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# QUANTITATIVE FATTY ACID SIGNATURE ANALYSIS: A NEW METHOD OF ESTIMATING PREDATOR DIETS

SARA J. IVERSON,[1,4] CHRIS FIELD,[2] W. DON BOWEN,[3] AND WADE BLANCHARD[2]

[1]*Department of Biology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J1*
[2]*Department of Mathematics and Statistics, Dalhousie University, Halifax, Nova Scotia, Canada B3H 3J5*
[3]*Marine Fish Division, Bedford Institute of Oceanography, Department of Fisheries and Oceans, Dartmouth, Nova Scotia, Canada B2Y 4A2*

*Abstract.* Accurate estimates of the diets of predators are required in many areas of ecology, but for many species current methods are imprecise, limited to the last meal, and often biased. The diversity of fatty acids and their patterns in organisms, coupled with the narrow limitations on their biosynthesis, properties of digestion in monogastric animals, and the prevalence of large storage reservoirs of lipid in many predators, led us to propose the use of quantitative fatty acid signature analysis (QFASA) to study predator diets. We present a statistical model that provides quantitative estimates of the proportions of prey species in the diets of individual predators using fatty acid signatures. We conducted simulation studies using a database of 28 prey species ($n = 954$ individuals) from the Scotian Shelf off eastern Canada to investigate properties of the model and to evaluate the reliability with which prey could be distinguished in the model. We then conducted experiments on grey seals (*Halichoerus grypus*, $n = 25$) and harp seals (*Phoca groenlandica*, $n = 5$) to assess quantitative characteristics of fatty acid deposition and to develop calibration coefficients for individual fatty acids to account for predator lipid metabolism. We then tested the model and calibration coefficients by estimating the diets of experimentally fed captive grey seals ($n = 6$, switched from herring to a mackerel/capelin diet) and mink kits (*Mustela vison*, $n = 46$, switched from milk to one of three oil-supplemented diets). The diets of all experimentally fed animals were generally well estimated using QFASA and were consistent with qualitative and quantitative expectations, provided that appropriate calibration coefficients were used. In a final case, we compared video data of foraging by individual free-ranging harbor seals (*Phoca vitulina*, $n = 23$) fitted with Crittercams and QFASA estimates of the diet of those same seals using a complex ecosystem-wide prey database. Among the 28 prey species in the database, QFASA estimated sandlance to be the dominant prey species in the diet of all seals (averaging 62% of diet), followed primarily by flounders, but also capelin and minor amounts of other species, although there was also considerable individual variability among seals. These estimates were consistent with video data showing sandlance to be the predominant prey, followed by flatfish. We conclude that QFASA provides estimates of diets for individuals at time scales that are relevant to the ecological processes affecting survival, and can be used to study diet variability within individuals over time, which will provide important opportunities rarely possible with other indirect methods. We propose that the QFASA model we have set forth will be applicable to a wide range of predators and ecosystems.

*Key words: feeding ecology; food webs; marine carnivores; pinnipeds; predator diets; predator–prey relationships; prey fatty acid composition and signatures; statistical model.*

## INTRODUCTION

The dynamics of predator–prey relationships, the structure of food webs, and the foraging behavior of individuals are central themes in ecology (e.g., Schoener 1971, Paine 1980, Stephens and Krebs 1986, Pimm et al. 1991, Sih et al. 1998). Accurate estimates of predator diets are required to understand these areas of ecology. For some carnivores (e.g., lions [*Panthera leo*]; wolves [*Canis lupis*]; sea otters [*Enhydra lutris*]) direct observation of feeding can be used to estimate diet. However, for many carnivores, including cetaceans, pinnipeds, mustelids, and ursids, as well as for nonbreeding seabirds, direct observation of feeding is rarely possible and indirect methods must be used to reconstruct the diet. These indirect methods are based on the recovery of digestion-resistant prey structures from feces, stomach contents, or from spewings such as owl pellets (Gaston and Noble 1985, Pierce and Boyle 1991). While there are some differences in the way such methods are used across taxa, the principles are the same (e.g., Carss and Parkinson 1996).

Although much of our current understanding of predator diets is derived from these methods, such estimates

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[4] E-mail: Sara.Iverson@Dal.Ca

can be biased (e.g., Jobling and Breiby 1986, Jobling 1987, Carss and Parkinson 1996). Most soft-bodied prey are difficult to identify given their rapid digestion. The diagnostic hard parts of some prey (e.g., shells of crustaceans, heads of large fish) may not be consumed by the predator or may be eroded during digestion, such that the size of prey consumed may be underestimated or the identification of prey may not be possible. Furthermore, the degree of erosion of hard parts is species-specific and often a function of prey size within species (Bowen 2000). Thus, differential rates of digestion among prey species may seriously bias estimates in favor of species with large and robust hard parts. Finally, these methods provide only a snapshot of the most recent meal and may not be representative of the longer term diet.

These limitations have led to the development of techniques that do not depend on the recovery of digestion-resistant hard parts (e.g., antisera to Atlantic salmon, *Salmo salar*, with limited success [Boyle et al. 1990]; stable isotope ratios of carbon and nitrogen [Rau et al. 1992, Gannes et al. 1997, Kelly 1999]). Although stable isotope ratios are useful in estimating the trophic level of a predator, they usually cannot determine the species composition of the diet (e.g., Hobson 1993, Gilmore et al. 1995, Koch et al. 1995).

A third method involves the use of fatty acid signatures (Iverson 1993). Fatty acids are the main constituent of most lipids, and unlike other nutrients, such as proteins that are readily broken down during digestion, fatty acids are released from ingested lipid molecules (e.g., triacylglycerols) during digestion, but are not degraded. The fatty acids of carbon chain-length 14 or greater pass into the circulation intact and are generally taken up by tissues the same way. Since a relatively limited number of fatty acids can be biosynthesized by animals (Cook 1991), it is possible to distinguish dietary vs. nondietary components. Once taken up by tissues, fatty acids are either used for energy or re-esterified, primarily to triacylglycerols, and stored in adipose tissue. Although some metabolism of fatty acids occurs within the predator, such that the composition of predator tissue will not exactly match that of their prey, fatty acids can be deposited in adipose tissue with little modification and in a predictable way.

Fatty acids in marine organisms are extremely diverse and have high levels of long-chain, polyunsaturated fatty acids that originate from various unicellular phytoplankton and seaweeds (Ackman 1980). Numerous studies have demonstrated that specific fatty acid patterns are passed from prey to predator near the bottom of the food web (e.g., Sargent et al. 1988, Fraser et al. 1989, Graeve et al. 1994, Navarro et al. 1995, St. John and Lund 1996, Kirsch et al. 1998) and that the fatty acid composition of zooplankton directly influences the fatty acid composition of blubber lipids of baleen whales (e.g., Klem 1935, Ackman and Eaton 1966, Hooper et al. 1973). Fatty acids have also indicated the presence of fish or other prey in the diets of terrestrial and aquatic carnivores (e.g., Johnson and West 1973, Rouvinen and Kiiskinen 1989, Wamberg et al. 1992, Colby et al. 1993, Pond et al. 1995, Raclot et al. 1998), the degree to which plants have been consumed by terrestrial carnivores (Iverson and Oftedal 1992, Iverson et al. 2001*b*), and changes in the diets of pinnipeds (Iverson 1993, Iverson et al. 1997*a*, Kirsch et al. 2000).

To date, fatty acid signatures have been used qualitatively to infer trophic levels and spatial and temporal differences in diets both within and among species (e.g., Kakela et al. 1993, R. J. Smith et al. 1996, S. Smith et al. 1997, Iverson et al. 1997*a*, *b*). However, since the pattern of fatty acids found in some plants and in many fish and invertebrates can be used to accurately identify individual species (Iverson et al. 1997*b*, 2001*b*, 2002, Budge et al. 2002), prey fatty acid signatures might provide quantitative estimates of predator diets. To do this requires an understanding of the characteristics of prey fatty acid signatures and the extent to which they differ in a given ecosystem, an understanding of how ingested fatty acids are metabolized and deposited in various tissues of the predator, appropriate sampling of predator tissue, and a statistical model that relates the predator signature to a mixture of possible prey signatures. Here we present a statistical model that provides quantitative estimates of the proportions of prey species in the diets of individual predators using fatty acid signatures. We use simulation studies to investigate the properties of the model, and controlled feeding studies of grey seals (*Halichoerus grypus*) and harp seals (*Phoca groenlandica*) to assess quantitative characteristics of fatty acid deposition. We then test the model by estimating the diets of experimentally fed captive grey seals and mink (*Mustela vison*), and the diets of individual free-ranging harbor seals (*Phoca vitulina*) filmed during natural feeding events. We used each of these systems to represent increasing complexity of diet estimation.

## Methods

### *The model*

We refer to the quantitative distribution of all fatty acids measured in a predator or prey sample as its fatty acid signature. To estimate the composition of the predator's diet based on these signatures, we take a weighted mixture of the fatty acid signatures of the potential prey types and choose the weighting that minimizes a statistical distance from that of the predator. Each prey type (typically species, but potentially subsets of species or groupings of similar species; e.g., Iverson et al. 2002) is summarized by its mean fatty acid signature, and we estimate its proportional contribution to the predator's diet.

We proceed by first defining how close the predicted diet (i.e., the quantitative mixture of signatures) is from

the true diet. We then develop the concept of ''calibration coefficients,'' which are required to account for predator lipid metabolism and the fact that the fatty acid signature of the prey will not be laid down exactly in the predator (i.e., for some fatty acids the values observed in the predator may be always higher, or always lower, than that found in the diet; e.g., Kirsch et al. 2000). Related to the concept of calibration, is whether to estimate the diet using all fatty acids identified or a subset that might better reflect diet. Lastly, the estimated signature contribution from prey must be corrected to account for differences in fat content (and thus fatty acid contribution) among prey types. All else being equal, species with a higher fat content will contribute proportionately more to the predator signature than those with a lower fat content. However, given that we know the fat content of each prey, it is straightforward to translate the estimated signature contribution to the proportion of each prey type eaten.

*Model notation.*—To set the basic model notation, let $y_{ij}$ denote the proportion of the $j$th fatty acid of the $i$th predator. The $i$ notation will be dropped when it is clear we are referring to a single predator. Let $x_{klj}$ denote the proportion of the $j$th fatty acid from the $l$th prey of the $k$th prey type (in this case species) and $n_k$ the number of individual prey of type $k$. The mean $\bar{x}_{kj}$ is the mean of the prey of type $k$ for fatty acid $j$. The problem is to estimate $\pi_k$, the true proportion of the $k$th prey type found in the predator's diet with the estimate denoted by $p_k$. The estimated proportion of each prey in the diet, $\hat{y}$, over all fatty acids, is formed as follows:

$$\hat{y} = \sum_k p_k \bar{x}_k.$$

*Distance measures and estimation of $\pi_k$.*—The estimation problem is to choose $p_k$ such that $\hat{y}$ is ''close'' to $y$. Both $y$ and $\hat{y}$ sum to 1 and can be thought of as distributions over the fatty acids. In this context, the Kulback-Liebler (KL) distance (*Encyclopedia of Statistics* 1983), defined as

$$\text{KL} = \sum_j (y_j - \hat{y}_j)\log(y_j/\hat{y}_j)$$

is a natural choice, as it was developed to compare distributions. There are several other possible distances including the more usual squared error (SQ) distance, $\Sigma_j (y_j - \hat{y}_j)^2$, the squared relative error (REL), $\Sigma ((y_j - \hat{y}_j)/y_j)^2$ and the squared error distance of the logs (LSQ), $\Sigma_j (\log(y_j) - \log(\hat{y}_j))^2$. To understand the relative behavior of these distances, we considered an absolute difference of 0.01 between the true ($y$) and predicted ($\hat{y}$) proportion for a common, an intermediate, and a rarer fatty acid, respectively (i.e., true proportions: 0.20, 0.05, and 0.01; predicted proportions: 0.21, 0.06, and 0.02, respectively). The SQ distance attributes the same weight for all true values. However, an absolute error of 0.01 should be more serious in the rare as opposed to the common fatty acid. Hence, the other three distances, which give more weight to the differences in the rare fatty acids, are preferable; of these three distances, the KL distance does so most conservatively and proportionately.

To then estimate the $p_k$, we carried out an optimization over the number of prey types, $k$, with the $p_k$'s constrained to be positive and sum to 1. The starting values for the optimization have the $p_k$'s all equal. The optimization was carried out in S-Plus (S-Plus 2000) using the function nlminb, which is a local minimizer for smooth nonlinear functions subject to bound-constrained parameters, and uses a quasi-Newton method. However, to efficiently conduct the simulations on large, complex data sets, we used a FORTRAN optimizer from Netlib.

*Standard errors of estimates.*—A major source of variability comes from variation in fatty acid signatures among individuals of a particular prey type (e.g., Iverson et al. 1997*b*, 2002, Budge et al. 2002). To capture this variability, we carried out the following bootstrapping procedure in which we repeatedly create new prey means by sampling with replacement from the prey database.

For $b = 1, \ldots, B$, steps 1 and 2 below are carried out:

1) For each prey type $k$, randomly select $n_k$ individuals with replacement and create a new mean $\bar{x}_k^{*b}$.
2) Carry out the estimation procedure for the bootstrap prey means and compute $p_k^{*b}$. The estimate of the standard error (SE) is computed as

$$\text{SE}(p_k) = \sqrt{\frac{\sum_b [p_k^{*b} - \text{mean}(p_k^{*b})]^2}{B - 1}}.$$

*Calibration coefficients.*—Calibration coefficients, $c_j$, were computed as follows: for a particular fatty acid, $c_j$ is computed as the 10% trimmed mean of the following $r_{li}^j$'s:

$$r_{li}^j = \text{seal}_{ij}/\text{diet}_{lj}$$

for all $l$ and $i$. For example, to estimate the ''grey seal'' calibration coefficients, we had eight seals and 30 herring. Since we could not analyze the actual herring that individual seals ate, $i$ (1 to 8) indexes the seals and $j$ (1 to 30) indexes the herring. This gives 240 calibration coefficients for each fatty acid, for which the 10% trimmed mean is then computed. These coefficients are then included in the distance measures by replacing the predator's observed proportion of fatty acid of type $j$ by

$$z_j = \frac{y_j/c_j}{\sum_s y_s/c_s}.$$

Although we used the trimmed mean across all individuals in modeling, we also estimated the 10%

trimmed mean within each individual to estimate a within-study SE for coefficients.

