Alaska Fisheries Science Center

National Marine Fisheries Service

U.S DEPARTMENT OF COMMERCE

AFSC PROCESSED REPORT 2001-04

Pinniped Food Habits and Prey Identification Techniques Protocol

October 2001

This report does not constitute a publication and is for information only.
All data herein are to be considered provisional.

Notice to Users of this Document

In the process of converting the original printed document into Adobe Acrobat .PDF format, slight differences in formatting can occur; page numbers in the .PDF may not match the original printed document; and some characters or symbols may not translate.

This document is being made available in .PDF format for the convenience of users; however, the accuracy and correctness of the document can only be certified as was presented in the original hard copy format.

Pinniped Food Habits and Prey Identification Techniques Protocol

October 2001

Monique M. Lance[1], Anthony J. Orr[2], Susan D. Riemer[3], Michael J. Weise[4], and Jeffrey L. Laake[2]

[1]Washington Department of Fish and Wildlife, 7801 Phillips Rd. S.W. Tacoma, WA 98498
Email: lancemml@dfw.wa.gov

[2]National Marine Mammal Laboratory, Alaska Fisheries Science Center, 7600 Sand Point Way N.E. Seattle, WA 98115

[3]Oregon Department of Fish and Wildlife, P.O. Box 642. Gold Beach, OR 97444

[4]Moss Landing Marine Laboratories, 8272 Moss Landing Rd. Moss Landing, CA 95039

ABSTRACT

This protocol was developed in an effort to standardize methods of collection, processing, identification and analysis of food habit studies using fecal sampling techniques to characterize the diet of pinnipeds and to facilitate comparisons among studies. Advantages of fecal sample analysis and inherent biases and limitations of these data are identified. Fecal sample collection protocols are described qualitatively and quantitatively and new processing techniques are detailed. A recent approach to prey identification utilizes all structures recovered from fecal samples. This technique is particularly important for identification of species with low rates of bone and otolith recovery (e.g., salmonids). Lists are provided of the most frequently used skeletal structures for identification of families of fishes found in studies along the west coast of the U.S. and prey identification options depending on level of expertise, time, finances and specific research goals. Current research using molecular genetic techniques to identify Pacific salmon (*Oncorhynchus* spp.) to species using bone recovered from fecal samples and genetic fecal analysis used to identify the individual harbor seal associated with each fecal sample provide essential information regarding collection and analysis biases. In most cases, fecal sample analysis provides a qualitative summary of species composition and a general inference of the frequency of prey consumption by pinnipeds. Advantages and disadvantages of indices used to analyze food habits data and diet reconstruction models are presented. Through ongoing research with captive animals, molecular genetic techniques, and the refinement and development of new biomass reconstruction models, we strive to advance our understanding of pinniped foraging ecology using fecal sample analysis.

CONTENTS

ABSTRACT	iii
INTRODUCTION	1
FECAL SAMPLE COLLECTION	2
Scope of Inference	3
Sample Size Formulae	3
Empirical Methods	6
FECAL SAMPLE PROCESSING	8
DATA COLLECTION FOR FOOD HABIT ANALYSIS	9
Comparative Collection	9
Otolith Enumeration	10
Otolith Grading	11
Otolith Measurements	11
Cephalopod Beak Enumeration	13
Cephalopod Beak Measurements	13
Bone Identification and Enumeration	14
Size Determination Using Bone	15
Level of Identification	16
Molecular Genetic Techniques	17
Scales and Ageing Otoliths	17
REPORTING RESULTS	18
ACKNOWLEDGMENTS	24
LITERATURE CITED	25
APPENDIX 1. Prey Identification Options	30
APPENDIX 2. Diagnostic bones used for prey identification by family	33
APPENDIX 3. Sample prey identification data sheet	36

INTRODUCTION

Investigating pinniped diet is important, not only to determine the role of pinnipeds in marine ecosystems, but also to quantify pinniped-fishery interactions, which may have economic and ecological significance (Pierce and Boyle 1991). Dietary studies of pinnipeds have been based on the analysis of stomach contents of sacrificed or recently dead individuals and stomach lavage, or fecal (scat) samples. Each method has inherent problems.

Collecting stomachs of sacrificed animals is time consuming, sample sizes are usually small, and now is unlawful in the United States because pinnipeds are protected under the Marine Mammal Protection Act of 1972. Also, several investigators have reported that cephalopod beaks accumulate in the stomach and may lead to an overestimation of the consumption rate of cephalopods (Pitcher 1980, Bigg and Fawcett 1985). Analyzing contents of stomachs collected from recently dead animals may be biased because animals might not have been eating or they may have consumed prey that they normally do not (e.g., during El Niño events), which may have directly or indirectly caused their death. Employing stomach lavage requires animals to be immobilized using drugs, therefore, sample sizes are usually small and there is a possibility of mortality (Antonelis et al. 1987). Also, large whole prey items, as well as small items that are entrapped in stomach rugae might not pass in the lavage tube resulting in their misrepresentation.

Fecal samples are biased because some prey remains (predominantly otoliths) may be partially or completely digested in the gastrointestinal tract (da Silva and Neilson 1985, Murie and Lavigne 1986, Jobling 1987, Dellinger and Trillmich 1988, Harvey 1989, Cottrell et al. 1996, Tollit et al. 1997, Marcus et al. 1998, Bowen 2000). Also, they might only represent prey most recently consumed before an animal comes ashore from a feeding trip. Despite these shortcomings, fecal samples are now routinely collected because they are readily available, there is little or no impact on animals, and many can be collected in a relatively short period of time.

In the past, only teleost fish sagittal otoliths and cephalopod beaks collected from fecal samples were used to describe what prey species were consumed. In an effort to account for prey with completely digested otoliths or those not represented by otoliths (e.g., cartilaginous fishes), some investigators recently have used all structures recovered in fecal samples to identify consumed prey (e.g., Olesiuk et al. 1990, Cottrell et al.1996, Riemer and Brown 1997, Laake et al. 2001[1]). There are constraints to using all remains including: (1) time and effort necessary to set up a reference collection, (2) time needed to adequately train personnel to identify structures, and/or (3) costs required for outside assistance identifying samples (Cottrell et al. 1996). Accounting for these constraints, the following protocol is intended to standardize data collection, fecal sample processing and reporting of the

[1] Laake, J.L., P. Browne, R.L. DeLong and H.R. Huber. Natl. Marine Mammal Laboratory. 7600 Sand Point Way NE, Seattle WA. 98115. unpub. manuscript. Pinniped diet composition: a comparison of models. 34 p.

diet of pinnipeds inhabiting the waters of California, Oregon, Washington, British Columbia and Alaska. It is not our intent to dictate or replace methods that investigators are currently using. It should be stressed that this is a work in progress and part or all of the following procedures may be modified or removed and others may be included as we gain further insight from new or revised techniques. This protocol should serve as an example of techniques used for sample collection, processing, identification and analysis. Techniques can be used in combination depending on the goals of the study in question. Also, it is recognized that there may be limited time or resources available for a project, therefore only certain sections of the protocol may be pertinent (Appendix 1).

We hope that this protocol will help standardize the reporting of data on pinniped diet in general, as well as to assess the impact that pinnipeds may have on a particular prey species (e.g., salmonids). It is not our intent to include all biases, methods, or statistical analyses that have been presented elsewhere; we encourage researchers to be aware of the literature on these issues.

FECAL SAMPLE COLLECTION

Pinnipeds are marine mammals, but they require suitable substrate to rest, molt, mate (in some species), give birth and nurse their young. Pinniped fecal samples can be found and collected from pinniped haul-out sites along coastal areas or oceanic islands.

Fecal samples are collected using hand-trowels, spoons, tweezers, and spray bottles and are placed in plastic (e.g., Zip-lock or Whirl-pak) bags. Bags are labeled with data on pinniped species, date and location, and then frozen and stored for later analysis. Effort should be made to collect the entire fecal sample including all prey hard parts, and only recently deposited samples should be collected. How recently the fecal sample was deposited is of particular importance if the collection site is not washed over by the tide on a regular basis. Recently deposited fecal samples are defined as those samples that contain prey hard parts that have not been broken down as a result of decomposition and exposure to the elements. The time required to degrade hard parts exposed to the elements varies depending on temperature, precipitation, humidity, wind, and wave and tidal exposure.

Frequency of fecal collections and the number of fecal samples collected (sample size) depend on the focus of the investigation and the spatial and temporal scales at which parameters will be estimated. A sample size (n) is chosen to achieve a certain level of precision for a parameter to be estimated (e.g., frequency of occurrence (FO) in a sample of salmonids). In some pinniped food habit studies, we are interested in testing hypotheses such as whether the diet of pinnipeds varies by season, year, location, sex, age group, etc. In hypothesis testing it is always useful to consider the power for the test before data collection. Power will depend on precision and thus on sample size. We will focus here on some examples of sample size estimation and will ignore the question of power, but refer the reader to the following papers on power

analysis: Hayes and Steidl 1997, Reed and Blaustein 1997, and Thomas 1997 (but see Gerard et al. 1998 and Hoenig and Heisey 2001).

Scope of Inference

The first step is defining what parameters will be estimated and the scope of inference. The biological population of interest and the spatial and temporal scales of interest define the scope of inference. More specifically, it is useful to define both the target population and the sampled population. The target population is the population about which one would like to make an inference and the sampled population is the population actually sampled from. Ideally they would be the same but the latter is usually a subset of the former. Defining the sampled population means constructing a list of sampling units (i.e., 'frame'), which is often the most difficult aspect of sampling. To be in the sampled population, each individual sampling unit and each pair of sampling units must have nonzero probabilities of being selected (required in order to obtain unbiased estimates of parameters and their variance). For example, in pinniped-salmonid studies, we might decide that we want to estimate annual salmonid FO in the diet of harbor seals in the Columbia River. This is a fairly specific question, but leaves a lot of room for interpretation. Will we also want to estimate FO by salmonid species or by season? If the population of pinnipeds varies seasonally how do we define an annual FO? It is not possible to address all of these questions here and we only do so to point out the importance of clearly defining the target parameter. In most cases, we may be interested in estimating several parameters or the same parameter at various scales. In most cases, it will not be possible to choose a sample size that is optimal (i.e., provides desired precision at least cost) for each estimate. However, a series of requisite sample sizes can be computed and the largest used to ensure the target or "better" precision is achieved.

