



## Diet estimation in California sea lions, *Zalophus californianus*

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### ABSTRACT

We performed a captive feeding experiment using California sea lions to assess biases associated with estimating pinniped diet using scats and spews. Sea lions were fed nine of their natural prey species: anchovy, sardine, Pacific mackerel, jack mackerel, hake, steelhead smolts, shortbelly rockfish, pink salmon, and market squid. Recovery percentages varied among prey species using otoliths and were improved for adult salmon and sardine using the all-structure method. Numerical and graded length correction factors provided better estimates of number and size of prey consumed. Four models used to determine the proportions of prey species consumed by a sea lion population were tested. The all-structure method and variable biomass reconstruction model, in conjunction with numerical and graded length correction factors, provided more accurate estimates than without. We provide numerical correction factors for all prey species, including correction factors for specific salmon bones: vertebrae, branchials, radials, teeth, gill rakers, and hypurals.

Key words: pinniped, California sea lion, *Zalophus californianus*, diet, correction factors, Salmonidae, variable reconstruction model.

The California sea lion (CSL), *Zalophus californianus*, is a marine predator of fishes and cephalopods off the western coast of North and Central America (Reeves *et al.* 2002). Understanding sea lion food habits will better define their role as top-level predators in the marine ecosystem, particularly their potential impact on commercially important fish stocks (Trites *et al.* 1997). Methods for studying diets are inaccurate because of digestion biases, which this paper aims to improve.

Prey hard parts found in scats (feces), spews (regurgitation), and stomach contents are used to reconstruct the diet of pinnipeds (Da Silva and Neilson 1985, Murie

and Lavigne 1986, Harvey 1989), and scat analysis is the most widely used and least invasive technique. Certain variables can affect percentage recovery of prey hard parts, including the robustness of prey hard parts, different prey species, size of prey, and regurgitation of partial or entire meals (*e.g.*, Treacy 1981, Bigg and Fawcett 1985, Murie and Lavigne 1985, Harvey 1989, Tollit *et al.* 1997), which can bias estimates of number and size of prey eaten. Captive feeding studies have been conducted to assess these biases to improve the accuracy of determining diets of free-ranging pinnipeds (*e.g.*, Antonelis *et al.* 1984, Da Silva and Neilson 1985, Harvey 1989, Lowry *et al.* 1991).

Otoliths, the “ear bones” of fish, are used as the primary identifying structure of fish in scat analysis; however, reliance on otoliths alone can provide misleading results. Because otoliths can have varying degrees of erosion or are often completely dissolved in the gut, researchers also use skeletal structures specific to prey species (Cottrell *et al.* 1996, Cottrell and Trites 2002). This “all-structure” method increases detection rates of additional prey consumed that are not always represented by otoliths (Tollit *et al.* 2003).

Reconstruction of pinniped diet is typically achieved by enumerating individuals of each identified prey species recovered from a collection of scat and spew samples. Primarily, four models have been used to estimate diets of pinnipeds in the past, yet there have been few studies that have properly examined these models to accurately estimate diet in CSLs. These four models are: Modified Frequency of Occurrence (mFO; Croxall 1993), Split-Sample Frequency of Occurrence (SSFO; Olesiuk *et al.* 1990), Variable Biomass Reconstruction (VBR; Harvey 1987, Laake *et al.* 2002), and Fixed Biomass Reconstruction (FBR; Laake *et al.* 2002). Using recovered structures, mFO simply assesses presence/absence of a prey species within a scat, without quantifying the number and size of prey structures present (Croxall 1993), whereas SSFO considers presence/absence, but also assumes each prey was consumed in equal quantities in a given scat and that all meals are the same size (Olesiuk *et al.* 1990). VBR and FBR indices use the minimum number of individuals (MNIs) represented by a set of structures and numerical and length correction factors (NCFs and LCFs) to recreate the actual meal biomass by calculating prey size and mass (Harvey 1989, Laake *et al.* 2002). VBR and FBR differ in how they represent foraging behavior. VBR allows the amount of prey consumed to be dynamic by calculating a proportion of biomass of a single prey consumed divided by the total biomass of all prey consumed, reflecting times of successful foraging bouts. FBR divides the proportion of a single prey species of the total biomass of all prey consumed but then divides this by the number of scats collected. This model assumes each scat is equally weighted, contributing the same proportion of each prey species to the total consumed. Resolving, which method accurately estimates sea lion consumption, is central to the investigation of sea lion diet.

Here, we built upon the work conducted by Orr and Harvey (2001), which unsuccessfully attempted to test reliably the variability of percentage recovery and passage times of otolith and squid beaks in CSLs of different sex and age classes. We (1) examined the variables affecting percentage recovery of identifiable hard parts of each meal fed to sea lions; (2) related these percentage recoveries to sex, age, meal frequency, meal size, prey species, and size of prey; (3) validated the use of the all-structure method; and (4) created correction factors (CFs) for otolith length reduction and percentage recovery of various prey species. We then tested each model's efficacy in estimating the biomass of prey consumed by a sea lion population.

## METHODS

*Study Animals and Facility*

CSLs were housed at The Marine Mammal Center (TMMC), Sausalito, California, after rehabilitation from illness or injury. Individuals were determined to be in good health by veterinary staff, so digestion should have been unaffected by illness or by prescribed medication.

*Feeding Experiments*

Nine animals were sampled: two subadult females, two adult females, three subadult males, and two adult males, identified as CSL A through CSL I (Table 1). Eleven different meal compositions of natural prey items were fed to CSLs (Table S1, S2). The prey species included northern anchovy (*Engraulis mordax*), market squid (*Loligo opalescens*), Pacific sardine (*Sardinops sagax*), shortbelly rockfish (*Sebastes jordani*), jack mackerel (*Trachurus symmetricus*), Pacific mackerel (*Scomber japonicus*), Pacific hake (*Merluccius productus*), pink salmon (*O. gorbuscha*), and steelhead salmon smolts (*Oncorhynchus mykiss*). Before feeding, standard length of each prey item was measured to the nearest millimeter and mass was determined to the nearest gram. Two different sizes of sardine (small: mean = 18.0 cm, SE = 0.03 cm; medium: mean = 22.2 cm, SE = 0.1 cm) and two sizes of rockfish (small: mean = 13.4 cm, SE = 0.1 cm; medium: mean = 20.8 cm, SE = 0.2 cm) were used. Size classes were created to estimate relationships between otolith length reduction and recovery within a species. Each meal consisted of prey items within 2.0 cm in length of each other. Atlantic herring (*Clupea harengus*) with heads removed were fed to supplement caloric composition. Meals were fed once or twice per day, depending on meal size.

Experimental meals were either a single meal comprised of one of the nine target species of prey or a mixed meal, consisting of hake and sardine. Three replicates of each experimental meal were fed to each sea lion. Within a meal, sea lions were fed each prey item sequentially. If a prey item was not consumed, it was immediately removed and recorded as such. Seventy-two hours were allowed between experimental meals to ensure complete passage before the next meal of the same species was fed. Orr and

Table 1. California sea lion (*Zalophus californianus*) individuals used in the captive feeding experiment and their sex, age class and mass, number of experimental meals fed, number of samples collected, and duration of the trial.