*Fatty acid subsets*.—We refer to fatty acids by the standard nomenclature of carbon chain length:number of double bonds, and the location (n-x) of the double bond nearest the terminal methyl group. In analyses of marine lipids, over 70 fatty acids can be identified and quantified, depending on the analytical methods and gas chromatograph (GC) column used (Fig. 1). However, not all fatty acids provide equal information about diet due to predator metabolism (Iverson 1993). For instance, if short- or medium-chain fatty acids (i.e., <14 carbons; also including *iso5*:0 in some cetaceans) are found in predator adipose tissue, these could arise only from biosynthesis, since any of these consumed in the diet would be immediately oxidized (Jackson 1974). In contrast, fatty acids with n-6 or n-3 double bonds or components such as 22:1n-11 generally arise only from diet; however, 22:1n-11 may exhibit reduced deposition (Bremer and Norum 1982). Other fatty acids arise from a combination of diet and biosynthesis. For instance, although both are found in prey, in predators 14:1n-5 is produced predominantly from biosynthesis, while some 22:5n-3 arises from modification (Ackman et al. 1988, Iverson 1993, Iverson et al. 1995). Fatty acids such as 16:0, 16:1n-7, 18:0 and 18:1n-9, may arise to some extent from biosynthesis in the predator, but are also highly indicative of differences in various prey (e.g., Fig. 1; Iverson 1993, Iverson et al. 2001*b*). Thus, for both of these latter types of fatty acids (i.e., those that always occur at predictably higher or lower levels in the predator than in prey due to some biosynthesis or some reduced deposition, respectively), calibration coefficients can be used to reduce the influence of systematic deviations on diet estimation.

Finally, some fatty acids found at low or trace levels may not be correctly identified and separated from abundant nearby peaks (e.g., 18:1n-11 from 18:1n-9; Fig. 1) depending upon the nature of the chromatographic equipment used. Therefore their detection in chromatograms can be problematic or inconsistent. Since most such fatty acids occur at low levels in carnivore tissue, these can be removed from further analysis if necessary.

In the present study, we did not use the fatty acids that could only be present in the predator primarily from biosynthesis, nor any fatty acids that were inconsistently identified (Appendix A). Of the remaining fatty acids, we used two subsets for modeling: (1) ''dietary,'' which includes only those 33 fatty acids that could arise from dietary origin, and (2) ''extended-dietary'' (41 fatty acids), which includes all ''dietary'' fatty acids as well as eight fatty acids that could be biosynthesized by predators, but whose levels in a predator are also influenced by consumption of specific prey (Appendix A). The subsets of fatty acids used were renormalized to sum to 1 (after application of calibration coefficients if used) prior to modeling.

*Conversion from proportions in fatty acid signature to those in diet*.—Given the estimated proportions of each prey type in the predator's fatty acid signature, the $p_k$'s, and the average fat content of each prey type, the $f_k$'s, one can then express the proportion of the actual diet derived from the $k$th prey type, denoted by $a_k$, as follows:

$$a_k = \frac{p_k/f_k}{\sum_k p_k/f_k}.$$

### *The data*

The data used in the present study represent hundreds of samples analyzed and 67 fatty acids identified per sample, and cannot be presented in detail. Thus, where possible we show representative examples.

*Prey fatty acid signatures*.—Simulation studies of the estimation model were based on a prey database of 954 fatty acid signatures (e.g., Fig. 1) of 28 marine fish and invertebrate species collected on the Scotian Shelf off eastern Canada (from Budge et al. 2002).

*Calibration coefficients*.—To determine the extent to which specific fatty acids undergo selective deposition or metabolism, we conducted three feeding experiments. The aim of these experiments was to develop calibration coefficients to weight individual fatty acids according to how directly they were deposited from diet. The first two studies used eight juvenile (2–3 yr old) grey seals (''grey calibration'') and five juvenile harp seals (''harp calibration''), which were housed temporarily in large indoor seawater tanks at Dalhousie University's Aquatron facilities. The grey seals were maintained for at least five months on a diet consisting solely of Atlantic herring (*Clupea harengus*, 6.2 ± 0.30% fat). The harp seals were maintained for up to five months on the same herring, but these animals had been in captivity for less time than the grey seals. All herring fed during the five-month period had been collected from a single lot and, although variable in fat and fatty acid composition, were considered to be the most uniform diet we could feed. At the end of the five-month period, a full-depth (~5 cm) blubber biopsy was taken from the pelvic region of each seal using a sterile biopsy punch according to Kirsch et al. (2000). The blubber biopsy was placed in a glass vial containing chloroform with 0.01% BHT and stored frozen until analysis. Thirty herring were randomly collected throughout the feeding period and kept frozen until analysis (<six months). In these two studies, we used the initial assumption that in the approximate five-month period, the fatty acid composition of blubber would resemble that of the seal's diet as much as it ever would.

In the third calibration study, we examined the degree to which blubber fatty acid composition resembled the diet after a period of complete and rapid fattening on a high-fat diet. Grey seal pups are born with neg-

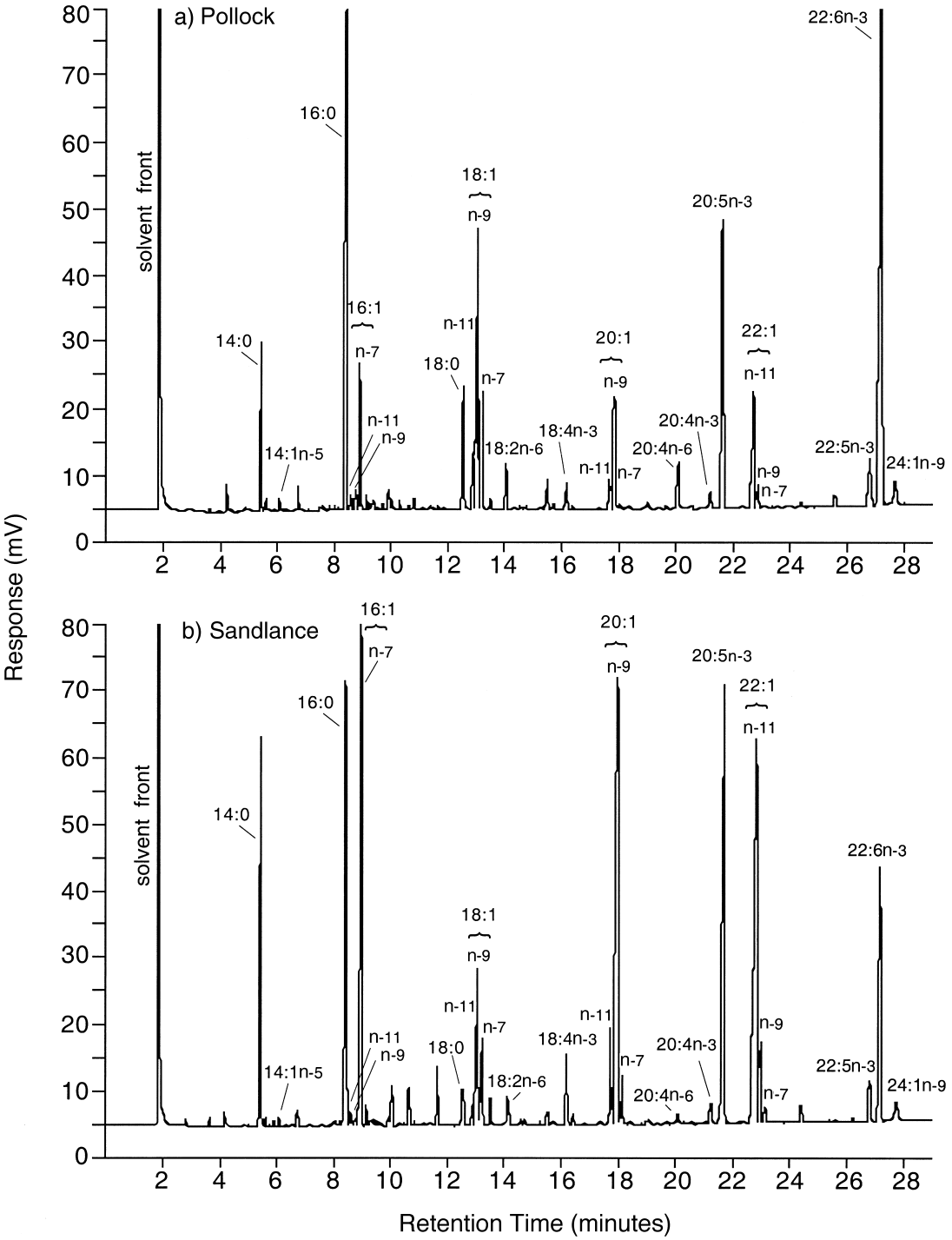


FIG. 1. Fatty acid chromatogram of one individual of each of two prey species, (a) pollock (*Pollachius virens*) and (b) sandlance (*Ammodytes dubius*), from the Scotian Shelf, illustrating relative differences between species. Here 67 fatty acids are identified and quantified in each chromatogram; however, only selected peaks are labeled on this plot. Fatty acids are eluted (''retention time'') in order of carbon chain length, number of double bonds, and position of double bonds on a polar capillary column (see *Methods*). The integrated area under each peak represents the relative mass percentage of each component.

ligible blubber, but at weaning (about 16 days postpartum [dpp]) they have deposited ~24 kg of fat in blubber from a milk-only diet, which is in turn produced completely from the blubber stores of the fasting mother (Iverson et al. 1993). Thus, virtually all blubber fatty acids in suckling pups arise from milk intake, permitting accurate estimation of calibration factors for individual fatty acids from a completely homogenous diet. Full-depth blubber biopsies were collected as described above from 17 grey seal pups at 15 dpp (i.e., immediately prior to weaning) on Sable Island, Nova Scotia, Canada (43°55′ N, 60°00′ W). Milk samples (40–60% fat; Iverson et al. 1993) were collected from each of these pups' mothers ($n = 17$) at 0, 5, 10, and 15 dpp, and the average milk fatty acid signature for each mother (i.e., here used as the ''prey'') was compared with that of her single pup (''pup calibration''). All samples were stored frozen in glass vials containing chloroform with 0.01% BHT until analysis.

*Experimental diet studies*.—We investigated the performance of the model using data from two captive feeding experiments (Kirsch 1997, Layton 1998). Both of these studies were designed to evaluate the effect of a known change in diet on the fatty acid signature of a predator. In one study, a second group of juvenile grey seals ($n = 6$, age 1–3 yr), housed temporarily in a seawater tank at the Aquatron facilities, had previously been maintained on a diet of Atlantic herring (averaging 5.1 ± 0.46% fat, from various lots) for up to five months. At the start of the diet trial, each seal was weighed, body composition was measured using isotope dilution (Oftedal and Iverson 1987, Bowen and Iverson 1998) and a full-depth blubber biopsy was taken and stored as described above. Seals were then fed an experimental diet, consisting of Atlantic mackerel (*Scomber scombrus*) and capelin (*Mallotus villosus*) for a period of 20 days. Atlantic herring, mackerel, and capelin share some similarities in fatty acid signatures (e.g., Budge et al. 2002), thus allowing evaluation of model performance when species in the diet do not differ markedly from one another. Due to the large size of the mackerel (averaging 38.1 cm, 0.5 kg), we removed the heads and cut the remainder of each into 5-cm thick cross-sections (i.e., including the viscera) for feeding. Seals were fed to satiation (or until they lost interest) twice daily; however, due to the constraints of this captive situation, it was not possible to determine individual intakes. As a result, some individuals undoubtedly consumed more and also different proportions of the prey species than others. Capelin (averaging 1.8 ± 0.23% fat) was offered only in the mornings and mackerel (averaging 18.3 ± 0.56% fat) only in the afternoons, in an attempt to get seals to eat the less-preferred capelin. The approximate daily ration offered averaged 5.4 $kg \cdot d^{-1} \cdot seal^{-1}$, comprising about three parts capelin to one part mackerel. At this daily ration, approximate fat intake would be 0.32 $kg \cdot d^{-1} \cdot seal^{-1}$ (Kirsch 1997). On days 12 and 20 of the experimental diet, seals were again weighed and a blubber biopsy was taken as described above; on day 20, body composition was again measured. Throughout the experiment, individual herring ($n = 15$), mackerel ($n = 25$), and capelin ($n = 25$) were randomly collected and stored frozen in airtight containers for analysis (<6 months).

In the second study, we used fatty acid data from fattening mink kits as an example of a terrestrial carnivore (Layton 1998). Briefly, until 21 dpp, 17 lactating females were fed primarily a wet diet (6.6% fat) along with some pelleted diet (17.3% fat), while kits consumed solely their mother's milk. Both the wet and pelleted diets consisted of primarily poultry offal (Layton 1998). Prior to feeding the experimental diets at 21 dpp, perirenal adipose tissue was sampled from 10 mink kits, euthanized in the course of other studies. The remainder of kits and their mothers were then fed one of three experimental wet diets. Each diet (6.6% fat) was composed of primarily poultry offal and fish meal, supplemented with either poultry fat, aquaculture herring oil, or seal oil (purchased from commercial sources) as 70% of the dietary fat source. Perirenal adipose tissue was sampled from six kits on each of the three wet diets at both 28 and 42 dpp (i.e., $n = 36$ total). Since diets were completely homogenous, a single sample of each was analyzed in duplicate for fat content and fatty acid composition. We were not able to obtain milk samples from the mothers. All samples were stored as described above.

*Free-ranging harbor seals filmed during foraging*.—In a final case, we studied 23 free-ranging adult male harbor seals during the breeding season of May–June 1997 on Sable Island. Throughout this period, males make routine foraging trips on the Scotian Shelf in the vicinity of Sable and reliably return to the island every few days (Walker and Bowen 1993, Coltman et al. 1997). Each male was fitted with an animal-borne video system (''Crittercam,'' [National Geographic Television, Washington, D.C., USA] Marshall 1998) for ≥3 d. The camera was positioned such that the animal's head was visible in the camera's field of view and programmed to film 10-min segments every 45 min during daylight, thus permitting the prey species that were eaten to be recorded (Bowen et al. 2002). At each deployment/recapture, a full-depth blubber biopsy was taken and diets were estimated using the model and the Scotian Shelf prey database. Since adult males remain in the vicinity of Sable for several months prior to reproduction, we assumed that prey eaten during these short-term studies would reflect the somewhat longer term diet inferred through blubber fatty acids. Thus, in one sense this was a validation experiment under free-ranging conditions, which employed a much more complex prey base than would have been possible in captive experiments.

