. Accordingly, the contents of the large intestine, and thus the faeces, contain most of the dietary information. However, in this study the prey composition of the stomach contents were characterized by a predominance of squid, while fish were more prevalent in the small and large intestines. This difference was mainly due to the higher occurrence of large beaks in the stomach.

Previously, Pitcher (1980) compared the prey composition of the stomach contents and faeces of the harbour seal and reported that squid were under-represented in the latter. Bigg and Fawcett (1985) also suggested that the accumulation of beaks in the stomach might result in the underestimation of squid in the diet of the northern fur seal when only scat analysis was used. They proposed two possible mechanisms for this accumulation: 1) irregular projections on the surface of squid beaks may anchor them to the stomach wall; and 2) squid beaks may tangle with one another and form a bolus near the pyloric sphincter, impeding their passage into the intestine. Although neither of these mechanisms was observed in this study, we did, however, discover that large beaks were present in the stomach but not the intestines, and that the small beaks of the firefly squid were present both in the stomach and the large intestine (Fig. 3). It seems, therefore, that the passage of larger beaks into the intestines may be restricted by the diameter of the pyloric region.

Squid beaks are largely chitinous (Bigg and Fawcett 1985) and may not be affected by digestive activity in the stomach. Clarke and Trillmich (1980) observed that the regurgitated stomach contents of Galapagos fur seals (*Zalophus californianus wollebaeki*) contained significant numbers of squid beaks, suggesting that large beaks do not accumulate in the stomach indefinitely, but are eventually regurgitated. Furthermore, Kiyota et al. (1999) noted that the ejecta of northern fur seals, collected from a breeding site, contained large fish otoliths.

It appears then, that the importance of squid as prey will be reflected very differently if only the contents of the stomach or large intestine are examined. Clearly, scat analysis alone will fail to provide accurate data on the distribution of prey with large hard parts in the diet. Supplementary information from the study of regurgitated stomach contents on land or of stomach contents following experimental lavage of live-captured animals,

is necessary to obtain unbiased estimates of prey composition.

The otoliths of the dominant prey fish (California headlanternfish and Patchwork lampfish) found in the stomach were significantly larger than those found in the large intestine. This may result from the erosion of  $\text{CaCO}_3$  by stomach acid, as previously described by Tollit et al. (1997), Orr and Harvey (2001), and Staniland (2002). Although otoliths may be affected by digestive action in the stomach, the digestive action for them could not confirm clear in this study.

In conclusion, estimates of the dietary composition of northern fur seals based separately upon the contents of the stomach or of the large intestine, differed significantly as a result of biased distributions of prey hard parts. The size of fish otoliths and squid beaks as major identification features influences dietary estimation by scat analysis alone. Although large otoliths may be affected by passage restriction, most are likely to be affected by digestive action. In addition, large beaks are particularly affected by passage restriction. In order to improve the accuracy of scat analysis, the following subjects should be investigated experimentally in captive animals: 1) difference in hard part sizes in ejecta and in scats; 2) the frequency of regurgitation of the accumulated hard remains of prey from the stomach; and 3) the influence of the digestive action to the relation of prey size and these hard parts size.

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