



## A new method to evaluate the nutritional composition of marine mammal diets from scats applied to harbor seals in the Gulf of Alaska



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### ABSTRACT

The nutritional profile of harbor seal (*Phoca vitulina* Linnaeus, 1758) diets was determined from scats using a new diet formulation software program and a prey nutritional database developed from proximate analyses of various prey found in Alaskan waters. Scats were collected from Tugidak Island, Alaska, during summers from 2001 to 2009 and prey composition of the diet was estimated using split-sample frequency of occurrence (ssFO) and biomass reconstruction (BR). While 60 different prey items were identified across the collection period, only 21 were found in >10% of scats during at least one sampling period, and an average of 2.6 prey species were identified per scat. Diet diversity was consistently lower in the breeding season than in the molting season. The estimated relative importance of some prey items was significantly different between methods; however, there were no significant differences in the overall estimated protein, lipid, or gross energy composition of the diets. The diet formulation software allows evaluation of the nutritional composition of free-ranging diets using the non-invasive collection of scat. Results from combining the prey database and nutritional composition of diets across years suggest that harbor seals have flexible diets and can maintain nutritional input by taking advantage of seasonally or periodically abundant prey.

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### 1. Introduction

Fecal (scat) analysis is a common, cost-effective, and relatively non-invasive method for describing the diet of marine mammals (Andersen et al., 2004; Berg et al., 2002; Casper et al., 2006; Cottrell et al., 1996; Merrick et al., 1997; Pitcher, 1980; Tollit et al., 2006). Diet composition is commonly estimated by evaluating the relative frequencies of prey species identified in scats, which is assumed to represent the relative importance of these prey in the diet (Andersen et al., 2004; Berg et al., 2002). Biases associated with dietary scat analysis, including underestimation of prey importance due to partial or complete digestive dissolution of prey with fragile parts or prey without hard parts, have been thoroughly reviewed and multiple studies have been conducted to quantify these biases (e.g., Bowen, 2000; Harvey, 1989; Tollit et al., 1997, 2006).

The metric used to assess the relative importance of prey in the diet may also skew the estimated diet composition. Frequency of occurrence (FO), split-sample frequency of occurrence (ssFO), and biomass reconstruction (BR) are three common metrics used for analyzing the dietary

composition of pinnipeds (Antonelis et al., 1997; Merrick et al., 1997; Sigler et al., 2009; Trites et al., 2007; Waite et al., 2012), each with its own set of associated biases and assumptions. A major source of bias with FO and ssFO calculations is that these methods only consider the presence or absence of prey in each sample, and function under the assumptions that 1) prey species identified in a single sample represent all prey items consumed during the last foraging bout, and 2) all of these prey species were consumed in equal quantities (Olesiuk et al., 1990). Such FO calculations tend to overestimate the importance of smaller prey items compared to larger prey items (e.g., one sand lance is not the dietary equivalent of one salmon). Similarly, prey items with robust hard-parts that are defecated over several scats may have higher prevalence across all collected scats (e.g., walleye pollock) (Arim and Naya, 2003; Tollit et al., 2003). On the other hand, estimates of diet composition using BR consider the differential quantities and mass of prey consumed. Biases associated with BR estimations include 1) the underestimation (or complete oversight) of prey with fragile hard parts or entirely soft bodies (da Silva and Neilson, 1985; Harvey, 1989; Orr and Harvey, 2001; Tollit et al., 1997, 2006), and 2) the overestimation of the relative importance of large prey items due to the assumption that the entire prey body was consumed, when in reality only a portion may have been eaten (Hauser et al., 2008; Laake et al., 2002; Phillips and Harvey, 2009; Wright et al., 2007). Use of BR estimations further requires that length-to-weight

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predictive equations for prey species are known for the region and time period studied, which is often not the case for less studied prey species or regions.

The study of nutrition in domestic animals has focused heavily on the use of techniques such as proximate composition of the diet, assimilation efficiency, and analysis of amino acid and fatty acid profiles to understand dietary requirements and deficiencies. These techniques have increasingly been used by wildlife researchers to evaluate the needs and consumption patterns of changing wildlife populations. More recently, ration formulation software programs, such as the MIXIT-WIN program (Agricultural Software Consultants, Inc., San Diego, California, USA) are used by agricultural industries to optimize the nutrient and energy content of livestock diets and balance them with costs of the ingredients. These more sophisticated modeling techniques will likely expand in their use with marine mammals to increase the breadth of knowledge that we can ascertain when the examination of scat samples is the most common form of dietary analysis.

Historically, Tugidak Island (56.51° N, 154.63° W) had one of the largest concentrations of harbor seals (*Phoca vitulina* Linnaeus, 1758) in the world with up to 17,000 seals present in the mid-1950s (Mathisen and Lopp, 1963; Pitcher, 1990). However, between the mid-1970s and the early 1980s, harbor seal numbers throughout Alaska, including those on Tugidak Island, declined substantially (Jemison et al., 2006; Pitcher, 1990). It is estimated that the Tugidak Island harbor seal population declined by as much as 85% between 1976 and 1988, with a maximum yearly population reduction of 19% per year from 1976 to 1982 (Pitcher, 1990). During the 1990s their numbers throughout the Kodiak Island archipelago stabilized and began to recover at about 6.6% per year from 1993 to 2001 (Small et al., 2003). Though this trend continues, the population is still reduced far below its pre-1970s' size (Jemison et al., 2006). The reasons for this dramatic decline are not fully understood. A dominant theory for the decline of pinnipeds is that a dietary shift from high-quality prey to nutritionally-inferior prey led to nutritional stress that contributed to population declines (Alverson, 1992; Trites and Donnelly, 2003). Harbor seals employ central-place foraging strategies around haul-out locations during early and late summer when they spend time ashore while pupping and molting (Burns, 2009; Thompson et al., 1989). Prey available to seals may be restricted by the seals' diminished ability to take extended foraging trips to foraging grounds due to this seasonal tie to rookies (Boness et al., 2006; Burns, 2009; Coltman et al., 1997; Thompson et al., 1994). As a result, this may increase the potential for localized prey depletion as intraspecific competition for nearby resources increases, such as has been observed with Pacific walrus (*Odobenus rosmarus divergens*) (Ray et al., 2006).