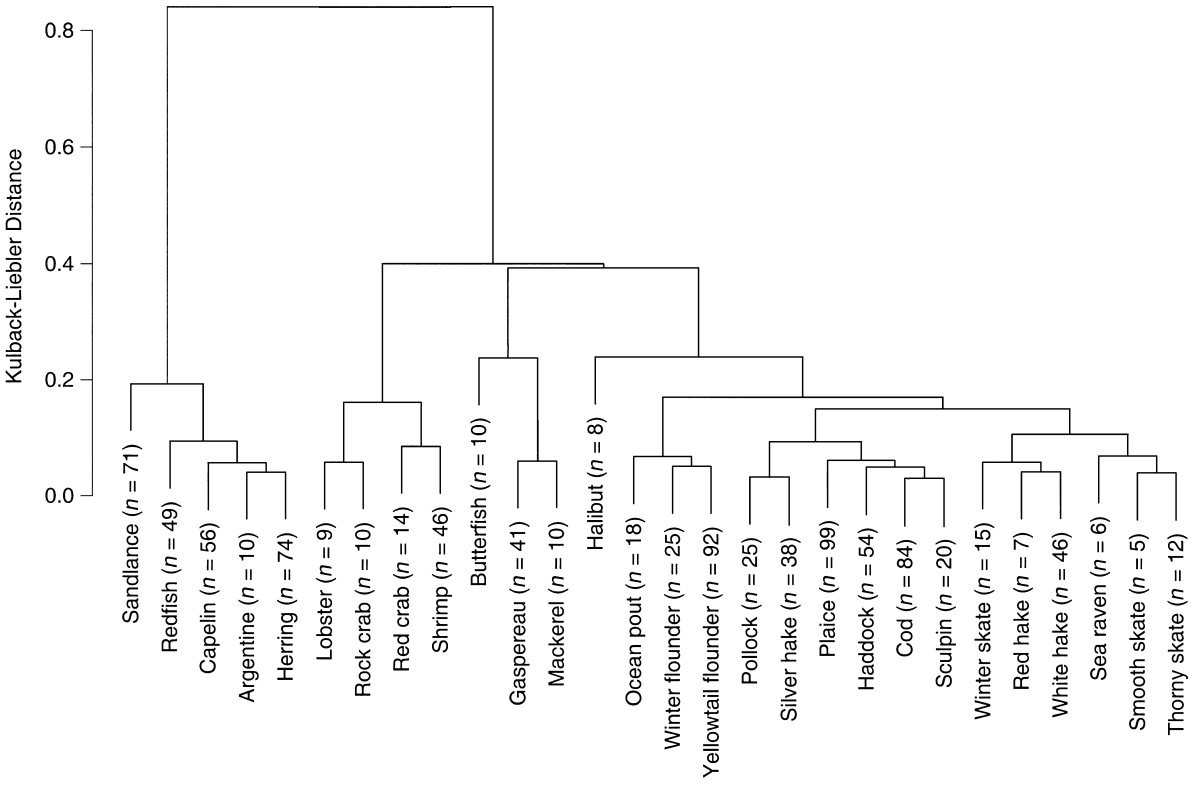


FIG. 2. Hierarchical cluster analysis on the mean fatty acid signatures (extended dietary subset) of 28 prey species ($n$ = 954 individuals) from the Scotian Shelf (Budge et al. 2002). Scientific names of all species not previously described in the text are as follows (in alphabetic order of teleosts, crustaceans): argentine (*Argentina silus*), butterfish (*Peprilus triacanthus*), gaspereau (*Alosa pseudoharengus*), halibut (*Hippoglossus hippoglossus*), ocean pout (*Macrozoarces americanus*), red hake (*Urophycis chuss*), redfish (*Sebastes* sp.), sculpin (*Myoxocephalus octodecemspinosus*), sea raven (*Hemitripterus americanus*), smooth skate (*Raja senta*), thorny skate (*Raja radiata*), white hake (*Urophycis tenuis*), winter skate (*Raja ocellata*), lobster (*Homarus americanus*), red crab (*Geryon quinquedens*), rock crab (*Cancer irroratus*), shrimp (*Pandalus borealis*). The Kulback-Liebler (KL) distance measure was used to determine how similar any two taxa were with respect to their fatty acid signatures. The average linkage method was used, which tends to identify spherical clusters.

### *Laboratory analyses*

Lipid was quantitatively extracted from all samples (Folch et al. 1957, Iverson et al. 2001*a*). Each whole prey was individually ground and homogenized prior to extraction. Milk and blubber samples were also homogenized prior to extraction. Fatty acid methyl esters were prepared using 1.5 mL of 8% boron trifluoride in methanol (Iverson et al. 1997*b*); this method in our laboratory produces identical results to that using Hilditch reagent (0.25 mol/L $H_2SO_4$ in methanol). Duplicate analyses of fatty acid composition were performed on all samples using temperature-programmed gas chromatography as described previously (Iverson et al. 1992, 1997*b*, Budge et al. 2002), on a Perkin Elmer Autosystem II Capillary FID (Perkin Elmer, Boston, Massachusetts, USA) gas chromatograph (GC) fitted with a 30 m $\times$ 0.25 mm ID column coated with 50% cyanopropyl polysiloxane (0.25 $\mu$m film thickness; J&W DB-23; Folsom, California, USA) and linked to a computerized integration system (Turbochrom 4 software, PE Nelson, San Jose, California, USA). Fatty acids and isomers were identified using the following methods: known standard mixtures (Nu Check Prep., Elysian, Minnesota, USA), silver-nitrate (argentation) chromatography, and GC-mass spectrometry (Hewlett Packard 6890 GC, 1:20 split injection, Micromass Autospec oa-TOF mass spectrometer, operated at 1000 resolution, scanning masses 120 to 450 [Hewlett Packard, Palo Alto, California, USA]). Fatty acid identifications on all chromatograms were checked, and corrected and reintegrated as necessary. Fatty acids are expressed as mass percent of total fatty acids.

### *Simulation studies*

*Simulation with no calibration coefficients.*—To investigate the properties of the estimation procedures and the robustness of the model in determining a given diet, we performed a number of simulation studies using the Scotian Shelf prey database. The first simulations were performed without calibration coefficients to assess the ability to estimate true diet based solely on differentiating and quantifying prey species by their fatty acid signatures. We used hierarchical cluster analysis to determine the relative similarity of prey species'

TABLE 1. Species composition of diets constructed for simulation studies.

| | Nonzero elements of the composition vector, $\pi$ (proportion of diet) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Diet | Cod | Haddock | Pollock | Silver hake | Plaice | Winter flounder | Yellowtail flounder | Sandlance |
| 1 | 0.333 | 0.333 | 0.167 | 0.167 | | | | |
| 2 | 0.200 | | 0.800 | | | | | |
| 3 | | 0.200 | | 0.800 | | | | |
| 4 | 0.100 | | | 0.100 | 0.100 | 0.100 | 0.100 | 0.500 |

*Notes:* Prey species used were based on 954 fatty acid signatures of 28 marine fish and invertebrate species collected on the Scotian Shelf off eastern Canada (Budge et al. 2002). Sample sizes of the above prey species were as follows: cod (*Gadus morhua*; $n = 84$), haddock (*Melanogrammus aeglefinus*; $n = 54$), pollock (*Pollachius virens;* $n = 25$), silver hake (*Merluccius bilinearis*; $n = 38$), plaice (*Hippoglossoides platessoides*; $n = 99$), winter flounder (*Pseudopleuronectes americanus*; $n = 25$), yellowtail flounder (*Limanda furruginea*; $n = 92$), and sandlance (*Ammodytes dubius;* $n = 71$).

signatures (Fig. 2). We then constructed four diets (Table 1): Diets 1–3 each contained two or four prey species that were more similar to one another than to all other species in the fatty acid database. These three diets represented difficult or, in some sense, ''worst case'' estimation scenarios. Diet 4 contained six species, some of which again were similar in fatty acid composition, and was constructed to represent the diet of a free-ranging grey seal based on results of fecal analysis (Bowen and Harrison 1994).

Simulations were used to evaluate how the accuracy of our estimates was affected by five factors: diet (four diets), fatty acid subset (dietary and extended-dietary), distance measure (KL, LSQ, SQ, REL), amount of ''noise'' in the simulated seal (0, 10%, 20%), and the number of individual prey ($n = 30$, 60, or 90) used in constructing the ''pseudo-seal'' fatty acid signature. Noise was meant to represent the proportion of the diet made up of incidental consumption of prey species that were not included in the assumed diet. The pseudo-seal fatty acid signature was constructed by sampling the prey database in the proportions specified by our simulated diet, with additional random prey added in to create the noise. Details of the simulation procedures are provided in Appendix B.

We calculated the relative mean squared error (RMSE) to measure how well simulations estimated the assumed diet. The RMSE was constructed by summing the relative squared deviations of the true diet from the estimated diet, $([\text{true} - \text{estimate}]/\text{true})^2$, for each simulation run and then averaging over the 1000 simulation runs for each factor setting.

*Simulation with calibration coefficients*.—To estimate the diet of a real predator, the effect of predator lipid metabolism on the deposition of dietary fatty acids must be included. Therefore, we also performed simulations using the three sets of calibration coefficients to examine how model estimates of diets were affected by the use of calibration coefficients and to test whether all sets of coefficients produced similar results. We used the grey seal calibration coefficients as the standard with which to compare the other two sets, as these should be applicable to the other experimental seal diet studies and to the free-ranging harbor seals and arose from the longer of the two seal feeding trials. The procedures for these simulations are described in Appendix B. We used the sum of the RMSEs of predicted diet from true diet (i.e., Table 1) of each pseudo-seal for the 1000 simulation runs and for the two fatty acid subsets to evaluate performance. These RMSEs were then compared to the RMSEs of predicted diet from true diet of the same pseudo (grey) seal using no calibration coefficients, and using harp and pup calibration coefficients in the fitting process.

## RESULTS

### *Calibration coefficients*

Despite large differences in fat content and homogeneity of the diet fed, in the known dietary history of the animals, and in the degree to which they fattened during the study, overall there was a reasonable degree of correspondence between the three sets of calibration coefficients and low within-study variability (Fig. 3). Calibration coefficients for most fatty acids were close to one, particularly in the case of suckling pups; however, there were notable exceptions. In general, the coefficients for the grey and harp seals fed herring were more similar to one another and deviated more from 1.0 than did the pup coefficients, but the pattern of deviations (Fig. 3) was similar in all three studies, suggesting that the underlying metabolic processes were common among animals and diets. The fatty acids with the 10 highest and 10 lowest calibration coefficients in both grey and harp seals, were also mostly among the highest and lowest in pups, although again the magnitude of deviation from 1.0 was smaller in pups (Fig. 3, Appendix A). Fatty acids such as 14:1n-5, 16:1n-11, 16:1n-9, 17:1 and 18:1n-11, with generally high coefficients, are predominantly biosynthesized by the predator and/or occur at low levels (generally occurring at $<0.8\%$ of total fatty acids in seals and/or prey). Because small errors in minor or trace fatty acids with large calibration coefficients might have large ef-

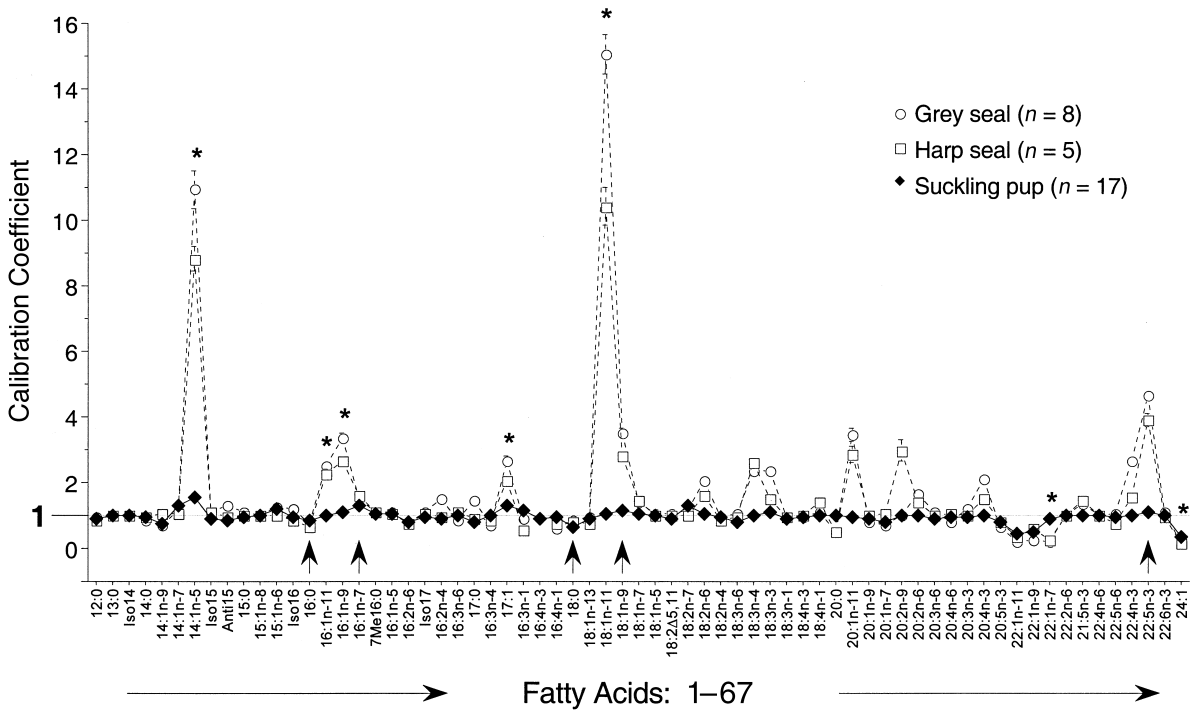


FIG. 3. Calibration coefficients (mean ± 1 SE) of the 10% trimmed means calculated within each individual (note that in most cases the standard error is too small to see) estimated for all 67 fatty acids quantified, using three different feeding studies: juvenile grey and harp seals maintained for five months on a diet of herring (6.2 ± 0.30% fat), and suckling grey seal pups at weaning having consumed only their mothers' milk (40–60% fat) and in which virtually all blubber fatty acids have arisen from milk intake. The 1:1 line is presented, which denotes the deviation of a given fatty acid in a predator from that consumed in its diet. Stars (⋆) indicate examples of fatty acids with large deviations from 1:1 but which usually occur at minor amounts (<0.5%) in seals and their prey. Arrows indicate common fatty acids that would be expected to have additional contribution from biosynthesis in predators, especially if on lower fat diets. See Appendix A for fatty acids used in modeling sets.

fects on estimates from the model, we removed these fatty acids from modeling subsets at the outset (see Appendix A). Relatively high coefficients of other fatty acids, such as 16:1n-7 and 18:1n-9 or 22:5n-3, are also consistent with the expected contribution from biosynthesis or metabolic modification, respectively, in the predator. However, these major fatty acids are good indicators of prey species (e.g., Fig. 1), and calibration coefficients provide a means of using them in the model.

