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# QUANTIFYING ERRORS ASSOCIATED WITH USING PREY SKELETAL STRUCTURES FROM FECAL SAMPLES TO DETERMINE THE DIET OF STELLER'S SEA LION (*EUMETOPIAS JUBATUS*)

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## ABSTRACT

We examined the digestion and passage times of bones and other hard parts from pollock, herring, salmon, and sandlance recovered from two juvenile captive Steller's sea lions (*Eumetopias jubatus*) subjected to varying activity levels. Key bones that could be identified to species were distributed over an average of 3.2 scats (range 1–6) following a single meal, with pollock remains occurring in significantly more scats than other species. Relying on otoliths alone to determine the presence of prey resulted in significantly fewer prey being identified than if other structures were also used (such as vertebrae, jaw bones, and teeth), particularly for salmon. Using either technique, there were significant differences in the likelihood that bones would be recovered from the series of scats produced following a meal, with pollock recovery exceeding herring (by three-fold) and sandlance (by eight-fold). Differences between species were reduced when recovery was calculated on a per scat basis rather than over multiple scats. Active animals passed greater numbers of bones, but the overall effect on prey recovery estimates was not significant. Defecation times of prey structures from a meal were variable and ranged from an initial 2–56 h to a final 28–148 h. The time interval to pass 95% of recovered structures varied by a factor of two among prey species, and was highest for pollock due to retention beyond 65 h.

Key words: Steller's sea lion, *Eumetopias jubatus*, diet, feces, scat, hard parts, bones, otoliths, passage rates, captive feeding.

The analysis of prey skeletal structures found in scats (feces) is now the most widely used technique for estimating the diet of pinnipeds, with sagittal otoliths being the most commonly used structure (Frost and Lowry 1980, Olesiuk *et al.* 1990, Bowen *et al.* 1993, Tollit and Thompson 1996). However, there remain a number of well-recognized problems related to differential rates of digestion (hence, recovery) and choice of skeletal structures used to identify prey (see reviews by Pierce and Boyle 1991, Bowen 2000). All available data indicate that the importance of species with small, fragile otoliths (*e.g.*, clupeids, smelts and salmonids) will be underestimated in a diet that also includes species with large, robust otoliths (*e.g.*, gadoids), if otoliths alone are enumerated and measured to reconstruct the biomass of each species consumed. The extent of this interspecific bias remains open to debate, mainly due to the wide range of factors that appear to influence digestion (Bowen 2000).

In theory it should be relatively simple to generate numerical correction factors (NCF) to correct for differential recovery, by comparing known numbers of fish fed, to estimates derived from reconstructing the number of prey consumed, by back-calculating from the number of paired (or unique) structures that survive digestion. However, recent captive feeding studies have indicated that, even within a single prey species, the digestion of paired structures such as otoliths is influenced by prey size (Tollit *et al.* 1997), meal size (Marcus *et al.* 1998), and size of the animal (Cottrell *et al.* 1996). A review of nine captive feeding studies also found differences in NCFs related to species of pinniped and level of activity, but clear interpretation was confounded, mainly by variability in experimental protocol (Bowen 2000). Clearly, experimental feeding protocols need to be standardized, realistic, and fine-scale enough to assess levels and causes of observed variability before results can be applied with confidence to scat data from the wild.

Low otolith recovery percentage of some prey species, plus the perceived difficulties in estimating reliable NCFs, has led many researchers to move from using exclusively fish otoliths and cephalopod beaks to what is termed the "all structure" technique (*i.e.*, all recovered skeletal hard remains such as vertebrae, gill rakers, and jaw bones are identified to their lowest possible taxonomic group). In one such study Olesiuk *et al.* (1990) demonstrated that Pacific herring (*Clupea pallasii*) and salmonids would have been underestimated by a factor of 1.6 and 10.4, respectively, had only otoliths been used to identify prey. Olesiuk *et al.* (1990) then calculated diet composition by applying a method termed split-sample frequency of occurrence (SSFO). An advantage of the SSFO method is that it does not require enumeration or size estimation of prey (as in biomass reconstruction). However, the method is potentially limited by assuming that hard remains in a scat represent each species eaten in the previous 24 h of feeding, and that the prey species contained within each scat were consumed in equal quantities. In accordance with all such dietary studies, it also tacitly assumes that meals of different prey species are equally represented in subsequent scats. However, no passage time data exist for Steller's sea lions (*Eumetopias jubatus*) or for many North Pacific prey, and no pinniped study to date has assessed passage times for all structures of any fish species. Similarly, no captive study has assessed the reliability of the all structure technique to enumerate the number of prey present in scats. Furthermore, data on the recovery percentage of skeletal structures other than otoliths surviving in scats remains scant.

The main objective of our study was to provide baseline values on the extent of inter- and intraspecific variability in the deposition, passage times, and recovery

percentage of diagnostic prey hard remains passed though the digestive tract of two juvenile female Steller's sea lions (SSL). We investigated four key prey: walleye pollock (*Theragra chalcogramma*), Pacific herring, sandlance (*Ammodytes* sp.), and pink salmon (*Oncorhynchus gorbuscha*). Factors investigated included activity level, study animal, and the impact of regurgitations on recovery of prey remains. Additionally, we assessed and compared estimates of the number of prey consumed using only otoliths with those derived from the all structure technique.

## Methods

### *Feeding Experiments*

Experiments were conducted with two 3-yr-old female Steller's sea lions (SSL1 #F97HA, mean mass 119 kg; SSL2 #F97SI, mean mass 139 kg) from 1 February to 6 June 2001 at the Vancouver Aquarium Marine Science Centre. Sea lions were maintained on a diet of Pacific herring at ~6% of their body mass (BM) per day, and were housed individually either in a continuously flowing saltwater swim tank ($5 \times 2 \times 2$ m, equipped with a $1 \times 2$-m haul-out platform) or in a grated dry run ($1.8 \times 2.5$ m). A swing door in the swim tank allowed the platform to be closed and a plastic sheet beneath the grated dry run allowed scats to be collected. Both animals undertook three 15-d feeding trials (Table 1). Each trial was designed to incorporate two identical 4-d bouts, differing only in the level of activity (one of the factors investigated in our study). Each trial began with a 68-h period with full access to water (days 1–3) and meals of filleted or headless herring to clear the digestive tract of diagnostic hard remains. From 1130 on day 3, animals were moved to a dry run and fasted for 24 h to simulate a resting period on land. In four trials an Actiware-Rhythm Monitor (Mini-mitter Co., U.S.A.) was glued to the fur on the animal's back to monitor activity levels (sampling rate = 2 min). The "active" bout of each trial began by moving the animal to the swim tank at 0830 on day 4. The first half of the first experimental meal (day 4, 1130, 3.5% BM) was comprised of 2.6% BM of pollock, followed 20 min later by 0.9% BM of herring (designed to simulate a successful foraging event on two prey patches). One herring contained 20 colored plastic beads (10 each of 2.3 and 4.2 mm diameter). Each animal then undertook a 3 h 50 min session in an enclosed swim mill designed to simulate active foraging behavior. Water flow speed was maintained at approximately 1.1 m/sec and the animal was observed for evidence of meal regurgitation. On exiting the swim mill, a meal of similar size, composition, and feeding frequency as the first was fed, and the animal was returned to the swim tank (which had been drained and cleaned during the swim mill session). The door allowing access to the haul-out platform was closed within 30 min to maximize overnight swimming time and hence high activity levels (Table 1).