Within a given year, harbor seal diets may vary as a consequence of life-history events, as well as seasonal availability of prey. Harbor seals are opportunistic predators, readily capitalizing on a wide variety of prey species from both benthic and pelagic habitats, including sculpins (Cottidae), flatfishes (*Pleuronectidae*), cephalopods, salmon (*Oncorhynchus* spp.), and Pacific sand lance (*Ammodytes hexapterus*) (Andersen et al., 2004; Berg et al., 2002; Herreman et al., 2009; Jemison, 2001; Lance and Jeffries, 2007; London et al., 2002; Pitcher, 1980; Tollit et al., 1998; Wright et al., 2007). Although many prey species have been identified in diets, often only a few of these prey items are present consistently enough to be considered principal prey items in the typical harbor seal diet (Andersen et al., 2004; Jemison, 2001; Pitcher, 1980). Prior studies of Tugidak Island harbor seals identified walleye pollock (*Theragra chalcogramma*), Irish lord (*Hemilepidotus* spp.), and Pacific sand lance as major prey items (Jemison, 2001; Pitcher, 1980).

The objectives of this study were to 1) describe the summer diet of harbor seals from Tugidak Island from 2001 to 2009, 2) estimate the nutritional profile of harbor seal diets, and 3) compare the relative importance of prey species in diets estimated using two different methods (i.e., ssFO and BR).

## 2. Methods

### 2.1. Sample collection and processing

Harbor seal scats were collected by the Alaska Department of Fish and Game (ADFG) from the western beaches of Tugidak Island, Alaska (Fig. 1), during summer (May to September) of 2001–2009 (Table 1). Samples collected between May 1 and July 14 were considered part of the breeding season (Jemison and Kelly, 2001), while scats collected between July 15 and September 30 were assigned to the molting period (Daniel et al., 2003). Breeding period samples were collected only during even-numbered years, while molting period scats were collected each year.

Fresh seal scats were collected into plastic zipper bags and frozen at  $-20^{\circ}\text{C}$  until further processing. Frozen scats were thawed, washed through a set of nested sieves (500  $\mu\text{m}$ , 710  $\mu\text{m}$ , and 1000  $\mu\text{m}$ ) using commercially-available dish soap to break up the scat without damaging the prey remains. Prey hard-parts were isolated and all hard-parts collected from each individual scat sample were dried and placed in individual bags.

Isolated prey remains were sent to Pacific IDentifications Inc. (PacID; Victoria, British Columbia, Canada) to be identified to the lowest possible taxonomic level. Prey items were enumerated and approximate size categories were assigned based on comparisons with an extensive reference collection. Samples not containing identifiable prey items (i.e., unidentified fish or “empty” samples) were not considered for further analyses.

Octopus beaks classified by PacID as “unidentified octopus” were individually re-examined and were all identified as giant Pacific octopus (*Enteroctopus dofleini*). As these beaks represented the vast majority of unidentified cephalopod prey remains, cephalopods will hereafter refer to giant Pacific octopus (GPO). Samples containing unidentified salmonids were also re-examined to increase taxonomic resolution using vertebrae width-to-height ratios (Huber et al., 2011). Salmon identified in samples could be placed in one of three categories: 1) Chinook (*Oncorhynchus tshawytscha*), 2) pink (*Oncorhynchus gorbuscha*) or coho (*Oncorhynchus kisutch*), or 3) chum (*Oncorhynchus keta*) or sockeye (*Oncorhynchus nerka*) (Huber et al., 2011). All salmon biomass estimates were based on a generic salmon length-to-weight ratio; however, nutritional composition was estimated using species-specific proximate composition data.

### 2.2. Diet assessment

A Shannon diversity index (SDI), a commonly used index for evaluating diet diversity of pinnipeds (Herreman et al., 2009; McKenzie and Wynne, 2008; Sigler et al., 2009; Sinclair and Zeppelin, 2002; Trites et al., 2007; Waite et al., 2012), was estimated for each collection period within each year as follows:

$$H = -\sum_{i=1}^k p_i \ln p_i$$

where  $p_i$  is the relative abundance of species  $i$ , and  $k$  is the total number of species identified (Shannon, 1949). A bootstrapping procedure was used to estimate a mean SDI for breeding and molting periods within each year and provide 95% confidence intervals so differences in diet diversity between breeding and molting periods could be evaluated.

The ssFO and BR methods of estimating prey relative importance were only applied to prey species that were identified in at least 10% of scats in at least one collection period. The ssFO was calculated for each prey species within a collection period as follows (Olesiuk et al., 1990):

$$\text{ssFO}_{jk} = \sum_{i=1}^N \left( O_{ik} / \sum_{k=1}^n O_{ik} \right) / N_j$$

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