PACIFIC ISLANDS FISHERIES SCIENCE CENTER

Report on Validation and Calibration of Fatty Acid Signatures in Blubber as Indicators of Prey in Hawaiian Monk Seal Diet

A report submitted under Contract No. AB133F-03-SE-1195
September 2003

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December 2010

Administrative Report H-10-05

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September 2003

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Preface

Fatty acid signature analysis is widely use in the study of marine mammal diets and trophic ecology. Several recent peer-reviewed articles and reference books on the subject cite an unpublished study carried out by scientists at Dalhousie University and Hubbs-SeaWorld Research Institute under Contract No. AB133F-03-SE-1195 with the NOAA Pacific Islands Fisheries Science Center. A report of the study was prepared under the contract and submitted to PIFSC in September 2003. The report describes experiments in 2001 using captive Hawaiian monk seals to validate and calibrate fatty acid signatures for use in ascertaining the feeding habits of this endangered species.

To make the study more readily accessible, the contract report is being issued here, as submitted (except for typographical corrections), in the form of a PIFSC Administrative Report. The findings, conclusions and opinions expressed in the contract report are those of the authors as independent investigators and do not necessarily reflect views of PIFSC, the National Marine Fisheries Service or NOAA.

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INTRODUCTION

Hawaiian monk seals, *Monachus schauinslandi*, have been listed as endangered since 1976. Since then, the overall population of monk seals has continued to decline (Ragen, 1993). One of the main hypotheses explaining this decline is reduced overall resources available and/or reduced specific prey species, which may affect individuals differently depending on their age and sex. Thus, understanding the foraging ecology of this species has become crucial, particularly if we are to understand the impact that commercial fishing and other human practices may have on this population.

Predator-prey dynamics, and thus diet composition, are key elements in the foraging ecology of a species. In marine mammals, information pertaining to diet composition and dominant prey species has generally been limited to measures that rely on the recovery of prey hard structures from feces and stomach content samples. These methods, while providing valuable information about prey species, are known to be biased. For example, prey species which do not contain hard parts or in which the hard parts are easily digested or not consumed are underrepresented in the estimation of diet composition. As well, due to the rapid digestion of food by carnivores, diet estimates based on fecal samples only represent the last meal consumed and may not represent foraging over a broad spatial or temporal scale (i.e., the longer-term diet). Furthermore, it is usually not possible to determine the age or sex of individuals that contribute fecal samples, such that comparison of diets among demographic groups is rarely possible.

With these biases in mind, a project was begun in 1998 to determine whether fatty acid signature analysis (Iverson, 1993) could be used to determine diet composition of free-ranging Hawaiian monk seals in the Northwestern Hawaiian Islands (S.J. Iverson and G. Antonelis, pers. comm.). Fatty acids are the largest constituent of lipids and those of carbon chain length 14 or greater are often deposited in animal tissue with minimal modification from diet. Lipids in the marine food web are exceptionally complex and diverse and since a relatively limited number of fatty acids can be biosynthesized by animals, it is possible to distinguish dietary versus non-dietary components. Once taken up by tissues, fatty acids are either used for energy or 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. By sampling a core of blubber from a free-ranging seal, one may relatively noninvasively obtain information about diet that is not dependent on prey with hard parts nor limited to nearshore influences.

To date, fatty acid signatures have been used qualitatively to infer trophic levels and spatial and temporal differences in diets both within and among various pinniped species, but have not previously been validated in Hawaiian monk seals. Over the past 4 years there has been a large amount of work devoted to the development of a quantitative method (quantitative fatty acid signature analysis, QFASA), to use fatty acids to provide quantitative estimates of predator diets; the development of this method has just recently been completed (Iverson et al., 2004). QFASA has been validated in experimentally fed captive grey seals (*Halichoerus grypus*) and mink (*Mustela vison*) and in individual free-ranging harbour seals (*Phoca vitulina*) filmed during natural feeding events (Iverson et al., 2004). However, several factors are critical to its use:

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 which relates the predator signature to a mixture of possible prey signatures. One of the critical issues with regard to understanding metabolism, is the development and use of appropriate calibration coefficients which weight individual fatty acids in the QFASA model as a function of how they are laid down in the predator. To date, these coefficients have only been estimated for captive grey seals and harp seals (*Phoca groenlandica*) (Iverson et al., 2004).

Thus, the aims of this project were (1) to determine whether the blubber fatty acid signatures of Hawaiian monk seals were predictably influenced by those of their prey, as has been shown for other species of seals and predators; (2) to estimate calibration coefficients and compare them to those estimated for other phocid seals; and (3) to determine whether QFASA could accurately predict the diets of individual monk seals using the calibration coefficients currently estimated.

METHODS

Sampling

Ten captive Hawaiian monk seals held at SeaWorld, San Antonio, Texas, were used for this experiment. All monk seals were females and had been at SeaWorld since 1999; prior to this, these individuals had been housed since pups at the Hawaii Sea Life Park. All seals had been maintained predominantly on a diet of Atlantic herring prior to the experiment, although occasionally other prey were fed.

The first sampling took place on 24 and 25 July 2001 (Table 1). Each seal was placed in a squeeze-cage and weighed to the nearest lb (subsequently converted to kg) by suspending the cage from a hanging scale. Four seals were chosen for administration of deuterium oxide (D_2O), a stable isotope of water, for measurement of dilution and thus body water pool, which can be used to estimate total body fat. Seals were given a weighed dose (at approx. 3 $g \cdot kg^{-1}$) of 99.8% deuterium oxide (D_2O; Sigma Aldrich) by gastric intubation, using a 16-French stomach tube inserted through a shortened foal tube (to pass through the throat). Seals were then moved to a holding area and two serial blood samples (10 mL) were taken $\geq$ 3 hr post-administration to determine D_2O concentration and to ensure that equilibration had occurred. Blood samples were taken from the rear proximal flipper sinus between the insertion of the rear flipper and the tail. In two individuals, a large and unaccountable fraction of the D_2O was lost during initial administration and, thus, these seals were not further sampled. In the other two individuals, administration was successful. Blood samples were centrifuged and collected serum was stored frozen in airtight vials until analysis.