Sample Size Formulae

Precise descriptions of the diet are necessary to make comparisons between or among the parameters of interest. Target precision can be specified as either a margin of error or a coefficient of variation (CV). The margin of error is an absolute value, which is the difference between the upper limit of a symmetric confidence interval (typically 95% interval) and the estimate. The CV is a relative measure of precision [(standard error)/estimate] that expresses the precision as a proportion or percentage of the estimate. Consider that a 10% CV on proportion data represents a margin of error of 0.02 if the proportion is 0.10 and it represents a margin of error of 0.16 if the proportion is 0.80. As an example, consider estimating FO of salmonids from 100 fecal samples. Assuming a randomly chosen sample, if 20 fecal samples contained salmonids, the estimate is $p = 20/100 = 0.20$. The variance of p is $p(1-p)/n$ which in this case is $0.2 \times 0.8/100 = 0.0016$ and the standard error (square root of the variance) is 0.04. A t-value with 99 degrees of freedom (df) is roughly 2, so the confidence interval for p is 0.20 + 2 x 0.04 = (0.12,0.28). The margin of error is 0.08. If we wanted the margin of

error to be 0.05, we could compute the necessary sample size by finding n such that the calculation gives a value of 0.05. This can be done algebraically and the solution is

$$n = \frac{4p(1-p)}{d^2},$$

where d is the desired margin of error and 4 in the numerator is the square of the t-value which is 2. Technically, the t-value depends on n (df = n-1) but as long as n > 25, a value of 2 is reasonable to use. For the example, we posed n = 4 x 0.16/0.0025 = 256) the sample size would have to be increased from 100 to 256 to achieve a margin of error of 0.05. If we chose to specify a target CV of 10%, this is equivalent to a specification that $0.10 = CV(p) = \sqrt{p(1-p)/n}\,/\,p$.
By solving for n we get the following equation:

$$n = \frac{1-p}{p\,CV^2},$$

which for our example would yield n = 0.8/(0.2 x 0.01) = 400 and an expected margin of error of 0.04. The above formulae have been stated specifically for proportions but the process is the same for any parameter estimate. For example, for a mean ($\bar{x}$) the formulae are

$$n = \frac{4s^2}{d^2}$$

$$n = \frac{s^2}{CV^2\bar{x}^2},$$

where s^2 is the variance of the data and *d* is the desired margin of error. In the case of binomial data (proportions),
$s^2 = p(1-p)$ and $\bar{x}^2 = p^2$.

The proper formulae for computing the sample size depends on the sampling design. The above formulae have all assumed a simple random sample of the population. If the sampling effort and the samples collected span the time frame of interest, then it is a random sample of the population. For example, if weekly fecal collections are conducted at a site that is not washed over by the tides regularly, and randomly selected fecal samples or all fecal samples are collected that are relatively fresh, approximately 1 to 7 days old, then the fecal samples represent the entire time frame of interest. Oftentimes, however, fecal samples are not collected as a simple random sample of all fecal samples that were deposited during a particular time frame of interest. The most likely scenario is collection of some or all fecal samples on a set of

dates on which the tide is sufficiently low to grant access to fecal samples. This is a form of cluster sampling. The first stage of sampling is the collection date and the second stage is fecal samples within date. Because diet is likely to be affected by the prey that is available, the fecal samples collected on a particular day will be more similar than fecal samples collected on different days. Thus, it is not reasonable to pool the entire sample of fecal samples and treat it as a random sample using the formulae above. It may be reasonable to treat each collection date as a random sample of dates, and the collection of fecal samples for each date as a random sample of those that could have been deposited (i.e., they are representative of the seal diet at that time). For cluster sampling, the appropriate variance contains two components, one for each stage of sampling. If there are *m* fecal samples collected on each of *n* dates out of a possible N dates, the variance for an average proportion (average FO) is

$$\mathrm{var}(\overline{p}) = (1 - n/N)\frac{\sum_{i=1}^{n}(p_i - \overline{p})^2}{n(n-1)} + \frac{n/N}{n^2}\frac{\sum_{i=1}^{n} p_i(1-p_i)}{m-1}.$$

In the second term, the finite correction factor is ignored because there is no way to enumerate the set of potential fecal samples from which the sample is derived. If *n* is small relative to *N*, the second term can be ignored and the variance simplifies to the standard variance formula of the observed proportions for the sample of *n* dates; however, *m*, the number of fecal samples collected on each date, does affect the variance because it introduces variability into $p_{i.}$. Thompson (1992) and Cochran (1977) and many other texts on sampling provide formulae for the optimal *m* given estimates of variability and cost of sampling at each stage and an adequate text or statistician should be consulted. If *n* is fixed by the availability of tides the standard formulae have to be adapted because they may predict an optimal *n* and *m*, which are not feasible. In that case, *n* should be fixed and a new *m* computed to achieve the desired precision. In most instances, it will not be possible to sample a fixed *m* because of the fluctuations in the availability of fecal samples. However, planning will be easiest by assuming a fixed target *m.*

All sample size calculations require an initial estimate of the parameter and its variance. The accuracy of the sample size prediction will hinge on the quality of the initial estimates. It is best if the estimates are derived from data that were obtained from the same or like system. Often it is necessary to use published values or best guesses. If a proportion *p* (as opposed to a mean or total) is estimated, and no estimate of *p* is available, a "worst case" value of $p = 0.5$ can be used in determining sample size. The quantity $p(1\text{-}p)$, and hence the value of *n* required by the formula, assumes its maximum value when *p* is one-half. If there are no published information or pilot data available, collect all available fecal samples on each sampling date. Sampling dates can be initially set every 2 weeks during low tidal cycles. Frequency of sampling may be modified depending on the number of fecal samples available. Process the fecal samples by choosing some initial value of *m* and analyzing *m* randomly chosen fecal samples from each collection date. Use this initial sample to estimate the sample size and then analyze the additional samples if more are needed.

Species composition can be plotted against cumulative number of fecal samples to determine when a sufficient number of samples have been analyzed (see cumulative species curve below). Fecal samples are typically relatively inexpensive to collect and expensive to analyze; therefore, if you haven't collected enough fecal samples there is no way to go back in time and collect them. While collecting fecal samples is inexpensive in terms of equipment and time, there is a 'cost' involved in the possible disturbance of pinnipeds. In determining required sample sizes and sampling frequency for a study, investigators must consider how many disturbances of pinnipeds are justified and tolerable. Pinnipeds may abandon a haul-out location if the frequency of disturbance becomes intolerable. Because the Marine Mammal Protection Act of 1972 protects all pinniped species from harassment and disturbance, permits must be obtained from the National Marine Fisheries Service to disturb pinnipeds for the purpose of fecal collection.

If no published information or pilot information is available on the sample size or frequency of fecal collections in a specific system, collecting 50 fecal samples every 2 weeks may be an appropriate goal. Fecal samples should be randomly processed and analyzed, as described above, and prey species should be plotted using a cumulative frequency curve or other method to determine if the number of fecal samples collected is adequate to address the question(s). Estimates of sample size should be reviewed seasonally to ensure that adequate numbers of samples are collected, especially in light of changing environmental conditions.

Empirical Methods

So far, we have described how to estimate sample size using formulae. An alternate empirical method is to resample a set of pilot data using different sample sizes. For example, a cumulative prey species curve can be used to determine whether a sufficient number of fecal samples have been collected to precisely describe the diet. The curve represents the cumulative number of new prey species against the cumulative number of randomly chosen fecal samples (Fig. 1). When all prey species are adequately represented in fecal samples for a given period of time the curve will reach its asymptote. At this point, an adequate number of samples has been reached. Numerous random samples can be selected, or resampled, for each consecutive fecal sample plotted creating a mean and an estimate of variability for each sample.

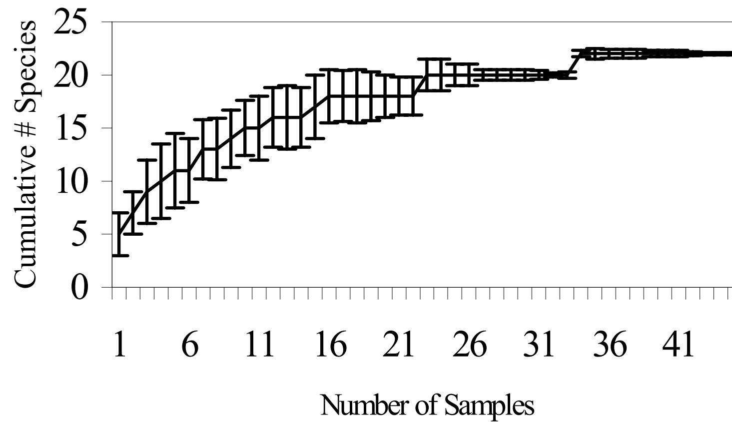

Figure 1. Cumulative prey species curve. The cumulative mean number of new prey species is plotted against the number of randomly chosen fecal samples. Error bars indicate one standard deviation.

To determine if an adequate number of fecal samples have been collected to assess the importance of one specific prey species several possible estimates exist. First, plot the frequency of occurrence (FO) of a target prey species for a cumulative number of randomly chosen fecal samples (Fig. 2). The graph will start with great fluctuations in the FO with a low number of samples, then level off when the number of fecal samples analyzed adequately describes a target prey species in the diet of pinnipeds.

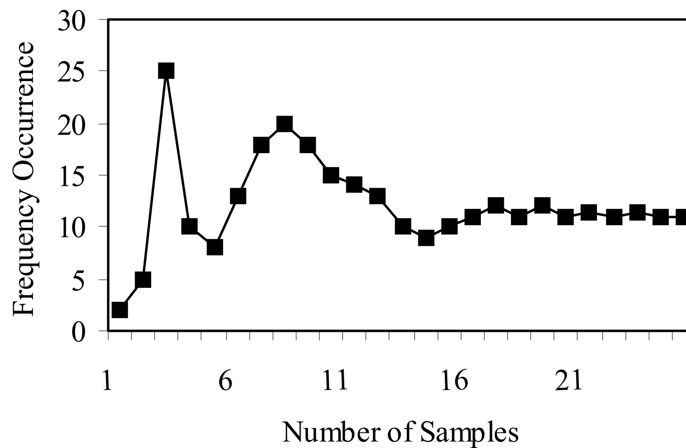

Figure 2. Frequency occurrence of target prey species plotted against randomly chosen fecal samples.