At 0830 on day 5, the animal was moved to the dry run, where individual scats were collected and frozen, and the time of defecation was recorded using a time-lapse video recorder. Concurrently, the tank was drained and cleaned, and the contents filtered through a 0.5-mm nylon mesh. At 1130, a single meal of sandlance was fed (2.25% BM), and at 1530 the animal was returned to the swim tank (again with no access to the haul-out). Day 6 was similar to day 5 except that two experimental meals of pink salmon were fed (3.5% BM) at 1130 and 1530. Overall, the feeding regime aimed to simulate short foraging trips as exhibited by free-ranging SSLs during summer (Higgins *et al.* 1988, Andrews *et al.* 2002).

*Table 1.* Example of feeding trial protocol. Meal sizes are expressed as a percentage of body mass (HH = headless or filleted herring, no HO = no access to the haul-out platform).

| | Time of day | | | | |
|---|---|---|---|---|---|
| Day | 0830 | 1130–1200 | 1530–1600 | Overnight | Notes |
| 1 | HH (3.5%)<br>Tank | | HH (3.5%)<br>Tank | Tank | Glue on activity monitor |
| 2 | HH (3.5%)<br>Tank | | HH (3.5%)<br>Tank | Tank | |
| 3 | | HH (5.0%)<br>Dry run | | Dry run | |
| 4 | Tank | Pollock (2.6%)<br>Herring (0.9%)<br>Swim mill | Pollock (2.6%)<br>Herring (0.9%)<br>Tank (no HO) | Tank (no HO) | 20 beads at 1130<br>Clean tank AM |
| 5 | Dry run | Sandlance (2.25%) | Tank (no HO) | Tank (no HO) | Clean tank AM |
| 6 | Dry run | Salmon (3.5%) | Salmon (3.5%)<br>Tank (no HO) | Tank (no HO) | Clean tank AM |
| 7 | | HH (5.0%)<br>Dry run | | Dry run | Clean tank AM |
| 8 | Dry run | Pollock (2.6%)<br>Herring (0.9%) | Pollock (2.6%)<br>Herring (0.9%) | Dry run | 20 beads at 1130 |
| 9 | Dry run | Sandlance (2.25%) | | Dry run | |
| 10 | Dry run | Salmon (3.5%) | Salmon (3.5%) | Dry run | |
| 11 | Dry run | HH (5.0%) | | Dry run | |
| 12 | Dry run | (HH 3.5%) | HH (3.5%) | Dry run | |
| 13 | Dry run | HH (3.5%) | HH (3.5%) | Dry run | Remove monitor |
| 14 | Dry run | Salmon fillets (3.0%) | HH (4.0%) | Tank | |
| 15 | Tank | End | | | Clean tank |

From day 7 onwards SSLs were maintained in an "inactive" state in the dry run and received analogous meals on days 7–11 as those fed on days 3–7, with the addition of 20 different colored beads on day 8 (Table 1).

Lengths and masses of pollock, herring, and salmon were recorded to $\pm 1$ mm and $\pm 1$ g, respectively. Size ranges were kept as narrow as possible to reduce the confounding effects of prey size. Sandlance were grouped in fixed proportions of three single cm size classes and weighed as a whole. Mean number and size ($\pm$SD) of each species fed to each animal are summarized across trials in Table 2. Between-trial consistency was good except for the final trial of SSL2, (during which only 1.5% BM of sandlance and a wider size range of salmon were fed).

Each sea lion was fed only fillets of herring or salmon, or headless herring for a further three-day period in the grated run (days 11–14), followed by a single night in the swim tank, to ensure that all experimental hard remains surviving digestion were collected. In addition to allowing the effects of activity level to be investigated, this inactive period (days 7–14) provided more accurate information on passage times and defecation patterns. The ability to recover all fish skeletal structures from the swim tank was tested by scattering either 30–36 marked otoliths and vertebrae of pollock, herring, and sandlance or 60 small (2.3–4.2 mm)

*Table 2.* The mean number (*n*), length (L) in cm and mass (M) in grams of individual prey fed to Steller's sea lions (SSL), and coefficient of variation of total meal size (CV) averaged across trials for each bout. Standard deviation (SD) is given in parentheses.

| SSL no. | Prey species | Bout | No. of trials | *n* | L[a] | M | CV |
|---|---|---|---|---|---|---|---|
| 1 | Pollock | Active | 3 | 15.0 (1.0) | 37.0 (0.5) | 398.7 (21.9) | 0.04 |
| | | Inactive | 3 | 16.3 (3.2) | 36.0 (1.6) | 367.2 (44.9) | 0.04 |
| | Herring | Active | 3 | 20.0 (0.0) | 19.5 (0.3) | 92.0 (3.8) | 0.02 |
| | | Inactive | 3 | 20.0 (0.0) | 19.6 (0.3) | 91.3 (5.6) | 0.02 |
| | Sandlance | Active | 3 | 744.3 (9.8) | 10.3 (0.7) | 3.6 – | 0.01 |
| | | Inactive | 3 | 748.0 (2.6) | 10.3 (0.7) | 3.5 – | 0.05 |
| | Salmon | Active | 3 | 6.7 (0.6) | 39.7 (2.2) | 1005.0 (186.6) | 0.07 |
| | | Inactive | 3 | 7.0 (1.0) | 41.0 (1.9) | 1111.9 (44.9) | 0.13 |
| 2 | Pollock | Active | 3 | 20.7 (1.2) | 36.3 (1.1) | 381.2 (37.8) | 0.04 |
| | | Inactive | 3 | 18.7 (1.2) | 37.0 (1.7) | 403.9 (19.6) | 0.07 |
| | Herring | Active | 3 | 20.0 (0.0) | 19.6 (0.3) | 92.6 (4.6) | <0.01 |
| | | Inactive | 3 | 20.0 (0.0) | 19.6 (0.3) | 93.1 (4.6) | <0.01 |
| | Sandlance | Active | 3 | 742.3 (228.1) | 10.3 (0.7) | 3.8 – | 0.24 |
| | | Inactive | 3 | 739.0 (233.8) | 10.3 (0.7) | 3.7 – | 0.24 |
| | Salmon | Active | 3 | 6.7 (1.5) | 43.3 (2.0) | 1302.5 (189.4) | 0.29 |
| | | Inactive | 3 | 7.0 (1.0) | 45.5 (2.1) | 1496.4 (227.2) | 0.15 |

[a] Fish lengths (L) are fork length for pollock and herring, total length for sandlance and standard length for salmon. See methods for details regarding estimates of sandlance length and mass.

plastic beads in the tank. This was done on six separate occasions, 1 h before draining.

