

# Journal of **Fisheries and Aquatic Science**

ISSN 1816-4927



Journal of Fisheries and Aquatic Science 6 (2): 186-193, 2011 ISSN 1816-4927 / DOI: 10.3923/jfas.2011.186.193 © 2011 Academic Journals Inc.

## A Comparison of Indexes for Prey Importance Inferred from Otoliths and Cephalopod Beaks Recovered from Pinniped Scats

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#### ABSTRACT

In this study a similar data set was used to compare results produced by using four indexes used for quantifying the California sea lion prey. Hard structures like Sagitta otoliths and cephalopod beaks have traditionally been used in pinniped diet analyses. They are important since their shape and inner features can be species or genus specific and aid in the identification of prey. Scats are currently the most used sample type in pinniped diet analyses because they can be easily collected, are abundant, noninvasive and provide enough otoliths and cephalopod beaks. Traditionally, food habit studies of the California sea lion Zalophus californianus based on scats have considered the Percent Number and Percent Occurrence as measures to determine prey importance. There are two other lesser-used indexes: Split-Sample Frequency of Occurrence and Index of Importance that have also been used for quantifying the California sea lion prey. A total of 251 hard structures (fish otoliths and cephalopod beaks) recovered from California sea lion scats collected at Isla San Pedro Mártir in the Gulf of California, México, were examined. Prey taxa were identified using voucher specimen material. The California sea lion diet was estimated using the four indices described above and the results were compared using  $\chi^2$  tests and correlation coefficients. Results obtained from four measurements were consistent in ranking the three main prey taxa but different in relative importance. A strong correlation was found among indexes, but the standardized residuals indicated that linear regression was not an adequate model to describe the relationship between PN and any other indexes. Representing the diet using a single measurement such as IIMP can facilitate the interpretation of results, allow comparisons and promote consistency in pinniped diet studies based on scats.

Key words: California sea lion, Zalophus californianus, diet, Mexico

#### INTRODUCTION

Describing the diet and quantifying prey importance in studies of marine predator feeding habits has been based on various measurements that rank prey species (Stott and Olson, 1973; Den-Hartog, 1979; Jones, 1981). In studies of fish diet, in which feeding habits are assessed using stomach contents, measurements used include the number, weight and volume of each prey category, expressed as a percentage of the total found in the sampled stomachs (Pinkas *et al.*, 1971). For other biological groups such as marine mammals and marine birds, alternative methods based on fecal samples or scats must be applied because obtaining stomachs is impractical or forbidden by law.

In particular, the pinniped diet has been analyzed mainly on the basis of Sagitta otoliths and other hard prey parts recovered from scats (Lowry et al., 1991; Hume et al., 2004; Orr et al., 2004; Garcia-Rodriguez and Aurioles-Gamboa, 2004). Sagitta otoliths are calcareous structures develop over a protein matrix located in the inner ear and bathed in endolymph (Payan et al., 1999). Otoliths have great importance to understand biological and ecological process occurring in the fishes since they storage information related to age and growth, geochemistry and morphological variations affected by environmental and genetic causes (Rooker et al., 2003; Volpedo and Cirelli, 2006; Gallardo-Cabello et al., 2007; Clardy et al., 2008; Santana-Hernandez et al., 2008; Ramirez-Perez et al., 2010). Because otoliths can be a tool for species identification, they are potentially useful in feeding studies of fish predators.

The current measurements used to quantify the prey importance in pinnipeds are the Percent Number (PN) and the Percent of Occurrence (PO). The PN takes into account the number of individuals but the frequency of occurrence does not. The PO considers only the presence or absent of a prey species but not the number of individuals. Olesiuk et al. (1990) proposed a measurement termed Split-Sample Frequency of Occurrence (SSFO). This index is based on two assumptions: 1) identified prey in scat samples represent all those consumed in the last meal and 2) all prey species constituting a meal had been consumed in equal volumes. Recently, an index proposed by Garcia-Rodriguez (1999), are being used to quantify prey importance in the California sea lion Zalophus californianus (Garcia-Rodriguez and Aurioles-Gamboa, 2004; Porras-Peters et al., 2008). This index, termed Index of Importance (IIMP), quantifies the contribution of each prey taxon by considering the relative abundance found in each scat sample. The IIMP assumes that prey found in each scat represents 100% of that consumed in the previous meal. This index was derived for taking in account the probability of finding one individual of taxon i in the sampling.