In all three studies, some of the lowest calibration coefficients were found for 20:0 (except in pups), 22:1n-11, 22:1n-9, 22:1n-7, and 24:1 (Fig. 3). Of these, 20:0 and 24:1 are either rare and not indicative of diet or inconsistently detectable (Appendix A) and thus were eliminated from use in the model at the outset. In contrast, 22:1n-11, 22:1n-9 and 22:1n-7 are important dietary indicators (e.g., Fig. 1; Iverson 1993, Iverson et al. 1997*b*). Again, for these and most other fatty acids with deviations from 1.0, calibration coefficients allow their use in the model.

### *Simulations with no calibration coefficients*

Our aim here was to determine the relative importance of diet complexity, fatty acid subset, distance measure, amount of "noise" in the simulated seal, and prey sample size in minimizing the RMSE of the estimated diet. Variation in RMSE due to sample size of individual prey (30, 60 or 90) was obtained by averaging over all the other factors. The RMSE decreased with increasing sample size by ~20% and 5% for the extended-dietary and dietary fatty acid subsets, respectively, indicating that a sample size of 30 individual prey would provide representative results. Variation in the average RMSE due to the level of "noise" used (0%, 10%, or 20%) did not exceed 10%. Thus, to assess the effect of the other three factors on the performance of the estimation model, we used a sample size of 30 and 10% noise in the other simulations.

We next considered the effects of the distance measure, fatty acid subset, and the complexity of the simulated diet on model performance. Significant effects were found for fatty acid subset, and diet, with a distance measure by diet interaction ($P < 0.05$, three-way ANOVA on the medians across the 1000 simulations), but not for distance measure alone. For the dietary fatty acid subset, SQ tended to perform somewhat worse than the other distance measures, whereas for the extended-dietary fatty acid subset, the KL distance generally performed best. Overall, the RMSEs were lowest

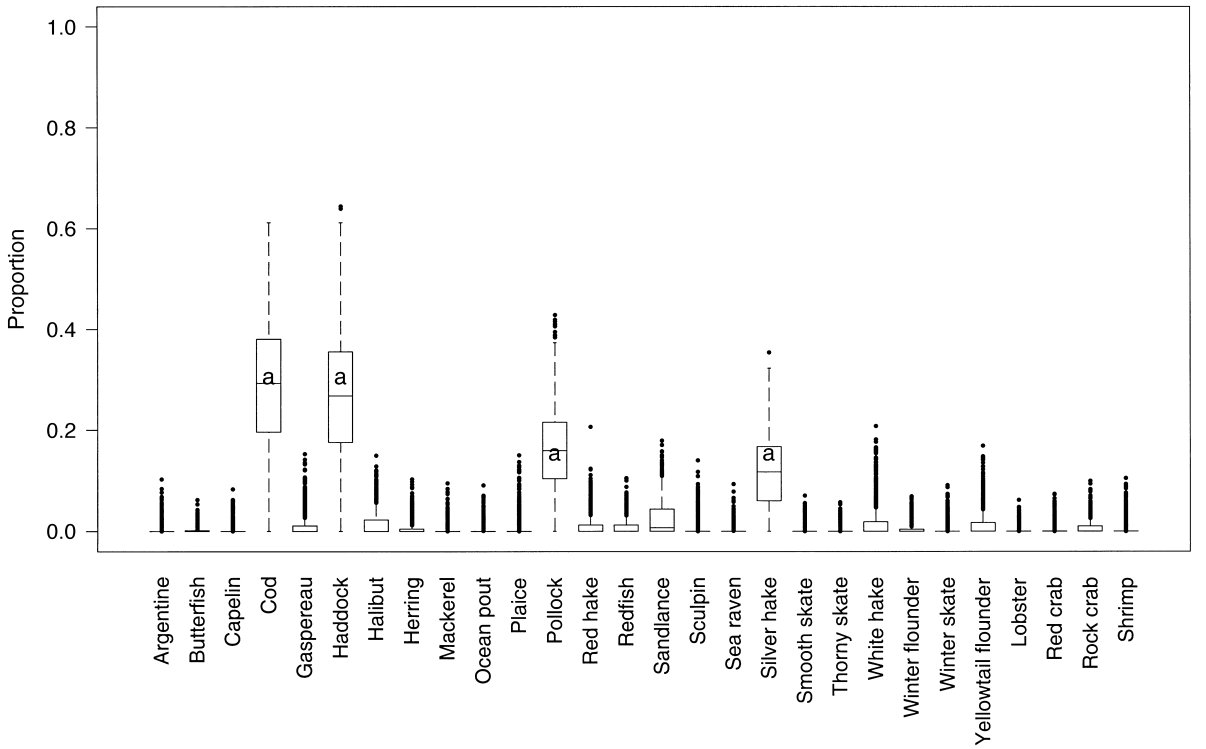


FIG. 4. Results of the simulation study for Diet 1 as defined in Table 1 with 10% error (noise) added, using the 28 Scotian Shelf prey species ($n$ = 954), the extended-dietary fatty acid subset, and no calibration coefficients, and with 30 individual prey used in constructing the pseudo-seal. Species are listed in alphabetic order (teleosts, crustaceans). In plots, "a" denotes the value (proportion) specified for each of the four prey species chosen for the diet. The simulation was run 1000 times, and estimated diet results are represented in box plots, as the median (middle horizontal bar), the 25th percentile (lower bar), and the 75th percentile (top bar) of the data distribution (i.e., the box contains 50% of the data). Dots represent outliers defined as being any value greater (or less) than 1.5 times the interquartile range (75th percentile–25th percentile) above the 75th (or below the 25th) percentile.

for the extended-dietary subset and KL distance, and highest for SQ. On the basis of these results, we concluded that any of the three distances, KL, REL, or LSQ, would generally give reasonable results. However, we have chosen to use the KL distance as this is a natural distance between two distributions, and arises in a number of statistical settings including the bootstrap (DiCiccio and Romano 1989).

Next we examined how well the model estimated each component of the simulated diets. As the noise was set at 10% for these simulations, accurate estimation would give a total of 10% other prey. Hence for Diet 1, we should estimate 30% each of cod and haddock and 15% each of pollock and silver hake, obtained by multiplying Diet 1 levels in Table 1 by 0.9. Using the extended-dietary fatty acid subset, the model estimated the true diet rather well (Fig. 4), with the major species in the diet distinguished from others in the prey database. Nevertheless, there was some misidentification (7%) of the diet composition to other prey types above the added noise. The results of simulations for all four diets and both fatty acid subsets are summarized in Table 2. Using the dietary fatty acid subset, although some species in each diet were reasonably estimated, others were not, resulting in a consistent overestimate of the other prey category. In contrast, using the extended-dietary subset, estimates of individual species within each diet were generally closer to the true values, but the other prey category still tended to be somewhat overestimated. When simulations of the same four diets were performed with no noise included, in all cases components of the diet were more accurately predicted and a lower proportion of the diet was attributed to other prey.

Patterns of values across these simulations provide insight into how the model performed within each diet (Fig. 5). For Diet 1, while the best fits corresponded closely to the specified diet, as the fit worsened the estimates became low for cod and high for pollock, suggesting that these two species may be difficult to distinguish. We also underestimated silver hake as the estimates deteriorated. In Diet 2, the estimates of pollock decreased as RMSE increased, with the balance going either to cod or other prey. In Diet 3, the estimates of haddock and silver hake decreased as the fit worsened, with the proportion attributed to other prey becoming very large in the worst fits. In contrast to the other diets, estimates did not change notably for Diet 4 as the fit worsened, except that yellowtail flounder became somewhat overestimated. In summary, especially for diets

TABLE 2. Mean estimated diets of pseudo-seals over the 1000 simulation runs for each of the four diets and two fatty acid subsets using the Kulback-Liebler (KL) distance and with noise set at 10%.

| Diet | Species | Specified diet | Dietary fatty acids | | Extended-dietary fatty acids | |
|---|---|---|---|---|---|---|
| | | | Estimate | 1 SD | Estimate | 1 SD |
| 1 | Cod | 0.30 | 0.37 | 0.150 | 0.29 | 0.129 |
| | Haddock | 0.30 | 0.14 | 0.130 | 0.26 | 0.127 |
| | Pollock | 0.15 | 0.18 | 0.085 | 0.16 | 0.085 |
| | Silver hake | 0.15 | 0.08 | 0.070 | 0.12 | 0.072 |
| | Other | 0.10 | 0.23 | 0.085 | 0.17 | 0.070 |
| 2 | Cod | 0.18 | 0.14 | 0.134 | 0.14 | 0.116 |
| | Pollock | 0.72 | 0.60 | 0.151 | 0.58 | 0.154 |
| | Other | 0.10 | 0.25 | 0.123 | 0.28 | 0.128 |
| 3 | Haddock | 0.18 | 0.01 | 0.034 | 0.12 | 0.089 |
| | Silver hake | 0.72 | 0.49 | 0.127 | 0.59 | 0.096 |
| | Other | 0.10 | 0.50 | 0.134 | 0.29 | 0.114 |
| 4 | Cod | 0.09 | 0.09 | 0.098 | 0.05 | 0.074 |
| | Silver hake | 0.09 | 0.02 | 0.036 | 0.03 | 0.039 |
| | Plaice | 0.09 | 0.05 | 0.064 | 0.06 | 0.066 |
| | Winter flounder | 0.09 | 0.06 | 0.048 | 0.07 | 0.047 |
| | Yellowtail flounder | 0.09 | 0.12 | 0.088 | 0.11 | 0.085 |
| | Sandlance | 0.45 | 0.39 | 0.090 | 0.42 | 0.079 |
| | Other | 0.10 | 0.27 | 0.106 | 0.26 | 0.100 |

*Note:* Pseudo-seals were created using 30 individual prey in the specified diet (no calibration) and modeled with the 28 Scotian Shelf prey species ($n = 954$, see Fig. 2).

that were specifically chosen to have species with similar signatures, in the worst cases it may be difficult always to separate these species, with an increasingly large percentage being attributed to other prey (i.e., Diets 1–3; Fig. 5a–c). However, as would be expected, fits are better and more consistent when diet items are more easily distinguished (i.e., Diet 4; Fig. 5d).

### *Simulations with calibration coefficients*

In the second set of simulation studies, calibration coefficients were used in the construction of pseudo-seals to mimic predator lipid biochemistry. Our primary interest in performing these simulations was to determine whether the three sets of calibration coefficients were comparable and whether they differed significantly from using no calibration. As before, we used a sample size of 30 prey and 10% noise. When pseudo-seals were created using calibration coefficients from a random grey seal and compared with pseudo-seals fitted using each of the four calibration scenarios (i.e., including no calibration, Appendix B), the RMSEs differed significantly among calibration coefficients and diets ($P < 0.01$, three-way ANOVA on the medians across the 1000 simulations), but not for fatty acid subset; there were no significant interactions. Overall, the RMSEs were lowest for the grey seal calibration coefficients and extended-dietary subset. Estimates of the simulated diets using no calibration coefficients differed most dramatically from those based on any of the three sets of coefficients, but differed less with pup coefficients. Although the grey and harp seal coefficients tended to give similar results, in a few cases the harp seal coefficients performed poorly compared to the grey seal ones. Simulations using the pup coefficients typically performed worse (higher RMSEs) compared to either grey or harp seal coefficients.

These results suggest that if differential lipid metabolism/deposition occurs in the predator (e.g., Fig. 3), calibration coefficients are needed to get accurate estimates of diets from the model. Different calibration coefficients produced similar, but not identical, results. Therefore, we assessed which coefficients were most applicable to the predator in question in modeling the diets of animals in the controlled feeding experiments.

### *Experimental diet studies and model application*

*Captive grey seals.*—Juvenile grey seals were fed a diet of herring prior to the start of this experiment. They were then offered a diet of ~3.4:1 mackerel/capelin, on a fat content (i.e., fatty acids) basis. Seals ate the mackerel readily and consumed all that was offered. However, they did not consume all the capelin that was offered. Thus, we assumed seals ate approximately half of the capelin, resulting in a ratio of mackerel to capelin fatty acids of 6.9:1.

Seals averaged 55.4 ± 4.31 kg and 33.0 ± 2.98% body fat (mean ± 1 SE) at the start of the experiment and gained 4.5 ± 0.67 kg over the 20-d feeding trial. Although all seals gained mass, they lost body fat (Kirsch 1997). Nevertheless, the fatty acid composition of blubber changed significantly ($P < 0.05$, MANOVA) over the course of the feeding trial (Fig. 6a) in the expected direction of the fatty acid patterns in the experimental diet. For example, the mackerel/capelin diet was somewhat lower in levels of 14:0, 16:0, and 22:1n-11 and higher in 18:1n-9, 20:5n-3, and 22:6n-3

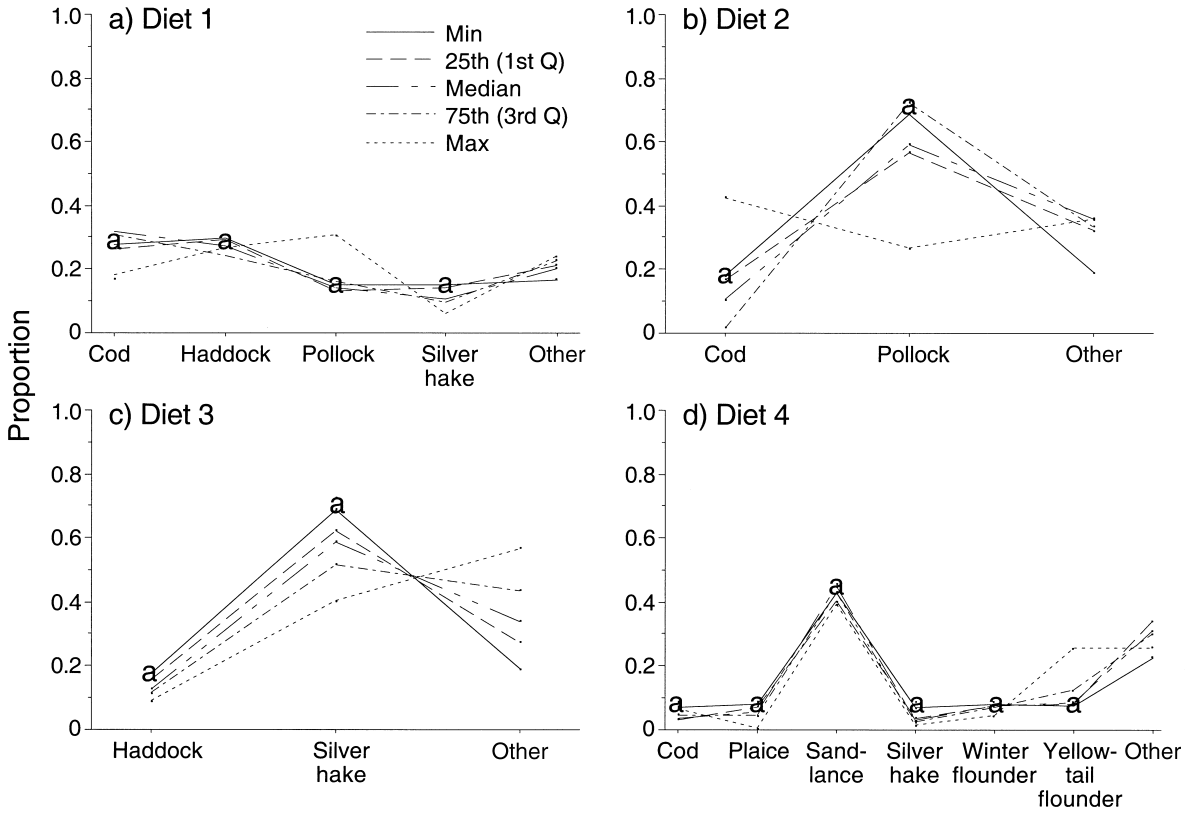


FIG. 5. Line plots of the simulation results for Diets 1–4 (a–d, respectively) yielding the best fits (minimum relative mean squared error, RMSE), the median fits (median RMSE) and the worst fits (maximum RMSE), as well as the runs at the 25th (first quartile) and 75th (third quartile) percentiles of the RMSE. Each plot represents the RMSE for mean diet for the 2% of the runs around the particular quartile. The quartiles of the RMSE are computed for the 1000 simulation runs using no calibration, 30 prey, 10% error, and the KL distance measure.

compared to the pre-experimental herring diet (Kirsch 1997). This corresponded to relative decreases and increases, respectively, in these components in blubber over the 20 days (Fig. 6a).