For blubber sampling, each seal was placed in a squeeze-cage and given an intramuscular injection of Midazolam. A small area on the flank was then sterilized and given an injection of 2% Lidocaine for freezing. A 1-cm slit was made in the skin with a no. 11 scalpel blade, through which a 6-mm biopsy punch was inserted. In each case, a full-depth blubber biopsy was taken. The biopsy was immediately placed in a glass vial containing chloroform with 0.01% BHT as an antioxidant and stored frozen until analysis.

After final sampling, all seals were returned to their exhibit areas and placed on experimental diets for 1 month. These diets were originally intended to be composed of both Spanish mackerel and California spiny lobster, but these changed according to what seals would consume (see Results). The second sampling took place on 21 and 22 August 2001 and proceeded as described above (Table 2). Only the 2 seals which were successfully administered D_2O initially, were re-administered D_2O. Since the interval between samplings was 1 month, it was assumed that all D_2O from the previous administration was washed out and thus a pre-administration blood sample was not taken from either animal to reduce stress of handling. Tables 1 and 2 provide all details of animals, handling and sampling at both time periods.

Table 1.-- Handling and sampling of Hawaiian monk seals at SeaWorld San Antonio, 24-25 July 2001.

Seal name	Flipper tag	ID #	Sex	Handling time (date)	Mass (kg)	Blubber biopsy (side)	D_2O (g)	Blood sample[1]
Kala	YC21	NOA0005655	F	1000-1020 (24 July)	187	Right	---	---
Kapuni	YC24	NOA0005659	F	0815-0920 (24 July)	216	Right	---	---
Koa	YC32	NOA0005656	F	0746-0753 (25 July)	213	Right	---	---
Laka	YC03	NOA0005657	F	0732-0742 (25 July)	229	Left	---	---
Nakui	YC04	NOA0005658	F	0756-0805 (25 July)	198	Right	---	---
Nani	YC28	NOA0005653	F	0917-0943; 1310-1335 (25 July)	208	Right	188.18 (@0942)	1313; 1334
Ola	YC14	NOA0005646	F	0930-0952 (24 July)	228	Right	---	---
Opua	YC35	NOA0005654	F	0719-0731 (25 July)	207	Right	---	---
Paki	YC16	NOA0005651	F	0807-0816 (25 July)	204	Right	---	---
Pulu	YC22	NOA0005652	F	0835-0914; 1345-1408 (25 July)	221	Left	187.12 (@0914)	1348, 1407

[1]Blood sample for D_2O analysis.

Table 2.--Handling and sampling of Hawaiian monk seals at SeaWorld San Antonio, 21-22 August 2001.

Seal name	Flipper tag	ID #	Sex	Handling time (date)	Mass (kg)	Blubber biopsy (side)	D_2O (g)	Blood sample[1]
Kala	YC21	NOA0005655	F	0830-0850 (21 Aug)	181	Left	---	---
Kapuni	YC24	NOA0005659	F	0810-0820 (21 Aug)	178	Right	---	---
Koa	YC32	NOA0005656	F	0915-0920 (22 Aug)	240	Left	---	---
Laka	YC03	NOA0005657	F	0820-0828 (22 Aug)	224	Left	---	---
Nakui	YC04	NOA0005658	F	0829-0835 (22 Aug)	193	Left	---	---
Nani	YC28	NOA0005653	F	0932-0958; 1313-1337 (22 Aug)	203	Left	185.54 (@0952)	1313, 1337
Ola	YC14	NOA0005646	F	0750-0806 (21 Aug)	207	Right	---	---
Opua	YC35	NOA0005654	F	0843-0848 (22 Aug)	208	Left	---	---
Paki	YC16	NOA0005651	F	0854-0900 (22 Aug)	201	Left	---	---
Pulu	YC22	NOA0005652	F	1004-1040; 1341-1403 (22 Aug)	214	Left	188.24 (@1035)	1341, 1407

[1]Blood sample for D_2O analysis.

Laboratory Analyses

Isotope Dilution

Serum samples from the two seals were distilled using the method of Oftedal and Iverson (1987). Distillates were analyzed in triplicate for D_2O concentration on a single-beam, fourier transform, infrared spectrophotometer (Perkin-Elmer FT-IR Paragon 1000), using gravimetrically prepared standards and distilled water as reference. Isotope dilution space (D) was calculated as given in Iverson et al. (1993). Dilution space was then converted to total body water (TBW) to correct for overestimation due to isotope loss to non-exchangeable body compartments using the specific relationship derived for pinnipeds by Bowen and Iverson (1998):

$$TBW\ (kg) = 0.003 + (0.968 \times D)$$

Total body fat (%TBF) was calculated using the equations derived for grey seals by Reilly and Fedak (1990) from TBW and body mass (BM):

$$\%TBF = 105.1 - (1.47 \times (100 \times TBW/BM))$$

Fat Content and Fatty Acid Composition

Samples (whole prey) of Atlantic herring (n = 25, from 5 different lots fed), mackerel (n = 30, from 4 different lots fed) and lobster (n = 10) were collected throughout the experiment; each whole prey was ground individually and lipids were quantitatively extracted in duplicate aliquots using a modified Folch method (Folch et al., 1957; Iverson et al., 2001); fat content was expressed as an average of the two duplicates. Lipid was extracted from blubber in the same manner after homogenization. Fatty acid methyl esters were prepared directly from 100 mg of the pure extracted lipid (filtered and dried over anhydrous sodium sulfate), using 1.5 ml 8% boron trifluoride in methanol (w/w) and 1.5 ml hexane, capped under nitrogen, and heated at 100°C for 1 hour. Fatty acid methyl esters were extracted into hexane, concentrated, and brought up to volume (50 mg/ml) with high purity hexane. This method of transesterification, as employed in our lab with fresh reagents, was routinely tested and found to produce identical results to that using Hilditch reagent (0.5 N H2SO4 in methanol).

