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An Enema Technique to Collect Dietary Information from Northern Fur Seals (*Callorhinus ursinus*) at Sea

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Abstract

An enema technique was developed to obtain dietary information on northern fur seals (*Callorhinus ursinus*) at sea. The enema apparatus consisted of a hand pump and a soft PVC tube connected to a plastic tank filled with seawater. The tube was inserted into the fur seal's anus, and fecal samples flushed out into a polyethylene bag enveloping the posterior of the fur seal. The efficacy of the enema sampling was tested on 23 fur seals captured and killed off the Pacific coast of northern Japan in April 1998. Twenty of 23 enema samples contained hard prey items such as fish otoliths and squid beaks. The average number (± SD) of hard prey items retrieved from the whole large intestine was 26.9 ± 33.0% for fish otoliths, 37.3 ± 38.5% for squid upper beaks, and 43.3 ± 40.9% for squid lower beaks. Size distributions of these prey items retrieved from the enema samples were similar to that remaining in the large intestines. These results indicated that the enema technique could provide dietary information that is comparable to intestinal contents and scat analysis. Combination of the enema technique with a pelagic live capture method using gillnets could provide a nondestructive way to investigate the feeding ecology of individual fur seals migrating offshore.

Key Words: northern fur seal, *Callorhinus ursinus*, diet analysis, enema technique, large intestine contents, scat analysis, live capture

Introduction

The diet of pinnipeds generally has been investigated based on the stomach contents of sacrificed animals (see Pierce & Boyle, 1991). With this method, the animal must be killed to collect stomach samples, and there are many empty stomachs sampled because the contents are emptied in few hours. Accordingly, the stomach contents analysis is an inefficient method. Scat analysis was applied as a nonlethal method to obtain dietary information from a wide range of pinnipeds (e.g., Daneri & Carlini, 1999; Dellinger & Trillmich, 1999; Gales & Cheal, 1992). Scat samples were collected on land or ice, which did not require capture or disturbance of the seals; however, it is not possible to assign scats to specific individual or even to specific subgroups of a population (i.e., adults or juveniles). To obtain dietary information from individuals, stomach lavage (Antonelis et al., 1987; Ferreira & Bester, 1999) and an enema technique (Staniland et al., 2003) can be applied to live pennipeds captured on land or ice. As scat samples collected on land retain dietary information of recent foraging, these methods were only applicable to those species that feed near the landing sites or to the later stages of foraging trips as those foraging further from the colony for extended times will undoubtedly defecate at sea (Naya et al., 2002).

Northern fur seals (*Callorhinus ursinus*) are one of the most pelagic species of pinnipeds. They have two distinct life phases: the breeding season on feeding sites and the migrating season when they wander thousands of kilometers without hauling sites. The diet of northern fur seals during the migration season has been examined through stomach contents analysis (Kajimura, 1985; Panina, 1966; Wada, 1971), and during the breeding season through scat analysis (Antonelis, 1996; Antonelis et al., 1997; Kiyota et al., 1999; Sinclair et al., 1994); however, even in the breeding season, scat may not completely contain the dietary information of fur seals while at sea, since the duration of a feeding trip for a lactating female fur seal ranges up to 15.3 days (Loughlin et al., 1987). To obtain more complete information on the feeding habits of northern fur seals, it is necessary to collect diet samples from individuals at sea, preferably through a nondestructive method.

In this study, we developed an enema apparatus to obtain fecal samples from northern fur seals captured at sea. To determine the efficacy of enema sampling, we also applied the enema technique to dead fur seals and compared the number and size of prey hard items in the enema samples with those remaining in the large intestines after sampling. Furthermore, we analyzed prey composition (e.g., fish and squid) between the enema samples and the large intestine contents.

Materials and Methods

To obtain enema samples from the fur seals, a plastic tank (3 l) containing seawater was connected to a hand pump and a soft PVC transparent hose (inner diameter, 6 mm; external diameter, 8 mm), separately (Figure 1). The pump discharged approximately 10 ml with every plunge.

