Impact of diet-index selection and the digestion of prey hard remains on determining the diet of the Steller sea lion (*Eumetopias jubatus*)

D.J. Tollit, S.G. Heaslip, R.L. Barrick, and A.W. Trites

Abstract: Nine prey species (n = 7431) were fed to four captive female Steller sea lions (*Eumetopias jubatus* (Schreber, 1776)) in 11 feeding trials over 75 days to investigate the effectiveness of different methods used to determine diet from prey hard remains. Trials aimed to replicate short (1–2 days) and long feeding bouts, and consisted of single species and mixed daily diets. Overall, 25.2% ± 22.2% (mean ± SD, range 0%–83%) otoliths were recovered, but recovery rates varied by species (ANOVA, P = 0.01) and were linearly related to otolith robustness (R^2 = 0.88). Squid beaks were recovered at higher frequencies (mean 96%) than the otoliths of all species. Enumerating both non-otolith skeletal structures and otoliths (together termed bones) increased species recovery rates by twofold, on average (P < 0.001), with increases up to 2.5 times for Pacific herring (*Clupea pallasii* Valenciennes in Cuvier and Valenciennes, 1847) and 3–4 times for salmonids. Using bones reduced interspecific differences (P = 0.08), but recovery varied among sea lions. Bones were distributed over more scats per meal (mean 2.9 scats, range 0–5) than otoliths (mean 1.9 scats, range 0–4). In three different 15-day mixed diet trials, biomass reconstruction (BR) indices performed better than frequency of occurrence indices in predicting diet fed. Applying our experimentally derived numerical correction factors (to account for species differences in complete prey digestion) further improved BR estimates, resulting in all 12 unweighted comparisons within 5% (for otoliths) and 12% (for bones) of the actual diet fed.

Résumé : Nous avons fourni neuf espèces de proies (n = 7431) à quatre lions de mer de Steller (*Eumetopias jubatus* (Schreber, 1776)) en captivité au cours de 11 expériences d'alimentation sur une période de 75 jours afin d'évaluer l'efficacité de plusieurs méthodes utilisées pour déterminer le régime alimentaire à partir des restes durs des proies. Les expériences cherchent à simuler des périodes courtes (1–2 jours) et prolongées d'alimentation et proposent des rations journalières monospécifiques et mixtes. Globalement, 25,2% ± 22,2% (moyenne ± ET, étendue 0%–83%) des otolithes ont été retrouvés, mais les taux de récupération varient en fonction de l'espèce (analyse de variance, P = 0,01) et sont en relation directe avec la robustesse des otolithes (R^2 = 0,88). Les becs de calmars ont été récupérés à des fréquences plus grandes (moyenne 96%) que les otolithes de toutes les espèces. L'énumération à la fois des otolithes et des autres structures squelettiques (conjointement « les os ») augmente les taux de récupération des espèces d'un facteur moyen de 2 (P < 0,001), atteignant 2,5 chez le hareng (*Clupea pallasii* Valenciennes in Cuvier and Valenciennes, 1847) et 3–4 chez les salmonidés. L'utilisation des os réduit les différences entre les espèces (P = 0,08), mais leur récupération varie d'un lion de mer à un autre. Les os se répartissent sur un nombre plus élevé de défécations par repas (moyenne 2,9 défécations, étendue 0–5) que les otolithes (moyenne 1,9 défécation, étendue 0–4). Dans trois expériences différentes de régimes mixtes, les indices de reconstitution de la biomasse (BR) fonctionnent mieux que les indices de fréquence d'occurrence pour prédire le régime alimentaire. L'utilisation des facteurs numériques de correction (qui tiennent compte des différences spécifiques de digestion totale des proies) que nous avons obtenu expérimentalement améliore encore plus les estimations de BR, ce qui a pour résultat que l'ensemble des douze comparaisons non pondérées se placent à moins de 5 % (pour les otolithes) et de 12 % (pour les os) des régimes alimentaires réels.

[Traduit par la Rédaction]

Introduction

The analysis of prey skeletal hard remains found in scats (feces) is now the most widely used technique for estimating the diet of pinnipeds, with sagittal otoliths and cephalopod beaks being the most commonly used structures because of their ease of identification and enumeration (Frost and Lowry 1980; Olesiuk et al. 1990; Bowen et al. 1993; Tollit and Thompson 1996). There remain a number of well-documented limitations, however, particularly related to differential rates of digestion and recovery across both prey and predator species (see reviews by Pierce and Boyle

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1991; Bowen 2000; Tollit et al. 2003). For example, fragile otoliths (e.g., from clupeids, osmerids, and salmonids) are generally recovered in lower relative numbers than large, robust otoliths (e.g., from gadids). Using captive feeding studies, numerical correction factors (NCFs) can be generated to correct for differential prey recovery by comparing known numbers of fish consumed with estimates derived from reconstructing the number of prey using the number of skeletal structures that survive digestion (Harvey 1989).

The wide range of factors that seem to influence digestion (Harvey 1989; Cottrell et al. 1996; Tollit et al. 1997; Marcus et al. 1998; Bowen 2000; Orr and Harvey 2001; Staniland 2002; Casper et al. 2006) require that feeding protocols be standardized and are fine scale enough to assess levels and causes of observed variability before results can be applied with confidence to scat data from the wild. Data are most lacking at the individual meal and scat level.

The dramatic decline of the western population of Steller sea lions (*Eumetopias jubatus* (Schreber, 1776)) in the 1980s (Loughlin et al. 1992; Trites and Larkin 1996) prompted a number of studies to determine what they eat and to explore the extent of dietary overlap with commercial fisheries (e.g., Merrick et al. 1997; Sinclair and Zeppelin 2002; Winship and Trites 2003; Tollit et al. 2004*a*; Zeppelin et al. 2004). To date, frequency of occurrence (FO) indices have been selected to describe Steller sea lion diet (Merrick et al. 1997; Sinclair and Zeppelin 2002; Trites et al. 2007). Identifying diagnostic skeletal structures in addition to otoliths (together termed as bones) is being used to ensure that prey species are not missed because of interspecific differences in the recovery of otoliths. Reconstructing the biomass of prey consumed is considered the most effective means of quantifying diet composition (Hammond and Rothery 1996; Bowen 2000), but it has not yet been applied to Steller sea lions, mainly because of a lack of species-specific numerical correction factors for many key prey species and the fact that appropriate bone to fish size allometric regressions have been only recently developed (Zeppelin et al. 2004).

Identifying diagnostic structures in addition to otoliths has clear value in increasing the probability of detecting the presence of many prey species (Olesiuk et al. 1990; Cottrell et al. 1996; Tollit et al. 2003). However, computer simulations suggest that using multiple structures to count the number of individuals consumed may cause biases in biomass reconstruction (BR) indices in certain cases (Joy et al. 2006). The simulations showed that biases that are more significant might also occur when using FO indices, particularly when small prey are consumed in small amounts. Captive feeding studies provide a unique ability to test diet estimates predicted by different indices (in addition to the use of NCFs) compared with the diet actually consumed. Results from such studies have relevance to all ecological studies that describe marine mammal diet using hard prey remains.

Passage times of otoliths and other structures can vary among prey species, potentially affecting recovery in subsequent scats found on haul-outs following foraging trips. Recovery and passage rate data exist for few prey species eaten by Steller sea lions, and are mainly restricted to one size class of prey (Cottrell et al. 1996; Tollit et al. 2003). Therefore, information regarding the impact prey size has on prey digestion, passage, and recovery is currently limited (Tollit et al. 1997; Bowen 2000). Potential impacts were highlighted by Tollit et al. (2004*a*), who noted that the virtual absence of juvenile (<20 cm fork length) walleye pollock (*Theragra chalcogramma* (Pallas, 1814)) in the scats of Steller sea lions from southeast Alaska may in part be because the relatively smaller structures of smaller fish were more likely completely digested and, therefore, underrepresented in the scats.

Regurgitation of prey remains occurs on land and at sea (e.g., Kirkman et al. 2000; Bowen et al. 2002). If regurgitation of prey is common in the wild and specific to certain prey or bone sizes, then estimates of diet based on recovery of hard parts in scats collected from haul-outs could be biased. Such biases have been previously highlighted for cephalopod beaks (Bigg and Fawcett 1985), which are often found in regurgitations (Fea et al. 1999), and may occur for bulky hard parts of larger fish (Kiyota et al. 1999; Tollit et al. 2003; Gudmundson et al. 2006).

The purpose of our study was to enumerate the level of complete digestion and subsequent recovery of otoliths and bones of key prey species and assess different diet reconstruction techniques for Steller sea lions. The specific objectives were to (*i*) compare the passage times and percent recoveries of eight key prey species when using sagittal otoliths with that of using all recovered skeletal hard remains; (*ii*) assess the influence of otolith robustness and prey size on prey recovery; (*iii*) provide robust otolith and bone NCFs for use in future BR diet composition studies; (*iv*) compare the reliability of diet predictions based on FO and BR indices (e.g., Olesiuk et al. 1990; Pierce and Boyle 1991; Laake et al. 2002) for three different mixed diet feeding scenarios when using different combinations of hard remains recovered from scats (otoliths vs. bones); and (*v*) examine the extent to which BR indices are improved when NCFs are applied.