Sea lion	Sex	Age class	Mass (kg)	No. of experimental meals	No. of scats	No. of spews	No. of trial days
CSL A	F	SA	35–55	18	34	0	35
CSL B	F	SA	35–55	35	113	3	40
CSL C	M	SA	53	32	96	15	32
CSL D	M	SA	105–119	30	116	3	24
CSL E	F	A	80	33	93	4	29
CSL F	M	SA	115	27	76	7	21
CSL G	M	A	190	28	95	5	18
CSL H	M	A	215	27	60	3	20
CSL I	F	A	81	25	78	5	23

Harvey (2001) estimated a minimum initial passage time of 16 h for otoliths, and 72% otolith recovery within 48 h of ingestion. In addition, they found the maximum passage time of squid beaks was 96 h. Therefore, replicate meals were fed after a minimum of 72 h for experimental fish meals and 170 h for squid meals to ensure a meal of the same species did not overlap in the samples. Ten percent of same-species meals were fed within 72 h and 74% within 96 h or more. Occasionally, a severely degraded otolith was found in a scat and attributed to the meal fed before the most recently fed meal (within 12 h). However, this rarely occurred and we are confident there was no confusion as from which meal the prey was consumed.

Following Worthy (2001), each sea lion was fed meals equivalent to 5%–10% of its body weight per day to maintain weight. Animals were fed once in the morning and once in the evening when fed small meals. The large meal consisted of the entire daily food weight allowance and was fed during late morning.

Each animal was housed in a 2.44 m tall, 6.1 m diameter circular enclosure containing an internal haul-out and uninhibited access to a 24,605 L pool, approximately 1.2 m deep. The floor of the pool was slanted to accumulate all fecal remains into the drainpipe when the pool was drained. A 0.5 mm mesh drain-screen basket external to the tank collected all the remains from the drainpipe. The effectiveness of the recovery system was tested by scattering a known number of different sized otoliths, similar to bones used to identify prey during the study, into the filled tank. The tank was drained, flushed with water to send any remaining material down the drainpipe to the basket strainer, and the basket strainer contents counted to assure 100% recovery (Harvey 1989). The presence of scats and spews were noted and animal behavior observations were recorded for health and activity levels of the sea lion every 30 min during daylight hours. Scat collection, cleaning, and bone enumeration followed Phillips and Harvey (2009; Appendix S1).

### *Data Analysis*

The time of scat collection was used to calculate passage time for each hard part. The mean initial defecation time (IDT) was determined as the time a structure from a meal was first found in a fecal sample; final defecation time (FDT; dependent variable) was determined as the last time a structure from a meal was found in a fecal sample. Percentage recovery was calculated by comparing the MNI of a prey species found in the samples to the original number of prey fed to the sea lion. Bones and otoliths identified to species were used to calculate MNI. Single and paired structures were the only bones to definitively determine MNI in scat (Lance *et al.* 2001). Each prey species had at least one type of diagnostic bone repeatedly present in scats and spews (Table S3, S4). Prootics of sardine and anchovy primarily contributed to MNI numbers. Multiple structures, such as gill rakers, vertebrae, and teeth, were also numerous in scats (Table S4). These structures contributed a presence or absence assessment, which is only valuable in the absence of other structures that determine MNI.

Equal variances and normality were tested with a Levene and Kolmogorov–Smirnov test, respectively (Zar 1999). Because homogeneity of variances was violated for most prey species, a randomization test of variables IDT and FDT was performed for each prey species and meal composition. For the nine animals, the values of the various response variables (*e.g.*, IDT) for replicate meals of the same type were averaged and the mean value for each sea lion was used in the statistical tests. Thus,

Table 2. Numerical correction factors (NCFs) using otoliths only and all-structures for prey species fed to California sea lions (*Zalophus californianus*). Prey items are listed with the total number fed, number of trials, the mean percentage recovery, and standard error values (SE). The NCFs were calculated as (1/total mean percent recovery of prey).

Species	Mean mass (kg)	No. of prey fed	No. of trials	Otoliths only			All-structure		
				Otoliths percent recovery	SE	NCF	All-structure recovery percent	SE	NCF
<i>Engraulis mordax</i>	20.07	1,052	26	57.5	4.5	1.74	58.6	4.5	1.7
<i>Merluccius productus</i>	441.32	143	39	77.5	5.4	1.34	87.6	3.2	1.2
<i>Oncorhynchus mykiss</i>	108.14	237	17	50.1	6.7	2.04	57.3	5.4	1.8
<i>Oncorhynchus gorbuscha</i>	1,814.63	27	26	74.1	8.6	1.37	see Table 3		
<i>Sardinops sagax</i>	87.33	891	48	31.7	3.5	3.17	43.5	3.1	3.0
<i>Scomber japonicus</i>	176.63	901	51	52.2	3.7	1.91	54.5	3.4	1.9
<i>Sebastes jordani</i>	80.79	88	6	68.4	8.3	1.46	68.4	8.3	1.5
<i>Trachurus symmetricus</i>	83.22	630	27	68.8	3.7	1.45	68.8	3.7	1.5
<i>Loligo opalescens</i>	45.08	703	26				89.5	3.0	1.1

we avoided any problems with pseudoreplication or a more complicated repeated measures analysis.

The percentage recovery of otoliths and beaks alone, and the all-structure MNIs were used to calculate NCF for each prey species. NCFs were calculated by the inverse of the total mean percentage recovery of a prey species (Harvey 1989, Lance *et al.* 2001; Table 2, 3). For each prey species, a Student's *t*-test tested differences between mean percentage recovery using otoliths and beaks alone and using the all-structure method to estimate diet. This test was performed with and without the addition of spew sample data to assess any differences between spews and scats.

Otolith length reduction due to digestion was determined by comparing the measured ventral length or width of digested otoliths found in samples with the length or width of an otolith from the mean size of prey fed (*e.g.*, Bigg and Fawcett 1985; Harvey and Antonelis 1994; Tollit *et al.* 1997, 2003; Cotrell and Trites 2002). Since otolith length has a positive linear relationship to fish length, a regression of fish standard length to otolith length or width was used to back calculate length or width of otoliths before ingestion from a representative sample of each prey species (Table S5). Mean percentage length reduction of otolith and squid beaks was calculated as:  $(1 - [\text{size egested}/\text{size ingested}] \times 100)$ . LCFs were calculated for each species as:  $100/(100 - \% \text{ length reduction})$  (Harvey 1989, Tollit *et al.* 1997). Otolith robustness of a species was determined by dividing the mass (mg) of the otolith by its ventral length (mm; Harvey 1989). A single-factor analysis of variance (ANOVA) was used to test if mean size of a prey species in one meal differed among the same species meals fed to all sea lions. We performed this ANOVA to assure that fish size among meals was not a confounding factor in our otolith reduction calculations.

Table 3. Percentage recovery (%) from  $n = 170$  scats containing salmon, standard error (SE), and numerical correction factors (NCFs) are reported for different structures used to identify and quantify adult pink salmon in California sea lion diet. Category 1 lists paired structures that are a more conservative estimate and category 2 bones are multiple structures used in a less conservative diet estimate.

Salmon structure	% Recovery	SE	NCF
Category 1			
Otoliths	55.56	0.03	1.80
Paired hypohyal	25.93	0.02	3.86
Scapula	22.22	0.02	4.50
Category 2			
Vertebrae	214.81	0.04	0.47
Teeth	225.93	0.04	0.44
Branchial	225.93	0.04	0.44
Gill Raker	159.26	0.04	0.63
Hypural	111.11	0.03	0.90
Radial	103.70	0.03	0.96
Hypohyal (pairs pooled)	44.44	0.02	2.25

An average LCF (aLCF) was calculated for each prey species using all otoliths in scats. A standardized otolith grading system was developed to assess the differential digestion of otoliths from prey species fed, based on Tollit *et al.* (1997; Table S6). Otoliths were categorized in grades 1–3 for all fish species, except pink salmon, medium sardine, and jack mackerel, which were categorized as grades 1 and 2, because of greater variability among grade 3 otoliths (Table S6). A grade of 0 was equivalent to the undigested otolith from a fresh fish skull. We compared the digested otolith found in scats and spews with an undigested otolith from the same species to determine the grade assignment. Subsequently, grade-specific LCFs (gLCFs) were calculated. Otoliths that could not be graded as 1, 2, or 3 were not measured to back-calculate prey length. Squid beaks were not given an erosion grade because beak material is resistant to digestion and does not significantly change from its original size (Harvey 1989). Approximately 75% of the measured otoliths, proportionately stratified among the different grades, were used to develop the LCFs for each species. The remaining 25% of otoliths/beaks were randomly resampled with replacement 1,000 times to test the accuracy of the LCFs. The accuracy of these LCFs was tested using a two-sample Student's *t*-test (Bonferroni adjusted  $\alpha = 0.017$ ), using these resampled otoliths/beaks and comparing the mean size of original prey ingested to the uncorrected, aLCF-, and gLCF-corrected prey size estimations.

