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Sources of Bias and Uncertainty in Seal Diet Composition: Hard Part and Fatty Acid Analysis

Sources de biais et d'incertitudes en ce qui concerne la composition du régime alimentaire du phoque : analyse des parties dures et des acides gras

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TABLE OF CONTENTS

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ABSTRACT

Diet estimation in marine mammals relies mostly on indirect methods. The most common methods and still widely used are the recovery of hard parts from stomach contents, intestines, and faeces. Several chemical methods also have been developed, including quantitative fatty acid signature analysis (QFASA). Both of these approaches have been used to estimate the diet of seals. Although based on different assumptions and methods, both approaches are subject to sources of variation and to potential biases. Experimental evidence shows that digestion strongly influences both the number and size of hard parts that can be recovery in stomachs, intestines and faeces. Number correction factors (NCF) and digestion coefficients have been developed to reduce the biases caused by the effects of digestion on hard parts recovered from faeces. Although more work needs to be done on sources of variation in the correction factors, experiment evidence clearly shows that reasonable estimates of diet are dependent on the application of such corrections. The use of fatty acids depends on prey having distinct fatty acids signatures and the application of calibration coefficients to account for differential metabolism of prey fatty acids which influence their deposition in predator fat stores, such as blubber. Quantitative estimates of diet composition are made using QFASA, a statistical model that estimates which prey species and amounts must have been eaten to account for the fatty composition of the predator. Experimental studies indicate that generally estimates of diet can be accurately made, but these studies also reveal that significant errors of the magnitude also seen using hard parts can occur. False positive and false negative errors can occur with both approaches and obtaining a representative sample from which to infer diet may be the most significant challenge in estimating diet regardless of the approach used. Further experiments are needed to better understand the sources of variation in otolith erosion and the effect of variation in calibration coefficients, number of prey seals and the fatty acid set used in QFASA on the accuracy of estimates.

RÉSUMÉ

L'estimation du régime alimentaire des mammifères marins repose essentiellement sur des méthodes indirectes. Les méthodes employées le plus couramment et le plus fréquemment consistent à récupérer des parties dures à partir des contenus stomacaux, des intestins et des fèces. Plusieurs méthodes d'analyse chimique ont également été mises au point, notamment l'analyse quantitative de la signature des acides gras (QFASA). Les deux approches ont été utilisées pour déterminer le régime alimentaire des phoques. Même si ces deux approches reposent sur des hypothèses et des méthodes différentes, les deux sont sujettes à des sources de variation et à des biais potentiels. Les preuves expérimentales montrent que la digestion influence fortement tant la taille que le nombre des parties dures qui peuvent être récupérées dans les estomacs, les intestins et les fèces. Divers facteurs de correction et des coefficients de digestion ont été mis au point pour atténuer les biais attribuables aux effets de la digestion sur les parties dures récupérées dans les fèces. Même s'il faut poursuivre les travaux sur les sources de variation dans les facteurs de correction, les preuves expérimentales démontrent clairement que des estimations raisonnables des régimes alimentaires nécessitent l'application de telles corrections. Pour utiliser les acides gras, les proies doivent avoir des signatures distinctes d'acides gras, et il faut appliquer des coefficients de calibration pour tenir compte des différences de métabolisme des acides gras des proies, ce qui influe sur le dépôt dans les réserves de graisse des prédateurs, telles que le petit lard. Les estimations quantitatives de la composition des régimes alimentaires sont établies au moyen de la QFASA, un modèle statistique qui évalue les espèces et les quantités de proies ayant pu être ingérées, selon la composition de la couche de gras du prédateur. Les études expérimentales indiquent que, de façon générale, les régimes alimentaires peuvent être déterminés avec exactitude, mais elles révèlent également que des erreurs graves concernant l'ampleur peuvent également se produire lorsque des parties dures sont utilisées. Les deux approches peuvent produire des erreurs de faux positifs et de faux négatifs; le défi le plus important, pour l'estimation des régimes alimentaires, réside dans l'obtention d'un échantillon représentatif duquel on peut inférer le régime alimentaire, et ce, peu importe l'approche employée. D'autres expérimentations devront être menées pour mieux comprendre les sources de variation dans l'érosion des otolithes et l'effet de la variation dans les coefficients de calibration, des nombres de proies des phoques et des ensembles d'acide gras utilisés dans la QFASA sur l'exactitude des estimations.