A second empirical approach is to plot the average number of a target species per fecal sample and 95% confidence intervals for every *x* number of randomly selected fecal samples (Fig. 3). Continue to collect and analyze additional samples until the average number of a target prey species per fecal sample becomes stable, and the confidence interval is judged to be sufficiently small. Another version of this second approach is to use resampling statistics to plot the average number of a target species per fecal sample for every *x* number randomly selected fecal samples, with the variability shown using error bars. Again, continue to collect and analyze additional samples until the average number of a target prey species per fecal sample becomes stable, and the variability is judged to be sufficiently small.

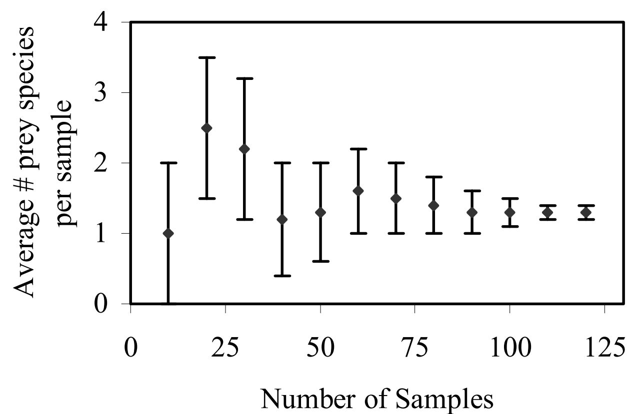

Figure 3. Average number of a target prey species per fecal sample plotted against the number of randomly chosen fecal samples. Error bars indicate one standard deviation.

FECAL SAMPLE PROCESSING

Fecal samples can be processed immediately or frozen until a later date. If the samples are frozen they should be thawed before processing. Fecal samples are processed by partially dissolving the sample in water, with or without detergent. The sample should be rinsed with running water through a series of nested sieves (e.g., 2 mm, 1mm, 0.5mm) or an elutriator (Bigg and Olesiuk 1990). All prey hard parts (i.e., fish bones, otoliths, cartilaginous parts, lenses, teeth and cephalopod beaks, lenses, and pens), should be recovered from the sieves, placed in vials, and labeled for storage and identification. Cephalopod beaks, lenses, and pens should be stored in a 50% isopropyl alcohol solution to prevent distortion. Fish remains should be stored dried. Samples may also be processed using an elutriator with a set of screens (minimum 0.5 mm) at the outflow to catch any prey remains that may have floated out of the sample.

Currently, the National Marine Mammal Laboratory (NMML) in Seattle is conducting a pilot study to process fecal samples in < 0.5 mm mesh bags using a washing machine. After data have been analyzed, information will be available indicating whether or not a washing machine may be a useful (alternative) method to process fecal samples. The value of collecting fecal volume data is being determined by examining a subsample of fecal samples collected by Washington Department of Fish and Wildlife (WDFW). Researchers at University of British Columbia (UBC) are currently developing a relationship between fecal sample volume and prey biomass. A volume (biomass)-weighted split sample frequency of occurrence model is being developed and is outlined in the workshop report (Olesiuk *in* Riemer and Lance 2001)

DATA COLLECTION FOR FOOD HABIT ANALYSIS

Comparative Collection

A comparative collection is essential for accurate bone and otolith identification. The collection should contain the prey taxon (species) most likely to be encountered in fecal samples. Ideally, the collection should contain several sizes of each species because the same structure can appear very different with size variations. Verified diagnostic structures from other fecal samples can be used as reference material in place of a comparative collection when known specimens are not available. Published otolith, bone, and cephalopod keys (e.g., Morrow 1979, Wolff 1982, Clarke 1986, Cannon 1987, Harvey et al. 2000) can also be useful tools to supplement a comparative collection.

When gathering specimens for a comparative collection, the species and age groups (length) should be considered. Ideally, a comparative collection contains a minimum of three fish per species (small, average and large). If possible, the fish should be collected from the area where the study is being conducted. In some cases, there are large numbers of species within a given family (e.g., sculpins) and collecting three of each species would be nearly impossible and very time consuming. In this example, the skeletal structures within the sculpin family are quite similar to one another and distinguishing among species within a genus can be difficult. When confronted with a situation like this, collecting a member from each genus found in the study area and reporting identifications to the genus or family level is the best option. If, however, the species in question is the target of the study, then a collection of as many species of these fish as possible should be made and identifications should be taken to the lowest possible taxonomic level using the skeletal structures or possibly even genetic analysis depending on the study objectives.

Fish collected for a skeletal comparative collection should be processed fresh or kept frozen in a sealed plastic bag until processing. Do not place the fish in formalin or alcohol because this will cause the flesh to adhere to the bones. The flesh can be removed in a number of ways including: Dermestid beetles (*Dermestes maculatus*), boiling in water, or using trypsin. Museums and larger institutions that process numerous samples typically maintain beetle boxes, but may or may not be accessible

for use. Cooking or boiling fish for a comparative collection simply requires slowly boiling a sample until the flesh can be easily removed or floats off of the bone. This technique is easy and chemical-free but does require frequent monitoring and often doesn't completely clean the bones. Trypsin is a stomach enzyme that dissolves the flesh while allowing the skeleton to remain in good condition. Concentrations of trypsin vary and should be considered when purchasing the product. The standard "recipe" for purified trypsin powder 1:100 (activity level) is: 2 cups warm water, ½ tsp. trypsin and 1 tsp. baking soda. This method works best when the fish is thawed and the above trypsin mixture is placed in a glass jar in a warm location or sunny window, which keeps the solution warm (below 90° F) and the enzyme active.

Prior to processing a specimen the following information should be collected:

- genus and species, common name
- fork length (tip of snout to fork in tail)
- standard length (tip of snout to caudal peduncle)
- total length (tip of snout to end of tail)
- weight (may not be accurate with frozen fish)
- sex and reproductive condition
- collection information, (location, date, collector, etc.)

After processing, the bones should be dried and separated by type for ease of use when identifying samples. Samples can be stored in plastic bags, jars or other containers that will ensure that the bones will not become wet and begin to degrade.

Otolith Enumeration

Separate left-, right-, and unknown sided otoliths of each species. Effort should be taken to determine the side of otoliths because this will affect the minimum number of individuals (MNI). The greatest number of left or right otoliths will be the minimum number as determined by these prey parts (this does not include skeletal elements). For otoliths of a given species where the side cannot be determined, add the unknown otoliths to the known side with the fewest otoliths until both sides are equal. If there are more unknown otoliths, divide their number by two and add the quotient to either known side. The result will be your MNI.

Example 1: a sample has 18 Pacific whiting (*Merluccius productus*) otoliths – 10 right otoliths (ROT), 5 left otoliths (LOT), and 3 unknown side otoliths (UOT)

ROT = 10 (ROT) = 10
LOT = 5 (LOT) + 3 (UOT) = 8
Minimum number = 10 (derived from ROT, which had the greatest number)

Example 2: a sample has 35 Pacific herring (*Clupea pallasii*) otoliths - 5 right otoliths (ROT), 10 left otoliths (LOT), and 20 unknown side otoliths (UOT)

5 (ROT) + 5 (UOT) + 15/2 (UOT) = 18
10 (LOT) + 15/2 (UOT) = 18
Minimum number = 18

Otolith Grading

Otoliths are graded so that species-specific correction factors can be applied to otoliths to improve estimates of prey size needed for consumption estimates. If an estimate of prey length and weight is not a part of the study, then otoliths do not need to be graded.

Otolith grading for these predation studies are consistent with those developed by Tollit et al. 1997 and are described below for Pacific whiting. Morphological features are listed in order of importance. Please see Tollit et al. 1997 for grading descriptions and photographs of additional prey species.

Grade (degree of digestion)	Distinctive external morphological features for each grade
1 (low)	Clear lobation on outside ventral and dorsal margins. Characteristic arrowhead shape with parallel margins. Square rostrum and lobate on outside. Sulcus distinct.
2 (medium)	Lobes less pronounced on margins. Posterior end less strongly pointed. Rostrum rounded or angled with no lobes evident. Sulcus indistinct.
3 (high)	No lobes evident, with margins smooth and/or jagged. No parallel margins, larger specimens lack arrowhead shape. Acutely angled rostrum, especially in larger specimens. No sulcus.

Otolith Measurements

Otoliths are measured so that their lengths and/or widths can be applied to species-specific regression equations, which can be used to estimate prey lengths and weights needed for consumption estimates. Otolith length and width measurements are recorded for only Grade 1 and Grade 2 condition (*see* "Otolith grading" section). Otoliths in Grade 3 condition are usually too worn and result in wide confidence intervals around prey length and weight (Tollit et al. 1997). If an estimate of prey length and weight is not part of the study, then otoliths do not need to be measured.

Otolith length regression equations exist for certain prey species (Harvey et al. 2000); however, regression equations for otolith width are still being refined.

Otolith length and width (if time allows) are recorded for the side (either left or right) that gave the highest MNI. If two size classes of a particular species are present in the sample, then measurements should represent both of those size classes. For example, if a sample contains two left and three right "large" hake otoliths and five left and three right "small" hake otoliths, then measurements would be recorded for three right "large" hake otoliths and five left "small" hake otoliths. The two different size classes (small and large) can be treated as two different "species" with respect to measuring criteria. Measure otolith length to at least the nearest 0.1 mm parallel to the sulcus from the anterior tip of the rostrum to the posterior edge using hand-held calipers, an image analysis system, or an ocular micrometer on a dissecting microscope (Fig. 4). Measure otolith width to at least the nearest 0.1 mm perpendicular to the sulcus at the widest point of the otolith (Fig. 4). Ocular micrometers should be calibrated for the magnification used to measure otoliths. Do not record otolith length if it is broken (unmeasurable). If the otolith is broken or chipped, then only measure the otolith width if intact.