### *Identification of Fish Hard Parts*

Individual scats were washed through a 0.5-mm sieve to recover and dry hard parts. Reconstructing prey biomass requires an estimate of the number of prey consumed from the structures recovered. The basic principle of using the Minimum Number of Individuals (MNI) technique is to avoid counting the same animal twice, by finding the minimum number of individuals represented by all structures in a sample (Ringrose 1993). We determined the key diagnostic structures to estimate MNI based on those used by Pacific IDentifications Inc. (Victoria, BC) for 3,720 scats collected from free-ranging SSLs in Southeast Alaska (Trites and Calkins, unpublished data). Using this information, two independent observers (DT and MW) identified and counted 2–16 key diagnostic structures per species using reference collections (Table 3). These structures accounted for 90%–97% of MNI estimates in the above analysis. Sorting continued until no new material was identified by either observer. One observer (DT) sought additional structures that might increase MNI estimates, but none was found. Verifications of all uncertain identifications were made by Pacific IDentifications Inc. Location of bones in fish skeletons are found in Cottrell and Trites (2002) and naming follows Rojo (1991).

We noted when samples contained bones found in a state that precluded passage though the digestive tract (*i.e.,* bones looked clean and undigested, vertebrae were

*Table 3.* Key diagnostic structures selected to estimate the number of fish recovered in experimental scats (presented in order of importance in their use in MNI estimates) based on their occurrence ($N_{prop}$) in 3,720 scats collected in Southeast Alaska (Trites and Calkins, unpublished data). *n* denotes the number of scats containing each prey species.

| Species | Key diagnostic structures selected | $N_{prop}$ | *n* |
|---|---|---|---|
| Pollock | *Category A*—angular, dentary, post cleithrum, quadrate, epihyal, vertebrae, hypobranchial, epibranchial, ethmoid, otolith, interhyal, pharyngobranchial, subopercle, vomer, premaxilla<br>*Category B*—gill raker | 0.94 | 1,618 |
| Herring | *Category A*—vertebrae, prootic, pterotic, otolith, opisthotic<br>*Category B*—gill raker | 0.90 | 846 |
| Sandlance | *Category A*—vertebrae, otolith | 0.97 | 482 |
| Salmon | *Category A*—vertebrae, hypercoracoid, radial, otolith, hypural, hypohyal, interhyal<br>*Category B*—gill raker, teeth, branchial | 0.91 | 774 |

Note: Category A structures can be used to estimate the number of prey, while category B structures indicate only a presence, and consequently a minimum of one individual.

still joined together with processes intact, bones were of a size to exclude passage though the pyloric sphincter, and/or small amounts of undigested flesh were present). These samples were considered to be from regurgitations. It is commonly accepted that data from scats and regurgitations should not be combined during data analysis (Fea *et al.* 1999). We therefore excluded regurgitations from further analyses (7 of 36 experimental meals) given that our primary goal was to analyze scats and not regurgitations. Nevertheless, we provide pertinent information on recovery percentage of prey in the 7 experimental meals that were regurgitated.

### *Estimates of Structure and Prey Recovery Percentages*

Recovery percentages (%) were calculated using two methods. The first provided information at the individual structure level, by dividing the number of structures recovered for a given prey species by the total number of structures fed, multiplied by 100 (Cottrell and Trites 2002). The second method uses MNI estimates for each key structure found in each scat or tank drain (see below and also Laake *et al.* 2002). The maximum MNI estimate (both from all key structures and, for comparison, from otoliths only) was then summed across scats. Prey recovery percentage was then calculated as the estimated total number of individual fish recovered divided by the number of fish fed, multiplied by 100. Different methods of calculating MNI are needed because skeletal structures can be individual, paired, or multiple. We determined left and right sides (where possible) of paired unique structures such as otoliths and considered the MNI to be the greatest number of left or right elements. Where we were unable to determine the side of a paired bone, we divided the number of hard parts recovered by two and rounded up to the nearest integer. If the sample contained a mix of known and unknown sides, the unknowns were added to the known side with the fewest number until both sides were balanced in number. The remaining number of unknown sides was divided by two and

the quotient added to the previously balanced total. Direct counts were used for individually occurring structures such as the atlas vertebrae. Only for sandlance (66 vertebrae/fish) and salmon (65 vertebrae/fish) were vertebrae used to calculate the proportion of prey recovered. Counts of vertebrae were divided by the average number of vertebrae per fish (Hart 1973). When prey were exclusively represented in a scat by other non-unique structures (*e.g.*, gill rakers, teeth), a MNI of one was applied.

On 3 of 164 occasions, a large scat was produced, followed by one or two very small ones less than 1 h later. In all three cases, the MNI from the "whole" scat was estimated from all scats combined. This is consistent with combining scats found in the field of similar color and freshness when in close proximity. In most cases (>83%), we used the presence of "empty" scats between the active and inactive feeding bouts to separate the origin of structures from the same species into either one bout or the other. Bead color present in scats, as well as the state of erosion, the number and species combination of structures allowed for a confident separation of the remainder of cases, where no "empty" scats were observed.

### *Passage Times and Scat Output*

Our experimental design allowed data to be collected on initial (IDT) and final (FDT) defecation times. We noted that FDT was greatly influenced by the recovery of one or two structures, a long period of time after the bulk of structures had been egested. While we collected data on FDT, we considered a more useful comparative measure to be the time in which we found 95% of all recovered bones (95% DT). We also calculated the "output interval" as 95% DT minus IDT to estimate the time span during which most bones were egested. We were not able to record the time of defecation while animals were in the tank. Therefore, we used time of tank exit (which represents the maximum possible passage time) for the analysis of passage times. Typically, this led to overestimating IDT, and underestimating the output interval (for active bouts) by 0–17 h, but this reduced precision should be consistent between experimental animals. We also assumed remains recovered from a tank drain originated from a single scat, leading to a possible underestimate in active trials of estimated "scat output," the term used to denote the number of scats in which remains from a single experimental ingestion event were recovered.

### *Statistical Analysis*

Our study provides informative fine-scale data on the digestion and passage of bones. Both animals undertook three repeated trials. However, sample size limitations (due to regurgitations in certain active bouts) required pooling data across trials prior to analysis and removed any within-animal effects. We recognize the loss of power in such an approach, and provide the standard deviation (SD) of the mean as a measure of variability across trials at the individual level in all relevant Tables and Figures. A repeated-measure ANOVA (with prey species and activity level as factors and individuals as the repeated measure) was used to test for differences in mean prey recovery percentages (all structures and otoliths only), passage times (IDT, FDT, 95% DT, and output interval) and scat output (Siegel and Castellan 1988). Some estimates of prey recovery percentage exceeded 100% because certain prey structures were deposited across multiple scats (see Discussion

*Table 4.* Recovery percentage (mean % ± SD) of structures for four prey species fed to two Steller's sea lions. Data are averaged across animals and trials (*n*) Active bouts, Inactive bouts, and active and inactive bouts combined (Pooled). Data are for structures with a pooled recovery percentage of >1.4%.