Duplicate analyses of fatty acid methyl esters were performed on samples using temperature-programmed gas liquid chromatography according to Iverson et al., 1997, on a Perkin Elmer Autosystem II Capillary FID gas chromatograph fitted with a 30 m x 0.25 mm id. column coated with 50% cyanopropyl polysiloxane (0.25µ film thickness; J&W DB-23; Folsom, CA) and linked to a computerized integration system (Turbochrom 4 software, PE Nelson). Identifications of fatty acids and isomers were determined from the following sources: known standard mixtures (Nu Check Prep., Elysian, MN), silver-nitrate (argentation) chromatography, and GC-mass spectrometry (Hewlett-Packard 6890 Gas Chromatograph, 1:20 split injection, Micromass Autospec oa-TOF mass spectrometer, operated at 1000 resolution, scanning masses 120 to 450). Individual fatty acids are expressed as weight percent of total fatty acids after employing mass response factors relative to 18:0. Theoretical relative response factors were

used for this purpose, with minor adjustments made after tests with accurate quantitative standard mixtures (Nu Check Prep., Elysian, MN). All sample chromatograms and identifications were individually checked to determine any column deterioration, replacement, or reprogramming of GC necessary. Fatty acids are expressed as weight percent of total fatty acids and are designated by shorthand IUPAC nomenclature of carbon chain length: number of double bonds and location (n-x) of the double bond nearest the terminal methyl group.

QFASA Modeling

The purpose of developing calibration coefficients is to be able to weight individual fatty acids according to how directly they are deposited from diet. Calibration coefficients for monk seals were calculated according to Iverson et al. (2004) using the lot of herring assumed to be fed closest to the start of the experiment and the initial samples of each seal. Here we made the assumption that all individuals had been on herring, and only herring, for an extended period of time (> 5 months) and that the fatty acid composition of blubber would resemble that of the seal's diet as much as it ever would. Although we did not have detailed records of prior feeding or analyses of prior lots of herring over the many months preexperiment, this seemed a reasonable starting assumption and the best we could work with.

The diets of seals were estimated according to Iverson et al. (2004): in summary, we refer to the quantitative distribution of all fatty acids measured in a seal or prey sample as its fatty acid signature. To estimate the composition of the seal'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 (Kulback-Liebler distance) from that of the predator. Each prey is summarized by its mean fatty acid signature, and we estimate its proportional contribution to the seal's signature after application of calibration coefficients. The proportions in signatures are then converted to proportions of diet after taking into account the relative fat contents (thus fatty acid contribution) of each prey.

RESULTS AND DISCUSSION

Body Mass, Composition and Food Consumption

The individuals of each prey species analyzed were quite variable in the fat content and fatty acid composition, especially between different lots. Unfortunately, we were not able to feed the animals from only one lot, and thus this certainly introduced substantial variability in signatures of seals. In general, however, the Atlantic herring averaged 8.8 ± 4.77 SD % fat (maximum 15% fat), while Spanish mackerel averaged only 2.6 ± 0.85 SD % fat (maximum 4% fat). California spiny lobster averaged 1.5 ± 0.59 SD % fat (maximum 2% fat).

At the start of the experiment, seals weighed 211 ± 13.2 SD kg (range: 187 - 229 kg). After this initial sampling, the seals were returned to their exhibit areas and were placed on experimental diets. Although the diets were originally intended to be composed of both mackerel and lobster, the seals would not eat the lobster offered and several seals refused the

mackerel. Thus, 2 seals (Laka and Kala) remained essentially only on herring, while the other 8 seals consumed 3-4 kg of mackerel per day.

After 1 month, at the end of the experiment, seals weighed 204 ± 18.7 SD kg (range: 178 - 240 kg). Thus, in general seals did not gain mass during the experiment and most lost mass. Most animals lost 3-7 kg over the experiment. However, it is unclear whether the masses recorded were actually accurate, as 2 animals (Kapuni and Ola) are reported as having lost 21 and 38 kg, respectively, while 1 animal (Koa) is reported to have gained 27 kg. These mass changes seem extraordinary in the course of only 4 weeks of feeding. These 3 animals were all on low-fat mackerel diets and consumed less prey than would be expected to meet daily rates of maintenance (e.g., captive grey seals of similar or smaller size consume at least twice those consumed here of a higher-fat prey). Thus, we would expect these animals to have lost some mass, but 20-40 kg seems quite high. Conversely, it would not be possible, at the levels consumed, for an animal to have gained 27 kg on this Spanish mackerel diet. Thus, unfortunately, it is unclear whether the masses recorded can be relied upon.

In the two animals given D_2O at the start of the experiment, both animals equilibrated within the bleeding times. Dilution space was measured at 132 kg and 125 kg in Pulu and Nani, respectively, and this equated to total body fat contents of 20.2% and 19.6%, respectively. These values are quite within expected ranges of adult phocid seals and indicate that the animals were reasonably fat. However, at the second sampling, the values are perplexing, and likely not accurate. Although both animals had equilibrated, both had lower dilution spaces and fat content was estimated to be 40-42%, despite both seals having lost 5-7 kg body mass. When seals are switched to a low fat diet, even if they are able to maintain body mass, they lose body fat (Kirsch et al., 2000; Iverson et al., 2004). Thus, these animals likely lost (perhaps substantial) fat. Additionally, body fat contents of 40% or more are only observed in newly weaned phocid pups. There are two possibilities for these latter results. Since total body mass is a critical value in calculating body fat content from dilution space (see equations above), if the masses recorded were inaccurate (see above) then so also would be the fat content calculations. Alternatively, if there was still D_2O remaining in the blood stream from the initial dosage, then this would have caused the estimates of fat content to be erroneously higher. While this seems unlikely, in that D_2O is usually washed out completely within 2 -3 weeks, it remains a possibility.