Fur seals were restrained on deck, and the lower half of the body placed in transparent polyethylene bag (90 x 100 cm). The PVC tube was then inserted into the fur seal's anus (insertion depth, 11.0-20.0 cm), and approximately 200 ml seawater were infused into the large intestine by 15-20 pressings of the hand pump (Figure 1). The enema sample was collected from the anus, along with the seawater, into the bag. The enema samples were later thawed in the laboratory and gently washed under running water 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 saggital otoliths (OT), squid upper beaks (UB), and squid lower beaks (LB) were sorted and counted using a binocular microscope. Fish species identification was based on OT morphology, following Ohizumi et al. (2001). Fish numbers were estimated using the maximum count of either left or right OTs, plus half of the total number of OTs of undetermined orientation. Squid species were identified, and individuals counted using LBs (Clarke, 1986). The occurrence, number, and percentage of prey species were determined separately for the enema samples and the large intestine contents. Subsequently, otolith length (OTL) was measured as the longest distance from the anterior rostrum to the posterior edge, parallel to the sulcus. The wing lengths of upper and lower squid beaks (UBL and LBL, respectively) were measured. The broken particles (OT, n = 122; UB, n = 28; LB, n = 46) were not measured and discarded.

To capture live fur seals at sea, we used a drifting gillnet (height, 8 m; length, 50 m; shrinkage, 60%; mesh size, 30 cm). When resting fur seals were sighted, the gillnets were floated in the shape of a horseshoe, and one fur seal was driven into the nets using a shotgun. With the fur seal entangled in the nets, the nets were hauled up onto the deck of the research vessel. The captured fur seal was caged while the nets were cut to remove it. Live captures were conducted off the Pacific coast of northern Japan (37.9º N, 141.8º E) on 18 April 1998.

We used these 23 northern fur seals (five male and 18 female, aged 1 to 21 years), which were collected pelagically in the reproduction monitoring program, to test the sampling efficacy of the enema method. Ages were derived from direct readings of canine tooth sections following Kubota et al. (1961). The fur seals were shot from a small boat off the Pacific coast of northern Japan (36.9-37.9º N, 141.5-142.1º E) during daylight hours on 18, 20, and 22 April 1998. After the fur seals were collected onto the deck of the research vessel, the enema technique was applied to the fur seal carcasses as described above. Thereafter, the fur seals were dissected, and the large intestines removed and preserved frozen at –20° C. The contents of

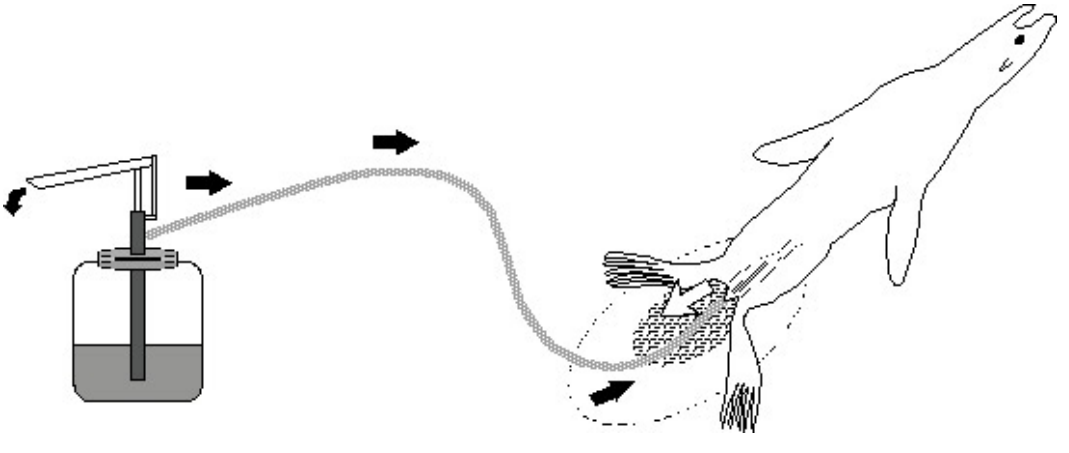

Figure 1. Illustration of the apparatus for obtaining the enema samples of northern fur seals

large intestines were analyzed in the same way as the enema samples, and the OTs, UBs, and LBs were counted and measured. The number and size of the food particles were compared between the enema (EM) samples and the remaining contents (RM) of the large intestines. Moreover, the prey composition of the EM were compared with that of the large intestine contents (LC).