In this study, we used real data of California sea lions as an example, to quantify the relative importance of prey by using PN, PO, SSFO and IIMP. We focused our attention on differences among the indexes, review why the relative importance may vary in relation to the index used and how these differences can affect interpretations of prey importance.

#### MATERIALS AND METHODS

California sea lion scats were collected at Isla San Pedro Mártir, Gulf of California, México, during June 1995 (Garcia-Rodriguez, 1999). This island is located at 28° 24′ 00″ N - 112° 25′ 30″ W and is inhabited by around 1,150 sea lions.

Samples were processed by soaking in biodegradable detergent solution and latter they were sifted through nested sieves of 2.00, 1.18 and 0.5 mm mesh size. Hard prey structures (otoliths and cephalopod beaks) were identified using voucher specimen from various institutions: Centro Interdisciplinario de Ciencias Marinas (CICIMAR), Instituto Tecnólogico y de Estudios Superiores de Monterrey Campus-Guaymas (ITESM), Centro de Investigación Cientifica y de Educación Superior de Ensenada (CICESE) and Los Angeles County Museum of Natural History (LACMNH). We calculated PN, PO, SSFO and IIMP for each prey taxon as follows:

$$PN_{i} = \frac{\sum_{j=1}^{U} x_{ij}}{\sum_{j=1}^{U} X_{ij}} \times 100 = \frac{x_{i}}{X} \times 100$$
(1)

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where,  $X_{ij}$ , is the number of individuals of taxon j in scat j, U is the total number of scats that contained detectable prey remains, xi is the total number of individuals of taxon i and X is the total number of individuals across all taxa.

$$PO_{i} = \frac{\sum_{k=1}^{U} O_{ij}}{U} \times 100$$

$$(2)$$

where,  $O_{jk}$  is the absence or presence of taxon i in scat j:

$$SSFO = \sum_{i=1}^{n} \left( O_{ijkm} / \sum_{k=1}^{N} O_{ijkm} \right) / n \tag{3}$$

i = 1,..., n samples collected; k=1..N different prey species where  $O_{ijkm}$  is a binary variate that indicates whether the kth prey species were absent or present in the ith sample collected in the jth strata in the mth month:

$$IIMPi = \frac{1}{U} \sum_{i=1}^{U} \frac{xij}{Xi}$$
 (4)

where  $x_{ij}$  is the number of individuals of taxon i in scat j, Xj is the total number of individuals in scat j across all taxa and U is the number of scats in the sample.

A chi-square test based on Monte Carlo simulations was done 1000 times to determine whether estimations using different indexes were statistically homogeneous. To show nearest similarities among indexes, a dendrogram based on UPGMA algorithm was constructed for using pair-wise  $\chi^2$ . We carried out a multiple correlation and regression the IIMP with each one index. According to Garcia-Rodriguez and Aurioles-Gamboa (2004) the IIMP can hold more information than other indexes; thus, we used it as reference in regression.

#### RESULTS

A total of 251 hard structures were recovered from 15 scats of California sea lion. Just two cephalopod beaks (Gonatus berryi) were found in the diet; the others were otoliths, indicating that the feeding was mainly piscivorous. Although results obtained from the four measurements (PN, PO, SSFO and IIMP) were consistent in ranking the three main prey taxa (Engraulis mordax, Myctophid 1 and Porichthys sp.), differences in relative importance were observed among measurements (Table 1). Principal difference among indexes was found by estimating the importance of E. mordax. Chi-squared test based on Monte Carlo simulations revealed significant differences among four data set ( $\chi^2 = 181.6$ , p = 0.0). Pair-wise  $\chi^2$  showed a significant difference manly in PN (Table 2). No statistical differences were found between IIMP and SSFO indexes ( $\chi^2 = 14.66$ , p = 0.3400) and between SSFO and PO indexes ( $\chi^2 = 18.32$ , p = 0.1510). Dendrogram shows that the results had mayor similarities when they were estimated by IIMP and SSPO. Most disparity was when prey importance was calculated by using PN (Fig. 1). High correlation was found among indexes (Table 3), but the standardized residuals obtained from the correlation between PN and IIMP were not homoscedastic (Fig. 2).