Because of these issues, we assumed that the initial values for the two seals were correct and assumed all seals to be similarly comprised of about 20% fat at the start of the experiment. We used these values for calculations of expected contribution to new signatures. Although we expect that most seals lost fat during the feeding trial, we did not attempt to estimate this value.

Fatty Acid Composition, Calibration Coefficients and QFASA Estimates of Diet

Approximately 71 fatty acids were identified and quantified in both seals and potential prey. The following figures use a representative number of those fatty acids to illustrate patterns. The first, and perhaps most obvious finding, in this study was the degree to which the fatty acid composition of the blubber of captive monk seals differed from that of their wild free-ranging counterparts (Fig. 1).

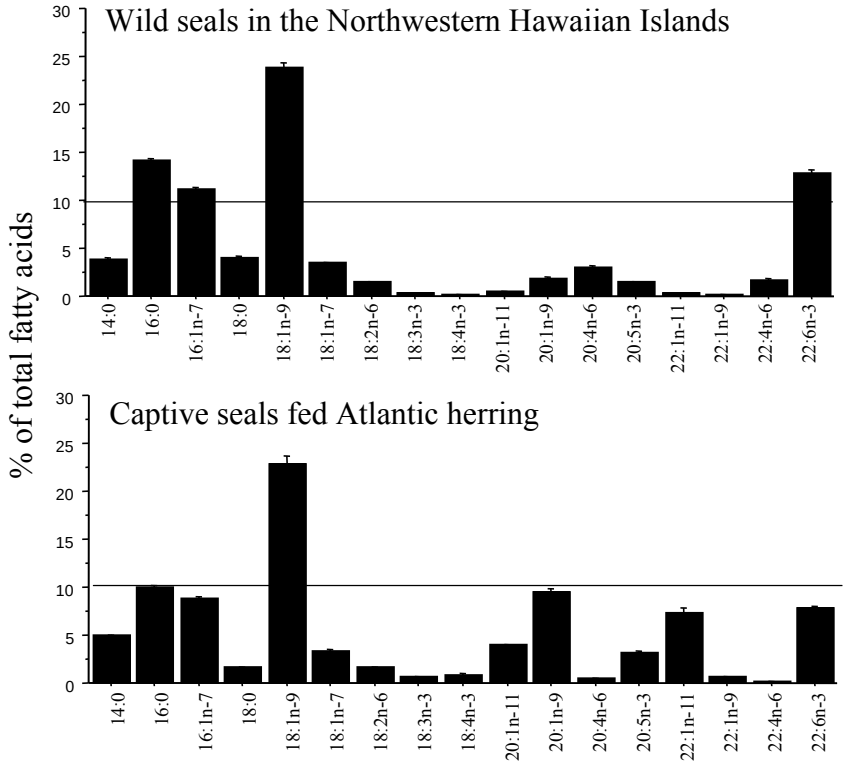

Figure 1.-- Selected fatty acids (mean ± SE) in the blubber of free-ranging monk seals in the Northwestern Hawaiian Islands (NWHI) in comparison to captive monk seals fed Atlantic herring. Values for free-ranging monk seals represent an average of 157 seals across the NWHI (S. J. Iverson and G. Antonelis, pers. comm.) and those for captive monk seals are the average of the 10 seals at the start of the experiment. A reference line at 10% is shown for illustrative purposes.

While the fatty acids 16:0, 16:1n-7, 18:0, 18:1n-9, and 18:1n-7 can arise from a mixture of both diet and biosynthesis within the seal, the rest of the fatty acids illustrated (especially ≥ 18:2n-6) are primarily dietarily derived. The fatty acid compositions are very different with the captive seals characterized, for instance, by high levels of 20:1n-11, 20:1n-9, 22:1n-11 and 22:1n-9, fatty acid indicators which are prevalent in the north Atlantic ecosystem and typically high in Atlantic herring (Budge et al., 2002). In contrast, monk seals feeding in the NWHI are characterized by much higher levels of fatty acids prevalent in the prey of that ecosystem, for example 20:4n-6, 22:4n-6 and 22:6n-3 (Fig. 1). Thus, clearly, monk seal blubber fatty acids differ with differing diets.

We can examine this further by comparing general prey vs. seal blubber signatures (Fig. 2).