INTRODUCTION

Diet estimation in marine mammals relies mostly on indirect methods because there are limited opportunities to directly observe what marine mammals eat (Table 1). Traditionally the most common methods and still widely used are the recovery of hard parts from stomach contents, intestines, and faeces. Less commonly, hard parts recovered from spewings are also used, particularly in fur seals and sea lions, but these represent prey not assimilated. Several chemical methods also have been developed. These include the analysis of stable isotopes of carbon and nitrogen, quantitative fatty acid signature analysis (QFASA), and the analysis prey DNA recovered from stomachs and faeces. All methods make assumptions, have requirements that must be met to generate the best estimates and have advantages and disadvantages (Table 1). These methods have been extensively reviewed (e.g., Pierce and Boyle 1991; Bowen and Siniff 1999; Bowen 2000; Santos et al. 2001; Pierce et al. 2004; Budge et al. 2006; Iverson 2009; Tollit et al. 2006; Tollit et al. 2010). Although the methods differ in many ways, it is important to remember that all of the indirect methods current in use are subject to bias arising from both features of the methods and our ability to sample the diet representatively from wild populations.

VALIDATION OF METHODS USED TO ESTIMATE DIET COMPOSITION

The value of prey hard parts recovered from gastrointestinal tracts and faeces in inferring the diet of marine mammals has been appreciated for decades, but possible limitations of their use only began to be evaluated in the late 1970s and 1980s (e.g., Prime 1979; da Silva and Neilsen 1985; Jobling and Breiby 1986; Jobling 1987). A number of feeding experiments with captive seals were conducted subsequently to better understand sources of variation, the nature of biases and to eliminate or reduce their influence on estimates of diet. Although chemical methods have a more recent history, experimental feeding studies have been conducted to validate their use (see below). In this paper, I focus on the feeding studies that have been done to validate the use of otoliths and prey fatty acids to estimate diets because they are the methods that have been used to generate estimates of diet in grey seals.

Fecal Contents

A growing number of experiments have been done to evaluate the extent to which hard parts, mainly otoliths and cephalopod beaks, recovered from pinniped faeces can be used to estimated diet (Table 2). Work by Prime (1979) appears to be among the first to experimentally evaluate the extent of digestion of otoliths recovered from seal faeces. A single harbour seal was fed four species of gadoid fishes revealing a high recovery rate of 86%, but leading to the inference that the remainder had been completely digested. Furthermore, those that were recovered were eroded and therefore smaller than those ingested resulting in an underestimation of the size of prey eaten. Recognizing the potential effect of otoliths robustness on digestion, da Silva and Neilson (1985) fed herring, a species with very fragile otoliths, to a harbour seal and found that only 4% of otoliths consumed were recovered in faeces. Although this recovery rate was negatively biased partly due to inactivity of seals during the experiment (see Bowen 2000), it serves to highlight the difficulty of estimating digestion coefficients. Prime and Hammond (1987) conducted a series of feeding trials using a single grey seal fed species which differed in the robustness of their otoliths and estimated species-specific digestion coefficients to account for partial erosion of otoliths.

To date, experimental work to validate the use of otoliths from faeces has been conducted on two phocid species and six otariid species (Table 2). In the first extensive examination of a phocid species, Harvey (1989) found that recovery rate of otoliths in faeces varied significantly by prey species as a function of otoliths robustness and confirmed that erosion also resulted in significant species-specific reduction in the size of otoliths. Using different prey species, Tollit et al. (2007) confirmed the sources of bias identified by Harvey, but also found that otolith recovery rates and degree of erosion differed by size within species. Again using harbour seals, Phillips and Harvey (2009) also found significant variation in both recovery rates and degree of otolith length reduction. Tollit et al. (2007) were the first to develop grade-specific digestion factors to account for the differing degrees of erosion observed among recovered otoliths. The extent to which experimentally derived factors are representative of those in the wild was assessed by comparing the distribution of grades from experimental and wild recovered otoliths. Although 34% of cod and whiting otoliths were graded high in the captive experiments, only 24% and 6% of otoliths recovered in the wild were given this grade suggesting that for some species experimentally derived coefficients may not be representative. Studies by Prime (1979), Berg et al. (2002) and Tollit et al. (2007) fed mixed diets, rather than feeding single species, showing that these sources of bias are robust to the method of feeding.

