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## Quantitative fatty acid signature analysis on New Zealand sea lions: model sensitivity and diet estimates

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We used quantitative fatty acid signature analysis (QFASA) to predict the long-term diet of New Zealand (NZ) sea lions (*Phocarctos hookeri*) incidentally caught in the NZ arrow squid (*Nototodarus* spp.) fishery. The QFASA model used fatty acid (FA) profiles based on 82 blubber samples of NZ sea lions bycaught between 2000 and 2006. First, the model was optimized by a series of simulations for which 1 model parameter—6 different sets of calibration coefficients (CCs) from different pinniped species and feeding regime, 2 sets of FAs, and the consideration of individual prey values, or mean prey values—varied each time. The best-fit parameters were those giving the lowest Kullback–Liebler distance values. Second, these parameters were used in a model to estimate the diet of NZ sea lions. QFASA was highly sensitive to the set of CCs applied. Across years the most important prey estimated with the best-fit CCs were southern arrow squid (*Nototodarus sloani*, 18–28% mass), hoki (*Macruronus novaezelandiae*, 10–27% mass), rattails (Macrouridae, 7–27% mass), and possibly scampi (*Metanephrops challengeri*, 1–19% mass). Despite the uncertainty on the accuracy of the match between the best-fit CCs used and the true FA metabolism of NZ sea lions, the variation of prey estimated among years was highly consistent with the trends of commercial catches during the same period, providing some confidence in the present QFASA predictions. The most important estimated prey were demersal species living mainly at depths >200 m that NZ sea lions encounter on the slopes of the Auckland Islands shelf. Our study emphasized the importance of these areas for bycaught NZ sea lions over the 1st half of the lactation period. DOI: 10.1644/09-MAMM-A-299.1.

Key words: dietary methods, feeding ecology, pinniped

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Accurate estimates of diet are fundamental in aiding conservation strategies and are of particular interest to managers when top predators, such as marine mammals, preying on commercial species are involved (Butler et al. 2006; Cornick et al. 2006). Traditional diet methods for marine species rely on the recovery of prey hard parts and are subject to numerous biases that are well documented in the literature (Pierce and Boyle 1991). For example, prey might not have any hard part resistant to digestion (e.g., crustaceans), and prey structures have species-specific digestion rates leading to the underestimation of fragile parts (e.g., small otoliths) or the overestimation of nondigestible parts (e.g., squid beaks—Bigg and Fawcett 1985; Dellinger and Trillmich 1988; Staniland 2002; Tollit et al. 2007a). Therefore, dietary

studies of marine mammals increasingly involve the combination of traditional techniques with methods such as fatty acid (FA) and stable isotope analyses, which overcome biases related to differential rates of digestion in the gut (Dehn et al. 2007; Grahl-Nielsen et al. 2005; Herman et al. 2005; Tucker et al. 2008). FAs are the main constituents of most lipids, and they are a source of stored energy situated mainly in the subcutaneous fat (blubber) in marine mammals (Iverson 2002). Long-chain FAs are deposited and metabolized mostly in a predictable manner (Iverson et al. 2004; Raclot and



Groscolas 1993; Summers et al. 2000), potentially reflecting the diet over weeks to months (Cooper et al. 2005; Iverson et al. 2004; Kirsch et al. 2000). FA analysis has been used extensively to assess geographical, temporal, and ontogenetic variation in the diet of a variety of marine mammals (Beck et al. 2007b; Herman et al. 2005; Smith and Worthy 2006; Staniland and Pond 2004; Walton et al. 2000). However, inference of prey species ingested by the comparison of the raw FA profiles of predator and prey alone is not sufficient, because the proportion of FAs deposited in the adipose tissue of the predator differ from that occurring in the prey because of differential metabolism of individual FAs in the predator (Iverson et al. 2004). Nevertheless, by using FA profiles in conjunction with a model taking into account FA metabolism (by including calibration coefficients [CCs]), it should be possible to estimate the species composition. Since the development of this method named quantitative fatty acid signature analysis (QFASA—Iverson et al. 2004), it has been used to infer the diet of several marine predators (Beck et al. 2007a; Iverson et al. 2006, 2007; Tucker et al. 2009). We propose to use QFASA to estimate the long-term diet of New Zealand (NZ) sea lions (*Phocarctos hookeri*), a pinniped endemic to NZ. Previous dietary studies on this species were based on the analyses of scat samples (Childerhouse et al. 2001; Lalas 1997; McMahon et al. 1999), regurgitates (Childerhouse et al. 2001), and stomach contents (Meynier et al. 2009) and defined NZ sea lions as generalist predators, preying on the most abundant prey at a particular location and depth. However, the diet information collected with these “hard part” methods is limited in time by the last meal ingested by sea lions and is associated with biases of differential digestion rates.