#### *Diet Reconstruction Models*

Four diet reconstruction indices are commonly used to determine the prey composition of a pinniped population (Appendix S2). Comparing hard part recovery to the known amount of prey fed evaluates each of these indices to determine the appropriate models for accurately reconstructing CSL diet. Furthermore, application of the appropriate CFs for degree of otolith erosion (LCF) and number of prey species (NCF) increases the accuracy of the biomass estimate. Ventral tips of Pacific

mackerel otoliths broke during digestion; therefore, we used CFs for otolith widths when testing models.

The estimated prey biomass of each scat was used to calculate the proportion of the total prey biomass consumed by the predator (Lance *et al.* 2001). Of all scats recovered during this study, including scats without any identifiable hard parts, sets of  $n = 50$  and  $n = 100$  scat samples were randomly selected without replacement 15 times for each sample size. Sample sets of  $n = 100$  were more accurate than those of  $n = 50$ ; therefore, only results from sample sets of  $n = 100$  are reported. A mean proportion of prey fed during this study was calculated from the 15 repetitions for each of the four models. A Dunnett's multiple comparisons test compared the calculated mean to the original prey proportions fed (Day and Quinn 1989, Zar 1999). The biomass reconstruction models (FBR and VBR) incorporated NCF and LCF; therefore, the accuracy of the models was assessed by comparing the uncorrected (no CFs) and corrected biomass (CFs applied) of the sample set to the original proportion.

## RESULTS

Two hundred and fifty-five experimental meals were fed to sea lions. The mean duration of a sea lion trial was 27 d (SE = 2.5 d), ranging from 18 to 40 d. A total of 778 scat and spew samples were collected, 23 of which were individual spews, 22 were scats and spews collected together in the drain screen, and the remainder were scats only. Hard parts from combined scat and spew samples were determined as originating from either a scat or spew by the orange/brown appearance of bones from scats and the white, clean appearance of bones from spews. Sea lions produced a mean 3.2 scats/d (SE = 0.1). The mean volume of a sample was 81.0 mL (SE = 5.0), with an average mass of 82.9 g (SE = 4.5 g) for scats and 109.9 g (SE = 8.9 g) for spews. There were 110 of 778 samples that did not contain diagnostic prey hard parts of interest and 16 of these samples were spews. Samples were collected on the drained floor (7.8%), on the haul-out (22.5%), on haul-out and in water (same sample; 3.2%), in the water (39.6%), and collected every 24 h in the drain screen (26.9%).

Hake and salmon, due to their large size, generally were torn into pieces by the sea lions during consumption. One adult male sea lion consumed two adult salmon whole. When hake were torn during consumption, otoliths sometimes fell out of the hake skull, so 17 of 286 hake otoliths were not ingested. Two of 27 pink salmon heads were not ingested. The number of prey ingested was reduced accordingly.

### *Passage Times*

Hard parts of a single meal were excreted through a mean of six scats (SE = 0.2), with a mean of two prey species/scat (SE = 0.04). A mean of 16.3% (SE = 0.2%) of all skeletal structures passed 20 h after ingestion, 27.9% (SE = 0.2%) in 40 h, and 45.8% (SE = 0.2%) in 60 h (Table 4). Recovery of prey species fed to sea lions was between 39.9% (*O. mykiss*) and 57.0% (*E. mordax*) after 40 h of ingestion, except for adult pink salmon (Fig. 1). Eighty-six percent of adult salmon hard parts were recovered within 20 h and 97.1% by 40 h. Variability in percentage recovery among the rest of the prey species increased 60 h after ingestion (Fig. 1).

Passage times were not significantly different between ages or sexes of the sea lion, meal composition (sardine and hake), or meal size (Pacific mackerel). However, FDT

Table 4. Mean, standard error, and range of initial and final passage times (IDT and FDT) in hours for all prey species fed to sea lions.

Species	Mean mass (kg)	Method	IDT	SE	FDT	SE	Range (min–max)	
<i>Engraulis mordax</i>	20.07	Otoliths	21.9	1.5	81.6	7.5	5.4–169.3	
		All-structure	21.9	1.5	81.6	7.5	5.4–169.3	
<i>Merluccius productus</i>	441.32	Otoliths	27.4	3.0	55.7	4.7	6.8– 69.5	
		All-structure	18.9	1.3	67.4	3.8	1.8–126.3	
<i>Oncorhynchus gorboscha</i>	1,814.63	Otoliths	30.6	2.4	35.8	4.0	17.7– 47.2	
		All-structure	18.7	1.2	62.4	4.3	3.5–120.8	
<i>Oncorhynchus mykiss</i>	108.14	Otoliths	25.3	4.6	59.1	7.8	6.3–157.3	
		All-structure	16.6	1.8	67.4	7.7	5.0–157.3	
<i>Sardinops sagax</i>	87.33	Otoliths	25.4	1.7	49.6	3.0	14.6– 90.0	
		All-structure	23.5	1.6	72.0	5.0	12.5–258.0	
<i>Scomber japonicus</i>	176.63	Otoliths	24.7	1.3	57.3	5.2	13.8–220.0	
		All-structure	20.9	1.3	68.9	5.1	5.0–220.0	
<i>Sebastes jordani</i>	80.79	Otoliths	22.6	1.4	62.0	4.6	18.8– 69.5	
		All-structure	16.0	2.8	62.0	4.6	6.7– 74.2	
<i>Trachurus symmetricus</i>	83.22	Otoliths	20.3	1.3	59.3	5.4	8.6–113.5	
		All-structure	20.3	1.3	70.6	6.5	8.6–165.7	
<i>Loligo opalescens</i>	45.08	Beaks	20.2	1.9	76.9	14.0	2.0–387.8	
		Mean	Otoliths	23.9	0.9	54.5	2.1	5.4–220.0
		Bones	18.9	0.7	58.2	2.0	1.8–258.0	

of steelhead smolt for subadult sea lions (80.1 h) was greater than adults (45.6 h). The significant difference in passage time between sea lion age for hake otoliths (ANOVA:  $P = 0.034$ ) and bones (ANOVA:  $P < 0.001$ ) was most likely due to only one adult sea lion receiving single meals of hake.

#### Prey Recovery

Mean percentage recovery was 57.4% (SE = 2.1%) for otoliths, 39.3% (SE = 1.9%) for other bones, and 88.5% (SE = 3.0%) for squid beaks. Mean percentage recovery increased to 67.7% (SE = 1.7%) using the all-structure method. Recovery of Pacific mackerel did not vary significantly across sex, age, meal composition, or meal size. Significant differences in percentage recovery for sardine (otolith; ANOVA  $P = 0.044$ ) and anchovy (bones; ANOVA  $P < 0.001$ ) were likely due to outliers (sardine and anchovy) and the differences in rockfish recovery (bones; ANOVA  $P < 0.001$ ) are most likely because of low sample size. Larger otoliths from hake, jack mackerel, and rockfish had a percentage recovery greater than 68% (Table 2). Mean percentage recovery of small rockfish otoliths was 55.0% (SE = 0.1%), and for medium fish was 81.9% (SE = 0.1%). Large fishes, eaten in smaller numbers (hake and salmon), had the greatest otolith percentage recovery for fish species (Table 2).