Materials and methods

Captive feeding trials

Eleven feeding trials were conducted with two juvenile (106–127 kg) and two adult (132–151 kg) female Steller sea lions (SSL1 #F97HA, SSL2 #F97SI, SSL3 #F00YA, SSL4 #F00NU) from 10 December 2001 to 22 July 2003 at the Vancouver Aquarium (Tables 1, S1[3]). All studies were conducted in accordance with guidelines of the Canadian Council on Animal Care, and with review and approval by The University of British Columbia Committee on Animal Care. Sea lions were maintained on a diet of Pacific herring (*Clupea pallasii* Valenciennes in Cuvier and Valenciennes, 1847) at ~6% of their body mass (BM) per day, and were housed individually either in a continuously flowing saltwater swim tank (minimum 20 000 L, equipped with a

[3] Tables S1 and S2 for this article are available on the journal Web site (http://cjz.nrc.ca) or may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Building M-55, 1200 Montreal Road, Ottawa, ON K1A 0R6, Canada. DUD 5119. For more information on obtaining material refer to http://cisti-icist.nrc-cnrc.gc.ca/irm/unpub_e.shtml.

2 m × 2 m haul-out area) or in a grated dry run (1.8 m × 2.5 m) when tanks were drained to recover scats. A metal tray beneath the grated dry run allowed scat collection.

Overall, we fed nine key prey species (walleye pollock; coho salmon, *Oncorhynchus kisutch* (Walbaum, 1792); Pacific cod, *Gadus macrocephalus* Tilesius, 1810; Atka mackerel, *Pleurogrammus monopterygius* (Pallas, 1810); Pacific herring; California market squid, *Loligo opalescens* Berry, 1911; Pacific sand lance, *Ammodytes hexapterus* Pallas, 1814; capelin, *Mallotus villosus* (Müller, 1776); eulachon, *Thaleichthys pacificus* (Richardson, 1836) (Tables 1, S1[3]). Our study focused primarily on five species — walleye pollock (pollock), coho salmon (salmon), Pacific cod, Atka mackerel, and Pacific herring (herring) — that are believed to be key prey species for Steller sea lions (e.g., Sinclair and Zeppelin 2002) and are commercially fished. To investigate intraspecific variability in the digestion of prey remains, different size classes of pollock, salmon, and Pacific cod were fed to sea lions (Table S1[3]).

Captive feeding trails aimed to simulate firstly short foraging trips as exhibited by free-ranging Steller sea lions during summer (Higgins et al. 1988). Typically, each trial began with a 68 h period with full access to water and meals of filleted or headless herring to clear the digestive tract of diagnostic hard remains (days 1–3). On the day following the last experiment meal, sea lions were maintained in an "inactive" state and fasted in the dry run for 24 h, and then fed only fillets of herring or headless herring for a further 3-day period (to ensure that all experimental hard remains surviving digestion were collected) during which they were housed either in the swim tank or in the dry run when the tank was being drained each day. All fecal material collected from swim-tank drains were cleaned and filtered through a 0.5 mm nylon mesh. The ability to recover all fish skeletal structures from the swim tanks was tested on 12 occasions by scattering 30–36 marked otoliths and vertebrae of pollock, herring, and sand lance, or 60–120 small (2.3–4.2 mm) plastic beads in the tank and around the haul-out area. During the experimental period, individual scat samples from an animal in the dry run were collected and frozen, and the time of defecation recorded. Animals undertook two different types of feeding trials: single and replicated. Details of each trail's feeding protocol are provided in Table S1.[3]

The eight single meal trials generally followed methods described in Tollit et al. (2003), which simply fed one (occasionally two or three) different prey items on each day over a 3-day experimental trial period. At ~1030 on day 3, animals were moved to a dry run and fasted for 24 h to simulate a resting period on land, then moved back to the swim tank and fed the first half of the first experimental meal (day 4, ~1030, ~2.5% BM). A meal of similar size and composition as the first was fed at ~1500 (Table S1[3]). At ~1030 on days 5 and 6, the animal was moved to the dry run, experimental meals of similar size (~2.5% BM) but different prey composition were fed at ~1030 and ~1500, with the animal returning to the swim tank between 1330 and 1500. Three trials differed from this protocol. Swim-tank drains were performed on days 2, 4, and 7 for the two trials conducted in April 2003. Therefore, there were no dry-run periods on days 1, 3, 5, and 6. Additionally, capelin was fed to SSL4 over 2 days, and meals of capelin and sand lance were fed hourly over a 5 h period. Meals were also spread over a 5 h period for the eulachon fed to SSL1. The trial conducted in September 2002 had just one drain at the end of the feeding study and therefore SSL1 had no enforced dry-run sessions.

Three replicated mixed meal feeding trials were undertaken, aiming to replicate bouts of consistent feeding. Each trial aimed to feed the same four species (pollock, salmon, herring, and capelin) at the same times (~1015 and ~1515) in the same quantity (7.5% BM / day) for 15 days, but in three different prey ratios (scenario 1, 25% of each species; scenario 2, 67.5%, 2.5% 22.5%, and 7.5%, respectively; scenario 3, 22.5%, 7.5% 67.5%, and 2.5%, respectively). On the first day of herring fillets at the end of the 15-day experimental period, an additional experimental meal of six Atka mackerel was fed. Scenario 1 resulted in four regurgitations in the first 5 days, so pollock were excluded from subsequent daily meals, and the remaining feedings consisted of the other three species in equal quantities. During the following 9 days, three small regurgitation events occurred. On the last day, pollock was again fed; however, an additional regurgitation occurred the following day. The resulting diet fed for scenario 1 was therefore 11.8%, 29.2%, 29.6%, and 29.4% in terms of mass for each prey species, respectively.

Fork length and (or) standard length of the eight fish species fed were measured to ±1 mm. Prey mass was typically recorded to ±1 g, but for a few meals, prey were subsampled and (or) were weighed as a whole. Size ranges in individual meals were kept as narrow as possible to reduce the confounding effects of prey size. No evidence of fish bones from secondary prey was found in random checks of fish and squid intestines. Mean (SD) number and size of each species fed to each animal are summarized for each trial in Table S1.[3]

Scats were washed individually through a 0.5 mm sieve (similar to tank drains). Otoliths, other fish hard remains, and beaks were recovered from fecal remains, left and right sides or upper and lower beaks were determined (when possible), and enumerated. Samples were considered from regurgitations if found in a state that precluded passage through the digestive tract (i.e., bones looked clean and undigested, vertebrae were still joined together with processes intact, bones were of a size to exclude passage through the pyloric sphincter, and (or) small amounts of undigested flesh were present). It is commonly accepted that data from scats and regurgitations should not be combined during data analysis (Fea et al. 1999; Kiyota et al. 1999). Therefore, we excluded regurgitations from certain analyses (13 of 75 experimental meals overall), since our primary goal was to analyze hard remains recovered from scats. Nevertheless, because regurgitation may result from size-selective accumulation of bulky hard parts (Kiyota et al. 1999; Tollit et al. 2003), we provide pertinent information on percent recoveries of prey in the 17.3% of experimental meals thought to have been regurgitated.

Estimates of structure and prey percent recoveries

Percent recoveries of otoliths and beaks were calculated using two methods. The first provided information at the individual structure level by dividing the number of structures

Table 1. Percent recovery of prey species fed to Steller sea lions (*Eumetopias jubatus*; SSL1–SSL4) based on otoliths or bones recovered over each feeding trial.