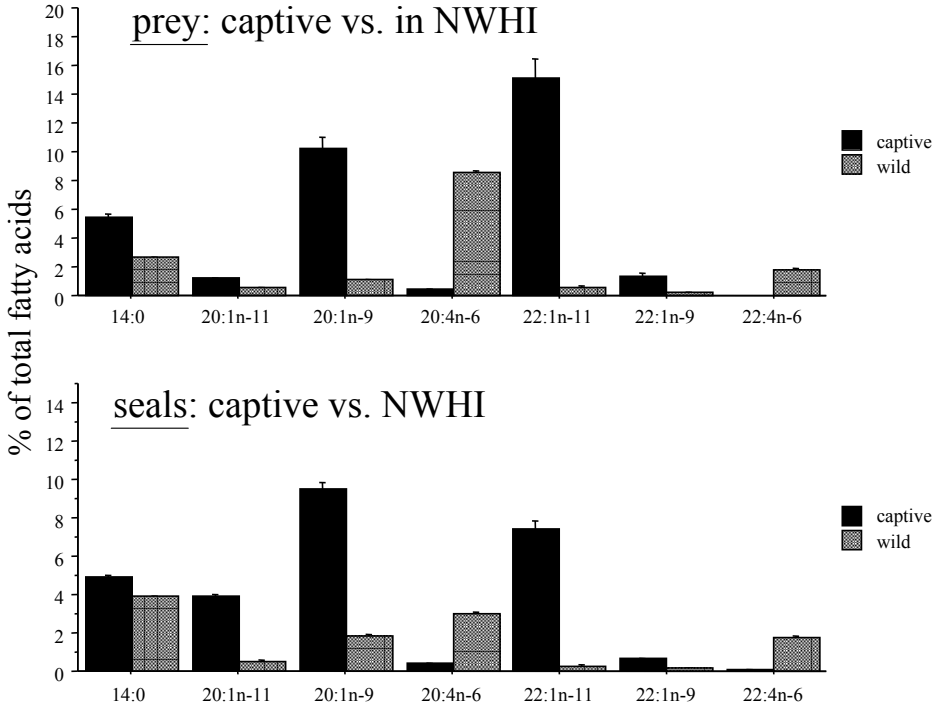

Figure 2.-- Selected dietary fatty acids (mean ± SE) in the (top panel) prey (herring) of captive monk seals fed herring in comparison to prey in the Northwestern Hawaiian Islands (NWHI) and (bottom panel) of captive monk seals fed Atlantic herring in comparison to that of the blubber of free-ranging monk seals in NWHI. Values for captive prey are the average of all herring analyzed (n = 25, from 5 different lots fed) and for wild prey are simply the average of all prey species previously analyzed in the NWHI data base (n = 1540 individuals; S. J. Iverson and G. Antonelis, pers. comm.) for comparison purposes. For seals analyzed, see Figure 1.

The general fatty acid signature of the two types of prey (captive-fed Atlantic herring vs. prey in the NWHI) are clearly reflected in the blubber signatures of monk seals. The high levels of 14:0, 20:1n-11, 20:1n-9, 22:1n-11 and 22:1n-9 of Atlantic herring are reflected in the captive seals, while much lower levels of these components in wild prey are reflected in the wild seals. Wild prey in the NWHI, in general, contain much higher levels of 20:4n-6 and 22:4n-6, which are also found in higher levels in the wild seals in the NWHI.

Thus, in answer to aim (1) to determine whether the blubber fatty acid signatures of Hawaiian monk seals were predictably influenced by those of their prey, as has been shown for other species of seals and predators: Clearly, monk seal blubber fatty acid signatures are predictably influenced by that of their diet (Figs. 2 and 3).

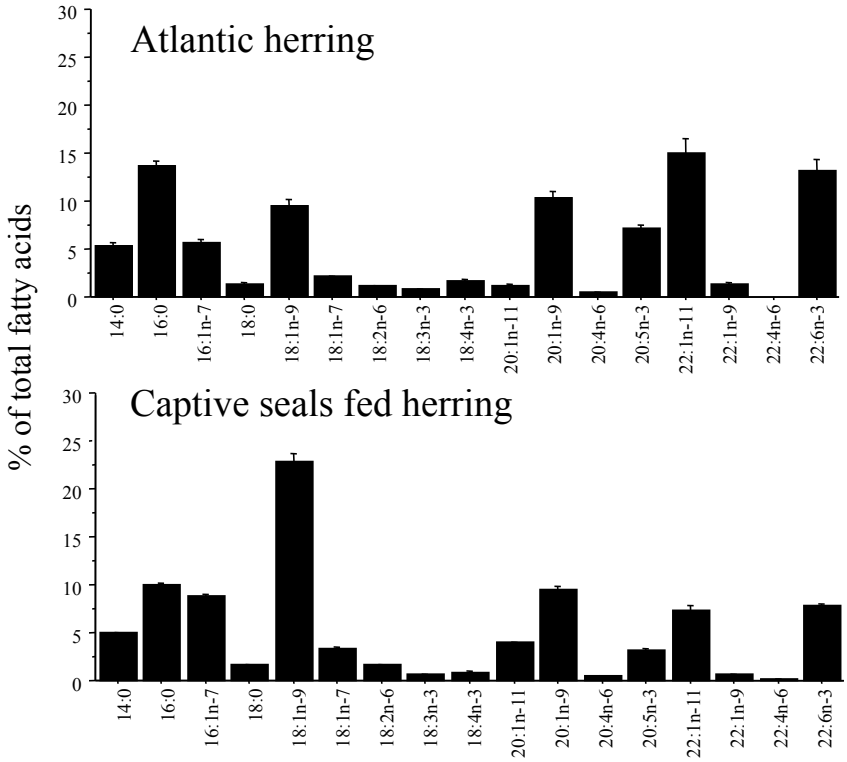

Figure 3.--Selected fatty acids (mean ± SE) in Atlantic herring (n = 25) and in the blubber of captive monk seals fed herring. Values for captive monk seals are the average of the 10 seals at the start of the experiment.

Figure 3 further illustrates the overall similarity in signatures between captive monk seals and the herring fed in captivity. Thus, the next step was to use these data to determine whether calibration coefficients could be estimated for monk seals and to compare these to those estimated for other phocid seals. For calculating monk seal, we used only the lot of herring assumed to be fed closest to the start of the experiment when all seals had been on herring, and used the initial blubber samples for each seal.

Overall, there was a reasonable degree of correspondence between the calibration coefficients estimated for monk seals and those previously estimated for grey and harp seals (Fig. 4).