Results

Of the 23 EM samples obtained from the dead fur seals, 20 (87.0%) contained hard prey remains. In total, 319 OTs, 125 UBs, and 73 LBs were collected from the EM samples. All the RM samples contained hard prey remains. The collection of hard prey parts by individual fur seal varied from 0-100%, averaging 26.9 $\pm$ 33.0%, OT; 37.3 $\pm$ 38.5%, UB; and 43.3 $\pm$ 40.9%, LB. The OTLs ranged from 0.97-6.21 mm in the EM and 0.99-6.09 mm in the RM. The OTLs showed complex bimodal frequency distributions, but were quite similar between the EM and RM samples (Figure 2). OTLs in EM samples were significantly smaller than in the RM samples ($p < 0.01$, Mann-Whitney *U*-test). The UBLs ranged from 2.95-4.09 mm in the EM samples and 2.97-6.94 mm in the RM, and LBLs ranged from 2.08-3.12 mm in the EM and 2.00-5.03 mm in RM samples. Frequency distributions of UBLs and LBLs showed unimodal distributions and were similar between the EM and RM samples (Figure 2). There were no significant differences in the UBLs and LBLs between EM and RM samples ($p > 0.05$, Mann-Whitney *U*-test).

It was easier to obtain the EM sample from the live fur seal than from the dead animals because the excretion of enema may stimulate reaction to muscle constriction of the anus and intestine. The enema contained 69 OTs, 10 UBs, and 10 LBs. The sizes of hard prey remains ranged from 1.13-4.28 mm in OTL, 3.32-3.76 mm in UBL, and 2.12-3.15 mm in LBL.

The prey compositions (percent number and occurrence) of the EM samples were similar to that of the LC samples (Figures 3 & 4). Ten species of fish and three species of squid were identified from the LC samples, while seven species of fish and one species of squid were present in the EM samples (Table 1). The dominant prey species in the EM and LC samples were California headlanternfish (*Diaphus theta*), patchwork lampfish (*Notoscopelus japonicus*), and firefly squid (*Watasenia scintillans*). In the EM samples of the live captured fur seal, California headlanternfish (n = 24.5) and firefly squid (n = 10) were present.

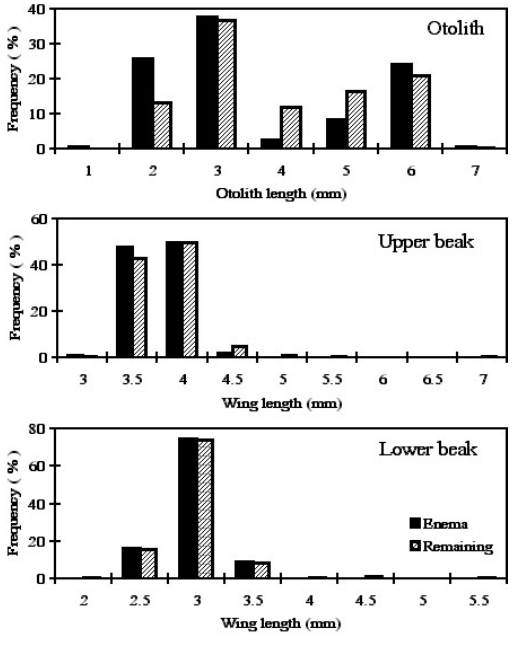

Figure 2. Histograms showing the percentage frequency of otolith length (OTL), upper beak length (UBL), and lower beak length (LBL) found in the enema (EM) samples and the remaining contents (RM) of the large intestines from dead northern fur seals collected at sea