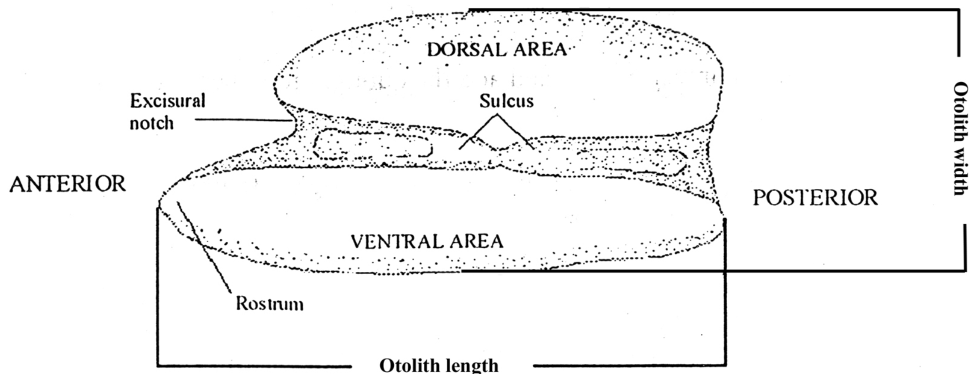

Figure 4. Right sagittal otolith from a teleost fish. Lengths should be measured parallel to the sulcus from the anterior tip of the rostrum to the posterior edge. Widths should be measured perpendicular to the sulcus at the widest point of the otolith.

There are instances when a sample might contain a large (>100) number of otoliths or beaks of a prey species. In such circumstances, one might consider measuring a subsample of the structures. Though we do not advocate subsampling, we realize that it might be appropriate due to time and resources that are available. If subsamples are used, then otoliths or beaks should be divided into different size classes (if necessary) and selected structures should be chosen randomly within each size class. Subsample size can be determined several different ways (should be determined by principal investigator or statistician), but for a rough estimate, one can readily plot a cumulative otolith size versus randomly selected otoliths curve similar to the cumulative prey species curve (see "Fecal Sample Collection" section; Fig. 1).

After lengths and/or widths have been determined, they can be used in indices such as index of relative importance or to calculate biomass. To account for degradation of otoliths during digestion, species-specific correction factors should be applied to otolith lengths (Harvey 1989) and widths when they are finalized.

Correction factors for otolith number in addition to otolith length are available for select species based on captive feeding trials (Harvey 1989). Use of these correction factors for otolith number for current studies warrants further discussion and will likely be refined with additional captive feeding studies in 2001-02 that will be conducted at UBC using Steller sea lions (*Eumetopias jubatus*) and at Moss Landing Marine Laboratory using harbor seals (*Phoca vitulina*).

Cephalopod Beak Enumeration

Cephalopod beak enumeration and MNI calculations should be consistent with otolith enumeration using upper and lower beaks. Some prey identifiers use statoliths for enumeration of cephalopods; however, differentiation between squid and octopus using statoliths is not possible so these data should be recorded as “cephalopod species”. Pens may be used for presence or absence of cephalopods, but should not be used for enumeration because they may break and split during digestion and thus artificially inflate the MNI.

Cephalopod Beak Measurements

Measure the rostral length of a beak to at least the nearest 0.1 mm using hand-held calipers or an ocular micrometer on a dissecting microscope (Fig. 5). It has been suggested that for market squid (*Loligo opalescens*) rostral width may yield a more accurate regression (Mark Lowry, NMFS pers. comm.). Rostral width of a beak is measured from the jaw angle on the left side to the jaw angle on the right side to at least the nearest 0.1 mm using hand-held calipers, an ocular micrometer on a dissecting microscope, or an image analysis system (Fig. 5). The size of beaks does not significantly reduce during digestion, so correction factors are not needed (Harvey 1989). Subsampling protocols for measuring beaks should be consistent with otolith subsampling described above. Regression equations for cephalopod beak lengths are available for select species (Kashiwada et al. 1979,Wolff 1982, Clarke 1986).

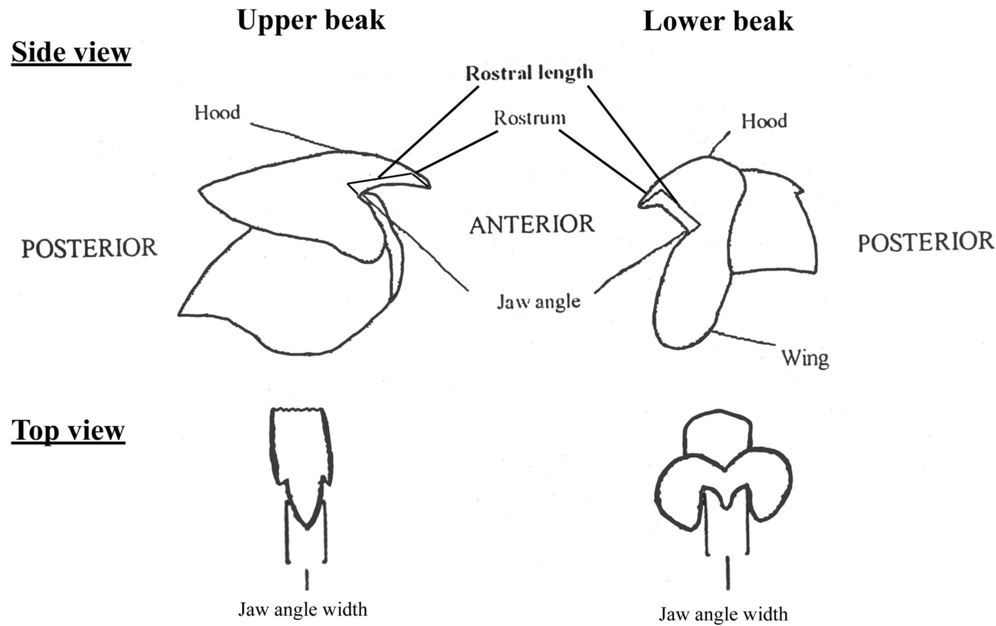

Figure 5. Upper and lower cephalopod beaks. The rostral lengths should be measured from the tip of the rostrum to the jaw angle of upper (preferred) or lower beaks. Rostral width measurements are useful for some cephalopod species.

Bone Identification and Enumeration

Previous pinniped food habit studies have relied solely on recovery of sagittal otoliths for species identification and only recently has it been documented that additional information can be obtained by using other diagnostic bones in addition to otoliths to identify prey (Olesiuk et al. 1990, Pierce and Boyle 1991, Cottrell et al. 1996). As a result, the "all structures" method was developed for fecal and gut analysis and incorporates both bone and otolith identification. Although the all structures method may increase detection of certain species for which otoliths are not commonly recovered, such as salmonids, this method can be time consuming and requires a significant amount of training. Otolith identification is still considered a useful method for determining the prey of pinnipeds in some instances, but if choosing to identify only otoliths, all structures should be recovered to allow for future analysis.

Samples collected in the Columbia River in the early 1980s by Beach et al. (1985) were originally identified using only otoliths. These samples were reexamined by Oregon Department of Fish and Wildlife (ODFW) for salmonids using the all structures method and although the results varied, FO of salmonids in most cases increased. For example, reexamination of one collection in August of 1980 resulted in a three-fold increase in the FO of salmonids from 13.8% (9 of 65) when only otoliths were used for identification to 50.8% (33 of 65) when all structures were used (Riemer and Brown 1996).

If personnel trained in bone identification are not available in-house, samples should be sent to an experienced outside source for identification (e.g., Pacific Identifications Inc., Victoria, B.C.). If identification is done in-house, we recommend a random sub-sample (minimum 10% of total samples) be identified and all of those samples be sent to experienced identifiers early in the identification process for an evaluation of identification techniques. Cross-checks throughout the learning process are useful for identifying errors. The all structures method is an ongoing learning process and thus cross-checks can also be a useful tool for experienced identifiers particularly when encountering "new" prey species or unfamiliar prey structures, or when there is a change in pinniped species or collection location. The identification method chosen should be explicitly stated to ensure comparability among studies.

When using the all structures method, prey should be enumerated by removing from the sample multiple diagnostic structures for each species (see Appendix 2 for commonly recovered structures for each family). Diagnostic bones (just as was described for otoliths above) should be identified by side (left or right) and enumerated to determine the structure that yields the highest MNI. When using vertebrae (or other numerous non-paired structures) for enumeration the vertebrae recovered in the sample should be counted or estimated using a subsample and then compared with the actual number of vertebrae recorded for the prey in question. For example if a species is reported to have 65-72 vertebrae and 135 vertebrae are counted or estimated for the sample then an MNI of 2 would be reported. We were unable to find a single reference that reported numbers of vertebrae for multiple species so we would suggest checking field guides and web sites (e.g., www.fishbase.com) dealing specifically with the prey in question to acquire this information. We recommend that all diagnostic bones that have been removed from the sample be placed in gel caps and labeled for future analysis.

Ongoing captive feeding studies at UBC are evaluating bone retention and levels of bone erosion from fecal samples to determine if certain bones (other than otoliths) can be used for species-specific regression equations. These equations could then be used to estimate the prey number (MNI), fish length and weight for consumption estimates.

Size Determination Using Bone

Determining prey size within a sample is necessary to calculate MNI (e.g., a large hake vertebrae and a very small hake basioccipital would represent two fish). Some species, such as salmonids, that are not commonly identified using otoliths have to be separated by bone size. In the majority of cases, juvenile salmonids can be separated from adults just by the size or type of structure recovered (e.g., vertebrae of smolts are much smaller than adult salmonids and teeth and gill rakers commonly recovered from adult fish are much too large to be smolts). While there is some information on using bone to determine the size of fish found in archaeological sites, using bone to determine fish size has not been undertaken in fecal analysis until recently. The NMML and Pacific Identifications Inc. have begun to investigate using bone size

versus length and weight to estimate size of walleye pollock (*Theragra chalcogramma*), Pacific cod (*Gadus macrocephalus*) and Atka mackerel (*Pleurogrammos monopterygius*) as well as *Sebastes* spp. and *Hexagrammos* spp. with bones recovered from Steller sea lion samples (Orchard 2001). We will continue to gather information on this topic and will try to come to some conclusions on whether these techniques can be used for fecal samples. Size determination may not be necessary for prey species that do not vary greatly in size (e.g., herring and smelt); however, for species such as, salmon, hake, flatfish and rockfish where juveniles and adults are both prey of pinnipeds, the determination of prey size consumed requires further attention. Addressing questions regarding bone size would require collecting fish samples of all sizes to investigate differences in the skeletal structures based on the size of the fish. The number of fish necessary is not known at this time and there is a possibility that for salmon there could be differences among river systems, which complicates this matter. In the mean time, identifiers should note the prey size-class when possible.