The NZ sea lion has been classified as “vulnerable in decline” by the International Union for Conservation of Nature (2009), with 86% of the entire pup production occurring at the Auckland Islands (50°30'S, 166°E—Chilvers et al. 2007). Its decreasing population (Chilvers et al. 2007) is in marked contrast with the large, increasing population of sympatric NZ fur seals (*Arctocephalus forsteri*—Harcourt 2001). The success of NZ fur seals has been attributed to their epipelagic foraging strategy (Harcourt et al. 2001; Mattlin et al. 1998) shared with other otariids with large populations (e.g., Boyd et al. 1994; Georges et al. 2000; Weise 2006). Benthic feeders such as the NZ sea lion tend to have smaller populations that are stable or in decline (e.g., Chilvers et al. 2006; Costa and Gales 2003; Thompson et al. 1998). The mean pupping date for this species is 26–27 December each year, and lactation lasts approximately 10 months, during which lactating females (LFs) alternate between foraging at sea and nursing their pup on land (Chilvers et al. 2007). Breeding and foraging-site fidelity in LFs is strong within a season and across years (Chilvers 2008; Chilvers and Wilkinson 2008). Nonlactating females (NLFs) also display high breeding-site fidelity (Chilvers and Wilkinson 2008), but their foraging behavior is unknown. Foraging habits of males (Ms) from the Auckland Islands have not been investigated,

but they tend to disperse to other breeding sites (Campbell Island, 52°33'S, 169°09'E; and Otago Peninsula, 46°S, 170°40'E) at the end of the female estrus in late January (Robertson et al. 2006). Accurate data on the long-term diet and prey availability are essential to better understand the energetics of the NZ sea lion and could provide insight into the low reproductive success of this marine mammal (67% of mature females—Childerhouse et al. 2010).

Our aims in the present study are to optimize the QFASA method on blubber of bycaught NZ sea lions already analyzed (Meynier et al. 2008a) by varying several parameters such as CC sets that are available in the literature (Iverson et al. 2004; Tollit et al. 2007b); and to estimate the long-term diet of NZ sea lions by using the best-fit QFASA parameters. QFASA estimates by year and by sex are discussed in terms of feeding ecology and biases related to different rates of FA metabolism between groups.

## MATERIALS AND METHODS

*Sample collection and lipid analysis.*—Blubber FAs from NZ sea lions have been analyzed in a previously published study in which sample collection and lipid analysis were detailed (Meynier et al. 2008a). In brief, a full blubber core was sampled from the midsternal body region of dead NZ sea lions captured incidentally in the NZ southern arrow squid (*Nototodarus sloani*) fishery that operates annually from February to May. FAs were analyzed following the method of Folch et al. (1957), with lipid extraction in a 8:4:3 volume of chloroform:methanol:saline mixture. FA methyl esters were prepared using 10% boron trifluoride in methanol and extracted into hexane. The relative contribution of identified FAs was determined using a flame ionization detector gas chromatography. FAs were designated by the shorthand notation of carbon chain length:number of double bonds and location (n-x) of the double bond nearest to the terminal methyl group. QFASA was applied on 82 blubber cores from 28 LFs, 23 NLFs, and 31 Ms bycaught between February and May from 2000 to 2006 (Meynier et al. 2008a). Blubber FAs are a combination of dietary FAs accumulated for several weeks to months and FA metabolism within the tissue (Iverson et al. 2004). Therefore, we believe that FA profiles in our study represented, to some extent, the diet ingested by bycaught sea lions from late austral summer to autumn (approximately January–May), which corresponds to the 1st half of the lactation period.

*Quantitative fatty acid signature analysis model.*—The diets of individual NZ sea lions were estimated using the model described by Iverson et al. (2004), and an FA library of prey from the Auckland Islands region (Meynier et al. 2008b). The model takes the mean FA profiles of each prey species in the prey library and estimates the mixture of prey FA profiles that comes closest to matching the FA profile of the predator's adipose tissue. Then, the best mixture is weighted by the fat content of each prey species and translated into a diet estimate (% mass).