Although the experiments with harbour seals provided considerable insight, a series of experiments on fur seals and sea lions indicated that there are species effects on the magnitude of bias caused by otolith digestion (Table 2). Recovery rates from several species of otariids were considerably lower than that found in harbour seals (e.g., Gales and Cheal 1992, Casper 2006). Other experiments on fur seals and sea lions added further support for the need to correct for the biases introduced by partial erosion and complete digestion of otoliths (Table 2). Grellier and Hammond (2006) extended experiments to grey seals and in a large series of feeding trials developed number correction factors (NCF) and digestion coefficients for 18 prey species.

The impact of attempting to correct for otoliths erosion bias has been investigated in several studies. Tollit et al. (2007) found that without correction, estimates of prey mass eaten by harbour seals were underestimated by up to 69%, but even when correction factors were used, estimates of the percentage of prey in the diet had errors of -11% to 18%. In simulations, Phillips and Harvey (2009) found that estimates of biomass of prey consumed by seals, corrected for otolith erosion, still differed from the true biomass by 3.4 % to 18.6%, depending on sample size. Tollit et al. (2007) applied Steller sea lion specific corrections for otolith erosion and found that estimates of diet composition were generally reasonably estimates (i.e., within 20% or less) of the true proportions, but large errors still occurred.

Stomach Contents

Stomach-content analysis has the longest history as a method to estimate diet. However, because it is necessary to sacrifice animals, few studies have been done on sources of variation and the nature of biases that may influence reliable estimation of diet, although the effects of digestion on otoliths will be much the same as in faeces, excepting the magnitude of the effects.

Only two studies have investigated the state of digestion of prey as a function of time in the stomach and intestines of seals. Bigg and Fawcett (1985) fed squid and herring to 5 northern fur seals and found passage rates of squid beaks and otoliths from the stomach differed significantly. Murie and Lavigne (1986) conducted experiments on 13 individuals of three species, mainly grey seals, to examine how otoliths recovered in the stomach could be used to infer food consumption and diet. They found that the number of herring otoliths recovered in the stomach declined linearly as a function of time and that none were found after about 12 h.

Furthermore, intact faeces did not contain the missing otoliths indicating that they had been completely digested in the stomach. They concluded that correction for complete digestion would be needed to accurately reconstruct the quantity of food eaten, but experiments to develop such factors have yet to be conducted.

Partial and differential erosion of otoliths in the stomach will introduce significant biases depending on the nature of the diet (i.e., variation in the robustness of otoliths ingested). In addition to the biases that can result from complete and partial erosion of otoliths, one additional source of bias can affect estimates from stomach contents that generally should not affect estimates derived from scats. Differential passage of otoliths of different species will result in bias in stomach analysis, but not in fecal analysis as all otoliths that survive digestion will ultimately be represented in faeces, providing enough are collected (Prime and Hammond 1987). In the case of stomach, once hard parts have left the stomach they can no longer be sampled.

A number of factors can influence the values of both digestion coefficients and NCFs (Table 5). Seal species appear to differ in the degree to which they erode otoliths consumed. This can be seen in Table 2, where the recovery rates (generally <10%) are much lower than that found in other pinniped species. Marcus et al. (1998) found that recovery rates in grey where significantly lower than in harbour seals, both species studies in the same experimental environment and fed the same species. Several studies have also found significant within species variation, at the level of individuals, on recovery rates and the reduction in size of otoliths (Grellier and Hammond 2006). The method used to feed otoliths to seals can also influence the extent of digestion. Grellier and Hammond (2005) compared an experimental otolith-carrier species to *in situ* otoliths of haddock, plaice, and sand eel otoliths fed to two captive gray seals. They found that method of feeding affected the amount of otolith digestion, and therefore our ability to estimate fish size and diet composition. Otolith recovery rates varied among the three prey species, but were not affected by feeding method. Recovery rates of otoliths from seal held in dry areas during feeding experiments are significantly lower that those provide the opportunity to swim during the experiment (Bowen 2000). Finally, a number of characteristics of meals, frequency, size, and composition, have all been shown to influence recovery rates (Table 3).

Fatty Acids

The idea that predator fatty acid composition of predator in fat stores might contain information about diet has been around for decades, but a method to use fatty acids to estimate the composition of the diet has only been developed recently (QFASA, Iverson et al. 2004). Like other methods, the use of fatty acids to estimate diet is based on assumptions which have been tested both through experimental feeding of seals in captivity, corroboration of results from other diet estimation methods (e.g. stomachs, animal-borne video) conducted simultaneously, and through computer simulations. Although the method is relatively new, captive validation experiments have now been conducted on 4 species of pinnipeds (6 studies), on one other mammal, and on four species of seabirds (Table 4).