The percentage recovery of adult pink salmon exceeded 100% (103.7%–225.9%). Using the all-structure method (Table 3) inflated the estimate of salmon consumed, compared with the recovery estimate of 74.1% when using only otoliths to calculate MNI (Student's  $t = 10.4$ ,  $df = 16$ ,  $P < 0.001$ ). Percentage of salmon estimated in the diet increased as the number of bone types used to estimate the percentage of salmon increased (Fig. 2). The all-structure method increased percentage recovery

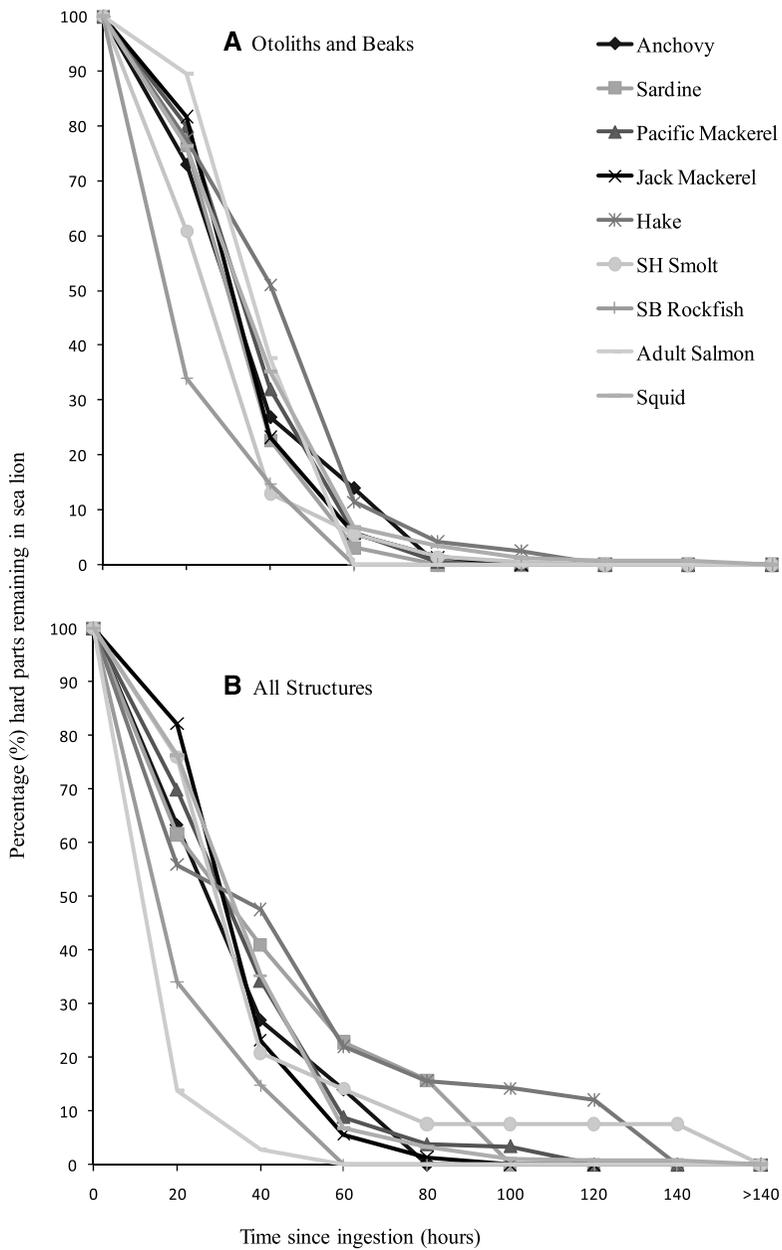


Figure 1. The mean percentage of (A) otoliths and beaks (%) and (B) all-structures (otoliths, bones, and beaks) retained in the sea lion at time since ingestion (hours).

for all species by a mean of 7.2% (SE = 1.3%). Sardines had the lesser percentage recovery using only otoliths and when using the all-structure percentage recovery with prootics (Table 2).

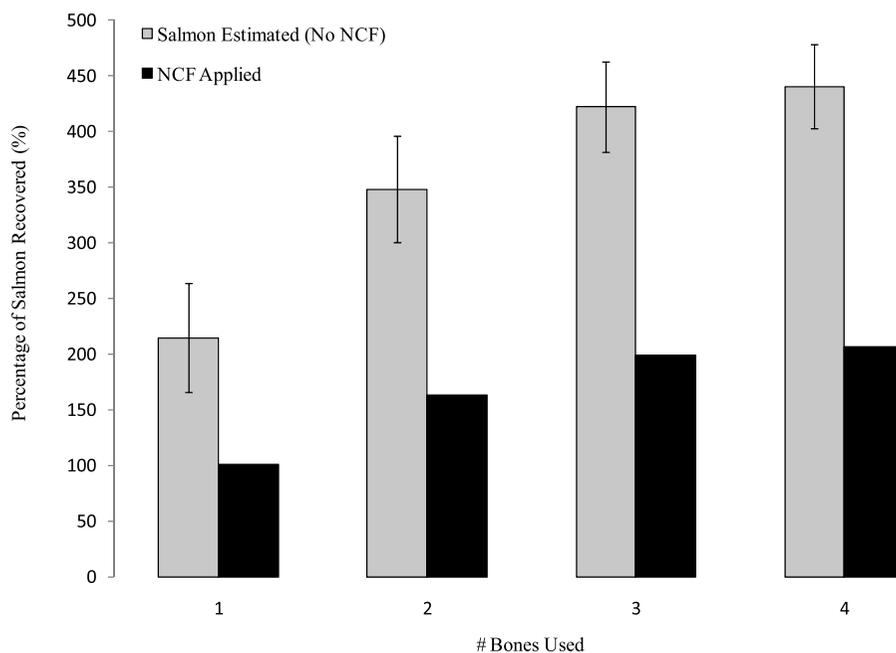


Figure 2. Calculated percentage of salmon recovered using an increasing number of bone types (vertebrae, branchial, teeth, gill raker, respectively) without an NCF applied and percentage of salmon estimated when the NCF for vertebrae is applied to the percentage recovery. (Error bars = standard deviation).

Estimates from spews used in conjunction with scat estimates did not significantly affect percentage recovery for anchovy (Student's  $t = 1.84$ ,  $df = 2$ ,  $P = 0.208$ ), sardine (Student's  $t = 0.95$ ,  $df = 14$ ,  $P = 0.358$ ), Pacific mackerel (Student's  $t = 1.08$ ,  $df = 4$ ,  $P = 0.342$ ), salmon smolts (Student's  $t = 2.68$ ,  $df = 4$ ,  $P = 0.055$ ), or hake (Student's  $t = 0.86$ ,  $df = 6$ ,  $P = 0.425$ ). Other species were not regurgitated during this study. Fish with large bones (*e.g.*, salmon and hake) were commonly regurgitated.

### Length Estimates

Graded otoliths underestimated the original prey length by a mean of 14.1% (SE = 0.3%) using otolith ventral length and a mean of 21.1% (SE = 0.8%) using otolith width. Prey length was underestimated using otolith ventral length assigned as grade 1 by an average of 6.8% (SE = 0.4%) and 4.0% (SE = 0.5%) using otolith width. Prey length was underestimated by an average of 19.5% (SE = 0.4%) using otolith length and 35.5% (SE = 1.2%) using otolith width for otoliths assigned as grade 2. Finally, prey length was underestimated by an average of 35.4% (SE = 1.06%) using otolith length and 36.8% (SE = 3.5%) using otolith width for otoliths assigned as grade 3.