In addition to using bone size to estimate fish size, captive feeding studies are underway to develop regression equations for species-specific diagnostic structures that will correct for erosion occurring during the digestive process. Regression equations will be developed from captive feeding experiments and bone-measuring protocols will be developed to determine size of prey. Appendix 2 contains a list of the most commonly occurring diagnostic skeletal structures for each family of fish species recovered from harbor seal and sea lion fecal samples collected in California, Oregon, Washington, Alaska and British Columbia pinniped diet projects. These structures can be used to determine prey composition within the samples and to calculate MNI. This list was developed to assist captive feeding researchers in selecting structures to develop regression equations and also to serve as a guide or starting point for new prey identifiers. The structures listed for each family are not exhaustive and additional structures may be added.

Level of Identification

Prey remains should be identified to the lowest taxonomic level possible. Researchers should work together to maintain accurate and consistent identification. Identifications to the lowest taxonomic level depend on the prey consumed, level of identification experience, completeness of the comparative collection and structure and erosion of the bone/otolith in question. In most cases, common prey can be identified with some practice. In some cases, the lowest taxonomic level possible may be only to family or order level. Identifiers must be confident in their identification regardless of the taxonomic level. Again, sending a sample early in the learning process to experienced identifiers is imperative for an evaluation of identification techniques and accuracy.

Level of identification may also depend on the objectives of the study, the analyses used, or the amount of time or funding allocated for prey identification. For example, if the focus of the study is to determine the importance of one particular prey taxon

(e.g., salmonids) in the diet of pinnipeds, then it may only be necessary to determine the identification of other prey taxa (e.g., flatfish and rockfish) to the family rather than species level when using some indices (e.g., reconstructed biomass proportion; *see* "Reporting Results"). However, if a given family or order is found to be a frequently occurring prey item regardless of the focus of the study, then an attempt to identify these prey to the genus or species level may also be required.

Molecular Genetic Techniques

The potential impacts of predation by U.S. West Coast pinniped species on various salmonid populations of concern in Washington, Oregon and California are the main drivers of these food habit studies; thus, identification of salmon to the species level is critical. Species identification is possible using salmon otoliths; however, very few salmon otoliths are recovered from fecal samples and species identification in most cases is not possible using current bone identification techniques. Development of molecular genetic techniques to identify Pacific salmon to species using bone recovered from fecal samples is an important tool for identifying salmon species. The NMML and the Conservation Biology Molecular Genetics Laboratory at Northwest Fisheries Science Center have used direct sequencing and restriction fragment length polymorphism to identify salmonid species and are working on techniques to identify salmonid stocks using microsatellites. The protocols for collecting these data (e.g., photographing bones before they are ground for genetic analysis and choosing specific bones) are in development. An update on these techniques may be found within the workshop proceedings (Huber et al. 2000 *in* Riemer and Lance 2001).

Genetic fecal analysis can be used to identify the individual harbor seal associated with each fecal sample collected. This provides crucial information about biases associated with fecal sample collection, namely whether the fecal sample collected is a random and representative sample of the population. The combination of genetic and prey hard part analysis can provide information on the number and genetic identity of seals involved in salmonid predation. This combination of analysis techniques allows determination of individual variation in overall prey selection and an insight into the foraging ecology and behavior of pinnipeds. WDFW is currently conducting genetic fecal analysis in Hood Canal. Genetic material is removed prior to fecal processing for food habits by dissolving the entire fecal sample in a buffer solution and removing four, one-milliliter aliquots, which contain sloughed epithelial cells (London 2000 *in* Riemer and Lance 2001).

Scales and Ageing Otoliths

Scales and ageing otoliths have been used in other disciplines to identify stocks of fish, age structure of commercially caught fish and age-length relationships, and may be useful for pinniped diet analysis studies.

Scales have been collected from spawning salmon and used to determine species and hatchery or wild stock based on growth rings evident on the surface of the scale (Lisa Borgerson, ODFW, pers. comm.). When dealing with target species, such as

salmonids, reading scales could prove to be a useful tool if a determination could be made to the species level and/or whether the fish were from a hatchery or wild stock. Bilton et al. (1964) describe scale differences among the five salmonid species and provide pictures of the scales. Unfortunately, due to the deterioration of most scales found in fecal samples the usefulness of this structure may be limited. Scales should be recovered from fecal samples and stored dry with other structures unless there is a chance they will be broken.

Ageing otoliths can provide information on prey consumed by pinnipeds and possibly give some length-age information on certain long-lived species such as rockfish. Ageing techniques differ depending on the fish species in question. A detailed manual developed by the Pacific Coast Groundfish Ageing Technicians documents the various ageing techniques, otolith storage and a summary of ongoing ageing studies and techniques. This document is a work in progress as techniques are changed or added and is an excellent source for information on otolith ageing (www.psmfc.org/care). Currently otoliths collected from fecal samples are not being aged.

REPORTING RESULTS

In order to ensure comparability of data, sample size reporting must be consistent. The total number of fecal samples collected is composed of samples with no prey remains (empty), samples with identifiable prey remains, and samples with prey remains that were "unidentifiable" (hard parts too eroded for identification) and "unidentified" (hard parts in good shape and could be identified if reference materials or expertise were available). Inclusion of "unidentifiable" and "unidentified" samples in calculations such as percent frequency of occurrence (FO) has varied in the past. Presenting these data in the form of a table will allow for comparisons among studies.

After prey remains have been identified to the lowest taxonomic level possible, and otolith/beak lengths have been measured, the information can be used in indices to determine the food habits of pinnipeds. A review of dietary, food habit, and food consumption studies of fish and other marine vertebrates reveals a lack of consistent methodological approaches and application of statistical tests (Cortes 1997). The relative importance of prey items in marine vertebrate diets can be evaluated in a variety of ways. The most common indices or measures used for quantitative description and evaluation of diet are percentage composition by number (%N), percentage composition by weight (%W), and percentage frequency of occurrence (%FO; Tirasin and Jorgensen 1999). Detailed discussions of the advantages and disadvantages of the various measures of diet were presented in Berg (1979), Hyslop (1980), and Bowen (1996). Until recently, little attention has been paid to evaluating the uncertainty associated with these measures (Ferry and Cailliet 1996, Tirasin and Jorgensen 1999). Discussions of estimating precision in dietary studies can be found in Ferry and Cailliet 1996, Cortes 1997, and Tirasin and Jorgensen 1999.

The following indices have been used in numerous studies to indicate the importance of prey collected in fecal samples of pinnipeds. This is a partial list of indices. The

appropriate index is determined depending on the question to be answered. Each index has its strengths and weaknesses, as well as what data are required to use them. In the following indices, we denote '*s*' as the total number of fecal samples that contained prey; however, this is not absolute (see previous discussion). Also, there is a bias associated with using minimum (MNI) rather than the actual number of individuals (usually an underestimation in counts). Using MNI is usually the best approximation that we have of actual counts; however, one should be aware of its potential shortcomings.

- Frequency of Occurrence (FO_i):

$$FO_i = \frac{\sum_{k=1}^{s} O_{ik}}{s},$$

where $O_i = 0$ if taxon *i* is absent in fecal *k*
1 if taxon *i* is present in fecal *k*
s = total number of fecal samples that contained prey

also expressed as: $\sum_{i=1}^{s}\sum_{k=1}^{n} O_{ik}$,

where $i = 1\ldots,s$ fecal samples and k = $1\ldots,n$ prey types (i.e., *n* times the mean number of prey items per fecal sample). This has no effect on the relative values of the index, rather it makes it sum to 100% so that it is directly comparable to other indices.

Frequency of occurrence is an index of presence or absence that indicates the proportion of time a certain taxon is consumed but not the number taken. It can also be expressed as a percentage allowing for statistical comparisons of diet composition among seasons and/or years. This index is useful if one wants to determine if a particular prey taxon is frequently or rarely consumed by a pinniped; however, it does not provide insight on the quantity of that prey that was consumed (i.e., presence = 1 to ∞). A major advantage of this index is that all structures do not have to be recovered from a sample and otoliths or beaks do not need to be measured. This index is similar to the average numerical importance index (3). Data necessary for this calculation include how often a prey item occurred in the samples and how many samples were collected with prey remains. Sample size does not include "empty" fecal samples that contain no prey hard parts and may or may not include samples that contain prey that is heavily eroded and cannot be identified ("unidentifiable"). Sample size should be explicitly defined in methods and results sections of reports.

- Relative Abundance (RA_i):

$$RA_i = \frac{\sum_{k=1}^{s} n_{ik}}{\sum_{k=1}^{s} n_k},$$

where n_{ik} = (minimum) number of individuals of taxon i in fecal k
n_k = (minimum) number of individuals of all taxa in fecal k
s = total number of fecal samples that contained prey.

Relative abundance is an index that expresses the numerical proportion of a prey taxon with respect to the other taxa without considering the number of times it appears in a sample (i.e., its frequency). It can also be expressed as a percentage, which allows for statistical comparisons of prey abundance among seasons and/or years. All structures do have to be recovered to determine MNI, but otoliths or beaks do not need to be measured to use this index. If all prey remains weighed the same, then relative abundance and biomass proportion indices would be equal. Data necessary for this calculation include the number and type of prey items in each sample (MNI) and the total number of prey items in the samples being analyzed.