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Table 1: Prey importance of California sea lions estimated by IIMP, PN, PO and SSFO

Species	N	0	IIMP	PN	PO	SSFO
Engraulis mordax	165	9	29.7	65.74	26.47	29.61
Mycthopid No. 1	39	6	29.0	15.54	17.65	22.55
Porichthys spp.	11	4	11.2	4.38	11.76	12.90
Trichiurus lepturus	8	3	8.3	3.19	8.82	7.84
Caelorinchus scaphopsis	1	1	7.1	0.40	2.94	7.23
Hemanthias peruanus	11	1	3.9	4.38	2.94	1.03
Gonatus berryi	2	2	2.1	0.80	5.88	2.84
Prionotus sp.	1	1	1.4	0.40	2.94	3.62
Symphurus sp.	3	1	1.1	1.20	2.94	1.03
Trachurus symmetricus	2	1	0.7	0.80	2.94	1.03
Physiculus nematopus	1	1	0.7	0.40	2.94	3.62
Prionotus stephanophrys	3	1	0.6	1.20	2.94	1.45
Merluccius sp.	1	1	0.4	0.40	2.94	1.03
Unid. Mycthophidae	3	2	3.8	1.20	5.88	4.22
Total	251		100.0	100.00	100.00	100.00

N: Total number of hard structures, O: Occurrence

Table 2: Pair-wise  $\chi^2$  (below) and corresponding p-values (above)

	PN	PO	SSFO	IIMP
PN	-	0.0	0.00	0.00
PO	101.0	-	0.151	0.00
SSFO	94.7	18.3	-	0.340
IIMP	79.9	34.4	14.70	-

Table 3: Correlation matrix

	PN	PO	SSFO	IIMP
PN	1.000	0.889	0.849	0.798
PO	0.889	1.000	0.972	0.947
SSFO	0.849	0.972	1.000	0.978
IIMP	0.798	0.947	0.978	1.000

All relationships were significantly positive (p<0.05)

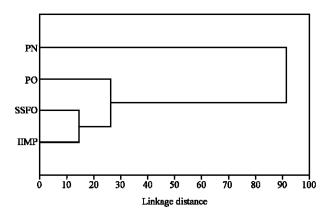
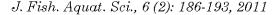


Fig. 1: UPGMA dendrograme based on pair-wise  $\chi^2$  obtained among four indexes



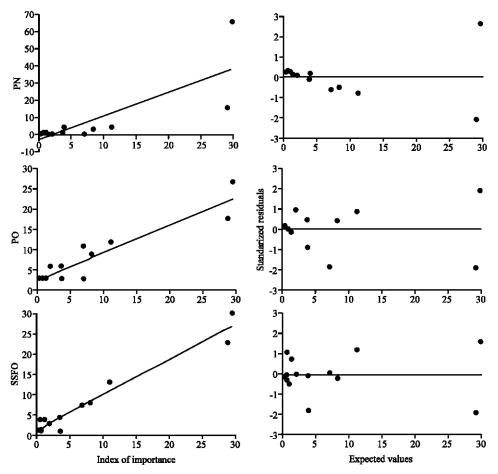


Fig. 2: Relationships between Index of Importance (IIMP), Percent number (PN), Percent of Occurrence (PO) and Split-Sample Frequency of Occurrence (SSFO), respectively. Lines come from fitted regression values

#### DISCUSSION

Results found in this study indicated that different values were obtained by using different indexes and that estimations obtained from PN were the most different. Our results suggest that when the number of individuals of given species varies among scats there is a tendency to overestimate its importance when PN is used, because the absolute number is considered for all samples. The IIMP showed for being much less sensitive than PN to changes in the number of prey in an individual scat. Thus, if a scat contained only one prey taxon, IIMP would be the same, regardless of the number of individuals of that prey taxon in that scat. However, as the number of individuals of a prey taxon in a scat increases, so does the PN for that prey taxon. This can be observed in the example (Table 1), where of a total of 165 E. mordax otoliths recovered, 132 of them were found in a single scat. Thus, the contribution of E. mordax estimated with IIMP was much less than with PN. Essentially, the IIMP allows each scat to contribute an equal amount of information, whereas PN can be dominated by a few scats with large numbers of prey.