| Prey species | Structure | Bout recovery percentage | | | | | |
|---|---|---|---|---|---|---|---|
| | | Inactive | *n* | Active | *n* | Pooled | *n* |
| Pollock | Otolith | 63.1 ± 1.5 | 6 | 95.5 ± 7.9 | 3 | 73.9 ± 30.0 | 9 |
| | Dentary | 9.5 ± 12.9 | 6 | 35.2 ± 18.6 | 3 | 18.1 ± 18.9 | 9 |
| | Angular | 8.2 ± 7.5 | 6 | 30.4 ± 13.3 | 3 | 15.6 ± 14.2 | 9 |
| | Interhyal | 11.0 ± 12.0 | 6 | 20.2 ± 11.0 | 3 | 14.0 ± 11.9 | 9 |
| | Pharyngobranchial #2 | 5.0 ± 6.9 | 6 | 19.7 ± 12.2 | 3 | 9.9 ± 11.0 | 9 |
| | Hypobranchial #3 | 4.5 ± 4.3 | 6 | 17.6 ± 8.8 | 3 | 8.9 ± 8.6 | 9 |
| | Quadrate | 7.6 ± 9.2 | 6 | 10.7 ± 6.9 | 3 | 8.7 ± 8.2 | 9 |
| | Epibranchial #4 | 2.7 ± 3.3 | 6 | 15.0 ± 8.0 | 3 | 6.8 ± 7.8 | 9 |
| Herring | Otolith | 12.9 ± 1.4 | 6 | 31.7 ± 18.8 | 3 | 19.2 ± 16.1 | 9 |
| | Prootic | 9.2 ± 10.3 | 6 | 13.3 ± 12.3 | 3 | 10.6 ± 10.4 | 9 |
| | Pterotic | 7.1 ± 6.2 | 6 | 14.2 ± 10.1 | 3 | 9.4 ± 7.9 | 9 |
| | Opisthotic | 4.6 ± 3.3 | 6 | 12.5 ± 8.7 | 3 | 7.2 ± 6.4 | 9 |
| Sandlance | Vertebrae | 10.8 ± 5.0 | 6 | 8.1 ± 4.7 | 6 | 9.5 ± 4.8 | 12 |
| | Otolith | 1.9 ± 1.2 | 6 | 10.7 ± 9.2 | 6 | 6.3 ± 7.8 | 12 |
| Salmon | Otoliths | 0.0 ± 0.0 | 6 | 22.6 ± 8.4 | 2 | 5.7 ± 10.9 | 8 |
| | Vertebrae | 0.5 ± 0.5 | 6 | 4.3 ± 6.1 | 2 | 1.4 ± 2.9 | 8 |
| | Teeth[a] | 18.4 ± 8.3 | 6 | 28.4 ± 35.4 | 2 | 26.5 ± 16.2 | 8 |
| | Gill rakers[a] | 26.5 ± 29.9 | 6 | 13.0 ± 9.1 | 2 | 22.8 ± 15.7 | 8 |

[a] For salmon teeth and gill rakers values represent number recovered per fish fed.

for details). Consequently, data transformation was considered inappropriate and only raw data were used. If main effects were significant, *post-hoc* multiple comparisons were made using Tukey tests. The null hypothesis that activity level had no effect on the recovery of individual structures was tested for pollock, herring, and salmon separately. Data for each animal were again pooled across trials. Structures with a pooled recovery estimate >1.4% were selected (Table 4) and mean values (converted to proportions and arcsine-transformed) were compared, for each animal separately, in paired *t*-tests for active and inactive bouts. Statistical tests were performed using Statview version 5.0.1 (SAS Institute Inc.).

## Results

All but one (95/96) of the bones (a sandlance vertebra) and all (180/180) beads that were scattered into the tank to test the ability to recover fish bones were recovered. We, therefore, assumed that all excreted structures were recovered during tank draining. The activity monitor indicated that animals were stationary for an average of 3.6 h ± 1.6 (5%) in the 72 h following the first meal of the "active" bout, while animals were stationary for an average of 41.3 h ± 1.2 (57%) in the same 72 h of the "inactive" bout (Mann-Whitney U, $P < 0.05$). Regurgitated material was observed from both animals, but only in tank drains. Regurgitations

occurred three times after meals of pollock and herring, and four times after meals of salmon. Unless stated explicitly, results in Tables and Figures exclude meals that were partly regurgitated.

*Structure Recovery Percentages*

Mean structure recovery percentages are presented across trials for active and inactive bouts, as well as for both bouts pooled (Table 4). When pooled, pollock had 4 structures with mean recovery percentage greater than 10%—otoliths (73.9%), dentary (18.1%), angular (15.6%), and interhyal (14.0%). Herring had only two structures recovered in high number—otoliths (19.2%) and prootics (10.6%)—whereas, overall, no structure exceeded 10% recovery for either sandlance or salmon. Mean recovery percentage for 17 of 18 structures was higher in active bouts (of which eight had more than a three-fold difference). The 0% recovery of salmon otoliths in inactive bouts compared with a recovery of 22.6 ± 8.4% in active trials was the most conspicuous result. In active bouts mean otolith recovery of pollock was 95.5 ± 7.9%, while sandlance was 10.7 ± 9.2%. Thus, at least in active bouts, all four prey species had structures that exceeded 10% recovery, although between-species differences in otolith recovery still varied by a factor of nine. The null hypothesis that activity had no effect on structure recovery was rejected in both animals for pollock (SSL1, paired $t_7 = 2.4$, $P = 0.02$; SSL2, paired $t_7 = 9.3$, $P < 0.001$), and was marginal in both animals for herring (SSL1, paired $t_3 = 2.2$, $P = 0.06$; SSL2, paired $t_3 = 2.4$, $P < 0.05$), and was also marginal in SSL2 for salmon (paired $t_3 = 2.2$, $P = 0.06$). Mean CVs across structures were high for inactive (CV = 0.96) and active bouts (CV = 0.63).

*Prey Recovery Percentages*

Prey recovery percentages based on otoliths and all key structures showed a number of clear trends when averaged across trials for each bout and animal (Table 5, Fig. 1). Importantly, identifiable hard remains were recovered for all species after every meal fed. Secondly, significantly more prey were identified using all key structures than when using otoliths alone (paired $t_{16} = 5.01$, $P < 0.001$). The clearest examples were for salmon (due to the additional use of teeth and gill rakers) and sandlance (due to the additional use of >59,000 vertebrae). Thirdly, although using all key structures increased the likelihood of detecting all species of prey, there were significant differences between species (repeated measures ANOVA, $F_{1,3} = 17.9$, $P = 0.02$), mainly due to recovering more hard parts from pollock compared with herring and sandlance (both Tukey test, $P < 0.05$). Recovery percentage using all key structures ranged widely among individual trials for each prey species (pollock, 22%–156%; herring, 15%–60%; sandlance, 3%–26%; salmon, 13%–86%). Causes of this intraspecific variability are examined in more detail below.