					Otolith			Bone		
SSL	Date of study	*N*	Prey species	Percent recovery (otolith or beak)	Percent recovery of prey	Output days	No. of days present	Percent recovery of prey	Output days	No. of days present
1	Dec. 2001	272	Walleye pollock*	65.8	66.2	4	3	66.5	4	4
		20	Coho salmon*	15.0	20.0	2	2	20.0	2	2
		18	Atka mackerel	41.7 (0)	44.4 (0)	(1)	(1)	50.0 (12.5)	(2)	(2)
3	Jan. 2002	158	Walleye pollock*	39.6	40.5	6	4	48.7	6	5
		20	Coho salmon*	5.0	5.0	1	1	15.0	2	2
		13	Atka mackerel	15.4	15.4	4	2	30.8	4	4
2	Feb. 2002	11	Pacific cod*	100.0 (0)	100.0 (0)	(1)	(1)	136.4 (36.4)	(2)	(2)
		180	Walleye pollock*	88.3 (41.4)	88.9 (41.6)	(4)	(4)	88.9 (41.6)	(4)	(4)
		8	Coho salmon	12.5	12.5	3	1	100.0	3	3
		17	Atka mackerel	26.5	29.4	2	2	52.9	2	2
1	Sept. 2002	38	Walleye pollock	82.1	82.1			82.1		
		180	Walleye pollock*							
		8	Coho salmon	6.3	12.5			12.5		
		17	Atka mackerel	5.9	5.9			5.9		
1	Feb. 2003	30	CA market squid	93.3 (38.3)				96.7 (43.3)	3	3
		4	Pacific cod	87.5 (25)	100.0 (25)	(3)	(2)	100.0 (25)	(3)	(2)
		30	Walleye pollock*	78.3 (31.6)	83.3 (36.7)	(5)	(4)	83.3 (36.7)	(5)	(4)
		60	Sand lance	1.7	3.3	3	2	6.7	3	2
		7	Coho salmon	7.1	14.3	2	1	85.7	3	3
		11	Atka mackerel	50.0	54.5	3	2	72.7	3	3
		30	Capelin	1.7	3.3	2	1	3.3	2	1
3	Feb. 2003	30	CA market squid	95.0				100.0	3	3
		3	Pacific cod	33.3	33.3	3	1	133.3	3	3
		30	Walleye pollock*	8.3	10.0	3	2	13.3	4	3
		6	Coho salmon*	41.7 (25)	50.0 (33.3)	(3)	(2)	166.7 (83.3)	(6)	(4)
		9	Atka mackerel	33.3	44.4	3	2	66.7	5	4
		30	Capelin	28.3	30.0	2	2	30.0	2	2
4	Apr. 2003	38	Pacific herring	18.4	18.4			73.7		
		30	CA market squid	96.7				96.7		
		722	Capelin	15.2	15.2			15.2		
		801	Sand lance	31.3	31.3			31.3		
1	Apr. 2003	32	Pacific herring	17.2	18.8			31.3		
		174	Eulachon	4.6	4.6			8.0		
		30	CA market squid	95.0				96.7		
		15	Atka mackerel	0.0	0.0	0	0	0.0	0	0
		17	Pacific cod*	100.0 (11.8)	100.0 (11.8)			105.9 (17.6)		
		212	Sand lance	0.7	0.9			2.4		
3	June 2003	141	Capelin	13.5	17.7			22.0		
		30	Coho salmon*	23.6	30.0			96.7		
		829	Pacific herring	24.5	25.2			47.4		

Table 1 (*concluded*).

SSL	Date of study	*N*	Prey species	Percent recovery (otolith or beak)	Otolith			Bone		
					Percent recovery of prey	Output days	No. of days present	Percent recovery of prey	Output days	No. of days present
		204	Walleye pollock	64.7	66.2			75.3		
		6	Atka mackerel	16.7	33.3			50.0		
4	July 2003	1205	Capelin	23.3 (13.2)	23.6 (13.3)			30.0 (16.6)		
		78	Coho salmon*	60.9 (17.3)	66.7 (21.8)			102.6 (38.5)		
		308	Pacific herring	38.6 (17.7)	40.9 (18.2)			82.7 (40.1)		
		90	Walleye pollock	100.0 (7.7)	104.4 (7.8)			128.9 (24.5)		
		6	Atka mackerel	66.7	66.7			83.3		
3	July 2003	393	Capelin	10.1	11.2			16.5		
		15	Coho salmon*	23.3	40.0			153.3		
		309	Pacific herring	20.1	22.0			57.9		
		497	Walleye pollock	68.1	68.8			78.5		
		6	Atka mackerel	50.0	50.0			83.3		

Note: The meal fed to SSL1 on Sept. 2002, consisting of walleye pollock, was from two size classes of fish. Recovery data including all recovered structures are provided for trials in which regurgitations occurred and the resulting percent recoveries are shown in parentheses if data from the regurgitated event were removed. The maximum number of days over which remains of experimental meal were egested (output days) and number of days that remains were present in drains or scats are also provided where recorded.

*Denotes small prey size class.

recovered for a specific prey species by the number of structures fed, multiplied by 100 (sensu Cottrell and Trites 2002). The second method used otoliths and beaks to calculate prey number using a minimum number of individual (MNI) technique (Ringrose 1993) for each scat or tank-drain sample. For paired structures such as otoliths, MNI is typically the greatest number of left or right elements for each species (see Tollit et al. 2003). Prey percent recovery was then calculated as the estimated number of individual fish recovered (MNI for each sample summed) divided by the number of fish fed, multiplied by 100. Prey percent recoveries were also calculated in an identical manner for bones.

Otolith robustness

In harbour seals (*Phoca vitulina* L., 1758), the robustness and recovery of otoliths seem to be related (Harvey 1989; Tollit et al. 1997). Consequently, recovery rate of otoliths can be predicted for prey species that have never been fed to captive pinnipeds. Otolith robustness (undigested otolith mass / otolith length) was calculated for each species and size class fed, with the exception of species with recovery data from a single experiment (Pacific cod and eulachon). Additional results were available for pink salmon (*Oncorhynchus gorbuscha* (Walbaum, 1792)) fed to the two adult Steller sea lions that also took part in our study (see Tollit et al. 2003), and for prey fed in the two previously published harbour seal studies (Harvey 1989; Tollit et al. 1997). Reliable recovery-rate data were only available from the latter study for species with larger otoliths, thus, we only included data for Atlantic cod (*Gadus morhua* L., 1758), and whiting (*Merlangius merlangus* (L., 1758)), plaice (*Pleuronectes platessa* L., 1758), and Atlantic herring (*Clupea harengus* L., 1758) recovery-rate data. The relationship between otolith robustness and recovery of otolith structures was tested using data from Steller sea lions and compared with combined data from both harbour seal studies (using a Student's *t* test; Zar 1984).

Passage times

Certain single meal feeding trials and the final meal of Atka mackerel in the replicated trials allowed the collection of crude passage-time data. The maximum number of days it took for otolith or bone egestion in scats following an experimental meal was calculated and termed output days. The number of days that scats were produced and contained otoliths or bones over this time was calculated and termed days present.

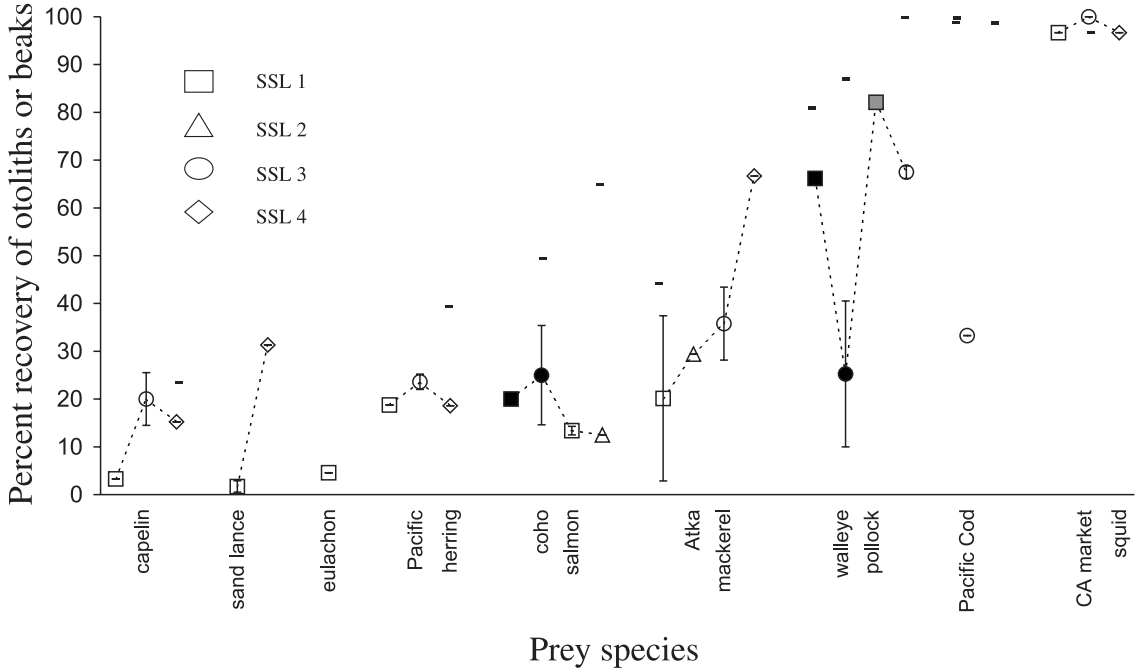
Numerical correction factors

Steller sea lion otolith and bone NCFs were calculated as the inverse of mean prey percent recoveries, where sufficient experimental data existed. Comparable data from Tollit et al. (2003) were available for three of the species (walleye pollock, Pacific sand lance, and Pacific herring) fed in this study, as well as pink salmon, and so were included in the study. Prey size-specific NCFs were calculated where possible and equal weighting across trials was given to each animal.