Estimated lengths of prey that were not corrected with LCFs were significantly less than estimates of prey size using aLCF or gLCFs (Table 5). Estimated mean fish length (without LCF) after digestion using otolith length was 16.5% (SE = 0.35%) less than the original fish length. Mean corrected fish length using aLCF differed by

Table 5. Uncorrected, average, and graded standard lengths and widths (cm) of prey fed to California sea lions (*Zalophus californianus*) tested statistically with a one-sample *t*-test to determine the efficacy of the correction factors ( $\alpha = 0.017$ , Bonferroni adjusted value).

Species	Original			Uncorrected			aLCF corrected			gLCF corrected		
	Mean length (cm)	Mean mass (kg)		Mean length (cm)	<i>t</i>	<i>P</i>	Mean length (cm)	<i>t</i>	<i>P</i>	Mean length (cm)	<i>t</i>	<i>P</i>
<b>Otolith length</b>												
<i>Engraulis mordax</i>	12.27	20.07		8.85	114.3	<0.001	12.32	1.3	0.211 <sup>a</sup>	12.32	1.6	0.101 <sup>a</sup>
<i>Sardinops sagax</i>	18.48	87.33		17.31	16.0	<0.001	18.90	4.4	<0.001	18.99	5.9	<0.001
<i>Scomber japonicus</i>	23.45	176.63		19.88	22.1	<0.001	24.05	7.4	<0.001	23.92	6.1	<0.001
<i>Trachurus symmetricus</i>	18.33	83.22		17.49	14.4	<0.001	18.36	0.5	0.650 <sup>a</sup>	18.33	0.0	0.966 <sup>a</sup>
<i>Merluccius productus</i>	37.02	441.32		26.15	43.5	<0.001	39.90	7.7	<0.001	37.50	2.9	0.003
<i>Oncorhynchus mykiss</i>	19.10	108.14		14.96	66.0	<0.001	19.95	10.7	<0.001	19.39	4.4	<0.001
<i>Sebastes jordani</i>	16.16	80.79		12.20	27.1	<0.001	16.39	1.6	0.122 <sup>a</sup>	16.00	1.9	0.284 <sup>a</sup>
<i>Oncorhynchus gorbascba</i>	50.03	1,814.63		43.30	43.5	<0.001	49.37	4.2	<0.001	49.52	3.3	0.001
<i>Loligo opalescens</i> (URL)	12.61	45.08		12.82	4.2	<0.001	12.82	4.2	<0.001			
<i>Loligo opalescens</i> (LRL)	12.78	45.08		12.02	12.6	<0.001	12.73	0.8	0.410 <sup>a</sup>			
<b>Otolith width</b>												
<i>Sardinops sagax</i>	18.48	87.33		16.20	30.7	<0.001	18.57	0.8	0.445 <sup>a</sup>	18.52	0.3	0.738 <sup>a</sup>
<i>Scomber japonicus</i>	23.35	176.63		20.51	22.1	<0.001	24.40	7.4	<0.001	24.00	6.1	<0.001
<i>Trachurus symmetricus</i>	18.30	83.22		17.78	8.9	<0.001	18.52	3.6	<0.001	18.60	5.3	<0.001
<i>Merluccius productus</i>	37.45	441.32		26.33	38.8	<0.001	48.09	21.2	<0.001	39.42	8.3	<0.001

<sup>a</sup>Non-significant values that signify the estimated length is similar to the original length fed.

4.5% (SE = 0.6%) from the original fish length (Table 5), and estimated prey length using gLCF had a mean difference of 2.4% (SE = 0.5%; Table 5) from the original fish length. Estimated dorsal mantle length (DML; without LCF) of squid using upper rostral lengths differed by 9.4% (SE = 0.4%) from the original squid mantle length fed to sea lions, and mean DML estimations using aLCF differed by 2.3% (SE = 0.7%) from the original mantle length (Table 5). Pacific mackerel prey lengths were underestimated more for large meals (14.3%) than smaller meals (10.3%,  $t = 3.5$ ,  $df = 622$ ,  $P = 0.001$ ) using otolith widths.

### *Consumption*

The mFO model estimated hake, salmon smolts, Pacific mackerel, sardine, and jack mackerel within 12.0% of the original proportion consumed using only otoliths/beaks (Fig. 3A). Estimates of hake, adult salmon, rockfish, sardine, and jack mackerel proportions using the all-structure method were within 4.0% of the original proportions (Fig. 3A). Using the otolith/beak method, squid was overestimated by 7.5% of the original proportion fed, yet when the all-structure method was implemented, this decreased to 2.0% of the original proportion fed. Only beaks were used to estimate squid proportions of diet for the otolith and the all-structure methods. However, the all-structure method more accurately estimated proportions of some species, such as adult salmon, while proportions of other species retained the same accuracy.

The SSFO model estimated the proportion of hake, salmon smolts, rockfish, jack mackerel, and squid within 1.4% of the original proportion of prey consumed but overestimated salmon by 7.9% and anchovy by 6.9% when using only otoliths/beaks. Using the all-structure method, adult salmon, sardine, rockfish, and jack mackerel were estimated within 1.0% of the original prey consumed and the remaining prey were estimated within 14.7% (Fig. 3B) of the original proportions.

Using NCFs and gLCFs with otolith and beak counts only, the FBR model estimated hake, salmon smolts, rockfish, and jack mackerel within 1.0% of the original proportion of prey consumed. The remaining prey species were estimated within 3.2% and 11.4% of the original proportion using otoliths and beaks (Fig. 3C). The FBR model estimated rockfish, anchovy, sardine, and squid within 0.4% and 1.7% of the original proportions using the all-structure method. Estimated biomass of hake and adult salmon was 7.8% greater than the original proportion of prey consumed (Fig. 3C).

The VBR model correctly estimated proportions of five of the nine prey species using NCFs and gLCFs, according to the Dunnett's multiple-comparisons test (Fig. 3D). Pacific mackerel, sardine, and jack mackerel proportions were underestimated. Most of the prey species using the otolith/beak method were estimated within approximately 2.0% of the original proportion consumed. The VBR using only otoliths and beaks and gLCF overestimated hake by 8.2%, and underestimated sardine by 4.3% and Pacific mackerel by 5.1% from the original proportion. Yet, when using the all-structure method, hake was overestimated by 8.5%, and sardine and jack mackerel were underestimated by 4.1% and 4.8%. Results from the VBR model without using LCFs differed when compared with the model's results when LCFs were applied. Using only otoliths/beaks without LCFs, hake had a percentage difference of 40.2%, and the following species had negative differences than when applying LCFs: salmon smolts 41.3%, adult salmon 26.2%, rockfish 77.9%, and Spanish mackerel 37.4%.