The PO was positively correlated with IIMP (Fig. 2), but they showed differences mainly in those species with a great number of hard structures (*E. mordax*) because, in comparison to PN and PO does not consider prey abundance. The SSFO was also correlated with IIMP and although both

indexes treat each scat as a sampling unit, in the SSFO index each prey taxon in any scat is given an equal weight for that scat, regardless of the number of individuals of each taxon in the scat. In this manner, the IIMP index incorporates more information about the number of prey in a scat than the SSFO index, regardless of the number of individuals of each taxon in the scat.

According to Liao et al. (2001) some authors supported the conclusion that mixed indexes as the IRI, based on the combination of different measures, to provide a better view of the importance of preys (Cortes, 1998). Other authors do not agree with that idea. Macdonald and Green (1983) stated that it might not be required to join several measurements into one index (e.g., IRI, Pinkas et al., 1971) if they are correlated, because it could lead to redundancy of variables. Compared to other indexes (e.g., IRI) IIMP does not combine variables in an equation, it just takes account information from each scat and then assigns relative importance based on average relative abundance. Because the PN, PO, SSFO and IIMP do not consider prey weight, consumption can be overestimated when the preys are small (Hawes, 1983).

Representing the diet using a single measurement such as IIMP facilitates the interpretation of results, allows comparisons and promotes consistency especially in pinniped diet studies based on scats. Additionally, an approach could use regression methods to estimate prey size (length or weight) from the lengths of hard structures like fish otoliths and bones and cephalopod beaks (Pierce and Boyle, 1991).

At present the IIMP has been used in California sea lion studies to compare populations, in the determination of trophic diversity, to describe spatial patterns in dietary habits, in the calculation of the daily energy requirements and the spatial and temporal variability of diet (Garcia-Rodriguez, 1999; Camacho-Solis, 2004; Garcia-Rodriguez and Aurioles-Gamboa, 2004; Salazar-Pico, 2005; Salazar-Valenzuela, 2006; Porras-Peters *et al.*, 2008).

Garcia-Rodriguez and Aurioles-Gamboa (2004) carried out an intensive study that included the analysis of samples from the main Gulf of California sea lion colonies. Differences were observed in the diet and in trophic diversity among seasons and rookeries. Those findings were support later by Porras-Peters et al. (2008) who combined the results based on the diet using IIMP with the results obtained using isotope analysis. They support the existence of a spatial structure related to the availability of sea lion prey. Trace metals analyses indicated a consistent relation between the trophic level estimated from the diet of the mother sea lions and the levels of Hg and Se in the hair of their pups (Elorriaga-Verplancken and Aurioles-Gamboa, 2008). Those studies provided relevant information on the diet of California sea lion using the IIMP and allowed a better understanding of the feeding strategy and the microevolutive processes related to the population genetic structure, as suggested by Schramm et al. (2009).

Correctly quantifying the true importance of prey to understand the role of species consumed on the predator is relevant to support the effective management of fisheries resources (Bowen, 1996; Liao et al., 2001). Accurate quantification of the importance of prey is essential mainly when preys and predators interact with human activity. California sea lion consumes species commercially valuable as Scomber japonicus, Engraulis mordax (Lowry et al., 1991) and Sardinops sagax (Garcia-Rodriguez and Aurioles-Gamboa, 2004); thus, correct estimation of the importance of prey is relevant for assessment of marine resources.

#### **ACKNOWLEDGMENTS**

FJGR acknowledges to Dr. D. Aurioles-Gamboa for his teaching and guidance. The authors are grateful with the CONACyT Project (2643-N) for the logistical support. We thank the officers and

crew of SEMAR (Army of México). Samples were collected under SEMARNAT permits: #DOO-700-(2)01104 and DOO-700(2)-1917. FJGR and JDA also thank the grants from SNI-CONACyT, COFAA-IPN and EDI-IPN.

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