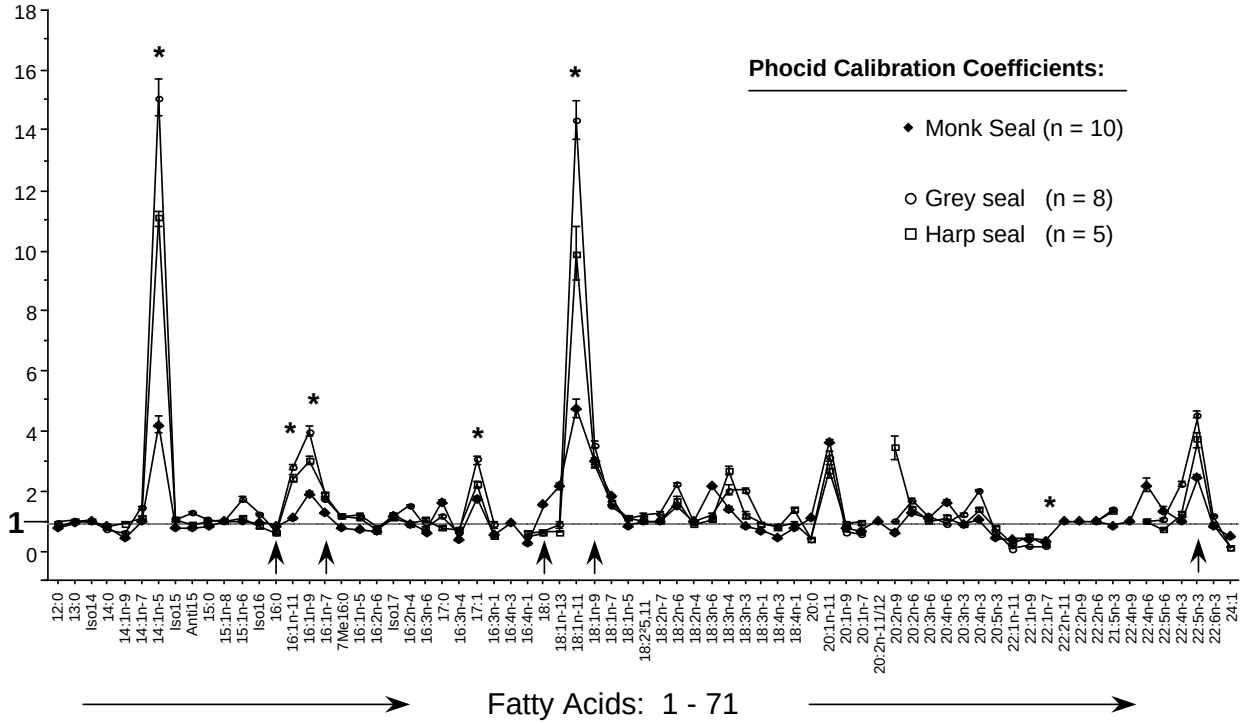

Figure 4.--Calibration coefficients (mean ± SE of the 10% trimmed means calculated within each individual; note: in most cases the SE is too small to see) estimated for all 71 fatty acids quantified in monk seals in comparison to those estimated from two previous feeding studies on grey and harp seals (Iverson et al., 2004). The 1:1 line is presented which denotes the deviation of a given fatty acid in a predator from that consumed in its diet.

*indicates 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.

Calibration coefficients for most fatty acids were close to one, however, there were notable exceptions. 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 < 0.5% 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 effects on estimates from the model, we removed these fatty acids from modeling subsets at the outset. 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. Thus, the calibration coefficients provide a means of using these fatty acids in the model.

Thus, in answer to aim (2) to estimate calibration coefficients and compare them to those estimated for other phocid seals: We conclude that monk seals are similar to other phocid seals in how they deposit and metabolize dietary fatty acids.

At present, it is not clear whether the differences observed in calibration coefficients between species (Fig. 4) are real or whether they may be the result of imperfect feeding/sampling regimes. That is, all three studies had limitations concerning our ability to sample the experimental diet, controlling the type of diet that was fed, or the duration of the experiment. First, none of the studies may have been long enough (e.g., 5 months on the same diet for grey and harp seals) to completely eliminate the signature from a previous diet or prey. In addition, the diet fed to seals is not homogeneous and we could only sample a subset of individual herring not actually fed to seals, with the assumption that these were representative all herring fed over the previous months. Unfortunately, certainly in the case of monk seals this is not a truly valid assumption, as is illustrated by the large variation in herring fatty acid signatures between lots of herring even within the 1-month period of our experiment (see Fig. 5b). Nevertheless, we feel these are a good starting point in accounting for effects of predator metabolism and can be used in preliminary modeling of monk seal diets using the QFASA model.

After being maintained on a fairly long-term diet of herring, 8 monk seals were switched to a diet of mackerel and 2 monk seals were continued on a diet of herring. The fatty acid signatures of these prey differed in a number of components. Although we attempted to feed seals some lobster, this was not eaten; nevertheless the fatty acid data for lobster are presented (Fig. 5a). While the overall signatures of herring and mackerel differed, there was substantial variability especially within herring, as illustrated by the differing signatures of the 5 different lots (Fig. 5b). Thus, even the 2 seals maintained on herring during the experiment would not necessarily be expected to retain the same signature between initial and final sampling.