- Average numerical importance ($\overline{NI_i}$):

$$\overline{NI_i} = \frac{\sum_{k=1}^{s} n_{ik}}{s},$$

where n_{ik} = (minimum) number of individuals of prey taxon i in fecal k
s = total number of fecal samples that contained prey.

Average numerical importance is similar to frequency of occurrence, but numerical importance considers the minimum number of individuals of a prey taxon in a fecal sample rather than just its presence or absence. It is also directly correlated (however, not redundant) with relative abundance, but the average number of a prey taxon per fecal sample is determined rather than its abundance relative to total abundance of prey. All structures have to be recovered to determine MNI; however, otoliths or beaks do not need to be measured to use this index. Data necessary for this calculation include the number of a given taxon in each sample and how many samples were collected with remains.

- Index of relative importance (IRI):

$$IRI_i = \left(\left[\sum_{k=1}^{s} \frac{n_{ik}}{n_k} + \sum_{k=1}^{s} \frac{m_{ik}}{m_k} \right] * FO_i \right) / s \quad ,$$

where n_{ik} = (minimum) number of individuals of prey taxon *i* in fecal *k*
n_k = (minimum) number of all prey taxa in fecal *k*
m_ik = mass of prey taxon *i* in fecal *k*
m_k = total mass of prey taxa in fecal *k*
s = total number of fecal samples that contained prey.

The IRI was originally expressed as (% number + % volume) x %FO. Pinkas et al. (1971) originated the IRI using percent volume, but it was replaced with percent mass by Hyslop (1980). Index of relative importance takes into consideration not only the abundance and occurrence of prey, but also prey size. This is important because larger prey, which may occur less often in the diet, may provide as much energy or be as important as more frequently consumed smaller prey. All structures need to be recovered to determine MNI, and otoliths and beaks need to be measured to use this index. It can also be expressed as a percentage. Data needed for calculating this index include the frequency of occurrence, minimum number of individuals of each prey taxon in the sample, and their mass, which is estimated from using species-specific regressions of otolith or beak rostral length to prey length and prey length to prey mass. IRI values cannot be compared among seasons or years, or among food types. Cortes (1997) suggested that IRI values be expressed on a percentage basis as a standardized measure in dietary analysis, so that comparisons among prey species within season, and among season and years are possible.

Percentage IRI (%IRI) for a specific prey species *i* (IRI_i) becomes:

$$\%IRI_i = 100 IRI_i / \sum_{i=1}^{n} IRI_i \ ,$$

where *n* is the total number of prey species.

The following two indices are used to calculate the proportion of a prey species in the diet of a particular pinniped species at a location over a period of time. These proportions can then be used to estimate the biomass of a prey species consumed by a pinniped species during a specific time frame of interest. Other factors that need to be evaluated to estimate biomass of prey species consumed by a pinniped species are the number of pinnipeds per age group present during a time frame of interest, age-group specific daily energetic requirements of pinniped species, proportion of the pinniped species at sea during surveys, number of days in the time frame of interest, and the energetic value of the prey species. There are biases associated with each index. For further discussion see Riemer and Lance 2001.

- Split-sample frequency of occurrence (*SSFO*; Olesiuk et al. 1990):

$$SSFO_i = \frac{\sum_{k=1}^{s}\left(O_{ik}/O_i\right)}{s},$$

where O_{ik} = 0 if taxon i is absent in fecal k
1 if taxon i is present in fecal k
O_k = total number of all taxa present in fecal k
s = total number of fecal samples that contained prey;

thus, if one prey taxon occurred in a fecal sample it is scored as 1, if two taxa occurred each are scored as 0.5, and so on.

When using *SSFO*, it is assumed that prey identified in fecal samples represent all prey consumed in the most recent meal, and all prey taxa comprising a meal (fecal sample) are consumed in equal volumes. The split frequency of occurrence is similar to frequency of occurrence because only the presence or absence of a prey taxon is required. All structures do not need to be recovered and otoliths or beaks do not need to be measured to use this index. Potential biases associated with *SSFO* are the proportion of small size prey may be overestimated and the proportion of large prey might be underestimated. Data necessary for this calculation include how often a prey item occurred in the samples and how many samples were collected with prey remains.

- Reconstructed biomass proportion (π_i; Harvey 1988; Hammond and Rothery 1996):

$$\pi_i = \frac{\sum_{k=1}^{s} b_{ik}}{\sum_{k=1}^{s} b_k},$$

where b_{ik} = biomass of prey taxon i in fecal k
b_k = biomass of all prey taxa in fecal k
s = total number of fecal samples that contained prey.

For some sample of fecal samples, π_i is the biomass of prey taxon i divided by the biomass of all prey taxa. This index is similar to relative abundance. All structures need to be recovered and otoliths and beaks need to be measured to use this index. This index would be ideal if actual (rather than minimum) prey consumed could be determined but this is usually not the case. One potential bias is that the proportion of large prey might be overestimated and the proportion of smaller size prey may be underestimated.

Estimates of pinniped consumption on commercially important fish species have been conducted for years using similar variations on the models described above. We do

not intend to imply that the *SSFO* and BR models are the only approaches to estimating pinniped consumption. For discussions on other models of pinniped consumption estimates please refer to Antonelis and Perez 1984, Mohn and Bowen 1996 and several papers with varied approaches that were presented at a Symposium for the Northwest Atlantic Fisheries Organization in September 1995 titled "The role of marine mammals in the ecosystem" include Hammill et al. 1997, Shelton et al. 1997, Stenson et al. 1997 and Warren et al. 1997.

ACKNOWLEDGMENTS

We are grateful to Joe Scordino (NMFS) and Al Didier (PSMFC) for funding and their continual encouragement and support. We appreciate the extensive wisdom and experience of Jim Harvey (MLML), Robin Brown (ODFW), Steve Jeffries (WDFW) and Bob DeLong (NMML) that helped us address these pinniped food habits questions and contributed greatly to the development of this protocol. Sincere thanks to all those involved in the West Coast Pinniped Predation studies and the many helpful discussions at the Newport meeting in 1999, conference calls throughout the year and the Prey Identification and Quantitative Analysis workshop in Seattle in 2000. Special thanks to Dom Tollit (UBC), Mark Lowry (SWFSC/NMFS), Bryan Wright (ODFW), Harriet Huber (NMML), Adria Banks (NMML), Mark Dhruv (NMML) and Josh London (UW) for their input on specific sections of this protocol and ongoing research. We acknowledge Susan Crockford and Becky Wigen of Pacific Identifications for their contributions to this protocol. This document was greatly improved with reviews by Bryan Wright, Susan Crockford, Harriet Huber and Gary Duker. We believe we have proven that this is in fact “rocket science”.

LITERATURE CITED

Antonelis, G.A., and M.A. Perez. 1984. Estimated annual food consumption by northern fur seals in the California Current. CalCOFI Rep, 25:135-145.

Antonelis, G.A., Jr., M.S. Lowry, D.P. DeMaster, and C.H. Fiscus. 1987. Assessing northern elephant seal food habits by stomach lavage. Mar. Mammal Sci. 3:308-322.

Beach, R.J., A.C. Geiger, S.J. Jeffries, S.D. Treacy, and B.L. Troutman. 1985. Marine mammals and their interaction with fisheries of the Columbia River and adjacent waters, 1980 - 1982. Processed Rep. 85-04. 316 p. Alaska Fish. Sci. Cent., Natl. Mar. Fish. Serv., NOAA, 7600 Sand Point Way NE, Seattle WA. 98115.

Berg, J. 1979. Discussion of methods of investigating the food of fishes, with reference to a preliminary study of the prey of *Gobiusculus flavescens* (Gobiidae). Mar. Biol. 50:263-273.

Bigg, M.A., and I. Fawcett. 1985. Two biases in diet determination of northern fur seals (*Callorhinus ursinus*). Pages 284-291 *in* Beddington, J.R., R.J.H. Beverton, and D.M. Lavigne (eds.), Marine mammals and fisheries. George Allen and Unwin, London.

Bigg, M.A., and P.F. Olesiuk. 1990. An enclosed elutriator for processing marine mammal scats. Mar. Mammal Sci. 6(4): 350-355.

Bilton, H.T., D.W. Jenkinson, and M.P. Shepard. 1964. A key to five species of Pacific salmon (Genus *Oncorhynchus*) based on scale characters. J. Fish. Res. Bd. Canada, 21(5), 1267-1288.

Bowen, S.H. 1996. Quantitative description of the diet. Pages 513-532 *in* B.R. Murphy and D.W. Willis (eds.), Fisheries Techniques, 2nd ed. American Fisheries Society, Bethesda, Maryland.

Bowen, W.D. 2000. Reconstruction of pinniped diets: accounting for complete digestion of otoliths and cephalopod beaks. Can. J. Fish. Aquat. Sci. 57:898-905.

Cannon, D.Y. 1987. Marine fish osteology: A manual for archaeologists. Dept. of Archaeology, Simon Fraser University, Burnaby, B.C. Pub. No. 18. 133 p.

Clarke, M. R. (ed.). 1986. A handbook for the identification of cephalopod beaks. Clarendon Press, Oxford. 273 p.

Cochran, W.G. 1977. Sampling Techniques. John Wiley & Sons, Inc., New York. 428 p.

Cortes, E. 1997. A critical review of methods of studying fish feeding based on analysis of stomach contents: application to elasmobranch fishes. Can. J. Fish. Aquat. Sci. 54:726-738.

Cottrell, P.E., A.W. Trites, and E.H. Miller. 1996. Assessing the use of hard parts in faeces to identify harbour seal prey: results of captive-feeding trials. Can. J. Zool. 74: 875-880.

da Silva, J., and J.D. Neilson. 1985. Limitations of using otoliths recovered in fecal samples to estimate prey consumption in seals. Can. J. Fish. Aquat. Sci., 42:1439-1442.

Dellinger, T., and F. Trillmich. 1988. Estimating diet composition from fecal analysis in otariid seals (Otariidae): Is it reliable? Can. J. Zool. 66:1865-1870.

Ferry, L.A., and G.M. Cailliet. 1996. Sample size and data analysis: Are we characterizing and comparing diet properly? Pages 71-80 *in* D. MacKinlay and K. Shearer (eds.), Feeding ecology and nutrition of fish. Proceedings of the Symposium of the Feeding and Ecology and Nutrition in Fish, International Congress on the Biology of Fishes. Am. Fish. Soc., San Francisco, CA.