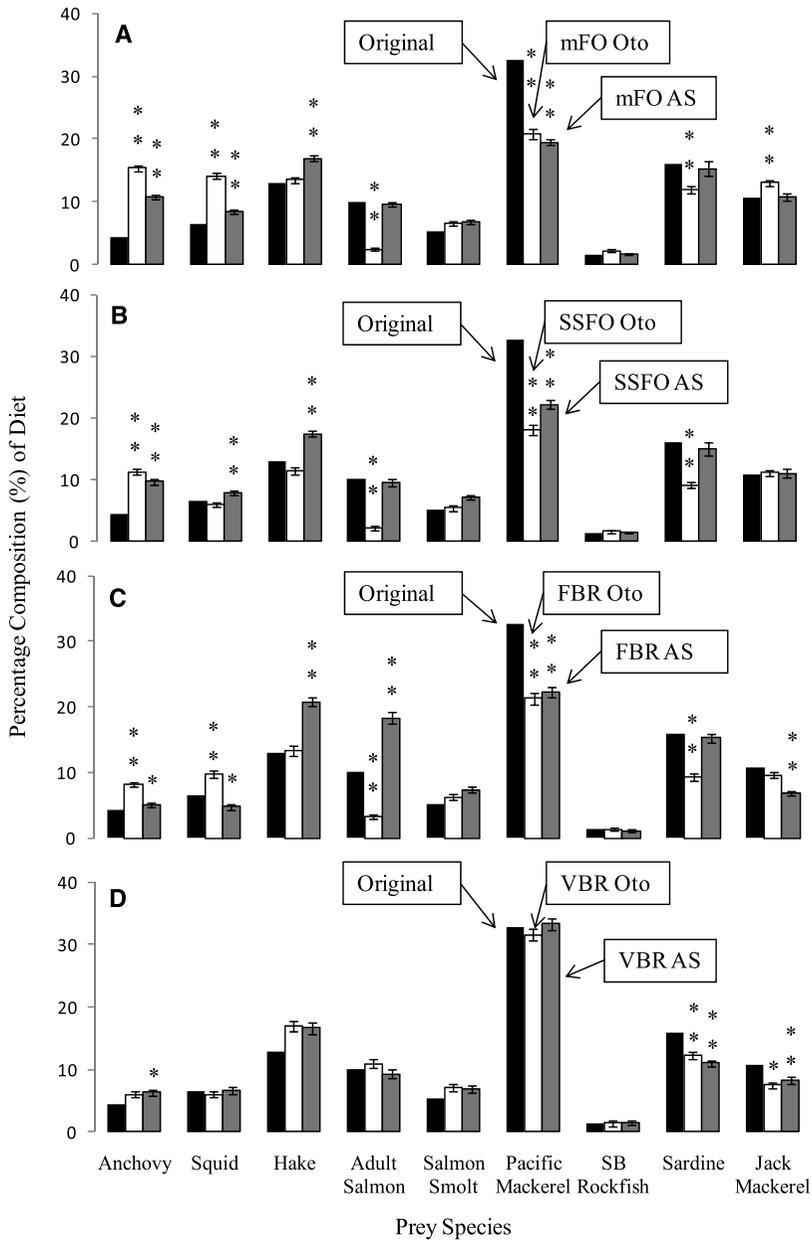


Figure 3. The four models, (A) modified Frequency of Occurrence (mFO), (B) Split-Sample Frequency of Occurrence (SSFO), (C) Fixed Biomass Reconstruction (FBR), and (D) Variable Biomass Reconstruction (VBR), are presented comparing the otolith and all-structure methods to compare model predictions to the original prey composition fed to sea lions using  $n = 100$  samples. The FBR and VBR models are corrected by numerical and graded length correction factors for each prey species.  $\alpha = 0.05$ ,  $P \leq 0.05$  (\*),  $P \leq 0.01$  (\*\*); error bars = standard error.

## DISCUSSION

Location of the animal at the time of scat deposition affects accurate assessment of sea lion diet, which can be problematic when studying free-ranging sea lion populations. Free-ranging adult male sea lions spend a mean of 51.3% of time at sea and 49.7% of time on land (Costa *et al.* 2007). If defecation occurs at sea, a scat from an animal in the water en route to or from haul-out sites would be unattainable from free-ranging sea lions but were quantified in this study. Approximately 49% of samples were collected from the water during this captive study, not including samples collected by the drain screen. Percentage recovery of prey from scats and spews found on haul-outs of free-ranging sea lions depend on how recently the meal was eaten before the sea lion returned to shore.

### *Passage Times*

Our findings were similar to past studies of passage time. Passage times for otoliths were 5.4–220.0 h, close to the range reported by Orr and Harvey (2001). The least IDT for all-structures in this study was similar to the 2 h value reported by Tollit *et al.* (2003), yet less than the 5 h reported by Staniland (2002). A single beak of *L. opalescens* had the greatest FDT of 387.8 h. We did not find differences in passage times between males and females, though Helm (1984) did find females had greater passage times than males. Helm (1984), however, only studied two sea lions of each sex; therefore, these differences could be due to individual variability. Even with a fast initial elimination time of hard parts from a meal, the greater FDTs of prey species can allow the collection and identification of prey consumed coastally and offshore.

The fragile nature of otoliths from some prey species likely affects the accuracy of diet estimation. Jobling (1987) found that prey with smaller, more fragile otoliths were dissolved and underrepresented in scats after 24 h of ingestion. Sardines are one such species, and consequently had the least otolith passage time in this study, which would decrease the probability of detecting sardine in scats deposited on land. However, mean passage time of sardine increased when using the all-structure method, thereby increasing the probability of sardine being detected in scats on land.

### *Prey Recovery*

Percentage recovery of otoliths varied for different prey species, supporting previous findings (Da Silva and Neilson 1985, Prime and Hammond 1987, Harvey 1989, Tollit *et al.* 1997, 2003; Grellier and Hammond 2005, Phillips and Harvey 2009). Hard parts not recovered were presumably digested completely or degraded to the extent they were unrecognizable. Larger, more robust otoliths of hake and rockfish had greater percentage recovery than smaller, more fragile otoliths, similar to previous studies (Da Silva and Neilson 1985; Harvey 1989; Tollit *et al.* 1997, 2003). The recovery of hake in this study was comparable with 71.24% of Tollit *et al.* (1997) and 77.20% (SE = 13.3) of Orr and Harvey (2001). This emphasizes the increased detection of prey with robust otoliths, such as hake, compared with prey with more fragile otoliths, such as sardine.

Fragmentation of a meal over multiple scats presents a dichotomy when calculating percentage consumption of salmon using the all-structure method. It complicates diet

assessment because a proportion of scats are defecated at sea, eliminating a percentage of the meal from the diet estimate. The all-structure method increases the probability of a prey item being detected, yet this prey item can be counted more than once. Overcounting of a prey item can be avoided by identifying structures to the lateral body side (right or left) and by pooling all scats together instead of treating each scat as a separate sample. In addition, researchers most likely do not collect multiple scats from a single individual in free-ranging sea lion studies; therefore, the potential to overestimate salmon in these studies is reduced. Nevertheless, fragmentation can cause inflated estimates of predation on adult salmon because most egested salmon bones are multiple structures, such as vertebrae. Given this difficulty, evidence of salmon predation using vertebrae can only be scored as presence/absence, not allowing determination of number consumed. Unless there are different sizes of the same bone type, each scat containing salmon bones is counted as one salmon, increasing the probability of detecting salmon. Therefore, the number of salmon detected using multiple structures will only correspond to the number of scats containing salmon parts, which will not accurately represent the number of salmon actually consumed.

Fragmentation of a meal over multiple scats and prey with fragile otoliths (Jobling 1987, Tollit *et al.* 2003) supports using the all-structure method for diet estimation. It is of the utmost importance to use only one bone type to identify adult salmon (*e.g.*, vertebrae) and species with fragile otoliths, such as sardine (*e.g.*, prootics), for calculating more accurate estimates. Predation on adult salmon and sardine was best estimated using the all-structure method in this study, supporting work by Tollit *et al.* (2003; Fig. S1). The all-structure method did not significantly improve estimates for other prey species, although other researchers have indicated this method improves estimated percentage recovery in pinnipeds (Cottrell *et al.* 1996; Tollit *et al.* 2003, 2007). To accurately estimate the percentage of salmon consumed by a population, it is important to use only one structure when using the all-structure method and to use the calculated NCF for that structure (Table 3).