Prey recovery percentages were less in trials where regurgitated bones were excluded from the analysis (Table 5), but were only statistically significant for pollock (Mann-Whitney U, $P < 0.05$). In general, regurgitated material consisted of larger bones such as jawbones, post cleithrums, vertebrae, and pollock otoliths, but also included smaller bones such as herring otoliths (Table 5). The larger bones of salmon also were regurgitated, but their impact on recovery was insignificant,

*Table 5.* Comparison of prey recovery percentage (mean % ± SD) for four prey species fed to two Steller's sea lions. Data have been averaged across trials for each animal and bout and presented for all key structures and otoliths only. Data from active bouts in which a meal was then regurgitated (R) have been presented separately and include data recovered postregurgitation with data in parentheses below for all bones including regurgitated bones.

| | | | | Recovery percentage in trials without R | | | Recovery percentage in trials with R | |
|---|---|---|---|---|---|---|---|---|
| SSL no. | Prey species | Bout | No. of trials | All key structures | Otoliths | No. of trials (R) | All key structures | Otoliths |
| 1 | Pollock | Active | 2 | 137.9 ± 9.7 | 109.6 ± 4.1 | 1 | 64.3 | 50.0 |
| | | Inactive | 3 | 110.1 ± 17.1 | 83.3 ± 15.3 | – | (135.7) | (92.9) |
| | Herring | Active | 2 | 45.0 ± 0.0 | 30.0 ± 14.1 | 1 | 25.0 | 25.0 |
| | | Inactive | 3 | 23.3 ± 14.4 | 10.0 ± 0.0 | – | (75.0) | (75.0) |
| | Sandlance | Active | 3 | 10.2 ± 4.7 | 3.5 ± 2.2 | – | | |
| | | Inactive | 3 | 11.0 ± 2.7 | 2.8 ± 1.5 | – | | |
| | Salmon | Active | 1 | 42.9 | 33.3 | 2 | 32.2 ± 25.2 | 8.4 ± 11.8 |
| | | Inactive | 3 | 52.2 ± 29.1 | 0.0 ± 0.0 | – | (70.2 ± 18.5) | (31.0 ± 3.3) |
| 2 | Pollock | Active | 1 | 131.8 | 90.9 | 2 | 42.5 ± 10.6 | 5.0 ± 7.1 |
| | | Inactive | 3 | 60.4 ± 34.3 | 51.3 ± 35.4 | – | (135.0 ± 7.1) | (97.5 ± 10.6) |
| | Herring | Active | 1 | 60.0 | 55.0 | 2 | 42.5 ± 3.5 | 37.5 ± 10.6 |
| | | Inactive | 3 | 28.3 ± 14.4 | 18.3 ± 15.3 | – | (77.5 ± 10.6) | (70.0 ± 7.1) |
| | Sandlance | Active | 3 | 18.3 ± 6.8 | 18.1 ± 7.0 | – | | |
| | | Inactive | 3 | 11.9 ± 8.1 | 1.1 ± 0.2 | – | | |
| | Salmon | Active | 1 | 85.7 | 57.1 | 2 | 78.8 ± 58.3 | 50.0 ± 70.7 |
| | | Inactive | 3 | 39.9 ± 24.0 | 0.0 ± 0.0 | – | (107.5 ± 46.0) | (62.5 ± 53.0) |

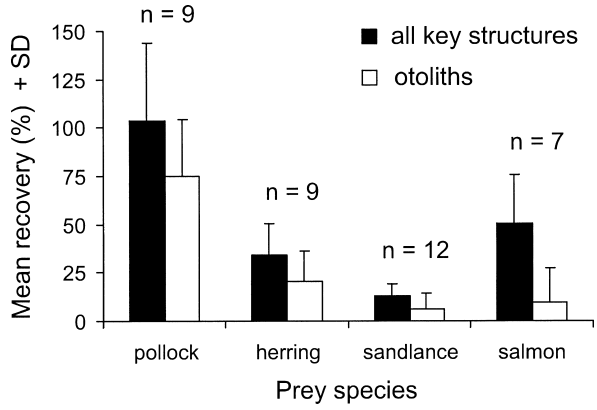


*Figure 1.* Between-species comparison of mean prey recovery percentage of all key structures versus otoliths only (all data combined, regurgitated meals excluded).

largely because smaller structures (such as teeth, gill rakers and otoliths) were later recovered in scats and were the structures generally used to calculate MNI.

The estimated number of pollock consumed based on MNI estimates from all key structures recovered in scats exceeded 128% in all active bouts (maximum = 156%, *i.e.*, estimates of prey number consumed exceeded the actual number fed). With few exceptions, prey recovery percentage in active bouts exceeded those in inactive bouts (Table 5). This was the same for otoliths and all key structures, but there was no significant difference in the estimated number of prey consumed among bouts ($P = 0.18$ for otoliths and $P = 0.34$ for all structures), despite clear trends for pollock and herring (Fig. 2). Interestingly, both of these species were fed during the most active phase (the swim mill session) of the active bout. For otoliths, there was a significant interaction between species and activity ($F_{1,3} = 46.08$, $P = 0.005$) with activity effects most pronounced for salmon and least for sandlance (Table 5). In the inactive bouts all key structure CVs exceeded 0.5 in all species for SSL2 and in herring and salmon for SSL1. CVs were less in active bouts, with a maximum of 0.37 for SSL2, and 0.46 for SSL1 fed sandlance.

Pooling data from both animals and all trials, and plotting the cumulative contribution of each scat or drain for active and inactive bouts (Fig. 2) indicated that hard parts of each prey species was generally found within 21 h and continued to be present up to 65 h. Beyond this time, recovery of pollock bones continued, whereas the remaining prey species leveled off (Fig. 2). Consequently, observed between-species differences in recovery percentage varied depending on the time since ingestion. Similarly, if mean prey recovery percentage was divided by the mean number of scats produced (scat output), values for pollock and salmon were similar in both active (26.3% *vs.* 24.7%, respectively) and inactive (18.3% *vs.* 19.7%, respectively) bouts. Values for herring were intermediate (active, 12.5%; inactive, 10.0%) and lowest for sandlance (active, 3.9%; inactive, 4.5%). The fastest time to the first appearance of identifiable remains (IDT) was 2 h for sandlance (in an active bout), while maximum FDT was observed for pollock (recovered 148.3 h post-ingestion). In 83% of the inactive bouts, small numbers of both experimental fish structures and beads were egested following the final overnight tank session. This was despite previously finding "empty" scats in the dry run and suggests that activity can dislodge bones retained in the intestine during inactivity.

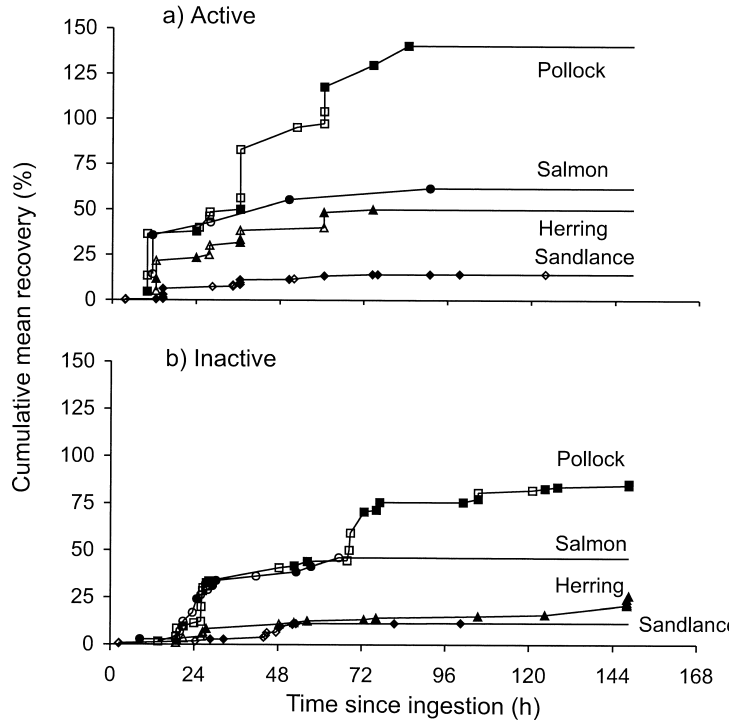


*Figure 2.* Between-species comparison of cumulative mean prey recovery percentage since time of ingestion for (a) active and (b) inactive bouts (all key structures, regurgitated meals excluded). Open symbols denote SSL1, filled symbols denote SSL2.