Gerard, P.D., D.R. Smith, and G. Weerakkody. 1998. Limits of retrospective power analysis. J. Wildl. Manage. 62(2): 801-807.

Hammill, M.O., C. Lydersen, K.M. Kovacs, and B. Sjare. 1997. Estimated fish consumption by hooded seals (*Cystophora cristata*) in the Gulf of St. Lawerence. J. Northwest Atl. Fish. Sci. 22:249:257.

Hammond, P.S., and P. Rothery. 1996. Application of computer sampling in the estimation of seal diet. J. Appl. Stat. 23:525-533.

Harvey, J.T. 1988. Population dynamics, annual food consumption, movements and dive behaviors of harbor seals *Phoca vitulina richardsi,* in Oregon. Ph.D. Dissertation, Oregon State University. 177 p.

Harvey, J.T. 1989. Assessment of errors associated with harbor seals *Phoca vitulina* faecal samples. J. Zool. (Lond.) 219:101-111.

Harvey, J.T., T.R. Loughin, M.A. Perez, and D.S. Oxman. 2000. Relationship between fish size and otolith length for 62 species of fishes from the eastern north Pacific Ocean. U.S. Dep. Commer. NOAA Tech. Rept., NMFS circular 150:1-36.

Harvey, J. T., G. A. Antonelis, and C. J. Casson. *unpublished data*. Quantifying errors associated with sampling pinniped food habits. 28 p.

Hayes, J.P., and R.J. Steidl. 1997. Statistical power analysis and amphibian population trends. Conserv. Biol. 11(1): 273-275.

Hoenig J.M., and D.M. Heisey. 2001. The abuse of power: the pervasive fallacy of power calculations for data analysis. Amer. Stat. 55(1):19-24.

Hyslop, E.J. 1980. Stomach content analysis - a review of methods and their applications. J. Fish. Biol. 17:411-429.

Jobling, M. 1987. Marine mammal faeces as indicators of prey importance – a source of error in bioenergetics studies. Sarsia 72:255-260.

Kashiwada, J., C.W. Recksiek, K.A. Karpov. 1979. Beaks of the market squid, *Loligo opalescens*, as tools for predator studies. CalCOFI 20:65-69.

Marcus, J., W.D. Bowen, and J.D. Eddington. 1998. Effects of meal size on otolith recovery from fecal samples of gray and harbor seal pups. Mar. Mammal Sci. 14(4):789-802.

Mohn, R., and W.D. Bowen. 1996. Grey seal predation on the eastern Scotian Shelf: modeling the impact on the Atlantic cod. Can. J. Fish. Aquat. Sci. 53:2722-2738.

Morrow, J.E. 1979. Preliminary keys to otoliths of some adult fishes of the Gulf of Alaska, Bering Sea, and Beaufort Sea. U.S. Dep. Commer., NOAA Tech. Report NMFS circular 420, 32 p.

Murie, D.J., and D.M. Lavigne. 1986. Interpretation of otoliths in stomach content analyses of phocid seals: quantifying fish composition. Can. J. Zool. 64:1152-1157.

Olesiuk, P.F, M.A. Bigg, G.M. Ellis, S.J. Crockford, and R.J. Wigen. 1990. An assessment of the feeding habits of harbour seals (*Phoca vitulina*) in the Strait of Georgia, British Columbia, based on fecal analysis. Can. Tech. Rep. Fish. Aquat. Sci. No. 1730, 135 p.

Orchard, T.J. 2001. The role of selected fish species in Aleut Paleo diet. M.A. thesis. University of Victoria. 228 p.

Pierce, G.J., and P.R. Boyle. 1991. A review of methods for diet analysis in piscivorous marine mammals. Oceanogr. Mar. Biol. Ann. Rev., 29: 409-486.

Pinkas, L., M.S. Oliphant, and I.L.K. Iverson. 1971. Food habits of albacore, bluefin tuna, and bonito in California waters. Calif. Fish Game Fish. Bull. 152:1-105.

Pitcher, K.W. 1980. Stomach contents and feces as indicators of harbor seal, *Phoca vitulina*, foods in the Gulf of Alaska. Fish. Bull., U.S. 78:797-798.

Reed, J.M., and A.R. Baustein. 1997. Biologically significant population declines and statistical power. Conserv. Biol. 11(1): 281-282.

Riemer, S.D., and M.M. Lance. 2001. Pinniped prey identification and quantitative workshop: proceedings of the December 2-4, 2000 workshop in Seattle, WA. Ore. Dept. Fish Wildl., Wash. Dept. Fish Wildl. and Natl. Mar. Fish. Serv., NOAA, 7600 Sand Pt. Way NE, Seattle WA. 98115. 46 p.

Riemer, S.D., and R.F. Brown. 1996. Marine Mammal (Pinniped) Food Habits in Oregon. Contract Report to Pacific States Marine Fisheries Commission. Contract number 95-97, 26 p. Report available at ODFW, Gold Beach OR. 97444.

Riemer, S.D., and R.F. Brown. 1997. Prey of pinnipeds at selected sites in Oregon identified by fecal (scat) analysis, 1983-1996. Ore. Dept. Fish Wildl. Tech. Rep. 97-6-02. 34 p.

Shelton, P.A., W.G. Warren, G.B. Stenson, and J.W. Lawson. 1997. Quantifying some of the major sources of uncertainty associated with the estimates of harp seal prey consumption Part II: Uncertainty in consumption estimates associated with population size, residency, energy requirement, and diet. J. Northwest Atl. Fish. Sci. 22:303-315.

Stenson, G.B., M.O. Hammill, and J.W. Lawson. 1997. Predation of harp seals in Atlantic Canada: Preliminary consumption estimates for the Arctic cod, capelin, and Atlantic cod. J. Northwest Atl. Fish. Sci. 22:137-154.

Thomas, L. 1997. Retrospective power analysis. Conserv. Biol. 11(1): 276- 280.

Thompson, S. K. 1992. Sampling, New York: John Wiley & Sons, Inc. 339 p.

Tirasin, E.M., and T. Jorgensen. 1999. An evaluation of the precision of diet description. Mar. Ecol. Prog. Ser. 182:243-252.

Tollit, D.J., M.J. Steward, P.M. Thompson, G.J. Pierce, M.B. Santos, and S. Hughes. 1997. Species and size differences in the digestion of otoliths and beaks: implications for estimates of pinniped diet composition. Can. J. Fish. Aquat. Sci. 54:105-119.

Warren, W.G., P.A. Shelton, and G.B. Stenson. 1997. Quantifying some of the major sources of uncertainty associated with the estimates of harp seal prey consumption Part I: Uncertainty in the estimates of harp seal population size. J. Northwest Atl. Fish. Sci. 22:289-302.

Wolff, G. 1982. A beak key for eight eastern tropical Pacific cephalopod species with relationships between beak dimensions and size. Fish. Bull. U.S. 80:357-370.

APPENDIX 1. Prey Identification Options

When designing a study to determine the diet of pinnipeds, time frame, focus of the study, financial constraints, prey identification expertise and availability of comparative collections all need to be considered. We developed the following list as a guide. Steps can be done in or out of sequence depending on the study design; however, we recommend each step be undertaken with other steps in mind. We strongly suggest that data are collected and samples are stored with the possibility of reanalysis. Although many of the steps below list analyses similar to one another, the level of identification will ultimately drive the analyses. For example, split sample frequency of occurrence analysis using only otoliths as suggested in Step 1 is quite different than this same analysis completed after Step 4 when all bone is identified for presence or absence within the samples.

Currently, Steps 1, 3 and 7 are the most common levels of identification carried out by the authors. In some cases, Step 8 has also been undertaken when more information is needed on a target prey species, such as the case of salmonids in the Columbia River.

1. Identify and enumerate teleost fish sagittal otoliths, cephalopod beaks and cartilaginous fish structures (e.g., mouthparts), identify cephalopod pens and statoliths, record presence or absence of fish lenses and grade otoliths. This step identifies and enumerates teleost fish using the otolith identification method and identifies and enumerates cephalopods and cartilaginous fishes. Grading the otoliths will allow for measurement to determine prey size, and the presence of fish lenses will indicate consumption of teleost fish when only cephalopod or cartilaginous fishes are present in the sample. Analysis at this level includes Frequency of Occurrence, Relative Abundance, Average Numerical Importance and Split-Frequency of Occurrence for otoliths only.

2. Identify the study's focus prey species, if any, using the "all structures" identification method. Combined with Step 1, this step will provide a more complete picture of the target species by using all structures and also include information on the other prey recovered in the sample using otoliths/beaks. In particular, this step should be considered in cases where the target species is not commonly identified using only otoliths (e.g., salmonids). This allows analysis similar to Step 1, but would include otoliths and all structures for the target species only.

3. Measure lengths of fish otoliths and cephalopod beaks. This step combined with Step 1 will prepare samples for Index of Relative Importance and Reconstruction Biomass proportion analysis using only otoliths and beaks. This step can also be combined with Steps 4, 5, 6, 7 or 8 for a look at the prey species using a combination of otolith and bone identification techniques.

4. Use the all structures identification method and record presence or absence of prey species. This step could be combined with Step 1 for a more detailed analysis of the

samples using all structures; however, numbers of prey within the samples (MNI) would not be available based on bone enumeration. Analysis would be the same as for Step 1

5. Enumerate "key" diagnostic structures to determine MNI for those prey species that commonly occur (>10% FO) in the samples. This step would use Appendix 2, which lists "key" diagnostic structures identified from fecal samples, other structures recovered but not listed in Appendix 2 would also be included. This MNI determination includes classifying bone by size. This will provide information on numbers of prey occurring in 10% or greater within a given sample based on the all structures method. Combining this data with Step 3 will prepare the data for use in Reconstruction Biomass proportion and Index of Relative Importance analyses.

6. Enumerate "key" diagnostic structures (Appendix 2) to determine MNI for all prey species regardless of their occurrence within the samples. This step is the same as Step 5 but includes all prey recovered in the samples not just the most frequently occurring species; analysis would also be the same if combined with Step 3.