Reliability of diet indices in replicated mixed meal feeding trails

We used the three different replicated mixed meal feeding

Fig. 1. Percent recovery of otoliths or beaks across nine prey species fed to four Steller sea lions (*Eumetopias jubatus*; SSL1–SSL4). Hyphens (above relevant animals) indicate percent recovery in trials where a regurgitation event occurred and includes all recovered structures (see also Table 1). Solid symbols depict small prey size class and the shaded symbol depicts a combination of medium-sized and small prey size classes.



trials to assess the reliability of four different indices in predicting diet. Both modified frequency of occurrence (FO modified to total 100%, MFO) and split sample frequency of occurrence (SSFO; for details see Olesiuk et al. 1990) indices are based on prey presence and absence, whereas the two different BR indices both require counts of prey and an estimate of prey size (for a full description and relevant equations for each index see Joy et al. 2006). The BR-fixed (BR-F) index gives equal weighting to each scat (as does SSFO), whereas the BR-variable (BR-V) index allows for individual variability in the weight of prey that each scat contributes to the overall total weight of prey (unweighted scats). We calculated diet estimates using each index when using only otoliths and again when using bones. We then recalculated BR indices after the application of our experimentally derived Steller sea lion specific NCFs (see the previous section Numerical correction factors). The underestimation of prey size because of partial digestion of skeletal structures can be accounted for using appropriate grading keys and size correction factors (see Tollit et al. 2004*b*; Grellier 2006). Therefore, in our analysis we limited our assessment to the impact of prey recovery and enumeration on diet estimates by assuming that egested prey mass equaled ingested prey mass. We estimated resampling errors (95% confidence intervals (CI)) related to variability across scats (*n*) using bootstrap techniques by repeatedly (1000 times) selecting *n* scats at random with replacement from the original sample set of scats (for details see Tollit et al. 2004*b*).

Results

All but one of the bones (95/96, a sand lance vertebra), and all the 4.2 mm beads (100/100), and 99.2% of the 2.3 mm beads (761/767) scattered into swim tanks to test the ability to recover fish bones were recovered. We therefore assumed that all excreted structures were recovered during tank draining.

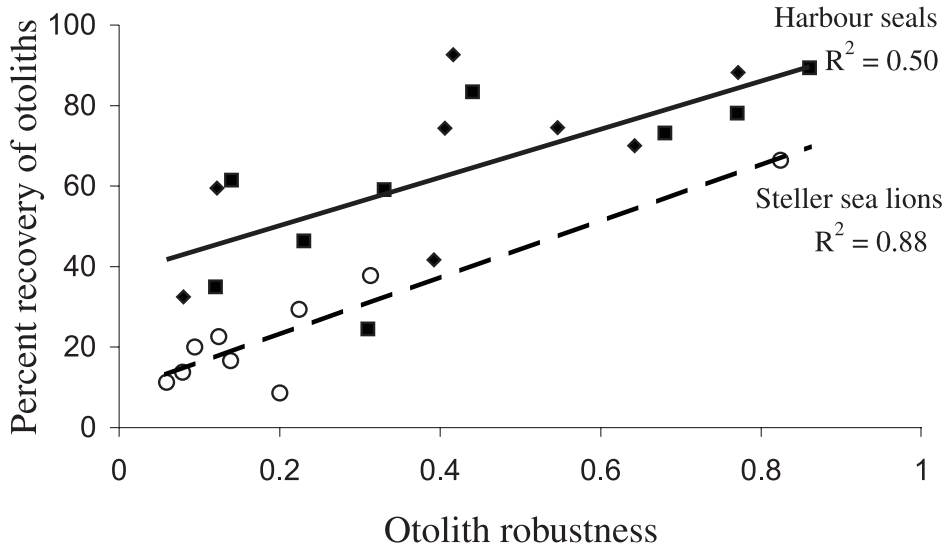
Regurgitated material was observed at least once for each animal in a total of 13 tank drains, and notably for one animal (SSL 4). All regurgitation events followed meals that included the larger sized fish, such as pollock, Pacific cod, salmon, and Atka mackerel (Table S1[3]). On days for which regurgitations were observed, soft fecal material was always found; therefore, it was impossible to be precise about which otoliths, bones, and beaks had been defecated and which had been regurgitated. In two samples, no sand lance or salmon otoliths were found in a tank drain that contained evidence of other regurgitated prey. In a third sample, a single sand lance otolith was found in a tank-drain sample that contained regurgitated Pacific cod bones. The total recovery of sand lance for this feeding trail was 0.7% (i.e., two otoliths, SSL1, Apr. 2003; Table 1). Thus if this single sand lance otolith was indeed regurgitated, the influence of regurgitation on the proportion recovered was deemed negligent. With the exception of these three instances, results in text, tables, and figures exclude partly regurgitated experimental meals unless explicitly stated.

The inclusion or exclusion of data from remaining suspected regurgitation events dramatically influenced the estimated rate of prey recovery from a particular meal. For example, regurgitations were suspected following three of four meals of Pacific cod fed to SSL1 (Feb. 2003). For these three regurgitated samples, Pacific cod otolith recovery for the feeding trial was 100% (Fig. 1) and the regurgitated samples accounted for 87.5% of otoliths recovered (Table 1).

Structure and prey percent recoveries

A total of 7431 fish and squid were fed across 11 feeding

Fig. 2. Relationship between percent recovery of otoliths and robustness of otoliths (undigested otolith mass / length) for Steller sea lion prey ($Y = 70.6X + 9.1$, mean $X = 0.23$, RMS = 47.0; ○), together with comparative data for harbour seal (*Phoca vitulina*) prey ($Y = 59.9X + 38.2$, mean $X = 0.41$, RMS = 241.2, with data based on Harvey 1989 (■) and Tollit et al. 1997 (◆)).



trials in 75 experimental days (Table 1). Averaged across all trials, the recovery of otoliths were 25.2% ± 22.2% (mean ± SD), but ranged between 0% and 83%. Otolith recovery of pollock (54.9 ± 26.8, $n = 6$) exceeded that of Atka mackerel (29.4 ± 22.5, $n = 9$), capelin (13.8 ± 9.7, $n = 5$), salmon (13.2 ± 7.8, $n = 7$), herring (20.1 ± 3.2, $n = 4$), and sand lance (11.2 ± 17.4, $n = 3$) (ANOVA, $F_{[1,5]} = 11.1$, $P = 0.01$). When trials with regurgitation events were removed, only one value was available for percent recovery of Pacific cod otoliths (33.3%, $n = 3$ fish) and eulachon otoliths (4.6%, $n = 174$ fish). The recovery of beaks from small (<50 g) squid was consistently high (95.6% ± 1.0%, $n = 3$) and exceeded otolith recovery in all six paired comparisons (Fisher's tests, all $P < 0.002$).

No significant differences in otolith recovery were detected comparing the two size classes of pollock (13–15 vs. 26–30 cm) or salmon (28–32 vs. 42–43 cm). However, the inclusion of recovery data from larger (~37 cm fork length) pollock (see Tollit et al. 2003) resulted in a significant size effect (ANOVA, $F_{[1,2]} = 8.3$, $P = 0.03$), with recovery of large fish exceeding that of small fish. When the four different animals were considered to be treatments, we failed to reject the null hypothesis ($P = 0.07$) that recovery across animals was similar. Study animal, and to a lesser degree prey size differences, feeding protocol, and natural variation, influenced the intraspecific variation observed for many species (Fig. 1).

Structure recovery rates were significantly greater for fish species with more robust otoliths than for species with thinner, fragile otoliths ($F_{[1,7]} = 51.2$, $P = 0.002$; Fig. 2). The larger size class of coho salmon made a poor fit, with recovery slightly less than that found for smaller salmon. Comparison with the captive harbour seal studies indicated that the slopes of the regressions were the same (Student's t test, $t_{[22]} = 0.45$, $P = 0.66$), but that the intercept for harbour seals was significantly larger (Student's t test, $t_{[23]} = 4.21$, $P < 0.001$).

Overall, fish recovery rates based on bones was 21.4% (SD = 30.4%) larger than recovery rates based only on otoliths (paired t test, $t_{[35]} = 4.22$, $P < 0.001$). In 10 out of 36 comparisons, values were the same (Table 1), but differences across prey species were clear (Fig. 3) with the impact of counting bones most dramatic for salmon (with counts based on otoliths typically 3–4 times lower than those based on bones) and herring (~2.5 times lower), and least dramatic for pollock and capelin (~1.15 times lower). Prey recovery

Fig. 3. Box plot of percent recovery of otoliths as a proportion of percent recovery of bones for each trial (Table 1, excluding regurgitations). Boxes denote 25th and 75th percentiles, lines denote to the 90th percentile, and ○ are outliers beyond the 90th percentile.