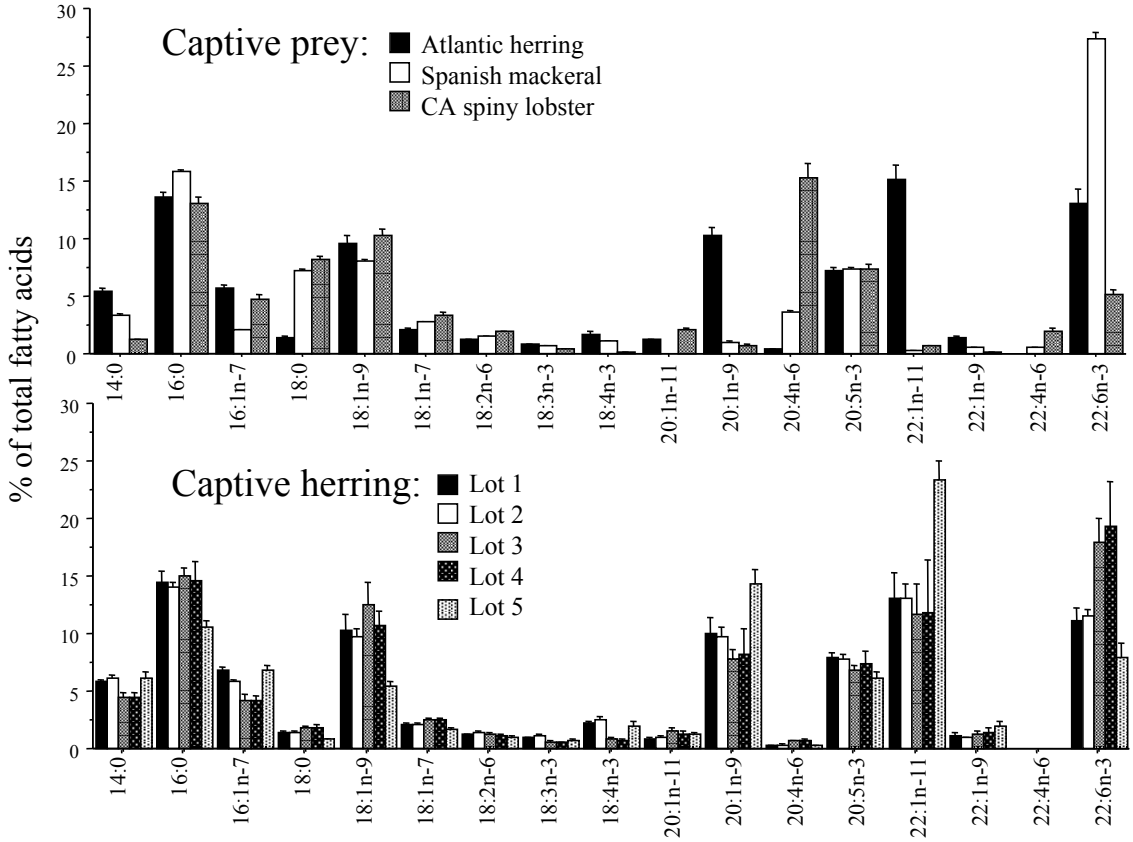

Figure 5.--Top panel: selected fatty acids (mean ± SE) in the three species of prey offered to captive monk seals: Atlantic herring (n = 25), Spanish mackerel (n = 30) and lobster (n = 10). Bottom panel: selected fatty acids in the 5 different lots of Atlantic herring sampled during the course of the 1-month feeding experiment.

Figure 6 illustrates the changes that occurred in the signatures of two seals switched to a diet of mackerel and two seals fed the different lots of herring. In the case of all seals switched to a diet of mackerel, signatures changed, but generally only to a small degree. This would be expected given the large starting body fat content and the change to reduced consumption of a low fat diet. As expected, the two seals which were fed the different lots of herring also changed somewhat between initial and final sampling (Fig. 6).

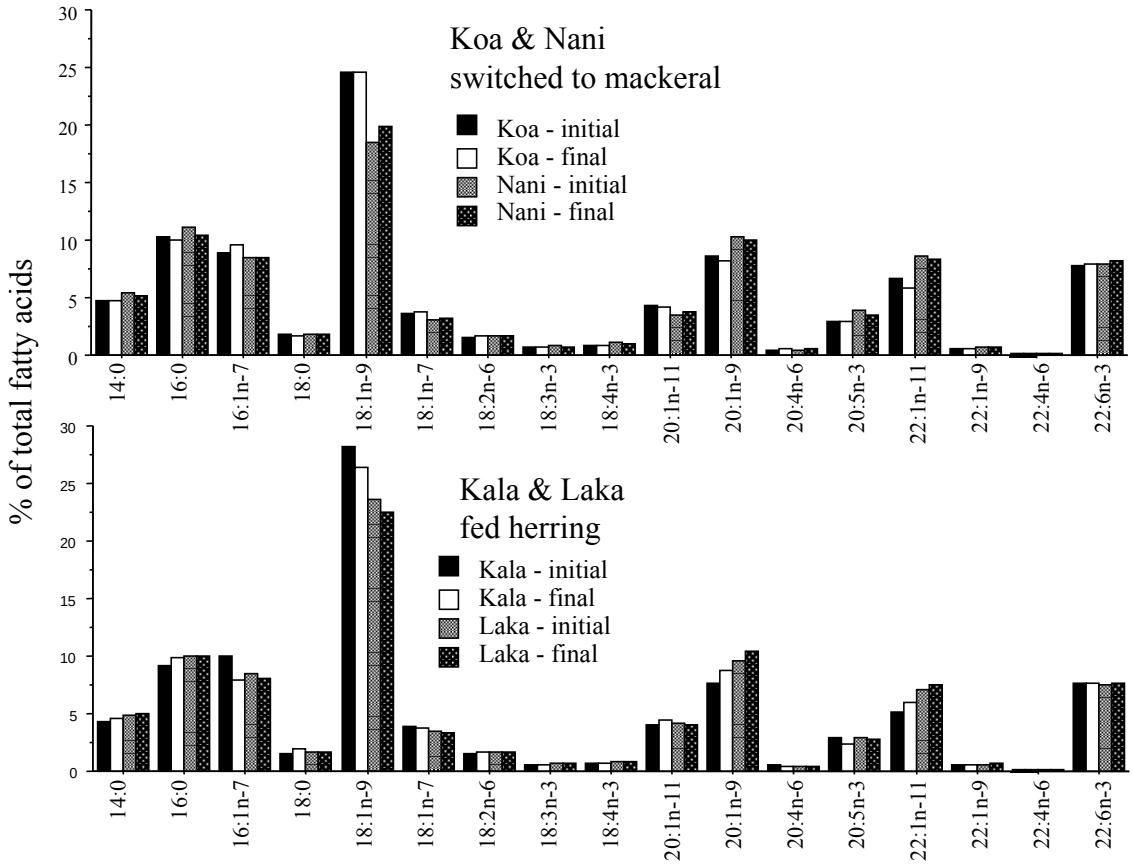

Figure 6.--Top panel: changes in selected fatty acids (mean ± SE) in two monk seals switched from a high-fat diet of herring to a low-fat diet of mackerel for 1 month. Bottom panel: changes in selected fatty acids in two monk seals fed the 5 different lots of Atlantic herring during the course of the 1-month feeding experiment.

Given the complexity of 71 total fatty acids and the number of prey or prey lot choices to consider, 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. Thus, as in previous cases, the QFASA model allows us here to use a mixture model of prey species signatures to asses that which most closely resembles that of the predator and thereby estimate its diet.

In general, the diets of monk seals were well predicted using the QFASA model, providing that appropriate calibration coefficients were used (Fig. 7).