Iverson et al. (2004) conducted a feeding experiment with grey seals fed on a maintenance diet of herring and then switched to a mixed diet of mackerel and capelin. Using calibration coefficients (CC) derived from grey or harp seals fed herring, predictions of the percentages of prey in the diet were within 5% of the true values. However, the results did depend on which CC were used. Cooper (2004) conducted three independent experiments on juvenile (6-10 month old) wild-caught grey seals brought into captivity and fed homogenate test diets for periods of 10-20 days. In all cases, by the end of the experiment, the estimated levels (using QFASA) of

the mix of species in wild diets declined to be proportionately replaced by the expected input level of the introduced diet. Iverson et al. (2003) studied 10 captive monk seals fed NW Atlantic herring of which 8 were then switched to a diet of two species, while 2 remained as controls on herring. No false positive identifications occurred in any seal at initial or final sampling times and the new diet was accurately predicted, as were the controls. Two independent experiments have been conducted with the Steller sea lion (Table 4). Tollit et al. (2006) fed 5 prey species in mixed diets and included pulses of single species and using a prey database much larger than that actually fed to test the accuracy and resolution of QFASA. Predictions were promising overall. In one experiment, over- or underestimations of prey averaged <2-5% in 16 of 18 prey species, but were higher (10%) for two species and in a second experiment were <2-5% in 17 of 18 species and ~10% in 1 species (Tollit et al. unpubl). Hoberecht (2006) conducted similar experiments with 7 species of prey again in mixed and pulsed diets. Overall, she found the QFASA predictions were good, but that there were some false positives, that species <5%, and pulse feeding was not always reliably detected. Nordstrom et al. (2007) conducted a series of feeding trials using 21 harbour seals and two main prey species with traces of 9 other species. Using harbour seal specific CC and a reduced fatty acid set, and the full prey library of 11 species, they found error rate averaged 12%. Reducing the size of the prey library also reduced the error rate. False positives included capelin selected instead of herring and salmon, and sandlance but some of these could have reflected the diet of seals prior to the start of the experiment. However, one of 9 experiments, herring-smelt-herring, revealed a large error indicating that more tests are needed to better understand why this occurred.

Experiments on other species of mammals and birds have provided further support for the accuracy of QFASA (Table 4). Experiments on mink found the QFASA estimated of diet were within 5% of that expected in two experiments but underestimated the fed diet by 30% in the third experiment when no CC where used. When CCs from grey seals were used errors of 10-15% were observed for all three diets, as would be expected given the difference between adipose tissue (mink) and blubber (seals). Iverson et al. (2007) conducted feeding trials on two species and found the QFASA estimates of diet where within 2-5% of expected diets. In both species, there was a small false positive in one of the diets. Wang et al. (2009) found that QFASA accurately predicted the initial diet and diet switches in two species of eiders but that there were large errors in one of five prey species when using the CC of another seabird species. However, the direct application of the results and eider CC to the wild is somewhat difficult as the test diets fed, both initially and during the diet switches, were extraordinarily high in carbohydrate (from a commercial duck feed), which would never be encountered in a naturally feeding marine piscivore. The degree to which unnatural carbohydrate diets affect fatty acid metabolism requires further investigation.

SOURCES OF VARIATION AND POTENTIAL BIASES COMMON TO ALL METHODS

There are several sources of variation and potential biases that are common to all methods used to estimate diet although the mechanisms that might produce those biases are different (Table 5). False positive can occur in hard-part analysis by incorrectly identifying the species. Freshly removed otoliths are easily identified to species in most cases, although small gadoid and flatfishes can be difficult or impossible to identify to species. The situation becomes much more difficult with partially eroded otoliths and there is some subjectivity in the assignment to species and thus some level of error can be expected. However, error rates associated with species identification have not been reported. In the cases of fatty acids, fatty acid identification is based on chemical standards and column retention times relative to those standards. Quantification is computer based, but some level subjectivity is used to separate a few fatty

acids. Samples are run in duplicate or triplicate to reduce errors. False positives can also occur in the QFASA model where a species not eaten is identified in the diet. We know this occurs from experimental studies, but how common this is and the circumstances causing them to happen are still not well understood.