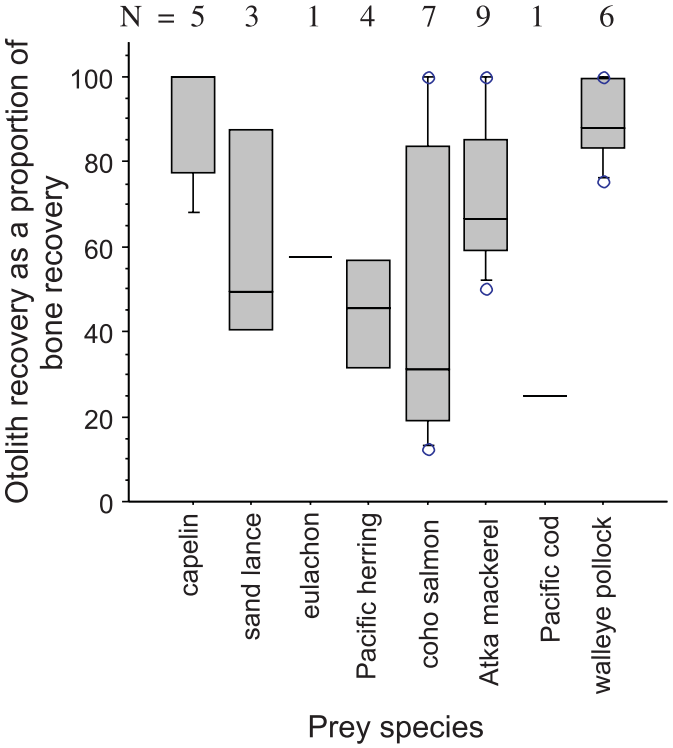
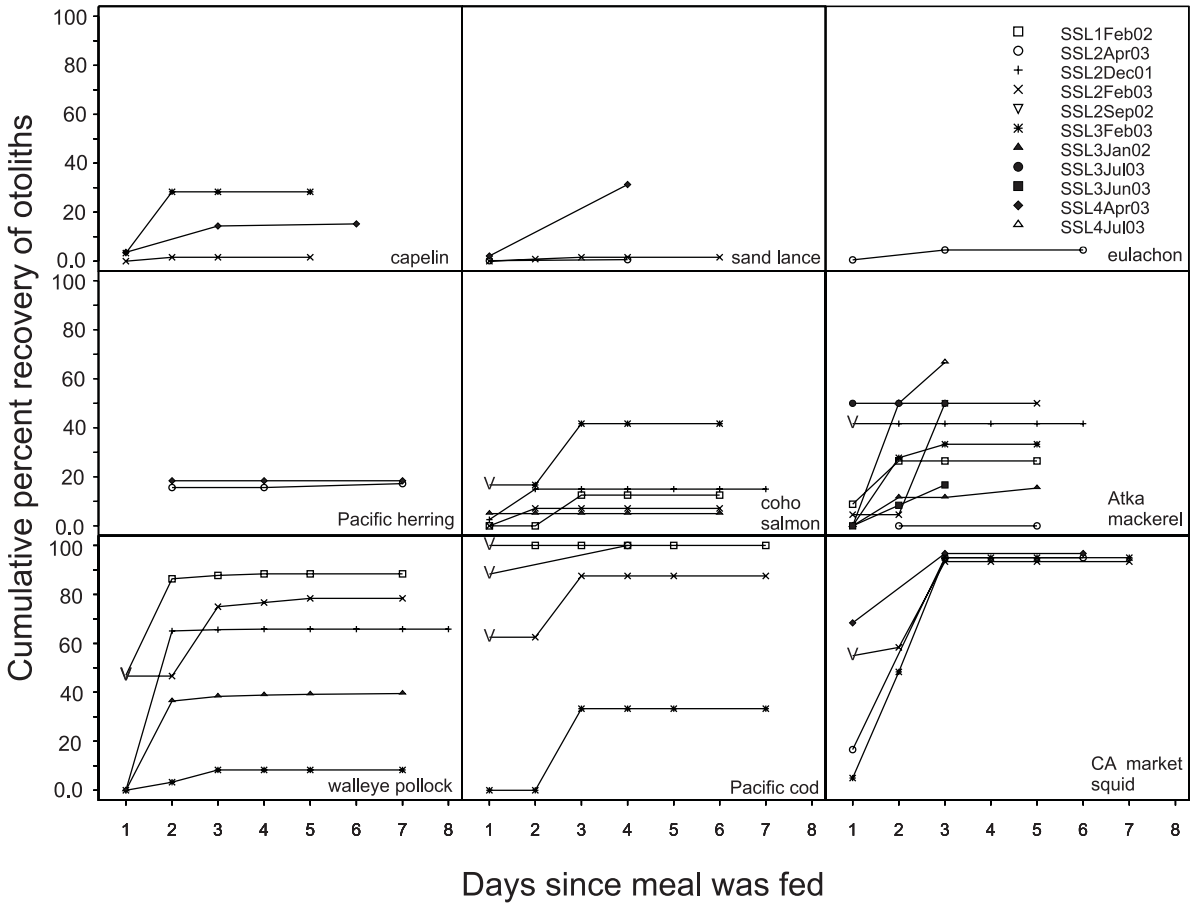


Fig. 4. Cumulative percent recovery of otoliths across days for each prey species. Each line depicts individual animal trials and a V depicts experiments in which a regurgitation event occurred.



using bones ranged between 0%–153%. In two trials where for which small numbers of fish were fed, counts of bones led to recovery rates in excess of 100%, a result of double counting fish in sequential scats from the same meal. This enumeration bias also frequently occurred in trials where regurgitations were observed. Overall, bone percent recovery of pollock (60.7 ± 26.1, n = 6), salmon (69.0 ± 54.1, n = 7), Atka mackerel (49.5 ± 31.3, n = 9), and herring (52.6 ± 17.8, n = 4) were clearly higher than for capelin (17.4 ± 9.8, n = 5) and sand lance (13.5 ± 15.6, n = 3), but differences were not significant (ANOVA, $F_{[1,5]}$ = 2.3, P = 0.08) (Table 1). Prey size effects were similar to that described for otoliths (recovery of small pollock was lower than for large pollock). When the four different animals were considered to be treatments, there was evidence to reject the null hypothesis that recovery among animals was similar (P = 0.04).

Passage rates

Overall, otoliths were egested over 2.9 ± 1.2 days (Table 1, Fig. 4), which was similar to bones recovered over 3.2 ± 1.2 days. The number of days otoliths were present in scats (1.9 ± 0.8 days, range 0–4 days) was less than that for bones (2.9 ± 1.1 days, range 0–5 days, P < 0.001). Species differences were plainly evident, with pollock otoliths occurring in more scats than otoliths of salmon or capelin (ANOVA, $F_{[1,5]}$ = 3.5, P < 0.05). However, otoliths and bones collected beyond day 3 generally contributed little to the total percent recovery of the feeding trial for all species (Fig. 4). Modal output equaled 2–3 days.

Numerical correction factors

NCFs were calculated from both mean otolith and mean bone percent recoveries (Tollit et al. 2003; this study; Table 2). The largest NCFs were calculated for capelin and sand lance (species that also had the widest 95% CIs) and lowest for large pollock and squid. Twofold to threefold differences were evident between otolith and bone NCFs for salmon and herring (Table 2).

Reliability of diet indices in replicated mixed meal feeding trails

Three 15-day mixed meal feeding trials were used to assess the reliability of two FO indices and two BR indices in predicting diet actually fed. Predictions are based on a relatively small sample size of scats per tank drains (~20–30 per trial) and relatively consistent diets. Both predictions of FO indices provided similar estimates (Table 3), but both suffered from clear limitations. Bones, but not otoliths, from all four species were found in the majority of scats and drains, and consequently, FO estimates based on bones tended towards 25% for all four species. Consequently, in both scenarios (2 and 3) for which a single prey dominated, FO underestimated the importance of the dominant prey by ~50% to 60%, and typically overestimated the importance of

Table 2. Numerical correction factors (NCF) with 95% confidence intervals (CI) for prey species and size class combinations based on percent recoveries of prey calculated from otoliths and bones.