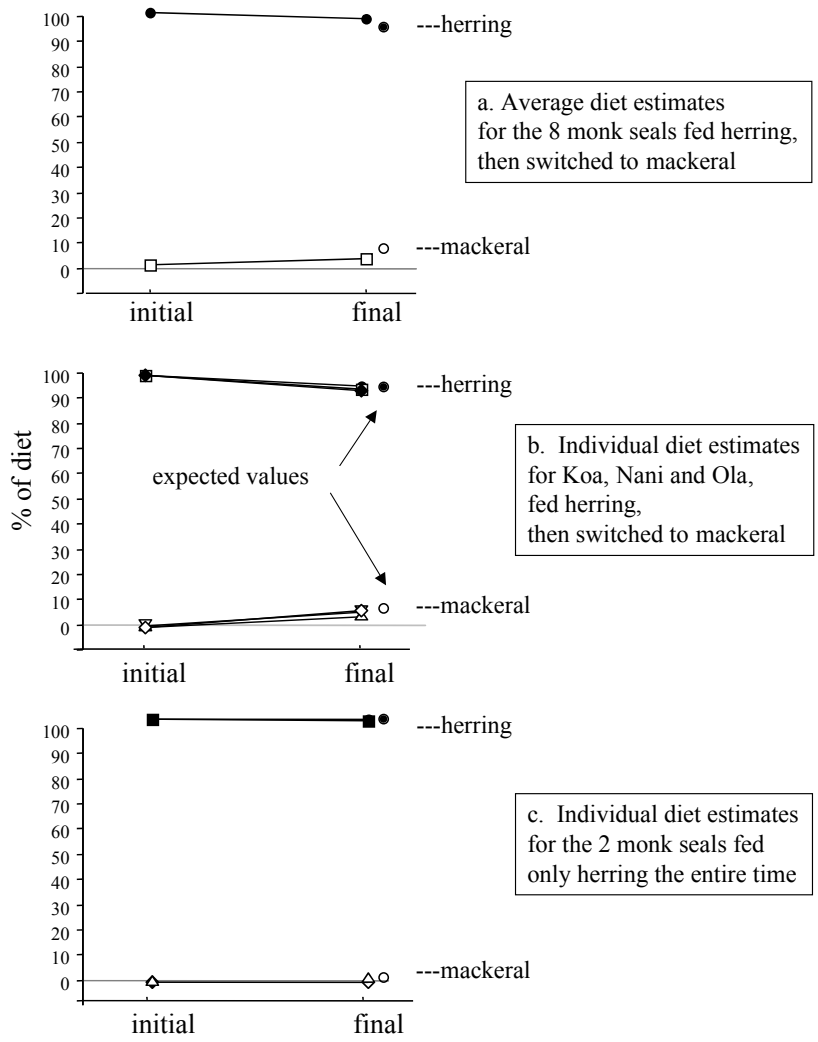

Figure 7.--Preliminary model estimates of the contribution of prey species to diets of captive monk seals (after taking into account relative fat contents of prey) previously fed herring and switched to a diet of mackerel for 1 month (top panel: average values for all 8 seals; middle panel: individual values for the 3 seals best predicted) and in monk seals continued on the different lots of herring for the month (bottom panel). Results are presented as the mean ± SE (often too small to see visually). The model inputs presented used the estimated monk seal calibration coefficients (Fig. 4) and the extended-dietary subset of fatty acids. Separated circles to the right of each plot are the average of the expected values for each prey item at the start and end of the experiment. Even though all seals lost fat, we used a simple approach for this estimation just for the sake of comparison:
Turnover of blubber fatty acids occurs even in a nonfattening animal (Kirsch et al., 2000). Thus, here, we assumed that all seals initially contained 20% fat (blubber fatty acids). At an average of 211 kg, this equates to about 42 kg fat. We then took the total amount of fish consumed during the feeding trial (about 200 kg) x the % fat of that fish (i.e., 2.6% for mackerel) = 5.2 kg fat consumed. We then assumed either 1) that all the new fatty acids consumed were deposited with existing fat and then used by the animal as a single pool (100% deposition) or 2) that some fraction (in this case, 50%) of the fatty acids consumed were immediately oxidized and not deposited. We then averaged the results of these two values for presentation.

When monk seals were modeled with the currently estimated monk seal calibration coefficients, overall, the seals that were switched to mackerel were estimated to have diets which increased in mackerel. However, the average for all 8 seals was somewhat below that expected. All seals lost fat and thus likely did not deposit a great deal of what they ate and thus some signatures did not change substantially. Nevertheless, mackerel came through and in at least three seals it was quite well predicted in the diet (Fig. 7). Correspondingly herring was reduced in the diet. Mackerel was correctly not predicted in any of the initial diets of all 10 seals. In the two seals that remained on herring, despite the fact that their signatures changed slightly (Fig. 6), likely with changes in lots of herring fed (Fig. 5), their diets were correctly predicted to be comprised of 100% herring both initially and finally (Fig. 7). Although not actually consumed by the seals, lobster was included in all modeling exercises, but correctly did appear in the diets.

In contrast to these results, when monk seals were modeled without the use of calibration coefficients, estimates of the percent contribution to diets did not correspond to either known or expected diet contributions at any time. Although the model performed better when using grey and harp seal calibration coefficients than with no coefficients, it did not perform as well as when using monk seal calibration coefficients.

Thus, in answer to aim (3) to determine whether QFASA could accurately predict the diets of individual monk seals using the calibration coefficients currently estimated: these preliminary results indicate that the diets of monk seals can be well predicted using QFASA. However, clearly the use of the correct set of calibration coefficients is critical to making accurate estimates of diet. Thus, we feel more work should be done to refine this current set of coefficients. Although aspects of the QFASA model are still in development, especially with respect to monk seals, this study suggests that QFASA can be a powerful tool to try to understand the diets of free-ranging monk seals, as has been found in other seals (Iverson et al., 2004).

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