False negatives can also occur in hard-part analysis as number of otoliths usually cannot be identified and therefore some species that are eaten may not be detected. Also species without hard parts or those whose hard-parts are completely digested cannot be detected in the diet (e.g., Dellinger and Trillmich 1988). Using QFASA, false negative can occur if a species that is consumed is not included in the prey library or if two species have fatty acid compositions that cannot be reliably distinguished. The former is more difficult to guard against than the latter which can be tested empirically. To date, prey species used to estimate diets of grey seals are able to be statistically identified with high accuracy (e.g., Budge et al. 2002).

Obtaining a representative sample of the population is both a source of variation and another potential bias common to all methods and in many ways perhaps the most important. Pinnipeds are abundant and wide-ranging species that typically forage in the water column or near the bottom of the sea floor. The difficulty of obtaining a representative sample of the diet of grey seals in Canada, and most likely many other marine mammals, can be illustrated as follows. Austin et al. (2006) used stomach temperature telemetry to estimate meal frequency in adult grey seals and determined that on average seals ate 1.7 meals per day. Grey seals feed during foraging trips with a mean duration of about 9 days at sea followed by about 1 day hauled out on land (Beck et al. 2003; Breed et al. 2009). Based on movement and diving behaviour (Breed et al 2006, Beck et al. 2003), it appears that grey seals feed throughout the year with the exception of about a month each associated with the spring moult and winter breeding season. Although both foraging trip duration and diving behaviour show strong seasonality (Beck et al. 2003, Breed et al. 2009), if we ignore this detail for the purposes of illustration, then I calculate that each individual in the population might consume about 467 meals per year. Thus, a population of 250,000 grey seals would eat about 117 million meals per year. We are not really interested in meals per se but in our ability to sample meals to estimate the resulting diet.

Using the recovery of hard parts in scats as the basis for estimating the diet, we assume that each sample represents parts of 2 meals on average. This assumption would presumably be similar for stomachs. This may be an overestimate as recent data from harbour seals indicates that a single meal is distributed over about 4 scats (Phillips and Harvey 2009). Nevertheless, we use our assumption for the purpose of illustration. In the case of fatty acids, each sample will represent more meals because of the integration of prey fatty acids into the blubber layer over periods of say several months (Iverson et al. 2004, Nordstrom et al. 2007) – so say about 100 meals. Passage rate of a meal from the stomach typically occurs within 48-72 h in grey seals (Grellier and Hammond 2007). Therefore, with foraging trips of 9 days we should only expect to see a small fraction of meals deposited on the beach in the form of scats – maximum of ~25%. Phillips and Harvey (2009) found that only 5% scats were recovery on land in a captive setting, suggesting that faeces may be preferentially passed at sea. Nevertheless, assuming that this is not the case, of the 117 million meals, only about 25%% might see the beach, or about 29 million. During the course of a year, an ambitious program might collect monthly, but on Sable Island we typically collected 3 times a year for about 300 samples per year. This represents about 0.002% of meals on the beach, but only 0.0005% of meal eaten because a large fraction of scats must be passed at sea.

The situation with fatty acids is somewhat better, but still our sampling rate is very low. The major difference is that we do not, at least in principle, miss a large fraction of the meals eaten because of the length of foraging trips and the rate of passage of digested food. Meals are

stored in the blubber over time. Even so, 240 fatty acid samples collected in a year on Sable Island, or elsewhere, represent only about 100X240/117,000,000 or 0.02% of meals consumed.

This low sampling rate would not be an issue if the diet was relatively homogenous. In this case, sampling just a few scats or individuals for fatty acids might accurate represent the diet of the population and increasing sample size would increase precision. However, there is considerable evidence from field studies that diet varies, seasonally, inter-annually, geographically, and by age and sex in grey seals and other pinnipeds (e.g., Bowen and Siniff 1999). Therefore, we need to be concerned that most of the meals eaten and perhaps a portion of the diet might not be sampled. All this serves to underscore the difficulty faced in attempting to reliably estimate the diet of a marine mammal, particularly if the proportions we are interesting in are small.

These calculations are clearly preliminary and could be refined considerably to account for correlation in the diet within and among individuals which could reduce the effective number of meals/indivduals that need to be sampled to estimate the species composition of the diet. Of course the situation maybe better if the population of interest is much smaller and foraging trips are short relative to passage time of food. Nevertheless, refinements in the assumption are unlikely to change the conclusion that our ability to obtain a representative sample of the diet of grey seals in Canadian waters may be very difficult given that such a large fraction of scats must be lost at sea and the low sampling rate in the face of multiple sources of variation.