Using these data, we estimated the possible contributions of the experimental diet to the overall blubber fatty acid signature for comparison with model estimates. The average seal started this experiment with ~18.3 kg blubber and consumed a total of 5–6 kg of new dietary fat in 20 days. Turnover of blubber fatty acids occurs even in a nonfattening animal (Kirsch et al. 2000). However, the actual turnover in our study animals was unknown. Thus, we used several scenarios to bracket the probable response of seal blubber fatty acids to the experimental diet. In one scenario, we assumed that all the new fatty acids consumed were deposited with existing fat and then used by the animal as a single pool. In this case, ~24% of the experimental diet signature (~21% mackerel and ~3% capelin) would have been represented in the seal's blubber signature, with ~76% of the pre-experimental herring signature remaining. If we assumed that some fraction of the fatty acids consumed were immediately oxidized and not deposited, this generates correspondingly lower estimated contributions of the experimental diet. A simpler scenario assumed that seals consumed similar daily rations before and during the feeding trial, and thus that the experimental diet represents a proportion of days fed. Assuming blubber represented an integration of diet over the previous 3–5 months (i.e., 91–152 days), we predicted the experimental diet would constitute 13–22% of total diet at day 20.

We modeled the grey seals using the two fatty acid subsets and six options of calibration (no calibration, grey, harp, and pup coefficients alone, the grey/harp average, and the grey/harp/pup average). At each blubber sampling (0, 12, and 20 days), the estimated contributions of each prey to the fatty acid signature of seals were significantly affected by the set of calibration coefficients used ($P < 0.001$), but not consistently by fatty acid subset ($P > 0.135$), as there was a significant interaction of the two effects ($P < 0.001$, two-way repeated measures ANOVA on arcsin-transformed data). Grey or grey/harp average coefficients tended to give similar results, as did those of harp or grey/harp/pup average, but all other sets differed significantly from one another ($P < 0.05$, Tukey-Kramer multiple comparisons). Nevertheless, the experimental diet was

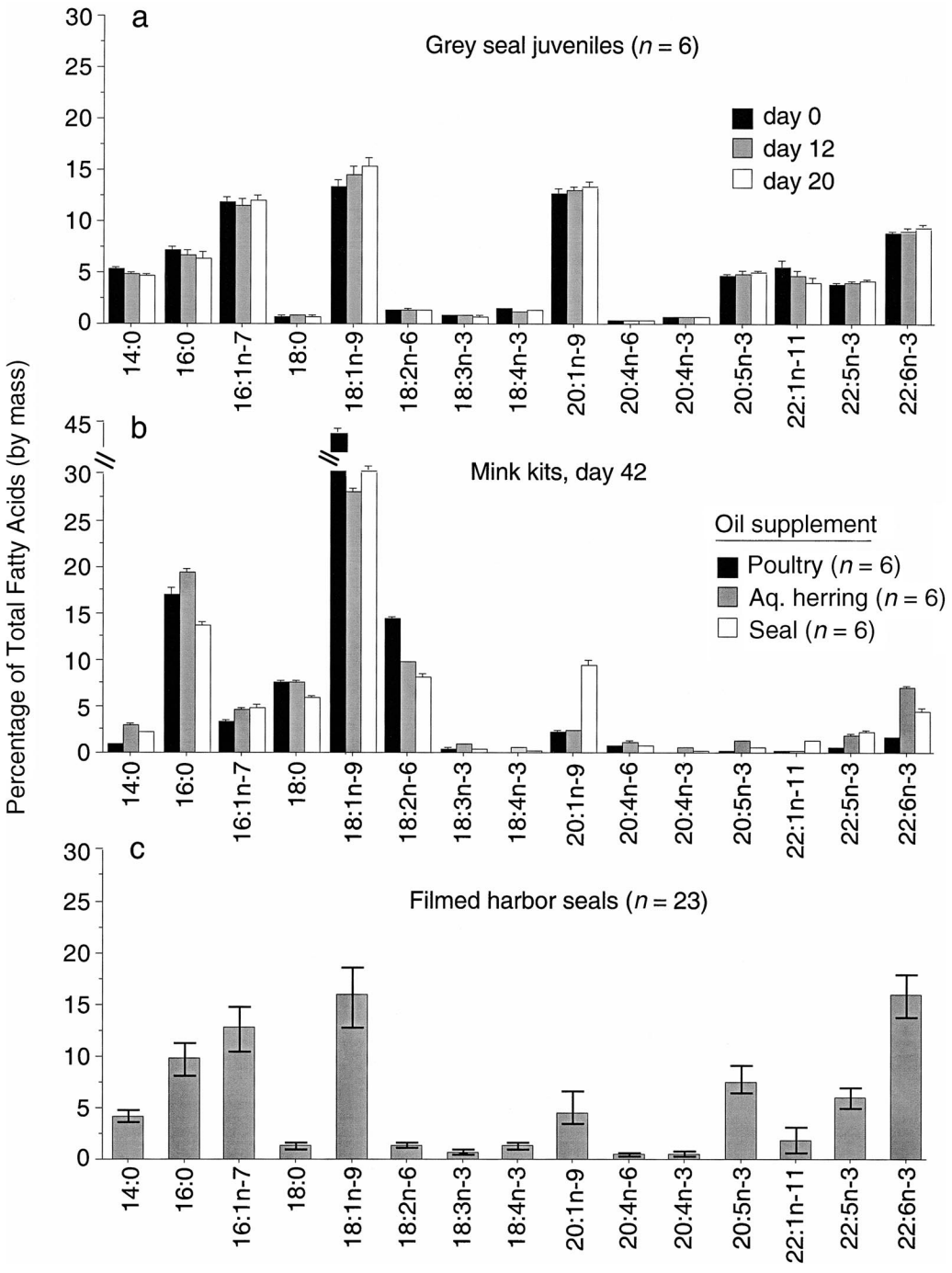


FIG. 6. Selected abundant fatty acids (15 of the 67 quantified) in blubber or adipose stores of the three case-study species: (a) captive juvenile grey seals previously fed herring and switched to a diet of mackerel and capelin for 20 days; (b) 42-day-old mink kits that had been raised until 21 days postpartum (dpp) on their mothers' milk and then switched to one of three diets supplemented with either poultry fat, aquaculture herring oil, or seal oil as the primary dietary fat sources; and (c) free-ranging adult male harbor seals filmed during natural feeding events. Bars are means, and vertical lines show $\pm 1$ SE except for harbor seals (c), where vertical lines show minimum and maximum values measured among individuals.

always better predicted using any of the sets of calibration coefficients than when no calibration was used.

Overall, the diet was best predicted using either the grey or the grey/harp average coefficients and the extended-dietary fatty acid subset. Using extended-dietary fatty acids and the grey seal calibration coefficients, the seals' fatty acid signatures were estimated to be composed of >95% herring at the start of the

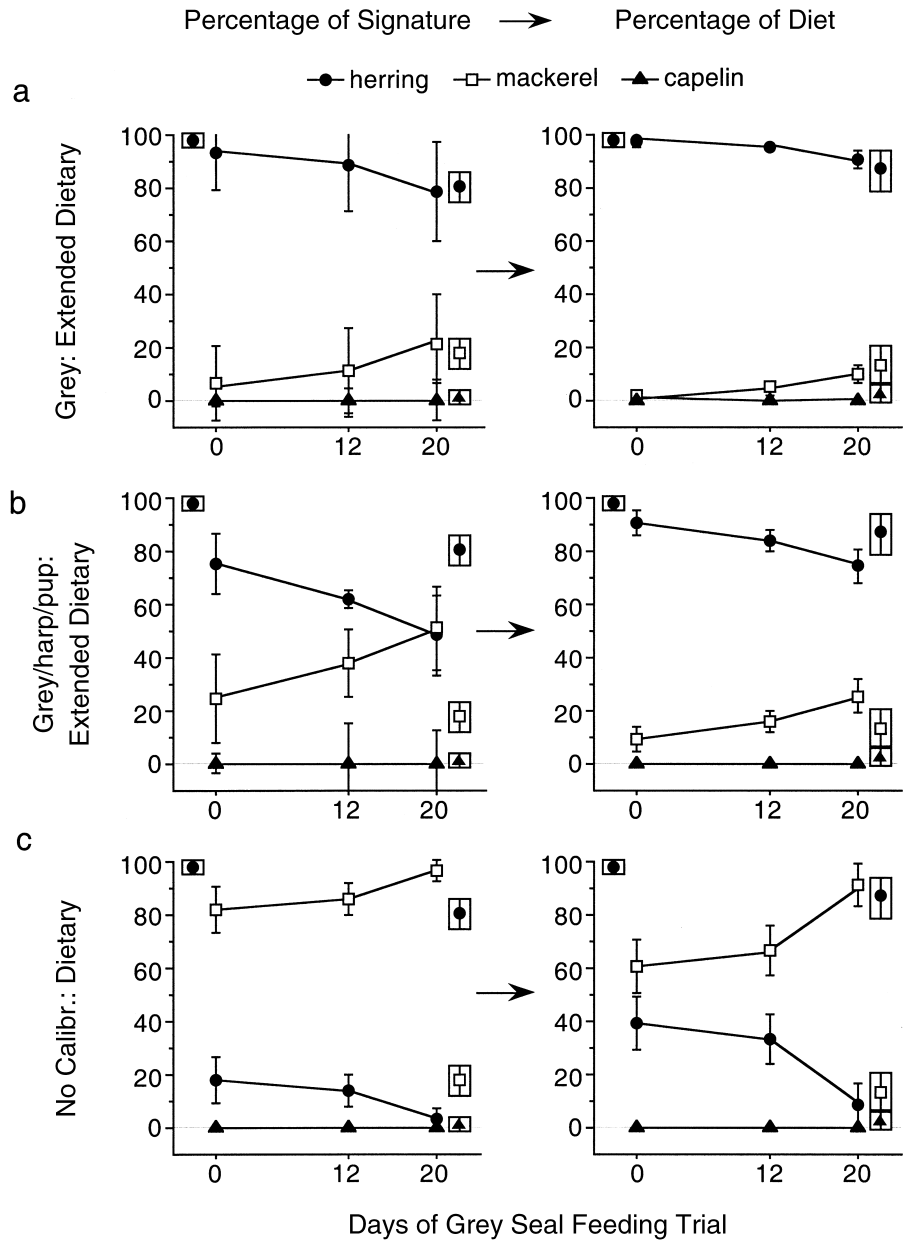


FIG. 7. Model estimates of the contribution of prey species to blubber fatty acid signatures (left) and to diets (right; i.e., after taking into account relative fat contents of prey) of captive grey seals previously fed herring and switched to a diet of mackerel and capelin for 20 days. Results are presented as the mean ± the average within-seal standard error (from bootstrapping) for percentage of signature estimates (left) and as the mean ± the average among-seal standard error for diet estimates. Results from three different model inputs are presented: (a) grey seal calibration coefficients (Fig. 3) and the extended-dietary fatty acid subset, (b) the average of the grey/harp/pup calibration coefficients and the extended-dietary fatty acid subset, and (c) no calibration coefficients and the dietary fatty acid subset. Vertical rectangles (with symbols enclosed) in each graph bracket the expected values for each prey item at the start and end of the experiment assuming deposition of 100% or 50% of dietary fatty acids at a ratio of mackerel to capelin fat of 6.9:1, or assuming blubber represents an integration of diet over 3–5 months (see text); the range of all three scenarios is included in vertical rectangles.

experiment (Fig. 7a). By day 20, herring had declined to 79%, with mackerel accounting for 21% of the signature. Although capelin was detected among the 1000 bootstrap estimates, especially by day 20, the average estimate of capelin in signatures was 0% at these times. After taking into account the relative fat contents of the prey fed, these signature values corresponded to average diet estimates of 98% herring and 2% mackerel initially, and 91% herring and 9% mackerel at 20 d. Using the grey/harp average coefficients, signatures

also were estimated to have changed gradually over the experiment to 69% herring and 31% mackerel at 20 d. In this case, diets were estimated to be composed of 96% herring and 4% mackerel initially and of 87% herring and 13% mackerel at 20 d.

Estimates of diet using the same extended-dietary fatty acids and the average of grey/harp/pup coefficients (Fig. 7b) differed significantly from the grey or grey/harp results, although overall patterns were similar. Herring and mackerel accounted for 90% and 10%, respectively, of the estimated diet at day 0 and 74% and 26%, respectively, at day 20. Capelin was again not detected as a significant component of the diet. Overall, estimates using grey, grey/harp, or grey/harp/pup coefficients corresponded well with the range of expected responses (e.g., Fig. 7a,b). While capelin did not appear in average estimates, we did not know the amount of capelin actually consumed by the seals; the maximum that could have been represented in signatures by day 20 even if seals had consumed all capelin offered was only 3–6%.

In contrast to any model using calibration, when the seals were modeled using no calibration coefficients and either fatty acid subset, estimates of the percent contribution to signatures or to diets did not correspond to either known or expected diet contributions at any time (Fig. 7c).