Species	*N*	Mean size class fed (cm)	Otolith		Bone			*N* (across experiments)	Otolith		Bone	
			Percent recovery of prey	SD	Percent recovery of prey	SD	*N* (each animal)		NCF	CI	NCF	CI
Capelin	1316	14.3–14.8	12.7	8.4	13.8	9.8	5	3	7.87	4.98–18.74	7.26	4.47–19.19
Sand lance*	5593	10.3–11.2	17.4	14.2	19.7	11.0	8	3	5.75	3.67–13.23	5.09	3.66–8.28
Pacific herring*	1268	19.6–19.5	28.7	14.4	56.7	13.9	7	4	3.48	2.54–5.55	1.76	1.49–2.16
Salmon spp.*	132	28.8–44.0	21.9	2.7	62.1	34.8	9	3	4.57	4.23–4.97	1.61	1.18–2.54
Coho salmon	85	28.8–31.8	22.5	3.5	54.2	48.3	4	2	4.44	3.86–5.24	1.85	0.98–14.56
Salmon spp.*	47	39.7–44.0	20.3	0.4	69.9	32.4	5	2	4.93	4.84–5.01	1.43	1.02–2.41
Atka mackerel	94	33.4–36.9	38.0	20.2	55.0	23.4	9	4	2.63	1.95–4.03	1.82	1.42–2.52
Walleye pollock	460	13.4–15.4	45.7	28.9	48.8	25.1	3	2	2.19	1.28–7.69	2.05	1.30–4.90
Walleye pollock	701	26.9–29.6	67.5	0.0	76.9	0.0	2	1	1.48	1.48–1.48	1.30	1.30–1.30
Walleye pollock*	53	36.3–37.0	93.2	9.6	138.3	9.2	3	2	1.07	0.96–1.21	0.72	0.67–0.78
CA market squid	90	36–43	—	—	97.8	1.9	3	3	—	—	1.02	1.00–1.05

Note: Mean values were calculated with equal weighting given to each animal across experiments.
*Includes recovery data from Tollit et al. (2003).

prey fed in small amounts, notably capelin (and particularly when using bones to detect prey presence).

The two BR indices performed substantially better than the FO indices (Table 3, Fig. 5), particularly after applying NCFs. Both BR-V and BR-F provided similar diet estimates before applying NCFs, but BR-V provided superior estimates after the application of NCFs particularly when using only otoliths (12 of 12 comparisons were within 5% absolute of diet fed). When using bones with the application of NCFs, BR-V estimates were within 5% of diet fed for 68% of comparisons (and 75% of comparisons for BR-F), with all 12 comparisons being within 12% of diet fed (and within 14% of diet fed for BR-F comparisons). As expected BR-F (with scats weighted, like SSFO) provided better estimates for scenario 1 than BR-V, with BR-V performing better in predicting the two trials in which one species dominated (Table 3).

Using bones proved slightly better (60% of estimates were within 5% of what was fed) than using only otoliths (50% of estimates within 5% fed) if NCFs were not used in determining diet composition. Although BR estimates were able to distinguish among the three diet scenarios before applying NCFs, there was a clear tendency (as expected) to underestimate capelin (by more than half), particularly when fed in large amounts (scenario 1). The same held true for herring, particularly for scenario 2 in which herring contributed 67% of the diet fed but was underestimated by around one-third (Fig. 5). Pollock and salmon were generally overestimated (by about two-thirds, on average, but by more than twice when fed in small amounts) when using bones. Confidence intervals varied across diet scenarios and species, and were generally wider when using otoliths rather than bones (Fig. 5, Table S2[3]). The application of NCFs to BR indices still resulted in some biases. For example, capelin (when fed as a large percentage of a meal) and salmon (when fed as a small percentage of a meal) were somewhat overestimated (by ~30% and 50%, respectively) by bones. Herring was consistently (~20%) underestimated when using bones, but in contrast was overestimated (<10%) when using otoliths only (Table 3).

Discussion

The use of prey remnants recovered from scat samples is presently the primary method used to describe the diet composition of Steller sea lions and other pinnipeds. Our study used scats collected during controlled captive feeding studies with active Steller sea lions to investigate how the recovery of otoliths and bones varied by prey species and affected diet estimates. We also sought to provide robust prey NCF values, regressions to predict otolith NCFs, and recommendations about the performance of different diet indices (particularly BR-based methods).

Our study, like all captive feeding studies, had methodological limitations. Our four study animals were all females between 100 and 150 kg in body mass. Feeding protocols differed somewhat (Table S1[3]), and despite the ≥72 h period after each experimental meal, it was possible for small numbers of bones to have been retained in the stomach ruggae or intestine, and excreted after our experiments. Activity has been shown to affect the digestion rates of fish bones in

Table 3. Prey species percent diet contributions in replicated mixed meal feeding trials comparing percent biomass (B) fed (In) with estimated diet (Out) calculated for each species using only otoliths or bones egested.

			Walleye pollock		Coho salmon		Pacific herring		Capelin	
Diet	Indices	Trial	Otolith	Bone	Otolith	Bone	Otolith	Bone	Otolith	Bone
Based on diet fed										
In	B	1	12	12	29	29	30	30	29	29
	B, R	1	7	7	31	31	31	31	31	31
	B	2	23	23	7	7	67	67	3	3
	B	3	66	66	3	3	23	23	7	7
Out	FO	1	17^{++} / 19^{+}	19^{+} / 21^{+}	21^{+} / 23^{+}	**24^{++}** / **25^{++}**	**34^{++}** / **30^{++}**	**31^{++}** / **28^{++}**	**27^{++}** / **28^{++}**	**26^{++}** / **26^{++}**
	FO, R	1	14^{+} / 16^{+}	16^{+} / 19	18 / 22^{+}	23^{+} / **25^{+}**	39^{+} / **32^{++}**	**34^{++}** / **29^{++}**	**28^{++}** / **30^{++}**	**27^{++}** / **27^{++}**
	FO	2	39 / 33^{+}	**26^{++}** / **26^{++}**	10^{++} / 14^{+}	25 / 25	33 / 32	28 / 27	18 / 21	21 / 22
	FO	3	30 / 31	24 / 25	7^{++} / 9^{+}	20 / 22	41 / 35	32^{+} / **27^{++}**	21 / 24	24 / 25
No NCFs applied										
Out	BR	1	21^{+} / 24	15^{++} / 19^{+}	33^{++} / 38^{+}	39^{+} / 38^{+}	**31^{++}** / **24^{+}**	**34^{++}** / **31^{++}**	15 / 14	11 / 11
	BR, R	1	13^{+} / 7^{++}	10^{++} / 11^{++}	**27^{++}** / **37^{+}**	40^{+} / 41^{+}	39^{+} / **33^{++}**	**36^{++}** / **33^{++}**	19 / 23	13 / 15
	BR	2	49 / 44	31^{+} / 31^{+}	5^{++} / **6^{++}**	17^{+} / 12^{++}	44 / 48	51 / **56**	**2^{++}** / 1^{++}	1^{++} / 1^{++}
	BR	3	**76** / 86	**65^{++}** / **73^{+}**	**2^{++}** / **2^{++}**	7^{++} / 7^{++}	**19^{++}** / 10	**26^{++}** / **18^{++}**	2^{++} / 2^{++}	2^{++} / 2^{++}
NCFs applied										
Out	BR	1	**10^{++}** / 9^{++}	**10^{++}** / **11^{++}**	**28^{++}** / **34^{++}**	**32^{++}** / **30^{++}**	37^{+} / **30^{++}**	**28^{++}** / **24^{+}**	**25^{++}** / **27^{++}**	**31^{++}** / **35^{+}**
	BR, R	1	**6^{++}** / 2^{++}	**6^{++}** / **5^{++}**	**22^{+}** / **27^{++}**	**30^{++}** / **29^{++}**	42 / **34^{++}**	**28^{++}** / 23^{+}	**30^{++}** / **36^{++}**	**35^{++}** / 43
	BR	2	**26^{++}** / **20^{++}**	**23^{++}** / **23^{++}**	5^{++} / **6^{++}**	18 / 13^{+}	**63^{++}** / **71^{++}**	53 / **59^{+}**	6^{++} / **3^{++}**	5^{++} / 4^{++}
	BR	3	**59^{+}** / **65^{++}**	**55** / **62^{++}**	**3^{++}** / 4^{++}	8^{++} / 8^{++}	31^{+} / **24^{++}**	**28^{++}** / **21^{++}**	**6^{++}** / **6^{++}**	9^{++} / **8^{++}**

Note: Values in boldface type denote estimates within 20% of actual fed values; $^{+}$ and $^{++}$ denote estimates within 10% and 5% absolute of actual fed values, respectively. Values that are underlined denote estimates that are higher than diet fed. Two frequency of occurrence (FO) indices are compared (SSFO / MFO) and two biomass reconstruction (BR) indices (Fixed / Variable) are compared (without and with the application of numerical correction factors (NCFs)). Data from trial 1 was subsampled and diet recalculated to provide contributions when no evidence of regurgitation (R) was observed. Confidence intervals are provided in Table S2[3].