Enclosures used to house pinnipeds in captive studies influence their activity level and can bias percentage recovery. Hawes (1983) found a direct relationship between percentage recovery and individual activity. We found greater mean percentage recovery for anchovy, Pacific mackerel, jack mackerel, pink salmon, steelhead salmon, hake, and squid than other researchers (Table S6, Appendix S2). In a captive study, increased activity level increased percentage recovery of otoliths for Steller sea lions (*Eumetopias jubatus*) (Tollit *et al.* 2003). Tollit *et al.* (2003) found greater percentage recovery of all tested species, especially salmon, when Steller sea lions swam for approximately 4 h, simulating a foraging trip. Free-ranging sea lions forage on prey patches in the open ocean many kilometers offshore. Costa *et al.* (2007) found female sea lions traveled up to 96.5 km off shore and male sea lions up to 650 km off shore during foraging trips. Confining a sea lion to a small swimming area does not mimic physiological demands of foraging behavior. Thus, greater mean percentage recovery in our study for the same prey items in studies by Harvey (1989), Orr and Harvey (2001), Tollit *et al.* (2003) could be due to individual variability in activity. Alternatively, contrasts could have arisen because of the greater number of replicates in this study, differences among pinniped species, or experimental design (Table S7).

The recovery of prey was not significantly affected by the 5.6% of samples regurgitated in this study. During 9 yr on St. Paul and St. George Island, Alaska, Gudmundson *et al.* (2006) found 12.9% of samples collected from northern fur seal (*Callorhinus ursinus*) were spews and contained primarily large cephalopod beaks. Studies of free-ranging CSL for 28 yr also have indicated spews contained predominantly

cephalopods.<sup>1</sup> We may have observed so few spews as a result of feeding only small sizes or small quantities of cephalopods during this study.

*Loligo opalescens* had the greatest FDT (387.8 h since ingestion), signifying an increased retention in the stomach, yet had a mean recovery of 89.5% (SE = 3.0). Previous researchers have observed a bias in frequency (Bigg and Fawcett 1985, Gales and Cheal 1992, Fea *et al.* 1999) and size (Gales *et al.* 1993, Lowry and Carretta 1999, Gudmundson *et al.* 2006) of squid beaks in spews. Tollit *et al.* (1997) found large cephalopod beaks were present in scats of harbor seals (*Phoca vitulina*). Two of the sea lions in this study had large beaks of the boreal clubhook and Humboldt squid present in both scats and spews with a minimum of 20–60 d since ingestion, having consumed them before entering the rehabilitation facility. This observation of large cephalopod beaks regurgitated and defecated does not support large squid beaks only being vomited, as Gales and Cheal (1992) proposed, yet this exemplifies the retention of cephalopod beaks in the digestive system of sea lions.

### *Length Estimates*

The otolith LCF we developed (Table 6, 7) better estimated length of prey consumed than the length estimates calculated with uncorrected otolith ventral lengths and widths (Table 5). aLCFs better estimated the size and biomass of prey, but gLCFs better estimated original prey size than aLCF, with decreased error. Relating diet analysis from captive studies to free-ranging pinniped populations has been problematic in the past because sea lion activity levels in captivity were not comparable with free-ranging sea lions. Incorporating the degree of digestion (grades 1, 2, or 3) standardizes otolith digestion, eliminating the bias of digestion time. Grading otoliths relate the degrees of otolith digestion found in this captive feeding study to otolith digestion found in free-ranging sea lions, and using gLCFs better estimates the otolith size before digestion.

Otolith erosion increased with meal size of Pacific mackerel, however, passage times did not differ between small and large meals. Jobling and Breiby (1986) performed dissolution tests at varying acidities and found erosion increased with increasing acidity. It is possible the pinniped stomach might increase acidity in response to a larger meal size; however, testing this hypothesis would require further study.

### *Consumption*

mFO and SSFO models misrepresent the amount of prey consumed (Fig. 3A, B). The SSFO is problematic because of the assumption that prey items are consumed in equal quantities. SSFO weights each scat; hence, a scat containing two species possibly consumed in different quantities will each be calculated as 50% of the diet. mFO and SSFO models are affected by skeletal fragmentation of one fish over multiple scats, increasing the possibility of counting the same fish more than once. Using multiple structures to identify prey items can exaggerate this error, though this might not be a problem in the wild because only a proportion of scats from a meal are collected. In addition, these models do not incorporate the biomass of prey

<sup>1</sup>Personal communication from Mark Lowry, Southwest Fisheries Science Center, 3333 North Torrey Pines Court, La Jolla, CA 92037-1022, March 2008.

Table 6. Average standard length correction factors (aLCFs) for prey species fed during captive study for the ventral otolith length and otolith width, the number of otoliths used to calculate aLCF ( $n$ ) is reported. The mean size of prey fed to sea lions (cm) and standard error (SE) is compared to the estimated prey length before correction with aLCF. The mean ventral or width reduction of otoliths and standard error are compared among prey species.

Species	$n$	Prey length (cm)			Percent of length reduction	SE	aLCF	SE
		Fed	SE	Estimated				
<b>Otolith ventral length</b>								
<i>Engraulis mordax</i>	558	12.29	0.02	8.81	28.31	0.54	1.30	0.01
<i>Mertuacius productus</i>	61	36.56	0.44	26.79	27.80	2.96	1.52	0.06
<i>Oncorhynchus mykiss</i>	128	20.03	0.12	14.56	23.23	1.12	1.33	0.02
<i>Oncorhynchus gorbuscha</i>	23	49.23	0.96	43.10	12.17	1.13	1.15	0.02
<i>Sardinops sagax</i>	247	18.48	0.09	17.31	6.59	0.74	1.09	0.01
<i>Scomber japonicus</i>	257	23.33	0.08	19.86	14.70	1.04	1.21	0.02
<i>Sebastes jordani</i>	56	16.16	0.47	12.20	23.32	1.67	1.34	0.03
<i>Trachurus symmetricus</i>	332	18.44	0.05	17.62	3.75	0.57	1.05	0.01
<i>Loligo opalescens</i> (URL)	598	12.94	0.07	12.92	-0.79	0.44	1.00	0.00
<i>Loligo opalescens</i> (URL)	514	12.94	0.07	12.16	4.65	0.52	1.06	0.01
<b>Otolith width</b>								
<i>Mertuacius productus</i>	109	36.56	0.44	24.73	32.82	2.86	1.79	0.09
<i>Sardinops sagax</i>	379	18.48	0.09	16.20	12.02	0.69	1.16	0.01
<i>Scomber japonicus</i>	716	23.33	0.08	20.26	12.94	0.65	1.18	0.01
<i>Trachurus symmetricus</i>	402	18.44	0.05	17.79	2.84	0.58	1.04	0.01

Table 7. Graded standard length correction factors (gLCFs) for prey species fed during captive study for the ventral otolith length and otolith width. Mean prey size (cm) and standard error (SE) sea lions and the estimated prey length from otoliths after digestion with standard error (SE), and percentage length reduction for each grade of otolith for prey species.