7. Enumerate the most numerous paired diagnostic structures for MNI calculations within each sample (consistent with Step 5) and also determine MNI based on an estimate of non-diagnostic numerous structures such as vertebrae. This step is similar to Step 6, but also includes the enumeration of the most numerous diagnostic structures, which in most cases is not attempted due to time constraints. Analysis would be the same as Step 5.

8. Conduct genetic analysis on bone recovered from target prey species. This step involves grinding bones for genetic analysis. It is suggested that these bones be photographed and measured prior to analysis. Genetic analysis can provide more information on target prey types such as species or stock information or even identification of individuals. This analysis can be expensive and should be used only when other forms of identification are not feasible.

9. Grade the level of erosion of "key" diagnostic structures recovered in samples. Grading techniques for "key" diagnostic structures are still being developed. It has not been determined if these structures (which do not include otoliths) can be used in regression equations. Ongoing captive feeding studies are addressing this issue.

10. Measure "key" diagnostic structures. The techniques for this step are being evaluated in captive feeding trials and will be developed in conjunction with Step 9. One concern with this technique is the potential for bone shrinkage during the drying process.

11. Age otoliths using techniques described by the Committee of Age Reading Experts (CARE). Ageing otoliths provides more information on the prey consumed by pinnipeds. Age and length relationships do exist for certain species. Ageing otoliths collected from fecal samples has not been attempted at this time and the

effect erosion may have on determining the age of the fish has not been evaluated. This may be a step to consider only for target prey species since it can be cost and time prohibitive.

12. Enumerate fish lenses. Currently this protocol suggests recording only presence or absence of fish lenses. Enumerating fish lenses may help with determining MNI of prey within the fecal sample, however fish lenses cannot be identified to any lower taxonomic level.

APPENDIX 2. Most frequently occurring diagnostic structures used for prey identification from harbor seal and sea lion fecal samples for families of fishes recovered from samples collected in California, Oregon, Washington, Alaska and British Columbia. Otoliths are used for identification of all families and species. Bolded structures are commonly used for identification and enumeration.

Acipenseridae (sturgeons) – scute

Agonidae (poachers) – vertebrae, scute

Ammodytidae (sandlances) – **basioccipital**, dentary, quadrate, palatine, **hyomandibular**, epihyal, **ceratohyal**, pharyngeal, posttemporal, vertebrae, **atlas**, ultimate vertebra, **hypural**

Anarhichadidae (wolf-eels) – teeth, vertebrae

Atherinidae (silversides) – vertebrae

Aulorhynchidae (tube snouts) – vertebrae

Bathylagidae (deep sea smelts) – vertebrae, preopercle, ceratohyal

Bathymasteridae (ronquils) – quadrate, ceratohyal

Batrachoididae (midshipman) – **vomer**, **basioccipital**, premaxilla, maxilla, teeth, dentary, quadrate, hyomandibular, epihyal, vertebrae

Carangidae (jackmackerels) – gill raker, quadrate, epihyal, pharyngeal plate, vertebrae, ultimate vertebrae, **scute**, branchials

Cephalopoda (squids/octopus) – beak, lenses (statoliths)

Chimaeridae (ratfish) – teeth, interhaemal spine

Clupeidae (herrings) – vomer, parasphenoid, basioccipital, **prootic/pterotic**, maxilla, preopercle, opercle, suboperculum, articular, **gill raker**, **quadrate**, hyomandibular, **epihyal**, **ceratohyal**, **hypohyal**, branchial, urohyal, scapula, radial, **vertebrae**, atlas, axis, caudal vertebrae, **ultimate vertebrae**, hypural

Cottidae (sculpins) – **vomer, basioccipital, premaxilla**, maxilla, dentary, articular, angular, operculum, preopercle, quadrate, hyomandibular, ceratohyal, epihyal, hypohyal, basihyal, gill raker, basibranchial, **radial**, vertebrae, atlas, ultimate vertebrae, **hypural, scute**, scale

Cyclopteridae (lumpfishes and snailfishes) – scute, palatine, premaxilla, quadrate, pharyngeal element, vertebrae, frontal, dentary, articular, hyomandibular, ceratohyal, pharyngobranchial

Cyprinidae (minnows) – branchial, vertebrae, atlas, hypural, secondary otolith

Embiotocidae (surfperches) – vomer, parasphenoid, basioccipital, **premaxilla, maxilla, teeth**, dentary, gill raker, quadrate, **pharyngeal plate, branchial**, urohyal, scapula, cleithrum, **vertebrae**, **atlas**, axis, ultimate vertebrae, hypural

Engraulidae (anchovies) – basioccipital, **prootic**, angular, opercle, quadrate, hyomandibular, ceratohyal, cleithrum, **vertebrae**, atlas, axis, thoracic vertebrae, caudal vertebrae, **ultimate vertebrae**, hypural

Gadidae and Merlucciidae (codfishes/whiting) – vomer, ethmoid, parasphenoid, suborbital, **basioccipital**, premaxilla, maxilla, teeth, dentary, **angular**, articular, opercle, interopercle, **gill raker**, palatine, **quadrate**, hyomandibular, interhyal, **epihyal**, ceratohyal, hypohyal, pharyngeal plate, pharyngobranchial, epibranchial, hypobranchial, posttemporal, cleithrum, supracleithrum, postcleithrum, radial, **vertebrae**, atlas, axis, caudal vertebrae, ultimate vertebrae, **hypural**

Gasterosteidae (sticklebacks) – vertebrae, ultimate vertebrae, **spine**, **spine holding plate**, scute

Gobiesocidae (Clingfishes) – quadrate, vertebrae

Hexagrammidae (greenlings) – **vomer**, premaxilla, maxilla, teeth, dentary, **angular**, articular, branchiostegal ray, **gill raker**, palatine, quadrate, pterygoid, epibranchial, hypobranchial, pharyngobranchial, **radial, vertebrae**, atlas, hypural, basihyal, epihyal, interhyal, ceratohyal, ultimate vertebrae

Myctophidae (lanternfish) - vertebrae

Myxinidae (hagfishes) - teeth

Ophidiidae (cusk-eels) – vomer, basioccipital, premaxilla, angular, quadrate, hypohyal, branchials, vertebrae, atlas

Osmeridae (smelts) – basioccipital, premaxilla, **maxilla, lingual plate**, dentary, articular, opercle, hyomandibular, **glossohyal**, ceratohyal, **vertebrae**, atlas, thoracic vertebrae, **ultimate vertebrae**, hypural

Paralichthyidae (large-tooth flounders) – vomer, basioccipital, premaxilla, maxilla, lingual plate, dentary, angular, gill raker, **palatine, epihyal**, ceratohyal, **hypohyal**, branchials, urohyal, **posttemporal, supracleithrum**, cleithrum, **vertebrae**, atlas, axis, ultimate vertebrae, hypural

Petromzontidae (lampreys) – circumoral teeth, teeth, tongue plate

Pholidae (gunnels) – parasphenoid, premaxilla, dentary, articular, hyomandibular, vertebrae

Pleuronectidae (right-eyed flounders) - vomer, basioccipital, premaxilla, maxilla, lingual plate, dentary, angular, gill raker, **palatine**, quadrate, pterygoid, **epihyal**, ceratohyal, **hypohyal**, basihyal, pharyngobranchial, hypobranchial, urohyal, interhyal pharyngeal, **posttemporal**, **supracleithrum**, cleithrum, hypercoracoid, **vertebrae**, atlas, axis, ultimate vertebrae, hypural, teeth, scales, interhaemal spine, scute

Rajidae (skates) – teeth, vertebrae, scute, denticle

Salmonidae (salmon - adult) – vomer, basioccipital, **teeth**, angular, suprapreopercle, branchiostegal ray, **gill raker**, palatine, quadrate, symplectic, **interhyal**, epihyal, ceratohyal, hypohyal (lower), branchial, urohyal, supracleithrum, scapula, postcleithrum, hypercoracoid, **radial**, basipterygium, **vertebrae**, atlas, thoracic vertebrae, ultimate vertebrae, hypural, scales, pectoral fin ray

Salmonidae (salmon - juvenile) – parasphenoid, **basioccipital**, teeth, **lingual plate**, dentary, gill raker, quadrate, **interhyal**, epihyal, ceratohyal, branchial, **radial**, basipterygium, **vertebrae, atlas**, thoracic vertebrae, ultimate vertebrae, hypural

Sciaenidae (croakers) – angular, gill raker, quadrate, vertebrae

Scomberesocidae (sauries) – vomer, basioccipital, maxilla, dentary, gill raker, palatine, branchials, vertebrae, atlas, hypural

Scombridae (mackerels and tunas) – vomer, basioccipital, premaxilla, maxilla, dentary, **gill raker**, palatine, **branchial, vertebrae**, atlas, spine, **hypural, scute**, scale

Squalidae (dogfishes) – teeth, interhaemal spine, vertebrae

Stichaeidae (pricklebacks) - vertebrae

Trichodontidae (sandfishes) – basioccipital, **preopercle**, premaxilla, pharyngobranchial, epihyal, vertebrae

Sebastidae (rockfishes) – **vomer**, basioccipital, premaxilla, maxilla, teeth, dentary, **angular**, articular, preopercle, branchiostegal ray, spacer, **gill raker**, palatine, quadrate, hyomandibular, symplectic, interhyal, epihyal, ceratohyal, hypohyal, basihyal, pharyngeal plate, pharyngobranchial, epibranchial, hypobranchial, urohyal, posttemporal, hypercoracoid, cleithrum, **radial**, interhaemal spine, **vertebrae, atlas**, axis, caudal vertebrae, **hypural**, scale, pterygiophores

Zoarcidae (eelpouts) – basioccipital, quadrate, vertebrae

APPENDIX 3. Sample prey identification data sheet

PREY IDENTIFICATION FORM

Sample Type ______________ **Identifier**______________ **Project** ______________

Sample Number	Collection Date MM/DD/YY	Prey Species	MNI oto/ beak	MNI other parts	Prey Part	Condition/Size (mm)				Bones/Comments	ID Date MM/DD/YY
						L/W	I	II	III		