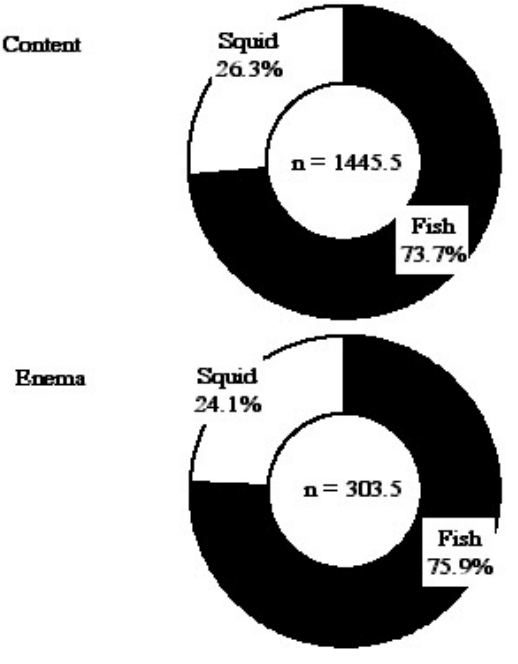

Figure 3. The proportions of fish and squid found in the enema samples and the large intestine contents of northern fur seals (n = total number of prey)

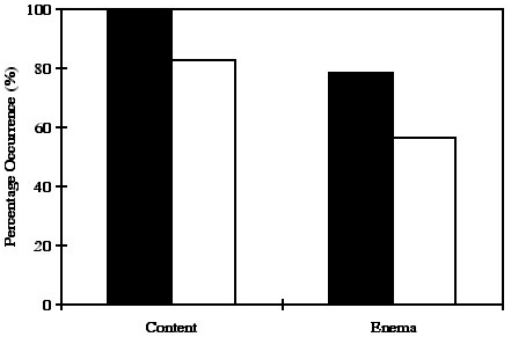

Figure 4. Percentage occurrence of fish and squids found in the enema samples and the large intestine contents of the northern fur seal (black columns, fish; white columns, squid)

Discussion

These results indicated that the enema technique was effective in obtaining contents of the large intestine. Dietary information obtained from the EM samples was similar to that of the content of the whole large intestine and likely to be equal to that gleaned from scat analyses as found for Antarctic fur seals (*Arctocephalus gazella*) by Staniland et al. (2003). The combination of pelagic live-capture and application of the enema technique provides a nondestructive method to obtain dietary information from a pinniped migrating offshore; however, scat (enema) samples have led to predictable bias in the quantitative estimation of diet composition (Jobling, 1987; Jobling & Breiby, 1986). The factors suggested the erosion (e.g., Arim & Naya, 2003; Bowen, 2000; Orr & Harvey, 2001) and passage restriction (Yonezaki et al., 2003) in prey hard items. Therefore, to obtain accurate dietary information, stomach contents should be obtained using the stomach lavage technique (e.g., Antonelis et al., 1987; Ferreira & Bester, 1999).

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Table 1. Percent number and percent occurrence values of prey species identified from hard remains in the enema samples and the large intestine contents of northern fur seals

	Percent number		Percent occurrence	
Prey species	Enema	Content	Enema	Content
Fish				
Engraulis japonicus	-	0.5	-	17.4
Lipolagus ochotensis	1.5	4.5	13.0	43.5
Scopelosaurus harryi	-	0.1	-	4.3
Symbolophorus californiensis	0.3	1.3	4.3	21.7
Tarletonbeania taylori	0.3	0.2	4.3	8.7
Ceratoscopelus warmingii	1.0	2.4	13.0	39.1
Diaphus theta	23.1	16.9	43.5	69.6
Notoscopelus japonicus	19.8	17.2	39.1	60.9
Paralepis sp.	-	0.1	-	4.3
Lestidum sp.	0.3	0.2	4.3	4.3
Unidentified fishes	29.7	30.3	69.6	100.0
Squids				
Watasenia scintillans	23.7	25.9	56.5	82.6
Gonatus pyros	-	0.1	-	8.7
Todarodes pacificus	-	0.1	-	4.3
Unidentified squids	0.3	0.1	4.3	4.3
Total number of prey	303.5	1,445.5		
Total number of fish	230.5	1,065.5		
Total number of squid	73.0	380.0		

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