Species	Grade	n	Prey length (cm)		% Length reduction		gLCF	SE	
			Fed	SE	Estimated	SE			
<b>Ventral length</b>									
<i>Engraulis mordax</i>			12.29	0.02					
	1	295			9.40	0.05	22.89	1.30	0.01
	2	247			8.39	0.05	31.87	1.48	0.01
	3	17			7.33	0.21	40.71	1.70	0.05
<i>Sardinops sagax</i>			18.48	0.09					
	1	174			17.89	0.15	3.90	1.04	0.01
	2	71			15.83	0.30	13.21	1.12	0.02
	3	2			15.67	0.00	29.43	1.35	0.07
<i>Scomber japonicus</i>			23.33	0.08					
	1	193			20.78	0.02	10.87	1.14	0.01
	2	64			17.05	0.06	26.41	1.42	0.03
<i>Trachurus symmetricus</i>			18.44	0.05					
	1	183			18.48	0.10	-1.45	0.99	0.00
	2	139			16.64	0.13	9.79	1.12	0.01
<i>Merluccius productus</i>			36.56	0.44					
	1	21			35.00	0.82	4.43	1.06	0.03
	2	27			24.85	0.77	33.75	1.56	0.05
	3	13			17.09	0.59	54.42	2.18	0.07
<i>Oncorhynchus mykiss</i>			20.03	0.12					
	1	45			15.97	0.23	14.68	1.17	0.02
	2	65			14.11	0.19	26.37	1.36	0.02
	3	17			11.93	0.44	37.70	1.63	0.05
<i>Oncorhynchus gorbuscha</i>			49.23	0.96					
	1	15			43.30	0.12	11.87	1.15	0.02
	2	8			42.75	0.27	12.73	1.13	0.02
<i>Sebastes jordani</i>			13.39	0.27					
Left otolith regression	1	5			18.22	1.39	5.87	1.06	0.01
	2	30			11.97	0.38	17.33	1.21	0.01
	3	21			11.14	0.33	34.76	1.56	0.01
<b>Width</b>									
<i>Scomber japonicus</i>			23.33	0.08					
	1	345			22.40	0.01	3.18	1.04	0.01
	2	314			18.16	0.01	20.49	1.27	0.01
	3	57			15.75	0.06	33.82	1.56	0.03
<i>Trachurus symmetricus</i>			18.44	0.05					
	1	216			18.61	0.11	-2.91	0.99	0.01
	2	153			16.96	0.12	8.13	1.09	0.01
<i>Merluccius productus</i>			36.56	0.44					
	1	32			35.02	1.17	4.24	1.08	0.03
	2	53			24.41	0.98	35.24	1.67	0.07
	3	24			13.32	1.07	64.73	3.02	0.18
<i>Sardinops sagax</i>			18.48	0.09					
	1	234			17.11	0.14	7.45	1.07	0.01
	2	123			14.80	0.15	18.71	1.24	0.01
	3	21			13.80	0.87	27.01	1.30	0.06

ingested and are vulnerable to error when larger individuals of a prey species are consumed (Laake *et al.* 2002). Prey consumed in lesser numbers, usually large prey with greater percentage recovery, will be overestimated and prey consumed in greater numbers, usually small prey with lesser percentage recovery, will be underestimated. Our mFO and SSFO simulations did not underestimate sardine or overestimate adult salmon using otoliths. The most important component to accurately predict diet of a population is biomass of the prey items, which is not accounted for in mFO and SSFO models.

Biomass reconstruction models (FBR and VBR, Fig. 3C, D) are more realistic calculations of a predator diet because the models enumerate number of prey by using MNI and incorporate estimates of prey biomass based on sizes of egested otoliths. However, similar to the SSFO model, the FBR model averages percentage consumption of each species over all the scats. Therefore, each scat represents the same proportion of a prey species of the total consumed biomass. This does not identify temporal variation in successful foraging on a particular prey item. The VBR model pools the contents of the scat samples, allowing for the variability in foraging success. The VBR model, in conjunction with NCFs and gLCFs, provided the best estimates of sea lion diet for the prey species fed during this study (Fig. 3D).

Other structures are useful when detecting the presence of some fish species. Because of species-specific differences in size of structures, smaller prey species with more fragile otoliths, such as sardine, may be underrepresented in scats. Hake have more robust otoliths and will be readily found in scats and counted more often than the sardine. Prootics of sardine are as the best alternative structure in detecting sardine in scats.

The number of scat samples collected can affect the performance of the model. Arim and Naya (2003) used simulations to determine that a greater sample size is necessary to detect otoliths recovered from larger rather than smaller prey with the same occurrence. Larger prey are consumed less frequently than smaller prey; therefore, a greater sample size is required to detect larger prey items eaten in small quantities. Our models calculated with 100 scats reduced error compared with using only 50 scats, supporting Hammond and Rothery (1996) who found 100 samples was most reliable when examining diet.

The VBR model requires the calculation of MNI, which uses otoliths and other identifiably diagnostic structures. Otoliths are more easily used to identify prey than other bones, saving the researcher time, and reducing the necessary level of taxonomic expertise. Linear relationships have been established between otolith length and standard fish length and are commonly used in estimating biomass of prey consumed (Harvey *et al.* 2000). Otoliths can be graded based on degree of digestion and gLCFs will most accurately estimate the original length and biomass of prey. Many researchers have recorded biases related to using only otoliths for determining diet (Hawes 1983; Jobling and Breiby 1986; Murie and Lavigne 1986; Harvey 1989; Gales and Cheal 1992; Cottrell *et al.* 1996; Tollit *et al.* 1997, 2003; Orr and Harvey 2001). Using paired structures other than otoliths is beneficial because certain bones have greater passage times (Joy *et al.* 2006; Table 2), increasing the probability of being detected in scats found on land. However, the all-structure technique is time consuming, is affected by skeletal fragmentation, introduces prey size biases, and can lead to an overestimate of larger prey by 30% (Tollit *et al.* 2003). We found the VBR model was more effective when incorporating the all-structure method, providing better estimates for most prey items (Fig. 3D). Our findings support the use of the all-structure method to increase percentage recovery by using

one structure to detect certain prey species, especially salmon (Tollit *et al.* 2003). Overall, we suggest using gLCFs of otoliths for most prey species but using other bone structures for adult salmon and sardine. Using the all-structure method for a few problematic and important species (*e.g.*, salmon) and only otoliths for all other species greatly increases accuracy while decreasing time and cost of hard part recovery and identification.

### Conclusion

This study demonstrated that hard parts of prey species had variable passage times and percentage recovery. Percentage recovery increased with larger, more robust otoliths. Otolith erosion was greater for larger meal sizes. This study has provided NCFs and gLCFs for different prey species to be used with otolith/beak and the all-structure methods. Without the numerical and size CFs, small species would be underestimated and large prey overestimated with the VBR model. Incorporating NCF and gLCF to the VBR model most accurately determined sea lion diet. It would be prudent to develop the VBR model to include the variability calculated with the CFs for a more robust estimate. Further research into the CSL physiological response of diving and digestion must be completed to address potential digestion biases associated with foraging behavior. Integrating scatological analysis and telemetry data can develop a broader understanding of the role of CSL the marine ecosystem.

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#### SUPPORTING INFORMATION

The following supporting information is available for this article online:

*Appendix S1.* Description of the scat collection and bone analysis methodology.

*Appendix S2.* Four diet reconstruction models tested in this study. Adapted from Joy *et al.* (2006).

*Figure S1.* Mean percentage recovery (%) using otolith and all-structure methods for fish species fed to California sea lions (*Zalophus californianus*). The mean percentage recovery reported for the all-structure method is calculated using only vertebrae. Error bars = standard error.

*Table S1.* A sample meal protocol followed during a week of a sea lion trial. All meals were small meals unless otherwise indicated. A small meal is approximately 2.5% of the sea lion mass and a large meal is 5% of the sea lion mass.

*Table S2.* California sea lions (CSL no.) included in the captive study and the prey species, number of prey fed per meal (*n*), and mean standard lengths (cm) and weights (g) of prey and meals.

*Table S3.* Single or paired structures (bones) found in greater numbers (*n*) in scat and spew of California sea lion (*Zalophus californianus*) in the captive study and the percentage recovered (%).

*Table S4.* Bones recovered in scat (with and without spew) that were identified to species.

*Table S5.* Least-squares linear regression equations used to calculate standard prey length (SL = standard length, DML = dorsal mantle length) and mass (M) from the ventral lengths (VL) or widths (W) of randomly selected left or right otoliths, and upper and lower rostral lengths (URL, LRL) from randomly selected upper or lower rostrums.

*Table S6.* Grading descriptions for changes in otolith morphology due to digestion: grades low, medium, and high for anchovy, hake, sardine, Pacific mackerel, rockfish, and steelhead smolts and grades low and medium for jack mackerel and pink salmon.

*Table S7.* Percentage recovery (%) of similar prey items compared with the activity levels of past pinniped captive studies; "na" = not available, "\*" = standard error.