DISCUSSION

Despite experimental evidence that otoliths were both partially eroded and completely digested by seals, application of factors to correct for the resulting biases was hampered by the lack of estimates for many fish species commonly consumed by seals in the North Atlantic. The situation has improved with the experimental work on grey seals by Grellier and Hammond (2007) and continued experimental work on harbour seals (e.g., Phillips and Harvey 2009). Nevertheless, empirically estimates correction factors for a number of species that are thought to be eaten by grey seals still have not been determined.

The highly variable nature of the digestion of otoliths in seals and the impossibility of collecting all fecal material from wild animals lead Dellinger and Trillmich (1988) to conclude that “reasonable estimates of absolute numbers of fish ingested by free-living seals from scat analysis” was precluded. Gales and Cheal (1992) concluded that “scat analysis is clearly a poor method for estimating the diet of the Australian sea-lion” as several species fed were not detected in scats. In both cases, recent research suggests that these conclusions are too pessimistic, but it is clear that estimating diet from the analysis of hard parts can lead to large errors.

To date, the greatest progress has been made in accounting for bias resulting from the effects of digestion on otoliths recovered from faecal samples. Progress has lagged with respect to developing approaches to reduce bias associated with the analysis of stomach contents. Nevertheless, it is clear that harp parts are both lost and eroded in the stomach and that correction for the differential effects of digestion on the otoliths of different species will need to be developed to increase confidence in the resulting estimates.

Experiment evidence on diet estimates from fatty acids indicates that predicted diets can be quite accurate. The experimental evidence also shows that predicted diets are more accurate when species-specific calibrations coefficients are used. However, they also illustrate that large errors and false positives do occur. Further experimental evidence is needed to better

understand both the resolution of the method and the circumstances which lead to significant errors.

Based on experimental evidence to date, it seem clear that both the analysis of hard parts, otoliths, and the chemical and statistical analysis of fatty acids of predator and prey contain useful information about the diets of pinnipeds. However, both approaches are dependent on assumptions that can or have not been adequately tested, and processes or factors that are know, in the case of otolith erosion, or could, in the case of omission of prey species fatty acid profile, cause bias in estimates of diet. While we should attempt to make both approaches better, the daunting task of representative sampling would suggest that it is a mistake to spend too much time debating which methods are reliable and which are not. I suggest that the most robust view of the diet of wide-ranging species, such as grey seals, will come from the use of multiple, independent methods.

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Table 1. Strengths and limitations of methods used to estimate the diets of marine mammals (after Tolitt et al. 2010).

Method	Dietary History	Species Composition	Prey Size	Requirements	Strengths	Limitations
Faeces, prey hard parts	Last few meals	yes	yes	• reference collection of prey species otoliths • otolith size measurements • otoliths-prey size regressions	• large sample size possible • non-lethal collection • relatively inexpensive to collect and process	• prey must have specific-specific hard parts and these must be ingested • hard parts must resist digestion • correction factors to reduce bias caused by partial erosion and complete digestion must be estimated • correction factors not available for all prey species • may not be representative of species with long foraging trips • demographic traits of individuals unknown
Stomachs, prey hard parts	Last few meals	yes	yes	• reference collection of prey species otoliths • otolith size measurements • otoliths-prey size regressions	• moderate - large sample sizes possible • demographic traits of individuals known • relatively expensive to collect samples	• animals must be killed • prey must have specific-specific hard parts and these must be ingested • hard parts must resist digestion • correction factors to reduce bias, but these are not usually available • may not be representative of species with long foraging trips • often many empty stomachs • differential digestion may further bias results
Stable isotopes	Days to years depending on tissue	generally no, but exceptions for simple diets	no	• fractionation factors for tissues • reference isotope levels from lower trophic levels	• integrates diet over time • used as independent check of trophic level	• trophic levels are relative to carbon source which must be measured • composition and size of prey not known

Method	Dietary History	Species Composition	Prey Size	Requirements	Strengths	Limitations
Fatty acids	Days to months, depending on species and life history	yes	some course resolution	• prey fatty acid signatures • calibration coefficients (CC) to account for predator metabolism • prey fat content • predator adipose tissue	• integrates diet over weeks-months • sampling location less likely to bias composition • demographic traits of individuals known	• detection level of rare prey still being evaluated • false positives possible • because of long integration time, location of foraging less well defined • only course resolution of prey size • estimates sensitive to CC and fatty acid set
DNA	Last few meals	Not currently, species can be identified but proportions not developed yet	no	• reference species-specific genetic primers • un-degraded prey DNA	• species identification with high accuracy possible • demographic traits can be determined	• can be difficult to isolate suitable DNA from stomach or faeces • presence only at this point

Table 2. Selective feeding experiments to evaluate the accuracy of diet composition estimates from recovered hard parts from faeces.