*Captive mink kits.*—Until 21 dpp, all mink kits had consumed only mothers' milk, while their mothers consumed a mixture of ''lactating pellets'' and ''wet diet.'' Milk fatty acids in carnivores, including mink, are largely derived from direct dietary intake (Wamberg et al. 1992, Iverson and Oftedal 1995). Since we were unable to sample milk for input into our model estimates, we assumed that the adipose tissue fatty acid composition of mink kits would resemble that of their mothers' diet (lactating pellets/wet diet) through ''indirect'' consumption. From 21 to 28 dpp, kits directly consumed one of three different oil-supplemented diets, in addition to milk from their mothers fed on these same oil-supplemented diets. By 42 dpp kits consumed primarily the oil-supplemented diets alone (Layton 1998). As expected, the fatty acid composition of adipose tissue of mink kits changed significantly over time ($P < 0.001$, MANOVA). The fatty acid composition also differed significantly among the kits fed the three different diets at both 28 and 42 dpp ($P < 0.001$, MANOVA, e.g., Fig. 6b).

Mink kits in this study contained an average of 7 g body fat at 21 dpp; by 42 dpp, after being switched to the oil-supplemented diets, kits had increased to an average of 27 g body fat (Layton et al. 2000). If fat deposited from new intake was roughly additive, the new oil-supplemented diet could comprise a maximum of 74% (i.e., (27 − 7)/27) of the overall dietary signature at 42 dpp, without accounting for milk also consumed or poultry and fish meal still in diets. We used this as an expected value to compare our results from modeling diets. Given that all oil-supplemented diets contained the same fat content and assuming all kits consumed similar quantities of milk and direct feeds, estimated signatures can be taken as the diet in this case.

Mink kits were modeled using the two fatty acid subsets and the six calibration sets described for grey seals. The estimated contributions of each diet type to the overall fatty acid signatures of mink were again significantly affected by the calibration coefficient set used, but also by the fatty acid subset, as well as an interaction of the two effects ($P < 0.001$, two-way repeated measures ANOVA on arcsine-transformed data). However, unlike grey seals, the major diet types were generally well estimated, as judged against our maximum estimated values, for all calibration sets and the two fatty acid subsets (e.g., Fig. 8). The largest errors occurred in differentiating the lactating pellets–wet diet from the poultry oil-supplemented diet. This was expected, as the lactating pellets and wet diets both were composed primarily of poultry offal and therefore had a similar signature to the poultry oil-supplemented diet. Signatures of kits were least accurately predicted at 21 dpp, both because of this similarity and because kits had only consumed the lactating pellets/wet diet ''indirectly'' though their mothers' milk, which was likely not identical to the diet. Since some fish meal was also contained in all diets, both before and after 21 dpp, minor amounts of seal oil and herring oil-supplemented diets (i.e., similar to a fish meal signature) appeared in modeled diets as expected.

Using the extended-dietary fatty acid subset and the average of the grey/harp/pup calibration coefficients (Fig. 8a), signatures of kits at 21 dpp were estimated to be composed of ~31% of the mix of lactating pellets/wet diet and 57% of the poultry oil-supplemented diet, or a total of ~88% of poultry-based diet. By 28–42 dpp, the poultry oil-supplemented diet accounted for 87–90% of the estimated signatures. Similarly, by 28–42 dpp, diet signatures of kits fed the herring oil and seal oil-supplemented diets, were estimated to be composed of 54–78% and 82–88% of each of these diets, respectively (Fig. 8a). In contrast to the grey seals, when the signatures of mink kits were modeled using no calibration (Fig. 8b), estimates of the percent contribution of the various formulated diets to the overall fatty acid signatures remained relatively consistent with expectation, and the indirect diets of kits at 21 dpp were actually better predicted. In each case, only the fed experimental diet appeared in the kit signatures at 28 and 42 dpp (Fig. 8b).

*Free-ranging harbor seals filmed while foraging.*—The fatty acid signatures of the 23 free-ranging adult male harbor seals were similar among individuals (Fig. 6c), suggesting similar diets, but also exhibited some variability. The diets of these individuals were modeled using the entire Scotian Shelf prey database of 954 individuals representing 28 species (e.g., Fig. 2). As in the captive grey seal feeding experiment, the estimated

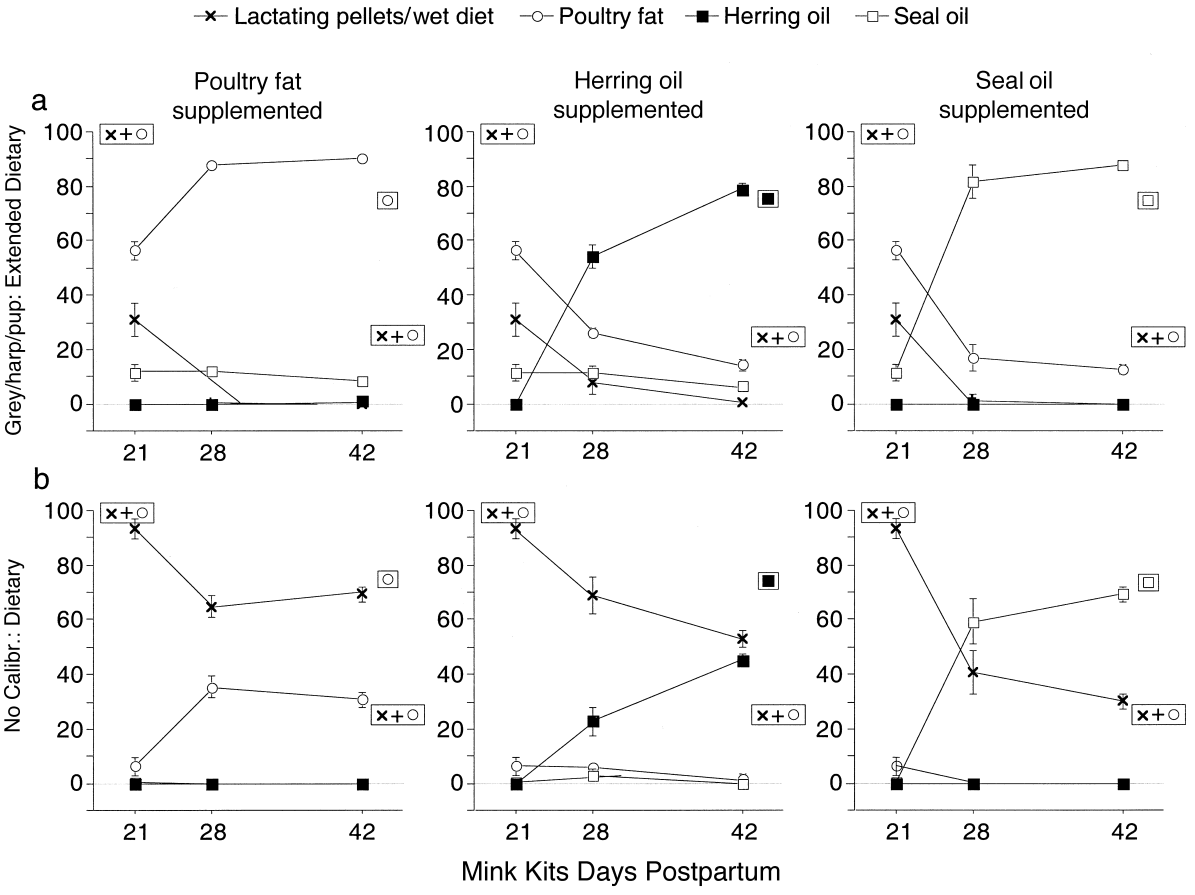


FIG. 8. Model estimates (mean ± 1 SE) of the relative contribution of experimental diets to the fatty acid signatures of adipose tissue in mink kits. Mink kits were raised until 21 days postpartum (dpp) on milk from their mothers (fed in turn on a mixture of ''lactating pellets'' and ''wet'' diet), and thereafter both mothers and kits were switched to one of three different oil-supplemented diets. Results are presented for the three different experimental diet groups (left, poultry fat; center, aquaculture herring oil; right, seal oil) and using two different model inputs: (a) the average of the grey/harp/pup calibration coefficients (Fig. 3) and the extended-dietary fatty acid subset, and (b) no calibration coefficients and the dietary fatty acid subset. Symbols within boxes at 21 and 42 dpp represent the maximum estimated contribution that oil-supplemented diets could be represented in signatures. Because lactating pellets and the wet diet were composed primarily of poultry offal, these are listed together with poultry oil-supplemented symbols due to similar signatures and thus overlap in model estimates. At 21 dpp, the results for the same 10 kits are presented in each graph for comparison with the latter treatment groups. At 28 and 42 dpp, data points represent results from six different mink kits in each graph (i.e., an additional 36 individuals). Note that bootstrapping of estimates was not possible as the diets were completely homogeneous.

proportional contributions of each prey type to the overall fatty acid signature of seals were significantly affected by the calibration coefficient set used, but not by fatty acid subset, and again there was an interaction of the two effects ($P < 0.001$, two-way repeated measures ANOVA on arcsine-transformed data).

Using the extended-dietary fatty acid subset and the grey seal calibration coefficients, all individuals were estimated to have consumed primarily sandlance (Fig. 9). Sandlance accounted for 37–90% of individuals' diets, averaging 62% of diets overall. This was followed by an average of 12% flounders (primarily yellowtail flounder) and 10% capelin. However, there was clearly variability among individuals; other prey items estimated for some individuals included varying amounts of cod, halibut, herring, skate, crab, and shrimp. Using the average of grey/harp seal calibration, sandlance was similarly estimated to comprise 63% of diets, followed by flounders, capelin, skate, halibut, and cod. Using the average of grey/harp/pup calibration resulted in the same predominant species, but lower estimates of sandlance and higher estimates of flounders and skate.

These results were consistent with qualitative expectations from filming these same individuals while foraging (Fig. 9, inset). Video recordings from 30 seals (including the 23 above), filmed intermittently over an average of three days each, showed all but one male foraging on sandlance. Of 223 10-minute video-sampling units filming identifiable prey captured, 91% were on sandlance, 7% on flatfish, and the remainder on gadoids and other prey (Bowen et al. 2002). In contrast

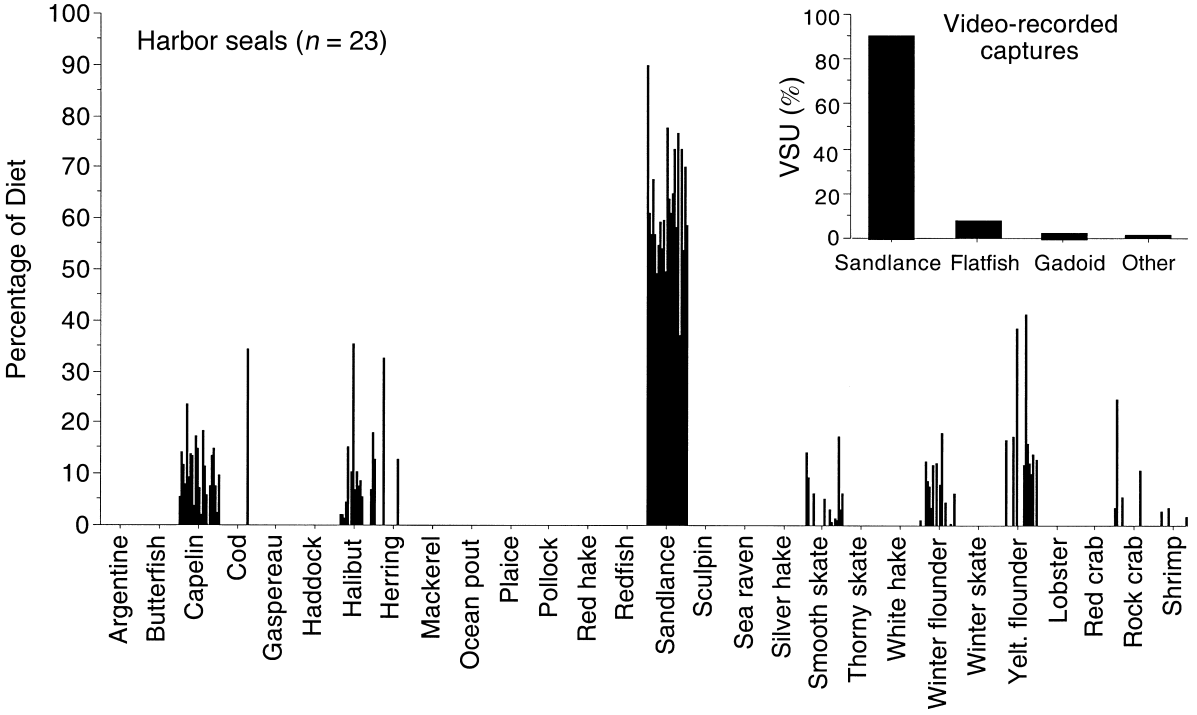


FIG. 9. Individual model estimates of the contribution of prey species to diets of 23 free-ranging adult male harbor seals deployed with an animal-borne video system (''Crittercam'') and filmed during feeding events. Seal signatures were modeled using the entire Scotian Shelf data base of 954 prey representing 28 species (e.g., Fig. 2), and proportions were converted to diet estimates after taking into account relative fat contents of each species. Results are presented using the extended-dietary fatty acid subset and the grey calibration coefficients (see Fig. 3). Inset: prey types consumed in video recordings of these seals, expressed as the percentage of all 10-minute video-sampling units (VSU) that filmed prey captures and that contained identifiable items ($n$ = 223, from Bowen et al. 2002).

to these results, completely different diets were estimated when no calibration coefficients were used: using either fatty acid set, sculpin dominated the diet, followed predominantly by gaspereau and skate, with $<1\%$ flounder and no sandlance.

## DISCUSSION

The use of quantitative fatty acid signature analysis (QFASA) to study predator diets relies upon the diversity of fatty acids and characteristic patterns among prey species, coupled with the narrow limitations on their biosynthesis in animals and the prevalence of storage depots of lipid in many predators. Our results demonstrate that QFASA is an effective tool for estimating pinniped and mink diets, and suggest that this approach could be widely applied to other predators. QFASA will enable us to study the foraging behavior of individuals and the structure of food webs in greater detail than has previously been possible in many ecosystems. We present here the first generation of this method, along with the underlying requirements that are essential to its use.