The predicted FA profile,  $\hat{S}_y$ , of the predator's adipose tissue  $y$  is calculated as:

$$\hat{S}_y = \sum_{i=1}^n p_i S_i,$$

where  $p_i$  is proportion of prey  $i$ ,  $S_i$  is FA profile of prey  $i$ , and  $n$  is number of prey. The optimization process chooses  $p$  such that  $\hat{S}_y$  is the closest solution to  $S_y$  (true FA profile of  $y$ ). The distance minimized between  $S_y$  and  $\hat{S}_y$  is the Kullback–Liebler (KL) distance, calculated over all FAs as:

$$KL = \sum_{j=1}^m (S_{yj} - \hat{S}_{yj}) \log \left( \frac{S_{yj}}{\hat{S}_{yj}} \right),$$

where  $S_{yj}$  is true value of FA  $j$  of predator  $y$ ,  $\hat{S}_{yj}$  is predicted value of FA  $j$  of predator  $y$ , and  $m$  is number of FAs (Iverson et al. 2004). KL values given in the outputs are an indication of fit, because the optimization minimizes this value. The optimization uses a quasi-Newton algorithm with a Broyden–Fletcher–Goldfarb–Shanno formula and was carried out with a package developed at Massey University (Fatty acid solution—R. Sherriff and P. C. H. Morel, pers. comm.). Results given by this package were verified using the package Fascal (Fascal version 1.11—M. J. Walton, University of St. Andrews, United Kingdom) already used in the literature (Nordstrom et al. 2008).

Calibration coefficients account for the differential deposition, mobilization, and biosynthesis of FAs during lipid metabolism occurring in the predator's adipose tissue and were determined previously from experiments during which captive seals were fed on diets of known FA composition for several months. They were calculated by dividing FA levels found in the blubber of seals by FA levels in the food. We used the CCs from gray seals (*Halichoerus grypus*) fed on herring (*Clupea harengus* [Iverson et al. 2004]—named CCs-GS/her), CCs from harp seals (*Pagophilus groenlandica*) fed on herring (Iverson et al. 2004—named CCs-HS/her), CCs from Steller sea lions (*Eumetopias jubatus*) fed on Pacific herring (*Clupea pallasii pallasii*, Tollit et al. 2007b—named CCs-SSL/her), CCs from Steller sea lions fed on eulachon (*Thaleichthys pacificus*, Tollit et al. 2007b—named CCs-SSL/eul), and CCs from Steller sea lions fed on a mixed diet (Tollit et al. 2007b—named CCs-SSL/mix). The mixed diet consisted of 64% mass herring, 15% eulachon, 14% squid, and 7% rockfish (Tollit et al. 2007b). These are the only available CCs that can be used for the application of QFASA on NZ sea lions because no NZ sea lion is in captivity. The CCs calculated from Steller sea lions were, for most FAs, similar to the CCs-GS/her and CCs-HS/her. Notable exceptions were the FAs 14:1n-5, 18:1n-9, and 20:1n-11, for which CCs calculated from Steller sea lions were ~2 times less than those from phocids (Tollit et al. 2007b). The main difference within coefficients calculated from Steller sea lions was for 22:5n-3, with a CC from the eulachon experiment 7 times greater than that from the herring experiment. Before each model

optimization,  $S_y$  is divided by CCs for each FA and renormalized to sum 100%.

The prey library was composed of fish and cephalopods known to be significant prey of NZ sea lions in the Auckland Islands region based on previous stomach content and fecal analyses (Childerhouse et al. 2001; Meynier et al. 2009). They included hoki (*Macruronus novaezelandiae*,  $n = 11$ ), javelin fish (*Lepidorhynchus denticulatus*,  $n = 10$ , representing the rattail group, Macrouridae), opalfish (*Hemerocoetes* spp.,  $n = 10$ ), red cod (*Pseudophycis bachus*,  $n = 10$ ), arrow squid ( $n = 10$ ), and octopus (*Enteroctopus zealandicus*,  $n = 7$ ). Scampi (*Metanephrops challenger*, a crustacean,  $n = 6$ ) and spiny dogfish (*Squalus acanthias*, a cartilaginous fish,  $n = 2$ ) were added as potential prey because they are abundant in the area where NZ sea lions forage (Jacob et al. 1998; O'Driscoll et al. 2003). They were not reported as prey from traditional methods, which could be due to the absence of parts hard enough to resist digestive juices. All specimens were collected around the Auckland Islands from December to April between 2004 and 2007, and their FA profiles and fat contents were analyzed previously (Meynier et al. 2008b).