Species	n	Diet	Results	Source
Phocidae				
Harbour seal, *Phoca vitulina*	6	11 species fed separately, substituted for maintenance meal	• recovery rate 24-89%, a function of otoliths robustness • recovery rates differed by species and seals • otolith length reduction 16-44% • passage time >90% recovery ≤ 24 h • unreliable without digestion coefficients and NCF	Harvey 1989
Harbour seal	7	9 species fed in mixed diets over 2-3 d	• recovery rates differed by species (7-91%)and size within species • otolith length reduced 27.5% (range 10-76.5%) differed by species • otoliths length reduction grade-specific • without correction mean mass of prey underestimated by 48% (-4 to -69%) • application of correction factors overestimates prey mass by 17% (-12% to 77%); graded factors had errors from -11% to 18%	Tollit et al. 2007
Harbour seal	1	3 species mixed diet	• recovery rates differed by species (4-48%)	Berg et al. 2002
Harbour seal	7	7 species fed separately	• recovery rates differed by species (30-90%) • mean reduction in otoliths length 20% and differed by species • mean percent difference in biomass consumed was 3.4 – 18.6%	Phillips and Harvey 2009
Grey seal, *Halichoerus grypus*	7	18 species separately or in pairs	• recovery rates differed by species 35-100% and size • digestion coefficients were species and grade specific • passage rates were highly species specific over the first 40 h	Grellier and Hammond 2006
Otariidae				
California sea lion/South American fur seal, *Arctocephalus*	2/2	2 species separately	• recovery rates differed by seal species • otolith length reduction of 12-17% • fish length underestimated by 16% and mass by 33-36%	Dellinger &Trillmich 1988

Species	n	Diet	Results	Source
australis			• unreliable reconstruction of biomass consumed	
New Zealand fur seal, *Arctocephalus forsteri*	3	7 species separately	• recovery rate varied by species (6 - 83%) • without correction for digestion all species underestimated	Fea and Harcourt 1997
Australian sea lion, *Neophoca cinerea*	2	5 species separately	• recovery rates differed by species, but < 4% for all species and zero for two species • reduction in otoliths length varied by species (14-40%)	Gales & Cheal 1992
California sea lion, *Zalophus californianus*	5	11 species, fed separately	• highly variable recovery rates by species (50.7%, 0-100%) • reduction in otolith lengths (30.1%, 17.1-47.6%) • passage rates vary among prey and highly variable among seals	Orr and Harvey 2001
Arctocephalus spp.	4	7 in two mixed diets	• only 64% of scat contained otoliths that could be used of diet estimation • recovery rates < 9% for all species with some species not represented • 27% of otoliths too eroded to identify • estimates of diet unreliable	Casper et al. 2006
Steller sea lion, *Eumetopias jubatus*	4	9 singly and mixed diets	• recovery rates differed by species (0-83%), by fish length, and robustness • without correction estimates of diet were unreliable • application of NCFs produced estimates that were usually within 20% of that fed	Tollit et al. 2007

Table 3. Factors the can influence estimates of digestion coefficients and number correction factors.

Factor	Effect	Source
Seal species	• recovery rates differ greatly by species	Dellinger and Trillmich 1988; Marcus et al. 1998; Tollit et al. 2007
Among individual variation	• large variation in recovery rate among individuals	Dellinger and Trillmich 1988; Grellier and Hammond 2006
Feeding method	• otoliths in carrier species were more eroded than those in situ, but recovery rate unaffected in some species, reduced in sand eels	Grellier and Hammond 2005
Seal activity	• reduced recovery rate non-swimming seals	da Silva and Nielsen 1985; Dellinger and Trillmich 1988; Harvey and Antonelis 1994; Bowen 2000
Meal frequency	• Reduced frequency associated with greater digestible dry matter	Trumble and Castellini 2005
Meal size	• greater recovery rate with larger meal	Marcus et al. 1998
Meal composition	• meal emptying and intestinal mobility decrease with increasing lipid content • mixed diet increase assimilation of nutrients	Lawson et al. 1997; Trumble et al. 2003

Table 4. Feeding experiments to evaluate the accuracy of diet composition estimates from fatty acid signature analysis.