Binning data from each bout/animal combination over consecutive 24 h periods (Fig. 3) highlights the level of variability across trials and between activity levels. High levels of variability across repeated trials were observed in both animals, but were highest for SSL2, particularly for salmon, sandlance, and pollock. Between-animal differences were most consistent in inactive trials with recovery percentage of SSL2 consistently less and lagging behind SSL1. The trend was most noticeable for pollock, where mean recovery for SSL2 was 60.4 ± 34.3% compared with 110.1 ± 17.1% for SSL1 (Fig. 3). Although we recognize the low sample size in some active bouts, activity appeared to increase the recovery percentage of both pollock and herring structures, especially for SSL2, where total recovery in active trials was twice that of the inactive trials. Overall, 88.8% (± 12.6) of beads were recovered (range 60%–100%), with identical recovery of the two different sizes. Nevertheless, bead egestion was highly variable, with higher recovery percentage in active bouts and a notable lag in peak recovery for SSL2 (Fig. 4).

*Passage Times and Defecation Rates*

Initial defecation time for all key structures varied between 2 and 56 h, but overall did not correlate with sea lion activity or species of prey consumed. However, mean inactive IDTs of SSL2 were notably greater than for SSL1 fed

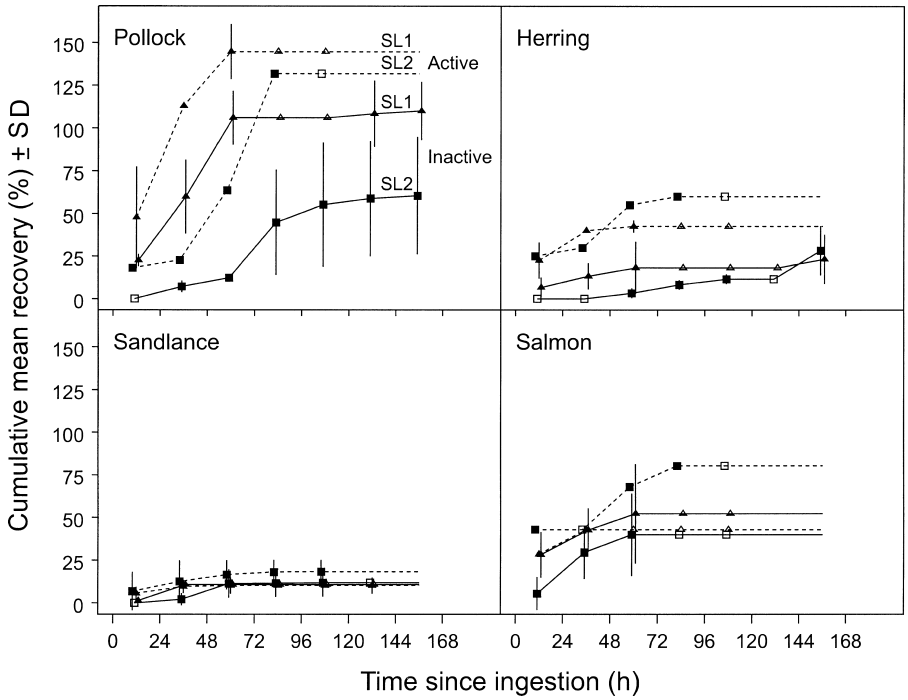


*Figure 3.* Between-animal and between-species comparison of cumulative mean prey recovery percentage since time of ingestion for active (dashed lines) and inactive (solid lines) bouts (scats binned on 24-h basis, regurgitated meals excluded). Triangles denote SSL1, squares denote SSL2, and open symbols denote no key structures identified in scat present. Symbols have been jittered in some cases for ease of interpretation.

pollock (by 11 h), herring (by 24 h) and sandlance (by 34 h) (Table 6). Mean FDT (82 ± 41 h) indicated a strong activity effect (repeated measures ANOVA, $F_{1,1} = 452929$, $P < 0.001$) and also a species effect ($F_{1,3} = 28.5$, $P = 0.01$), with both recovery of pollock and herring exceeding that of salmon, and herring exceeding sandlance (all Tukey tests, $P < 0.05$). No difference was found between FDT and the 95% DT for salmon. However, 95% DT was an average 23 ± 34 h less than FDT for the remaining species. Activity effects for the 95% DT were not significant ($F_{1,1} = 0.02$, $P = 0.91$), emphasizing the effects of long-term retention of a few hard remains in inactive bouts. In contrast, species effects on 95% DT were marginally insignificant ($F_{1,3} = 6.7$, $P = 0.08$). FDTs were shortest for salmon otoliths and longest for pollock otoliths, with an overall average of 67.7 ± 39.4 h (Table 6).

Output interval (95% DT – IDT) did not vary significantly among species or activity levels. Overall, however, the interval during which pollock bones were deposited was more than twice that of salmon (Table 6). The same trend was also observed in scat output (Table 6). In the latter case, species effects were significant ($F_{1,3} = 41.6$, $P = 0.006$), with the scat output from pollock meals exceeding that from the other three species of prey (all Tukey tests, $P < 0.05$). Overall, the mean number of scats containing prey from a single ingestion event was 3.2 ± 1.36 (range 1–6) when all key structures were used (Table 6). Otoliths appeared over an

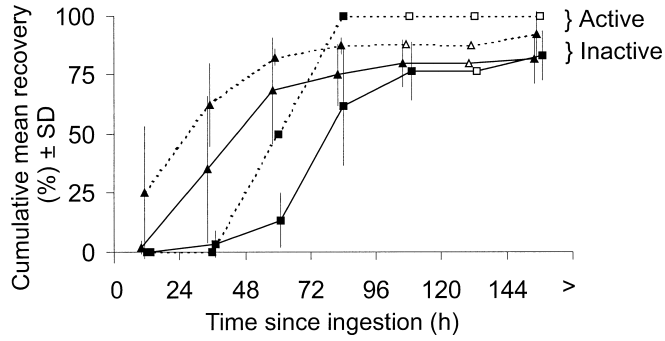


*Figure 4.* Between-animal comparison of cumulative mean bead recovery percentage since time of ingestion for active (dashed lines) and inactive (solid lines) bouts (scats binned on 24-h basis, regurgitated meals excluded). Triangles denote SSL1, squares denote SSL2 and open symbols denote no beads identified in scat present. Symbols have been jittered in some cases for ease of interpretation.

average 2.2 ± 1.45 scats per meal (range of 0–5). Species effects were again significant ($F_{1,3} = 201.7$, $P < 0.001$), with all species appearing more frequently than salmon, and pollock occurring more often than herring and sandlance (all Tukey tests, $P < 0.05$).

While in the dry run, the SSLs produced between 0 and 4 scats per day, with a mode of one per day and averaged 375 ± 221 ml (range 5–910). SSL1 defecated two or more times on 14 different days, as opposed to SSL2 who defecated two or more times on only two days. Total scat volume was similar between animals (SSL1, 8,365 ml; SSL2, 8,690 ml) despite SSL2 consuming ~15% more total food mass.