*Variation of QFASA parameters.*—The optimization involved the variation of 3 parameters, the FA set, the CC set, and whether the FA variation within a prey species was considered. Each specific combination of these 3 parameters is called a simulation, which implies 82 runs of the model (1 run per sea lion) resulting in 82 KL values.

Two sets of FAs were used. The 1st set of 27 FAs includes all FAs in common between prey and blubber samples identified previously by gas chromatography (Meynier et al. 2008a, 2008b). They are the saturated FAs 14:0, 15:0, 16:0, 17:0, and 18:0; the monounsaturated FAs 14:1n-5, 15:1, 16:1n-7, 18:1n-9, 18:1n-7, 18:1n-5, 20:1n-11, 20:1n-9, 22:1n-11, and 22:1n-9; and the polyunsaturated FAs 16:3n-4, 18:2n-6, 18:3, 20:2n-6, 20:3n-6, 20:4n-6, 20:3n-3, 20:5n-3, 21:5n-3, 22:5n-6, 22:5n-3, and 22:6n-3. FAs 15:0, 14:1, 15:1, and 18:1n-5 made only a minor contribution to the total mass and so were removed to create a 2nd set of 23 FAs. Deletion of these FAs generally improves the results obtained from the QFASA model (Iverson et al. 2004; Nordstrom et al. 2008).

For each FA set, QFASA was tested with different CCs available in the literature for the blubber of pinnipeds. Six scenarios were applied for this study: no CCs, CCs-GS/her, CCs-HS/her, CCs-SSL/her, CCs-SSL/eul, and CCs-SSL/mix.

For each set of FAs and CCs applied either the mean FA profiles of prey species or the FA profiles of individual prey were considered. Before using the model, prey FA profiles were visualized in a principal component analysis to assess the overlap between different prey species (Meynier et al. 2008b), and prey groups were redefined to avoid misclassification of species by the QFASA model. The regrouping of octopus (subdivided into 2 groups) and opalfish (removal of some individuals) was necessary because of great intraspecific variation in their FA profiles. Thus, 9 prey groups were available in the prey library. Variability of FA profiles within prey groups was considered by taking each prey individual as

**TABLE 1.**—Kullback–Liebler (KL) values (median and range) for the different simulations performed with quantitative fatty acid signature analysis on blubber fatty acid (FA) profiles from New Zealand sea lions (*Phocartos hookeri*). SSL is Steller sea lion (*Eumetopias jubatus*). Superscript letters show the most important significant results from statistical tests (Friedman tests and Wilcoxon signed-rank tests). CC = calibration coefficient.

Experiment on:	CCs					
	Prey fed:	Gray seal	Harp seal	SSL	SSL	SSL
		None	Herring	Herring	Herring	Eulachon
23 FAs						
Mean prey	20.6 (10.1–42.4)	19.3 (6.9–52.4)	10.5 (3.9–30.1)	5.1 <sup>c</sup> (1.4–15.0)	9.8 (4.8–30.1)	9.6 (3.8–29.0)
Individual prey	15.1 <sup>ab</sup> (5.6–31.4)	14.4 <sup>ab</sup> (4.4–44.6)	7.8 <sup>ab</sup> (2.3–24.4)	3.1 <sup>abc</sup> (0.8–9.4)	6.7 <sup>ab</sup> (4.0–15.7)	7.2 <sup>ab</sup> (2.5–15.5)
27 FAs						
Mean prey	20.4 (10.1–42.4)	19.6 (7.6–53.7)	11.0 (4.2–31.5)	5.2 <sup>c</sup> (1.5–15.0)	10.7 (6.1–30.2)	9.6 (2.9–23.3)
Individual prey	15.3 (6.1–31.2)	15.5 (5.4–44.7)	8.8 (2.6–25.6)	3.2 <sup>c</sup> (1.2–11.7)	7.2 (4.3–17.8)	7.3 (2.5–18.6)

<sup>a</sup> The KL values were significantly lower ( $P < 0.05$ ) than values with 27 FAs and individual prey for each CC scenario.

<sup>b</sup> The KL values were significantly lower ( $P < 0.05$ ) than values with 23 FAs and mean prey.

<sup>c</sup> The KL values were significantly lower ( $P < 0.05$ ) than values with other CC scenarios.

a different prey group. In other terms, instead of computing FA profiles from 9 prey groups, the model computed FA profiles from 63 groups (total of individuals in the prey library).