Fatty acids have previously been used to examine qualitative aspects of food webs. However, this is the first attempt to use fatty acids to provide quantitative estimates of predator diets. The problem is to match weighted patterns of possibly hundreds of individual prey samples with those of the predator, using up to 67 fatty acids in each sample of predator and prey (e.g., Fig. 1). Given this complexity, it is generally not possible to interpret fatty acid patterns in predators by visual inspection, especially when the number of potential prey choices is large, when significant within-species variability exists, and when aspects of lipid metabolism of the predator must be taken into account. Our approach has been to develop a mixture model of prey species signatures that most closely resembles that of the predator and thereby estimate its diet. The use of QFASA to accurately estimate predator diets has four fundamental requirements: (1) a quantitative model and an appropriate measure of its performance; (2) appropriate sampling, analysis, and evaluation of potential prey species; (3) appropriate sampling and analysis of predator tissue; and (4) an understanding of, and accounting for, lipid metabolism and deposition in the predator.

### *Statistical model parameters*

There are a number of ways to determine how close the predicted fatty acid signature is to the observed predator signature. We have used the KL distance, as it gives more weight to relatively larger errors from

the true value, does so more conservatively and proportionately than the other measures, and because it generally performed better than the other three distance measures in our simulations.

Our model currently uses the mean of each prey species to estimate its contribution to the predator's signature. However, free-ranging animals do not consume homogenous species, but rather individuals of various prey species. Although it may be possible to accurately distinguish species within an ecosystem by their fatty acid signatures, there can be considerable variability within species (Budge et al. 2002, Iverson et al. 2002). In some cases, this variability may correspond to predictable changes with age or size of the prey (e.g., Iverson et al. 1997*b*, 2002), such that species subgroups could be incorporated into the model to provide additional detail about the diet. However, even in these cases, we must find a way to incorporate the variability in prey into model estimates of diet. We have used a bootstrapping procedure to compute standard errors of individual estimates. At present, this seems the most appropriate way in which to calculate confidence limits on estimates of prey composition in the diet. Another approach would be to use each individual prey in the database (e.g., $n = 954$ for the harbor seal example). However, this would lead to computational problems and statistical issues, since we would be modeling on more prey than fatty acids.

Another source of variability arises from within-species differences in fat content. Prey species with higher fat contents will contribute proportionately more per unit intake to a predator's signature than species with a lower fat content; hence this must be factored into estimation of diet from signatures (e.g., see Fig. 7). For illustration, we have used prey species averages in these diet estimates. However, it would be straightforward to incorporate within-species variability in fat content into the standard errors of estimated diets using a similar bootstrapping procedure.

### *The prey database and simulation studies*

A prerequisite of QFASA is a database of potential prey species and an understanding of whether those species can be distinguished by their fatty acid signatures. Obviously, QFASA cannot detect a prey species in the diet of a predator if that species is not represented in the database. Rather, the model will produce an estimated diet that best matches that of the predator, given the available prey. The ''best'' fit will be found even if this fit is poor and key prey are missing. Thus, the onus is on the investigator to sample the appropriate species and to understand within-species variability. Nevertheless, sampling every species in the ecosystem is neither possible nor warranted. QFASA will not likely detect the occasional consumption of a prey species. Thus, species that are probably rare in the diet, either because they occur at low numbers or are not available to the predator (e.g., outside its foraging range or depth), need not be included in the fatty acid database. Although such decisions will ultimately depend on the complexity of the ecosystem and the question being asked, in general the potential loss of rare species in the estimated diet may be more than compensated by the ability to determine those prey that the predator depends upon for survival.

QFASA also requires that the potential prey can be reliably distinguished on the basis of their fatty acid patterns. Multivariate techniques such as discriminant analysis and classification trees are useful for this purpose (e.g., Smith et al. 1997). For instance, in two different ecosystems (the Scotian Shelf, the Gulf of Alaska), multivariate analyses revealed that >26 species could be distinguished by their fatty acid signatures with >95% accuracy. Nevertheless, some species with similar ecology and diets, such as certain flatfishes, can be somewhat difficult to distinguish from one another (Budge et al. 2002, Iverson et al. 2002). Although other multivariate methods, including hierarchical cluster analysis (Fig. 2), provide insight into overall relationships among species fatty acid signatures, model simulations provide a more powerful means for assessing which prey may be too similar to be reliably distinguished in the estimation model (e.g., Figs. 4 and 5). We also have found that sequentially removing prey species that arise in diet estimates and then rerunning the model can be quite informative. The newly estimated diet can then be used to determine which species are substituted for the missing species and therefore allow a deeper understanding of model diet estimates.

### *Biological issues: calibration coefficients, fatty acid subsets, and predator sampling*

Dietary fatty acids are directly incorporated into the lipid stores of predators across all trophic levels (see *Introduction*). But while many of the fatty acids in a predator's tissue provide information about diet, some fatty acids provide information less directly than others, as a consequence of their deposition characteristics and their ability to be biosynthesized. Thus, the fatty acid composition of a predator's lipid stores will never exactly match that of its prey. Our conception and use of calibration coefficients and fatty acid subsets recognizes this and assumes that, if physiological and biochemical processes are shared among animals, similar animals consuming similar diets should share similar characteristics of fatty acid deposition and biosynthesis. Understanding these characteristics, and which predator tissues to sample, are critical in using QFASA.

Several factors affect the deposition and biosynthesis of fatty acids. Fatty acid synthesis in animals is greatly reduced or absent, and dietary fatty acids tend to be stored directly in adipose tissue, when animals consume a high fat diet in excess of energy requirements (Nelson 1992). For instance, in seals consuming a diet in which fat comprised >95% of calories, blubber fatty

acid composition was not significantly different from that of the diet (Iverson et al. 1995). However, most animals do not eat a diet of almost pure fat, but instead consume a complex mixture of fat, protein, and carbohydrate. Although preformed dietary fatty acids are less likely to enter typical lipid synthetic pathways (Nelson 1992), carbohydrates or amino acids (protein) consumed in excess of requirements are used to synthesize fatty acids in the liver or adipose tissue. Thus, in carnivores, excess dietary amino acids are used to synthesize certain fatty acids, which will augment those directly deposited from diet, hence influencing tissue fatty acid signatures. These synthesized fatty acids are usually restricted to those with 16 or 18 carbon atoms and usually, at most, one double bond in specific positions (i.e., 16:0, 16:1n-7, 18:0, and 18:1n-9) (Volpe and Vagelos 1973, Wakil et al. 1983, Cook 1991, Nelson 1992). These fatty acids are also common in prey, and thus the proportions found in predators may reflect both differences among prey (e.g., Fig. 1) and biosynthesis. Therefore, proportions of some of these fatty acids found in predators will always be absolutely higher than those found in the prey (e.g., Kirsch et al. 2000). Other fatty acids may have reduced deposition in the predator (e.g., Bremer and Norum 1982, Lin and Connor 1990, Jandacek et al. 1991), but will still be reflective of differences among prey. In this case, the proportion in the predator will always be absolutely lower than that in prey (e.g., isomers of 22:1, Fig. 3).

The calibration coefficients we have developed to account for predator lipid metabolism are clearly an important component of estimating the diets of predators using QFASA (Figs. 7–9), but current estimates of these coefficients are by no means definitive. Each of our calibration experiments had limitations concerning our ability to sample the experimental diet, the type of diet that was fed, or the duration of the experiment. For example, we are not necessarily convinced that five months was long enough to eliminate the influence of the pre-experimental diet on the fatty acid pattern in blubber of grey and harp seals. In addition, the diet fed to seals was not homogeneous, and we could only sample a subset of individual herring not actually fed to seals, with the assumption that these were representative of the entire lot fed over the five months. In contrast, the pup calibration coefficients are based on a well-sampled homogeneous diet (i.e., milk) from birth. However, the potential problem of applying these coefficients more generally is that the diet was exceedingly high fat (i.e., 60% fat milk), which likely suppressed fatty acid biosynthesis fully. This, in addition to the high digestibility of milk in general, may explain why a larger number of the pup calibration coefficients were close to 1.0 compared to those measured in the seals fed fish.

Despite these limitations, we are confident that these sets of calibration coefficients are a good starting point in accounting for the effects of predator metabolism on fatty acid deposition, given the results of applying them in our model. Furthermore, the three sets of coefficients reveal some similarities among animals in patterns of fatty acid deposition and biosynthesis and, in general, are comparable to those recently calculated from feeding studies of other captive phocid and otarrid pinnipeds and seabirds (Iverson and Springer 2002; S. J. Iverson, *unpublished data*). Nevertheless, it will be important for the investigator to determine the most applicable set of coefficients for a given predator. For instance, most pup coefficients were closer to 1.0 than were those of grey and harp seals, and were generally more similar to those obtained from seabird adipose tissue. It may be that calibration coefficients in the more structural blubber of pinnipeds are characterized by generally greater deviations from 1.0 than are those in newly suckling pups or in the less structured adipose tissue of seabirds, and those of other mammals. Characteristics of calibration would also require investigation before they can be applied to modeling very different types of predators such as ectothermic fish.

In addition to calibration, there is also the possibility of further refining the subset of fatty acids used in the model (Appendix A). We have currently evaluated two subsets. Both in simulations and in modeling the diets of experimental animals, the model generally performed better with the extended-dietary subset. We expect that the extended fatty acid subset performed better simply because we are using more information (fatty acids) in the estimation of diet. Nevertheless, we believe there is room for fine-tuning the fatty acid subset(s) used to model diets. The most appropriate fatty acid subset may also vary with the type of predator (e.g., mammal vs. bird) and the ecosystem (i.e., temperate or tropical marine, freshwater, terrestrial) under study. We see this as an important area for further research.

A final issue in the use of QFASA, and somewhat related to the issue of calibration coefficients, is appropriate sampling of predator tissue and an understanding of the basic properties of the tissue sampled. Although fatty acids are stored in a number of tissues, the primary site of fat storage in most vertebrates is adipose tissue (Pond 1998). Adipose tissue is composed of numerous specialized cells called adipocytes, which are capable of storing massive amounts of triacylglycerols and thus, fatty acids. Adipose tissue is also extremely dynamic, as adipocytes alternately store or mobilize triacylglycerols largely depending on energy balance. Hence, adipose tissue will be most directly influenced by dietary fat intake and is the tissue that should generally be sampled for QFASA. However, not all adipose tissue behaves in the same way, and only those sites that represent the most metabolically active fat energy reservoir should be sampled. For example, in mammals, very small adipose depots are scattered throughout the body, many of which may have specialized functions (e.g., Pond 2000). In contrast, the fewer large adipose depots (e.g., visceral or subcutaneous fat, blubber) are likely to serve mainly

to store lipid and should be targeted for QFASA. Among three such large sites sampled in individual seabirds and polar bears (*Ursus maritimus*), the fatty acid composition did not differ significantly, indicating that any of these sites could be used (Iverson and Springer 2002; G. Thiemann, S. J. Iverson, and I. Stirling, *unpublished data*). Such verification may be important in other predators.

The blubber of marine mammals represents a specialized form of adipose tissue, whose function and fatty acid composition may differ over the body and by tissue depth (Iverson 2002). In particular, blubber taken near the skin is more structural in nature, and thus is less rapidly influenced by changes in diet. In pinnipeds, although sampling the full depth of the blubber layer provides accurate estimates of overall diet (e.g., this study), splitting blubber into inner and outer halves can reveal temporal change in diet, as the inner and outer halves provide estimates of more recent and less recent diet, respectively, even with use of currently available ''full-depth'' calibration coefficients (Cooper et al. 2001). However, in cetaceans, because their blubber is much more structured and stratified (e.g., Koopman et al. 1996, Iverson 2002), blubber samples taken only from the metabolically active inner layer (i.e., near the body core) are appropriate, as this is where dietary fatty acids are primarily deposited and mobilized (Koopman et al. 2002).

Finally, it is important to recognize that many animals undergo extended periods of fasting and depletion of fat stores, followed by replenishment. Some studies have indicated selective mobilization of fatty acids from adipose tissue during induced fasting (e.g., in rats, Raclot and Groscolas 1995), but studies of weaned grey seals showed no overall change in blubber fatty acid signatures after three weeks of natural fasting (S. J. Iverson and L. Rea, *unpublished data*). Although any such issues may be in part accounted for by calibration coefficients, the precise effects of modeling diets in species after extended fasting requires further research.

### *Experimental studies*

Our grey seal and mink experimental feeding studies, along with the filmed free-ranging harbor seals modeled with a complex ecosystem-wide prey database, provided validations of the QFASA model. In captive grey seals fed for some time on herring and then switched to a short-term diet of mackerel and capelin, diets were generally well predicted as long as appropriate calibration coefficients were used (Fig. 7). However, capelin were not eaten readily and seals did not consume all that was offered. This may account for the fact that the proportions of mackerel and herring predicted in QFASA diets were consistent with expectations, but capelin was not. Although we estimated that ~3% of capelin should have appeared in signatures (assuming seals ate half the capelin offered), it is also possible that not enough capelin was eaten to be reliably estimated by the model. As indicated previously, QFASA may not be able to detect trace levels of a prey in the diet. Further research is needed to determine the detection limit of QFASA and how this might vary among prey.

The diets of mink kits were remarkably well estimated, especially at the time when we could quantify direct consumption of oil-supplemented diets by kits. By 42 dpp, when kits had fed directly on diets for two weeks, the experimental diets were all well predicted by the model (Fig. 8). All oil-supplemented diets were estimated to comprise ~80% of diets, consistent with our calculations from total body fat deposition (~74%) during that period. Given that the initial lactating pellets and wet diet comprised primarily poultry offal, it is not surprising that this diet overlapped with estimates of the poultry oil-supplemented diet. Likewise, since all oil-supplemented diets contained some fish meal, small amounts of both seal- and fish-oil diets also appeared in signature estimates. In contrast to the seals, the estimates of mink diets without using calibration coefficients were still reasonable (Fig. 8b). This is likely due to the fact that true calibration coefficients for mink adipose tissue are generally closer to 1.0, as is the case for seabird adipose tissue (Iverson and Springer 2002) and suckling seal pups (Fig. 3).

The video-recorded harbor seals provided an opportunity to assess how QFASA performs in a free-ranging predator foraging in an ecosystem with many potential prey species. We also had the advantage that harbor seals in this population have been extensively studied, allowing us to carefully evaluate results of the model estimates. The video records from these males showed that their major prey was sandlance, and this has also been shown for animals in this population from both gastric lavage data (Bowen et al. 2001) and fecal analyses (W. D. Bowen, *unpublished data*). Sandlance is abundant near Sable Island and is the major prey of grey seals foraging in that area as well (Bowen and Harrison 1994). The fact that our model also estimated sandlance to be the major prey component of harbor seal diets (Fig. 9) provided a unique type of validation of QFASA. Other diet items estimated by QFASA included yellowtail flounder and other flatfish, capelin, gadoids, and crustaceans, all of which are known to be consumed by these harbor seals (Bowen et al. 2001, 2002; W. D. Bowen, *unpublished data*). The QFASA results also revealed considerable variation in diet among individuals. Although this variability was implied from earlier fecal or stomach contents analysis, these methods had previously provided only a snapshot of the last meal, limiting the ecological interpretation of individual variability.