## Discussion

The use of prey remnants recovered from fecal samples is the primary method now used to describe the diet of Steller's sea lions, a species which saw dramatic population declines in the 1980s (Loughlin *et al.* 1992, Trites and Larkin 1996). Our study attempted to simulate the activity patterns undertaken by wild Steller's sea lions and to provide the first experimental assessment of the all-structure technique to count prey in the scats of SSLs, as well as providing baseline values on the recovery and passage times of bones among prey species and animals.

Our study, like all captive feeding experiments, has methodological limitations. Meal size can affect the relative recovery of otoliths (Marcus *et al.* 1998). We therefore attempted to feed our two juvenile SSLs meals that were a constant proportion of their body size. However, this was not the case for meals of sandlance (*i.e.*, the second half of the experimental meal was not fed at 1530), which might explain our lower total recovery percentages for this species. Additionally, we found 11% of ingested beads remained in sea lion guts beyond the length of our study and found bones that were in the gut during more than 6 d of inactivity were egested after a period of activity (Fig. 3, 4). Thus, despite the 92-h collection period after each final meal (salmon), it is possible for small numbers of bones to have been retained in the stomach ruggae or intestine and excreted after our experiments concluded. Furthermore, we no not know how well we managed to replicate the

*Table 6.* Comparison of passage times for all key structures and scat output (mean h ± SD) for all key structures and otoliths only from four prey species fed to two Steller's sea lions. Data are averaged across trials for each animal and bout and presented for initial (IDT) and final (FDT) defecation times, as well as the time in which ≥95% of all bones recovered were egested (95% DT). Scat output denotes the mean number of scats in which species remains from a single experimental meal were recovered.

| | | | | Mean time (h)—all key structures | | | Scat output | |
|---|---|---|---|---|---|---|---|---|
| SSL no. | Prey species | Bout | Trials | IDT | FDT | 95% DT | All key structures | Otoliths |
| 1 | Pollock | Active | 2 | <21.0 ± 0.0 | 69.0 ± 0.0 | 60.8 ± 11.5 | 5.0 ± 1.4 | 4.0 ± 1.4 |
| | | Inactive | 3 | 17.2 ± 3.0 | 112.5 ± 40.4 | 69.1 ± 0.8 | 4.7 ± 1.5 | 3.0 ± 1.0 |
| | Herring | Active | 2 | <21.0 ± 0.0 | 69.0 ± 0.0 | 69.0 ± 0.0 | 3.5 ± 0.7 | 2.5 ± 0.7 |
| | | Inactive | 3 | 21.5 ± 4.4 | 107.3 ± 70.4 | 33.6 ± 12.7 | 2.3 ± 1.5 | 1.7 ± 0.6 |
| | Sandlance | Active | 3 | <9.5 ± 10.0 | 71.3 ± 45.6 | 47.3 ± 4.0 | 3.3 ± 1.2 | 2.7 ± 1.5 |
| | | Inactive | 3 | 2.3 ± 0.3 | 45.4 ± 1.9 | 45.4 ± 1.9 | 2.7 ± 0.6 | 2.0 ± 1.0 |
| | Salmon | Active | 1 | <21.0 | 28.0 | 28.0 | 2.0 | 1.0 |
| | | Inactive | 3 | 20.4 ± 0.5 | 39.5 ± 22.6 | 39.5 ± 22.6 | 2.7 ± 1.2 | 0.0 |
| 2 | Pollock | Active | 1 | <21.0 | 84.7 | 84.7 | 6.0 | 5.0 |
| | | Inactive | 3 | 27.7 ± 0.4 | 124.8 ± 23.6 | 118.0 ± 14.6 | 4.0 ± 1.0 | 2.7 ± 2.1 |
| | Herring | Active | 1 | <21.0 | 74.6 | 74.6 | 5.0 | 4.0 |
| | | Inactive | 3 | 45.6 ± 15.7 | 148.2 ± 0.3 | 108.5 ± 31.0 | 2.3 ± 0.6 | 1.7 ± 0.6 |
| | Sandlance | Active | 3 | <29.0 ± 13.9 | 78.7 ± 19.6 | 60.1 ± 14.8 | 3.7 ± 1.2 | 3.3 ± 0.6 |
| | | Inactive | 3 | 36.6 ± 10.7 | 76.9 ± 26.2 | 60.8 ± 18.0 | 2.3 ± 1.2 | 2.3 ± 0.6 |
| | Salmon | Active | 1 | <21.0 | 91.0 | 91.0 | 3.0 | 2.0 |
| | | Inactive | 3 | 20.5 ± 10.5 | 46.3 ± 15.8 | 46.3 ± 15.8 | 2.0 ± 1.0 | 0.0 |

conditions experienced by free-ranging sea lions. Consequently, it is probably wise to consider presented results as relative rather than absolute values, and that our results may be valid only for juvenile female Steller's sea lions.

Our study documented advantages of using multiple structures to assess prey numbers consumed. First, key structures of each prey species were found in scats after every experimental meal (even those meals that were partly regurgitated), but this was not the case when only otoliths were considered (Table 6). Using all key structures compared with using only otoliths increased prey recovery percentage for all species, particularly for salmon, due to the use of teeth and gill rakers—where the increase exceeded five-fold (Table 5). Although the use of all key structures lessened interspecific discrepancies (compared with using only otoliths), differences in overall prey recovery percentage were still significant, with pollock recovery exceeding herring and sandlance (Fig. 1). Nevertheless, otoliths were the most important structure for enumerating prey (Table 4), and our recovery percentages for pollock, herring, and salmon (Table 4) were within a few percent of those recorded by Cottrell and Trites (2002)—the only other SSL captive feeding study undertaken to date—and further corroborate that relative recovery of otoliths that are large and robust are higher than those that are small and/or fragile (Harvey 1989).

We observed high variability in defecation times (Table 6), the recovery percentages of individual structures (Table 4), and in prey recovery percentages when using otoliths or a combination of key structures (Table 5). Important sources of variability included both activity level and study animal (despite being the same age and sex, and similar in size). In addition, we observed high variability across repeated trials, particularly when animals were inactive (Table 4, 5; Fig. 3). Otolith and bone digestion rates appear to be subject to considerable natural variation that is impossible to control under standard captive conditions. Despite controlling for potential prey size and meal size effects (see Tollit *et al.* 1997, Marcus *et al.* 1998), intraspecific ranges in prey recovery percentages for sandlance and pollock varied more than eight-fold. Differences between the two individuals may reflect variability in gut lengths (Laws 1953) or simply differences in individual behavior and gut function. Clearly, more data from a range of ages and sexes must be collected to assess the full extent of variability in these parameters.

While the baseline recovery values we provide can be used to calculate NCFs for juvenile SSLs (see Bowen 2000), two factors, in addition to the high variability noted, confound their easy application. The first is regurgitation, as we observed in our active bouts. Notably, recovery percentage of pollock otoliths and other bones were significantly reduced when we excluded regurgitations (Table 5). Our captive animals may have behaved differently than those in the wild, but at-sea regurgitation of fish remains has been observed in another species (Bowen *et al.* 2002). We do not know why our captive animals regurgitated, but speculate that large deposits of undigested bones may have been uncomfortable in the stomach during swimming. Notably, many of the other pollock structures recovered in high numbers by Cottrell and Trites (2002) were overwhelmingly recovered in regurgitations in our study. 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). Information from head-mounted cameras may provide data on whether, and to what extent, free-ranging Steller's sea lions regurgitate at sea.