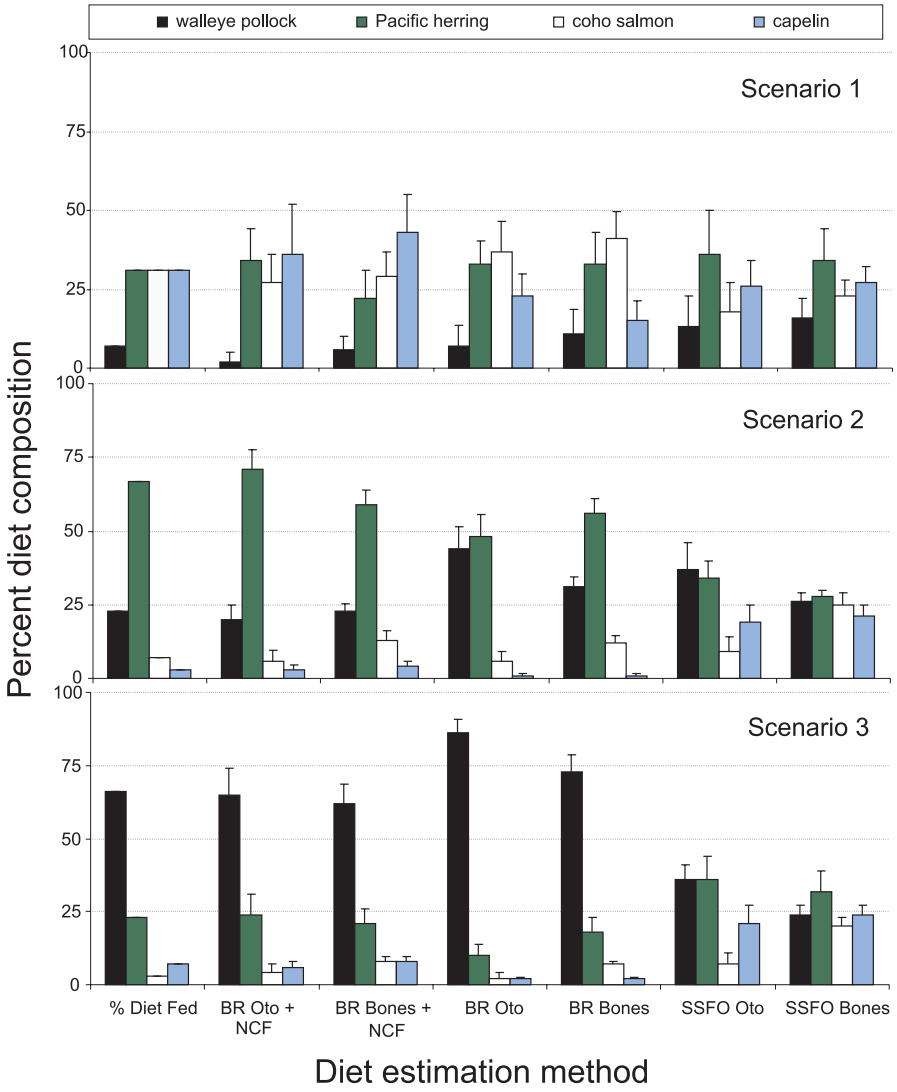
Steller sea lions, with lower recovery rates in inactive animals (Tollit et al. 2003). Animals in our study were typically kept in dry runs for a few hours during tank drains, with occasional longer standardized periods aiming to replicate an extended haul-out period in the single meal feeding trials. No extended haul-out periods were undertaken in the replicated feeding trials, but species recovery rates were comparable (Table 1), suggesting that these differences in our experimental protocols were not a major influence on the interspecific differences in prey recovery observed. Clearly, it is uncertain how well we managed to replicate the conditions experienced by free-ranging sea lions and further studies using various protocols are warranted. Overall, we consider our results relative rather than absolute values, and in calculating our final prey NCFs we have supplemented our study with comparable data from a previous published study (Tollit et al. 2003). Finally, we recognize care is needed to interpret data from some species for which the resulting sample sizes are still small.

Our study documented clear species differences in the recovery of prey and that these influence the ability to correctly determine diet when using bones or only otoliths. Only one meal (Atka mackerel) resulted in no identifiable structures recovered in subsequent scats. As has been demonstrated in many other captive feeding studies (Prime and Hammond 1987; Dellinger and Trillmich 1988; Harvey 1989; Cottrell et al. 1996; Tollit et al. 1997, 2003; Orr and Harvey 2001; Grellier 2006), prey species with large, robust otoliths, such as pollock, had greater percent recoveries than species with small or fragile otoliths, such as capelin or sand lance. Across species, we found fivefold differences in average otolith percent recoveries (Table 2). Variability within a species was caused by study animal effects (though statistical significance was marginal; Fig. 1), prey size effects, natural and experimental feeding variations, and because of regurgitations, exclusively when relatively large fish were fed (which occurred after 17.3% of experimental meals; Fig. 1). Small (<50 g) market squid were consistently recovered in large proportions (>93%), exceeding that of all other species fed (Table 1). In captive feeding trials of northern fur seals (*Callorhinus ursinus* (L., 1758)), Yonezaki et al. (2003) recovered large proportions (70%–100%) of small squid beaks in scats, and all larger beaks (dorsal mantle length > 160 mm) in regurgitation events. Clearly, regurgitated material should be collected if observed at haul-outs, because size-specific biases may often occur in fish prey as well (e.g., Gudmundson et al. 2006).

We found no statistical difference in otolith (or bone) percent recoveries between the two size classes of pollock or salmon fed. Prey size differences were only obvious if recovery data from larger adult pollock were included (Tollit et al. 2003). Recovery of juvenile (~14 cm) pollock was 2–2.5 times less than the recovery of adult pollock. Given that the mass of one adult fish represents >20 juvenile fish, our results indicate that the low prevalence of juvenile pollock found in the scats of free-ranging Steller sea lions in the Gulf of Alaska (Zeppelin et al. 2004) and southeast Alaska (Tollit et al. 2004*a*) during the 1990s was unlikely because of differences in digestion and subsequent recovery rates.

Steller sea lions consume many different fish prey species (e.g., Sinclair and Zeppelin 2002), but not all species are suitable for captive feeding studies. Thus, we attempted to

Fig. 5. Biomass reconstruction variable (BR-V) and split sample frequency of occurrence (SSFO) percent diet composition estimates for three replicated mixed meal feeding trials, depicting percent diet fed (regurgitated meals excluded in scenario 1) and diet predictions based on otoliths (Oto) and bones with and without the application of experimentally derived numerical correction factors (+NCF). Error bars reflect 95% confidence intervals.



use otolith robustness as an index to predict otolith percent recoveries, and hence NCF values. We found a significant linear relationship using our Steller sea lion data (Fig. 2), but although the slopes of the regressions were similar, recovery was lower for sea lions than for captive harbour seals (Harvey 1989; Tollit et al. 1997) (Fig. 2). A number of captive studies with otariids have highlighted the scarcity of otoliths in scats (Gales and Cheal 1992; Casper et al. 2006), and the latter study proposes that the mixed diets fed to animals in these studies may result in reduced recovery rates compared with studies on feeding single-species diets. A wide range of factors can affect digestion (see reviews by Pierce and Boyle 1991; Bowen 2000; Tollit et al. 2006), but available data (including our analysis) suggest that direct extrapolation of otolith NCF values between phocid and otariid diet studies should not be done.

Our study documented certain advantages of using bones to assess the number of prey consumed. First, using bones compared with using only otoliths increased the mean prey percent recoveries for all species, but not consistently across species (Fig. 3). The additional use of bones had little effect on recovery for pollock and capelin (~15%) compared with the increase in recovery of herring (by ~2.5 times) and salmon (typically by 3–4 times). When only a small number of salmon were ingested, a relatively small increase in the estimate of the number of prey egested using bones (sometimes exacerbated by enumeration biases) resulted in large increases in our calculated percent recoveries. Consequently, across the nine salmon feeding trials with three animals (Table 2), otolith recovery was consistently lower (mean 22%) but also less variable than recovery rates using bones (mean 62%). Other than otoliths, the main structures used to determine prey number using bone MNI techniques were radial, hypercoracoid, vertebrae, hypohyal, and quadrate bones for coho salmon, and hypohyal, prootic or sphenotic, vertebrae, and pelvic bones for herring. As seen in Tollit et al. (2003), pollock were best enumerated using dentary, angular, quadrate, and interhyal bones, in addition to otoliths,

whereas for capelin, additional key structures to determine MNI were quadrate, vertebrae, operculum, and dentary bones.

Using bones increased fish recovery and reduced differences among species, and although resulting differences were still as large as fivefold (Tables 1, 2), they were no longer statistically significant ($P = 0.08$). However, recovery of the two smallest forage fish species (sand lance and capelin) clearly differed from the other seven prey species fed (Table 2). Bowen (2000) noted that forage fish will likely be underestimated if BR techniques are applied to otolith data without applying NCFs. Our study suggests that this is also likely to be the case if BR techniques are applied to bone data.

Our study has resulted in NCFs for both otolith and bones for seven key prey species of Steller sea lion (Table 2). Our NCFs take into account considerable species differences in the proportion of prey eaten that are completely digested. Differences across study animals were marginal for otolith recovery, but were significant for bone recovery. Therefore, NCF data were weighted evenly across study animals (Table 2).