### *Comparison with other methods*

Both direct and indirect methods are used to determine the diets of predators. Each of these methods has advantages and disadvantages, and some methods are

more applicable to some species. The diets of terrestrial and marine carnivores and seabirds are most often estimated from the identification of prey structures that are resistant to digestion. The obvious disadvantage of these methods is that not all prey may have such structures (or they are not consumed by the predator), and there may be differential digestion of structures among prey species, both leading to biased estimates. In addition, only the last meal is represented. Nevertheless, these methods have contributed greatly to our understanding of diets of many taxa. In many cases, results from these methods also provide an opportunity for both qualitative validation of, as well as useful comparison with, results from QFASA. However, QFASA offers several advantages over these methods, one being that prey without hard parts, or with easily digested parts, can be detected. QFASA also provides quantitative estimates of proportions of prey in diets, which is a more meaningful measure than the frequency-of-occurrence measure commonly obtained from the recovery of hard parts. But perhaps one of the more striking advantages of QFASA is that it provides estimates of diets for individual animals and at time scales (i.e., integrated over longer periods) that are relevant to the ecological processes affecting survival. Because sampling is nonlethal, QFASA can be used to study diet variability within individuals over time, providing opportunities rarely possible with other indirect methods. While we believe QFASA offers a number of such advantages, it is important to remember that wise application of QFASA requires a rather considerable investment in prey sampling and a recognition that some prey species may have fatty acid signatures that are too similar to permit their separate identification in the diet.

### *Conclusions and future directions*

We have presented a statistical model that provides quantitative estimates of the proportions of prey species in the diets of individual predators using fatty acid signatures. We have shown that predator fatty acid signatures respond rapidly to changes in diet, and that these changes are well estimated using QFASA. Nevertheless, we need to better understand how predator fatty acid signatures respond to changes in diet over longer time scales. For some animals, such as many bears and marine mammals, which go through annual periods of extensive depletion of fat stores during fasting followed by intensive fattening prior to the next breeding season, we currently have some insight into the likely time frame over which the fatty acid signatures are integrating the diet. However, for many other animals this may be less obvious. We suggest that the current QFASA model can be applied to a number of predators and ecosystems. However, as with any new method, additional experimental studies are needed to better understand aspects of the turnover and deposition of fatty acids (in both the blubber of marine mammals and in the adipose tissue of other predators), in order to provide robust quantitative estimates of predator diets.

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## APPENDIX A

Fatty acids routinely quantified in the current study, their predominant source in predator adipose tissue, those currently used in the two modeling sets, and their calibration coefficients estimated from three experimental studies.

| Fatty Acid† | Average in Scotian Shelf ecosystem‡ (%) | Predominant source in predator§ | "Dietary" fatty acids | "Extended-dietary" fatty acids | Calibration coefficients‖ | | |
|---|---|---|---|---|---|---|---|
| | | | | | Grey | Harp | Pup |
| 12:0 | 0.10 | B | | | 0.97 | 0.86 | 0.92 |
| 13:0 | 0.03 | b | | | 1.00 | 1.00 | 1.00 |
| *iso*-14:0 | 0.03 | b/? | | | 1.00 | 1.00 | 1.00 |
| 14:0 | 3.92 | b | | X | 0.86 | 0.93 | 0.95 |
| 14:1n-9 | 0.15 | b | | | 0.70 | 1.06 | 0.75 |
| 14:1n-7 | 0.05 | b | | | 1.14 | 1.03 | 1.26 |
| 14:1n-5 | 0.46 | B | | | 10.92 | 8.83 | 1.54 |
| *iso*-15:0 | 0.20 | b/? | | | 1.12 | 1.11 | 0.91 |
| *anteiso*-15:0 | 0.10 | b/? | | | 1.30 | 0.95 | 0.84 |
| 15:0 | 0.39 | b | | | 1.09 | 0.97 | 0.97 |
| 15:1n-8 | 0.03 | b | | | 1.00 | 1.00 | 1.00 |
| 15:1n-6 | 0.04 | b | | | 1.24 | 1.00 | 1.20 |
| *iso*-16:0 | 0.19 | b/? | | | 1.16 | 0.82 | 0.96 |
| 16:0 | 11.56 | b | | X | 0.74 | 0.63 | 0.83 |
| 16:1n-11 | 0.55 | b | | | 2.51 | 2.24 | 0.98 |
| 16:1n-9 | 0.34 | b | | | 3.37 | 2.64 | 1.11 |
| 16:1n-7 | 9.44 | b | | X | 1.52 | 1.61 | 1.30 |
| 7methyl16:0 | 0.25 | b | | | 1.10 | 1.08 | 1.04 |
| 16:1n-5 | 0.22 | b | | | 1.12 | 1.05 | 1.01 |
| 16:2n-6 | 0.12 | D | X | X | 0.76 | 0.74 | 0.81 |
| *iso*-17:0 | 0.18 | b/? | | | 1.09 | 1.05 | 0.96 |
| 16:2n-4 | 0.46 | D | X | X | 1.50 | 0.95 | 0.89 |
| 16:3n-6 | 0.37 | D | X | X | 0.86 | 1.12 | 1.00 |
| 17:0 | 0.23 | b | | X | 1.40 | 0.91 | 0.78 |
| 16:3n-4 | 0.24 | D | X | X | 0.68 | 0.87 | 0.98 |
| 17:1¶ | 0.36 | b | | | 2.67 | 2.04 | 1.27 |
| 16:3n-1 | 0.08 | D | X | X | 0.85 | 0.57 | 1.14 |
| 16:4n-3 | 0.12 | D | X | X | | | 0.90 |
| 16:4n-1 | 0.46 | D | X | X | 0.59 | 0.77 | 0.97 |
| 18:0 | 2.17 | b | | X | 0.84 | 0.79 | 0.64 |
| 18:1n-13 | 0.10 | D | | | 0.95 | 0.74 | 0.89 |
| 18:1n-11 | 1.63 | B | | | 15.04 | 10.40 | 1.04 |
| 18:1n-9 | 12.32 | b | | X | 3.46 | 2.79 | 1.15 |
| 18:1n-7 | 3.69 | b | | X | 1.41 | 1.44 | 1.04 |
| 18:1n-5 | 0.46 | b | | | 1.04 | 1.00 | 0.99 |
| 18:2Δ5,11 | 0.07 | D | | | 1.04 | 1.00 | 0.87 |
| 18:2n-7 | 0.06 | D | | | 1.13 | 1.00 | 1.26 |
| 18:2n-6 | 1.17 | D | X | X | 2.02 | 1.57 | 1.04 |
| 18:2n-4 | 0.13 | D | X | X | 0.98 | 0.86 | 0.94 |
| 18:3n-6 | 0.10 | D | X | X | 1.08 | 0.94 | 0.78 |
| 18:3n-4 | 0.12 | D | X | X | 2.32 | 2.59 | 1.01 |
| 18:3n-3 | 0.57 | D | X | X | 2.27 | 1.48 | 1.07 |
| 18:3n-1 | 0.10 | D | X | X | 0.95 | 0.95 | 0.88 |
| 18:4n-3 | 1.15 | D | X | X | 0.96 | 0.99 | 0.96 |
| 18:4n-1 | 0.16 | D | X | X | 1.10 | 1.39 | 1.01 |
| 20:0 | 0.09 | b | | | 0.50 | 0.50 | 1.00 |
| 20:1n-11 | 1.10 | D | X | X | 3.42 | 2.83 | 0.97 |
| 20:1n-9 | 6.30 | D | X | X | 0.81 | 1.00 | 0.91 |
| 20:1n-7 | 0.70 | D | X | X | 0.71 | 1.05 | 0.82 |
| 20:2n-9 | 0.05 | b | | | 1.00 | 2.93 | 1.00 |
| 20:2n-6 | 0.27 | D | X | X | 1.65 | 1.39 | 1.02 |
| 20:3n-6 | 0.06 | D | X | X | 1.07 | 1.00 | 0.91 |
| 20:4n-6 | 1.15 | D | X | X | 0.82 | 1.04 | 0.92 |
| 20:3n-3 | 0.11 | D | X | X | 1.16 | 0.98 | 0.98 |
| 20:4n-3 | 0.48 | D | X | X | 2.11 | 1.50 | 1.00 |
| 20:5n-3 | 9.51 | D | X | X | 0.65 | 0.80 | 0.82 |
| 22:1n-11 | 4.41 | D | X | X | 0.20 | 0.34 | 0.47 |
| 22:1n-9 | 0.62 | D | X | X | 0.27 | 0.59 | 0.49 |
| 22:1n-7 | 0.16 | D | X | X | 0.18 | 0.26 | 0.90 |
| 22:2n-6 | 0.02 | D | X | X | 1.00 | 1.00 | 1.00 |
| 21:5n-3 | 0.36 | D | X | X | 1.37 | 1.45 | 1.02 |
| 22:4n-6 | 0.17 | D | X | X | 1.00 | 1.00 | 1.03 |
| 22:5n-6 | 0.29 | D | X | X | 1.04 | 0.76 | 0.96 |
| 22:4n-3 | 0.09 | D | X | X | 2.58 | 1.55 | 1.01 |
| 22:5n-3 | 3.53 | b | | X | 4.64 | 3.91 | 1.09 |
| 22:6n-3 | 15.52 | D | X | X | 1.11 | 0.93 | 1.00 |
| 24:1¶ | 0.50 | D | | | 0.13 | 0.15 | 0.32 |

**APPENDIX A.** Continued.

| Fatty Acid† | Average in Scotian Shelf ecosystem‡ (%) | Predominant source in predator§ | ''Dietary'' fatty acids | ''Extended-dietary'' fatty acids | Calibration coefficients‖ | | |
|---|---|---|---|---|---|---|---|
| | | | | | Grey | Harp | Pup |
| Total used in current modeling sets:# | | | 33 | 41 | | | |

† Fatty acids are listed in order of elution on a polar capillary column. Although not detected in samples in the current study, shorter chain fatty acids routinely identified in other samples in our laboratory include *iso*-4:0, 4:0, *iso*-5:0, 6:0, 8:0, *iso*-10:0, 10:0, and *iso*-12:0. However, any of these present in a predator arise solely from biosynthesis, since fatty acids of chain length $\leq$ 12:0 consumed in the diet are immediately oxidized (e.g., Jackson 1974). Thus, these could not be used in modeling. Although very long chain fatty acids (>24 C) do exist, their occurrence in blubber or adipose tissue is rare and at trace levels only; *trans*-fatty acids also measured in other samples in our laboratory are equally rare in the animals used in this study.

‡ Levels of individual fatty acids, averaged across all prey and seals from the Scotian Shelf (SS) database in the current study, to provide an idea of relative abundance or rarity of fatty acids in this marine ecosystem.

§ Predominant source in a monogastric predator: B = all or primarily from biosynthesis; D = all or primarily from direct dietary intake; b = relatively large contributions from both biosynthesis and diet; ? = not fully understood. For instance, *iso-* and *anteiso-* fatty acids are produced primarily by bacterial biosynthesis from branched-chain amino acids; thus in mammals they are produced largely de novo (e.g., from gut bacteria and possibly other sources; Ackman et al. 1975, Gurr and James 1980); varying degrees for this capacity have also been demonstrated in cetacean blubber and melon (e.g., Morii and Kaneda 1982, Koopman et al. 1996, 2003).

‖ Calibration coefficients determined from three studies (see *Methods: The model*) using fish-fed grey seals (''Grey'') and harp seals (''Harp'') and suckling grey seal pups (''Pup''). Values are the 10% trimmed mean across all individuals as used in modeling. Values are absent for a fatty acid if it was not detected in either predator or prey in a given study.

¶ This fatty acid category represents several isomers combined, as their detection occurred with varying degrees of reliable separation on some individual GC columns due to slight stationary phase shifts in production.

# Fatty acids that arise in the predator largely from biosynthesis, or those that were generally found at trace levels or were inconsistently detected, were not used in modeling, since minor errors in fatty acids with large calibration coefficients that are present in small amounts would have large effects on the consistent performance of the model.

## APPENDIX B

### Procedures Used in Model Simulations

*Simulation with no calibration coefficients*

We construct a pseudo-seal from our specified diets (Table 1) as follows:

1) Choose the diet composition vector ($\pi$), the amount of noise ($e$), and the number of prey to be sampled ($n^s$).

2) For each prey type, split the samples into two sets: a simulation set $x^S_{klj}$ and a modeling set $x^M_{klj}$ with sample sizes $n^S_k$ and $n^M_k$, respectively. (The splitting process is only carried out for species with $n_k > 5$).

3) From the $k$th prey type, sample with replacement $n^S \times \pi_k$ from the $n^S_k$. Call the selected sample $x^{S*}_{klj}$.

4) To simulate noise, sample with replacement, $n^S \times e$, prey from prey types which are not part of the diet composition vector, $\pi$. Call this sample, $e^{S*}_{lj}$.

5) Adding each sampled prey from the prey types in the diet and the simulated noise from step 4 forms a pseudo-seal. We then divide by the total number of prey sampled:

$$y^*_j = \frac{\sum_k \sum_l x^{S*}_{klj} + \sum_l e^{S*}_{lj}}{(1 + e)n^S}.$$

6) Next using the modeling data set from step 2 plus all other species samples in the Scotian Shelf database, compute the composite prey mean for each of the $k$ prey types by averaging the $n^M_k$ prey of each type. This is expressed in the following formula:

$$\bar{x}^M_{kj} = \frac{1}{n^M_k} \sum_{l=1}^{n^M_k} x^M_{klj}.$$

7) Perform the estimation procedure using the simulated seal $y^*$ and $\bar{x}^M_k$ to get an estimated diet $p^r_k$.

8) Repeat steps 1–7 1000 times.

*Simulation with calibration coefficients*

A) Choose a seal at random from the eight available seals in the grey seal calibration study and compute its calibration coefficient as described previously.

B) Perform Steps 1 through 4, as detailed above.

C) Modify Step 5 as follows:

$$y^*_j = c_j \frac{\sum_k \sum_l x^{S*}_{klj} + \sum_k e^{S*}_{lj}}{(1 + e)n^S},$$

with re-normalization performed as follows:

$$y^*_j = \frac{y^*_j}{\sum_j y^*_j}.$$

D) Since the pseudo-seal now has simulated metabolism effects, we take this into account in the fitted procedure described in Step 7: since we used one of the eight grey seals to form the pseudo-seal, the average of the other seven grey seals is used in the fitting process.

E) Both the pseudo-seal and the other seven seals are randomly chosen in each of the 1000 repeated simulations.