Species	n	Diet	Results	Source
Pinnipeds				
Grey seal	6	• 3 species, mixed • maintenance diet of herring • CC estimates from seals fed herring	• using grey seal calibration coefficients (CC) predictions of fed diet within 5% of true • results did depend on CC set used • accurate estimates depending on the use of CC	Iverson et al. 2004
Grey seal (YOY)	28	• mixed wild species, replaced with homogenated test diets	• using grey seal calibration coefficients (CC) predictions of experimental diet contribution at 10-20 days consistent with that fed.	Coopeer 2004
Hawaiian monk seal, *Monachus*	10	• 3 species, mixed • maintenance diet of herring • CC estimates from monk seals fed herring	• fatty acid signatures remarkably altered by diet of Atlantic herring compared to Hawaiin Islands • diet switch accurately estimated by QFASA with no false positives	Iverson et al. 2003
Stellar sea lion	7	• 5 species, mixed diets • CC derived from 5 indivduals fed herring	• best results using SSL derived CC • quality of predictions depend on FA set used	Tollit et al. 2006
Steller sea lion	3	• 7 species in mixed diets • seals lost weight during experiments	• best results using SSL derived CC • Overall prediction good, some false positives and species <5% not reliably estimated • pulse feeding not reliably detected • results difficult to interpret as animals lost mass	Hoberecht 2006
Harbour seal	21	• 2 main singly 8 others at trace levels • no dietary history on seals, fed herring and salmon oil prior to expt • CC estimated from 4 seals fed herring	• harbour seal CC showed some differences from other phocids • fewest errors (13%) using harbour seal-specific CC and reduced FA sets • using full 11 species prey library, error rate was 12% • reducing size of library reduced error rate • false positives included capelin selected	Nordsrom et al. 2007

Species	n	Diet	Results	Source
			instead of herring, mean error 10%, salmon 15%, and sandlance 5%, but some of these could have reflected diet prior to the expt • QFASA reliably estimated diet of single substitution, control did not change • Large errors were observed in the switch treatment	
Other mammals				
Mink, *Mustela vison*	18	• 3 artifical diets, mixed	• best prediction without CC for 2 of 3 diets with errors <5%, for third diet prediction underestimated by 30% • with grey CC, errors of 10-15% for fed diet for all diets (but these are not appropriate for unstructured adipose tissue of mink) • two false positive using grey seal CC at ~5-8%	Iverson et al. 2004
Birds				
Murres	26	• 2 species, mixed • CC derived from fed constant diet of silverside from birth	• predicted diet within 2% of fed diets • 2% false positive in one diet	Iverson et al. 2007
Kittiwakes	13	• 3 species, mixed • CC derived from murres fed constant diet of silverside from birth	• predicted diet within 5% of fed diets • 5% false positive in one diet	Iverson et al. 2007
Steller's eider	8	• 5 species, 2 mixed expt diets • CC derived from Steller's eiders on constant diet	• CC from eider similar but differed from musses • accurate predictions on initial diet, but errors associated with change in diet reflecting integration time	Wang et al. 2009
Spectacled eider	8	• 5 species, 2 mixed expt diets • CC derived from Spectacled eiders on constant diet	• accurate predictions on initial diet, but errors associated with change in diet reflecting integration time	Wang et al. 2009

Table 5. Common sources of bias with methods based on hard parts and chemical identification of mammal diets.

Source of Bias	Details
False positives	• some eroded hard parts can be incorrectly assigned to species • fatty acids of some species may not be reliably discriminated
False negatives	• fraction of hard parts usually cannot be identified • species without hard parts will not be detected • species for which fatty acids are not available cannot be estimated • degraded DNA may fail to identify a species
Representative sampling	• hard parts generally represent the last few meals and therefore an unknown fraction of the diet might not be sampled at haul outs in marine mammals with long and wide-ranging foraging trips • sampling fraction is so small that it may be impossible to accurately characterize the diet which is know to vary with ecological and demographic factors