Numerical correction factors should ideally be applied as a multiplier to the estimated mass of each prey item before combining scat data and reconstructing the overall diet. However, alternative techniques can be adopted such as applying the NCF to the total number of otoliths or prey before integrating with a mean or median mass of prey (Harvey 1989; Browne et al. 2002).

Our capelin otolith and small squid beak NCF values were similar to those found for California sea lions by Orr and Harvey (2001), but our herring otolith NCF value was twice as large in comparison and was more comparable with herring NCF values estimated for harbour seals by Harvey (1989), Cottrell et al. (1996), and Tollit et al. (1997). The only other captive Steller sea lion feeding study (Cottrell and Trites 2002) calculated NCF values from recovery based on counts of otolith structures (not counts of prey). In comparison, our results for pollock, salmon, and herring recovery were similar, but results were not comparable for Atka mackerel, which were recovered seven times more frequently (29% vs. 4%) in our study. We saw large within and across animal variability in the recovery of Atka mackerel otoliths (Fig. 1) and study protocols varied. Cottrell and Trites (2002) also fed slightly larger sizes (40.4 ± 4.7 cm total length) of Atka mackerel to younger (age 1–3 years) study animals.

No study to date has calculated bone NCF values for comparison. Our baseline values were between 7.26 (capelin) and 0.72 (large pollock). Having the large pollock NCF value <1.0 was due to double-counting fish using different structures in sequential scats following a single meal (see Tollit et al. 2003). This shortcoming of using bones to enumerate small numbers of prey was shown theoretically in computer simulations by Joy et al. (2006) and also occurred in two of our trials (Table 1) in which small numbers of relatively large fish were consumed.

Our study confirmed the work of Tollit et al. (2003) that bones and otoliths from a single experimental meal are typically distributed in 2–3 scats over 2–3 days, with bones occurring in more scats compared with otoliths (Table 1). Collection of multiple scats from the same animal at a haulout has been shown to occur in scat DNA studies conducted by Kvitrud et al. (2005), with obvious consequences for possible sampling bias. Clearly, it should not be assumed that scats represent merely the last 24 h of feeding. Despite the relatively controlled nature of these studies, variation in prey percent recoveries for salmon, sand lance, Atka mackerel, and small pollock were all high — suggesting the need for collecting additional data using a variety of protocols, ages, and sexes to assess the full extent of variability.

The three replicated mixed meal studies were used to assess the reliability of different diet indices using different combinations of hard remains (only otoliths vs. bones). We recognize that although the NCF values are based on many different feeding trials (Table 2), the NCFs applied in this assessment were at least in part based on the percent recoveries from the replicated trials themselves, and from the same size and sex of animal. Nevertheless, we believe our results are useful for assessing levels of bias in each diet index under best case circumstances.

We found no support for the hypothesis that extremely low otolith recovery was linked with feeding mixed species meals to Steller sea lions, as was seen in *Arctocephalus* seals (Casper et al. 2006). Our findings agree with Casper et al. (2006) that FO indices were the least reliable in distinguishing among the three diet scenarios fed (Fig. 5, Table 3), particularly when we used bones. FO indices based on bones tended to conclude that all prey were equally consumed, because both of the FO indices we tested gave each scat equal weight, and a large proportion of scats contained at least some identifiable evidence of all four species. This resulted in underestimating prey when its contribution was large and overestimating prey when its contribution was small. Consequently, FO indices gave good predictions only when prey contributed to the diet in relatively similar amounts (scenario 1; see Table 3, Fig. 5). We recognize that FO indices are potentially useful in determining the predominance of prey in the diet given large sample sizes (see Olesiuk et al. 1990), and computer simulations indicate that around 100 scats are needed to assess geographical and temporal variations (Trites and Joy 2005).

FO indices are most useful when diet diversity of meals (number of species in a scat) is low. This implies that particular care should be taken in interpreting any analysis of prey occurrence to describe diet composition if initial analyses of scats indicate high species diversity per scat. Overall, the replicated and varied mixed diet scenarios in our study (scenarios 2 and 3) probably represent the greatest challenge to obtaining reliable diet predictions using FO indices. Our data does indicate that despite feeding relatively fragile prey in small quantities, the all structure approach does consistently identify prey presence. In studies based on scats from the field, Olesiuk (1993) reported that SSFO diet composition percentages for key prey varied by a factor of two or three, depending on the assumed composition within each scat. Clearly, occurrence indices are more readily produced, as they require no prey counts or prey size calculations.

In contrast to FO methods, BR methods were able to distinguish among the three diet scenarios (Fig. 5). Before the application of NCFs, bones provided slightly better estimates than using only otoliths, but both overestimated pol-

lock and underestimated capelin and herring, as might be expected given their relative percent recoveries. Laake et al. (2002) assessed harbour seal diet from bones and compared results using SSFO and BR-V indices. Their study found a clear relationship between prey size and diet estimates between indices, with SSFO relative to BR providing considerably smaller values for large fish (>1 kg) and larger values for small fish (<10–20 g). Differences in estimators were primarily the result of using a weighted vs. an unweighted diet model, but differences may have been reduced if NCFs had been applied. Our study only fed prey between 24 and 434 g, but generally showed a similar (though weaker) bias, with the exception of large prey fed in small amounts. The tendency of FO indices to underestimate the contribution of large prey (coupled with its skeletal fragility or robustness) suggests that Pacific salmon (*Oncorhynchus* spp.), which occurred in ~20% of western stock Steller sea lions scats through the 1990s and exceeded 40% occurrence in some regions (Sinclair and Zeppelin 2002), may be of primary importance in certain regions in terms of mass contribution to the summer diet. Similarly, the importance of Pacific cod in winter is likely to be magnified. The dominance of Atka mackerel in the Aleutians is unlikely to change if a mass-based approach was taken, given its overwhelming dominance by occurrence. Information on numbers and sizes of different prey is thus required to justify such speculation.

Application of NCFs resulted in improved diet estimates, especially those based on variable mass contribution per scat (unweighted) and otoliths alone, in which all 12 comparisons were within 5% (absolute value) of that fed. Bones provided diet estimates within 14% of the actual diet fed, with BR-V clearly a better estimator than BR-F for scenarios 2 and 3, in which one prey species dominated. Our results are consistent with the premise that output of prey remains were pulsed and random in nature and that diet models, such as BR-V, that are able to take this into account may provide more reliable estimates of diet. The BR-V model allows for the fact that pinnipeds are unlikely to consume the same amount of prey in each meal and that foraging success may vary through time and across individuals. The obvious limitation of an unweighted approach is the potential for the contribution of a few scats to outweigh the remaining scats. Computer simulations of scats collected in the field suggest that ~100 scats are necessary to reduce such sampling errors (Hammond and Rothery 1996), and the bootstrap techniques used in this study provide an effective (and important) method to describe the level of confidence around calculated diet estimates.

The identification and enumeration of bones in addition to otoliths is time consuming and requires considerable taxonomic experience. Use of additional bones can increase detection rates in many species (Olesiuk et al. 1990; Tollit et al. 2003; this study), decreasing interspecific differences, and for certain species (e.g., salmon and herring), dramatically improve estimates of the number of prey consumed. The results of our small-scale diet index comparison suggest that bones provide marginally better predictions of diet using BR indices than otoliths if NCFs are not applied. However, after NCFs are applied, the use of otoliths alone combined with a BR-V index provided better diet estimates that were generally similar to the diet actually fed. Applying NCFs to bones improved our estimates of diet. However, they were somewhat less reliable than otoliths, with notable over-estimates of salmon when fed in small amounts. The differences between the two combinations of structures used were likely related to MNI enumeration using bones, and factors such as the large variability around the salmon bone percent recovery (CV = 0.89) compared with the salmon otolith percent recovery (CV = 0.15) (Table 2).

Overall, BR models (coupled with NCFs) can theoretically provide satisfactory predictions of mixed prey diets; however, we recommend using an integrated approach to reconstructing diet and understanding the role of Steller sea lions as marine predators. An integrated approach is required because of the variability involved in estimating NCFs and enumerating prey, coupled with the hard to quantify limitations of hard remains analysis of Steller sea lion scat (such as the impacts of regurgitation, the frequent presence of rocks in the stomach, secondary prey issues, and identifying prey without hard parts). Fatty acid signature analysis of predator lipid stores (Iverson et al. 2004) can track major dietary shifts in captive Steller sea lions integrated over multiple months (D. Tollit and S. Iverson, unpublished data) and, together with molecular genetic approaches (Jarman et al. 2004; Purcell et al. 2004; Deagle et al. 2005), may be useful in highlighting important diet components that may be missed or underrepresented using only hard remains. New molecular genetic (Deagle et al. 2005; Deagle and Tollit 2007) and near infrared spectroscopy (Kaneko and Lawler 2006) techniques both merit further study in quantifying diets using fecal samples.

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