Rocks are apparently common in the stomachs of SSLs and theories for ingestion include the need for ballast, prevention of hunger pangs during fasting, and the destruction of parasites (Thorsteinson and Lensint 1962, Calkins 1998). Richardson and Gales (1987) suggested that stones provide mechanical assistance with the breakdown of hard food items. In support of this hypothesis, a number of objects similar to rocks were present in the stomach of a single captive SSL (#F97SI) during a pilot study. Otolith recovery averaged only 28% after feeding 396 pollock of 28–45 cm (D. Tollit, unpublished data), compared to >86% recovery found for the same animal in our study. Together, regurgitation and the presence of rocks may add additional and potentially large sources of variability to bone recovery percentage from free-ranging animals, particularly for prey species with large bones. However, Thorsteinson and Lensint (1962) suggested that rocks were probably regurgitated when foraging for food begins, since actively feeding animals did not contain them. A review of the presence of rocks in sea lions' stomachs is needed to provide information on these different hypotheses. These same two factors (presence of rocks and regurgitations) may explain the enigma of the surprisingly low representation of pollock otoliths in the scats of free-ranging SSLs. Pollock otoliths are the most frequently recovered structure in captive studies (Table 4, see also Cottrell and Trites 2002) representing ~38% of the bones recovered, yet are relatively rare in scats collected in the field, representing ~15% of bones recovered (Table 3, see also Sinclair and Zeppelin 2002). If a mix of rocks in stomachs and regurgitations do not explain this observation, then alternative possibilities may be that free-ranging animals retain food in the stomach for longer periods, consume smaller meal sizes (see Marcus *et al.* 1998), or eat less frequently. Future captive experiments should therefore consider designing increasingly realistic and standardized feeding protocols that synthesize information (such as from telemetric and stomach borne data loggers) from free-ranging animals.

The need to simulate realistic conditions was also highlighted by the greater proportion of structures that were recovered when animals were predominantly swimming in water (active) as opposed to when they were relatively sedentary in the dry run (inactive). This included even relatively large (>10 mm) structures such as pollock otoliths. As digestion of prey hard remains by pinnipeds occurs solely in stomachs (Frost and Lowry 1980), it appears that limited activity slows gastric emptying. This is supported by experiments with mice (Grunewald and Tucker 1985), otters (Carss *et al.* 1998) and by the lower otolith recovery percentage generally observed in pinnipeds kept in dry enclosures compared with those with access to water (Bowen 2000). While the same trend was apparent (particularly for pollock and herring) when we calculated prey recovery percentage for SSLs (Table 5; Fig. 2, 3), we did not observe statistically significant activity effects. Nevertheless, we suspect the recovery percentages we calculated from our active bouts may be more realistic than those from the inactive bouts.

Our study is the first to provide information on passage rates and deposition of multiple skeletal structures passing through the gut of a pinniped. Bones from fish fed to captive SSLs were deposited over an average of 3.2 scats and generally over periods of 0.1–2.7 d. However, deposition was not consistent among species or activity level. Both output interval and scat output varied by a factor of two (Table 6), although results were significant only for scat output (with pollock found in more scats per meal than any other species). Final defecation time (FDT) showed both a species and an activity effect, with faster passage times occurring when animals were active. Carss *et al.* (1998) also found activity quickened transit times

in otters. Overall, FDT averaged 82 h for all key structures, and fell to 65 h for 95% DT and 68 h for otoliths only. Our otolith transit times were similar to those recorded by Orr and Harvey (2001) for active Californian sea lions, but were notably longer than those recorded for harbor seals, for which 90% of the otoliths of various species were passed within 24 h (Harvey 1989). The extended output period of all four prey species in our study (Table 6) suggests that hard parts in SSL scats can represent, not just nearshore foraging, but potentially can represent animals returning from a distance of 228 km (at a conservative swimming speed of 3.5 km/h, Stelle *et al.* 2000).

Our study highlighted two limitations of using Minimum Number of Individuals (MNI) to estimate taxonomic abundance from multiple structures. The first limitation results from skeletal fragmentation. Over 92% of the time we found structures from experimental meals were distributed over 2–6 scats (Table 6). As each scat was considered a separate sample, this fragmentation across multiple scats resulted in double counting of fish, particularly when multiple structures were used to enumerate prey. For example, otoliths might be used to estimate MNI in one scat, and jawbones from the same fish might be used in a following scat, resulting in a combined MNI that exceeds the number fed. In our study this bias became apparent for the relatively robust bones of pollock, which were recovered in high numbers. The second limitation is that MNI yielded a better estimate of the actual number of individuals consumed (N) when the meal consisted of few individuals (as in the case of salmon in our study). All other things being equal, the reliability of MNI as numbers of individuals per meal increased depending on the number of unique (and recoverable) structures per taxon. Thus, if a small fish has few unique and identifiable structures (like sandlance) and is consumed in large numbers, then MNI will yield a poor prediction of N and likely lead to an underestimate in its contribution by weight or number.

Overall, our study emphasizes a number of problems associated with present models used to estimate diet from scats. We showed that single scats can contain the hard remains of prey consumed over a few days, but passage time varies among species, and clearly a single scat does not represent a single meal. We also showed that the all structure technique does not remove the effects of interspecifc differences in recovery, even though it significantly increases the recovery of prey compared to using only otoliths. Furthermore, using multiple structures to count prey using MNI is size-biased and is also influenced by the robustness of identifiable structures.

We recognize that our study collected all scats produced after each meal, which is unlikely to be the case when researchers visit haul-out sites when collecting scats in the wild. Biases counting prey using MNI will remain when using scats from the wild (Allen and Guy 1984, Ringrose 1993), but clearly the impact of double counting fish with robust bones (as well as the impact of interspecific differences in scat output) will depend on a number of factors, particularly the time periods animals spend on land, foraging trip times, and distances to the foraging areas. Similarly, it is important to note that if one considers all structure recovery percentages on a per scat basis, then interspecific differences are lessened and differences between pollock and salmon disappear.

Using scats to quantify the diet of Steller's sea lions is challenging considering the number of variables that appear to influence digestion (our study, Warner 1981, Tollit *et al.* 1997, Bowen 2000). Our study was based on two young females and needs to be extended to cover a greater number of animals. Digestion and passage

rate information needs to be collected at the most basic sampling unit (*i.e.*, the scat). Simulation studies are also needed to assess levels of bias by incorporating both captive and field data, as well as to provide confidence intervals around any diet estimates (see Hammond and Rothery 1996). While scats can provide researchers with estimates of prey size (Tollit *et al.* 1997), and can be used (if biases are assumed to remain consistent) to make temporal or geographical comparisons of prey importance, we believe a multifaceted approach to quantifying diet composition in pinnipeds is necessary. These include stable isotope analysis (Kurle 2002) and quantifying diet from blubber fatty acid signatures (see Iverson *et al.* 1997).

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