The distributions of KL values for each of the 24 simulations were compared using Wilcoxon signed-rank tests ( $Z$ ) between 2 simulations and Friedman tests ( $\chi^2$ ) between 3 and more simulations (with post hoc Wilcoxon tests for significant outcomes). Because KL is the distance minimized by the model, the parameters with low KL values were considered optimal and were kept to estimate the diet of NZ sea lions from blubber FA profiles.

*Diet estimates of bycaught NZ sea lions using QFASA.*—The best-fit model parameters were used to determine diet estimates of NZ sea lions from QFASA on blubber FA profiles. Diet estimates were compared between LFs, NLFs, and Ms, and among years. These groups were tested for significant variation of mass percentage by Kruskal–Wallis ( $H$ ) tests, and post hoc Mann–Whitney  $U$ -tests. All statistical tests, except post hoc tests, have an  $\alpha$  significance level of 0.05. For post hoc Mann–Whitney tests we adjusted significance levels according to the sequential Bonferroni method to reduce type I errors (Holm 1979). The comparison between sex groups involved 3 post hoc tests, therefore the lowest of the 3  $P$ -values was compared to  $\alpha = 0.017$  (0.05/3), the 2nd lowest  $P$  to  $\alpha = 0.025$  (0.05/2), and the 3rd lowest  $P$  to  $\alpha = 0.05$ . Six years were compared involving 15 post hoc tests, with readjusted  $\alpha$ -values of 0.003 (0.05/15), 0.004 (0.05/14), and so on, from the lowest to the highest  $P$ -value.

New Zealand sea lions are considered generalist predators (Childerhouse et al. 2001; Lalas 1997; McMahon et al. 1999; Meynier et al. 2009), and temporal variation in the diet is generally attributed to a change in prey availability. Abundance of marine species in the NZ subantarctic is not available, but reported commercial catches can be used as a proxy for variation in abundance if they are not limited by the Total Allowable Commercial Catch. Diet estimates among years were compared with commercial catches in the NZ subantarctic (Ministry of Fisheries 2007) using a Spearman's

rank test ( $r_s$ ). Statistical tests were run using MINITAB 15.0 (MINITAB, State College, Pennsylvania) and SPSS 16.0 (SPSS Inc., Chicago, Illinois).

## RESULTS

*Variation of QFASA parameters and KL values.*—The distribution of KL values varied greatly between simulations with a minimum value of 0.8 (23 FAs, individual prey, CCs-SSL/her) and a maximum value of 53.7 (27 FAs, mean prey, CCs-GS/her; Table 1). The predicted FA profile of the NZ sea lion with a KL value of 0.8 matched closely with the true FA profile, whereas large differences are visible between the predicted and true percentages of FAs for the NZ sea lion with a KL value of 53.7 (Fig. 1). Simulations using 23 FAs gave median KL values that were up to 15% lower than those with 27 FAs ( $Z = -7.881$  to  $-3.249$ ,  $P \leq 0.001$ ); except for simulations CCs-SSL/mix–mean prey ( $Z = -1.342$ ,  $P = 0.180$ ) and simulations CCs-SSL/her–mean prey ( $Z = -0.743$ ,  $P = 0.458$ ; Table 1). Likewise, simulations using FA profiles of individual prey showed lower KL values than those using mean profiles ( $Z = -7.867$  to  $-5.079$ ,  $P < 0.001$ ). KL values calculated using CCs-SSL/her were significantly lower than those using other CC scenarios, irrespective of the other parameters chosen ( $\chi^2_{5,82} = 290.564$ – $312.836$ ,  $P < 0.001$ ; post hoc Wilcoxon signed-rank tests,  $Z = -7.866$  to  $-7.508$ ,  $P < 0.001$ ; Table 1). Moreover, KL values between the CCs scenarios varied 4–5 times between the lowest and highest values (Table 1). To observe the consequences of different CCs on the diet estimated by QFASA, percentages of prey from the simulations with 23 FAs, individual prey, and the different CC scenarios are presented. When no lipid metabolism was taken into account (no CCs), the model predicted 70% by mass of rattails in the diet with a median KL value of 15.1 (Fig. 2a; Table 1). A high dominance of rattails (>70% by mass) also was found with the CCs from Steller sea lions fed on eulachon and mixed diet (Fig. 2a), whereas the model estimated a diet with several major prey species when the CCs from Steller sea lions fed on herring (CCs-SSL/her) and the CCs from phocids

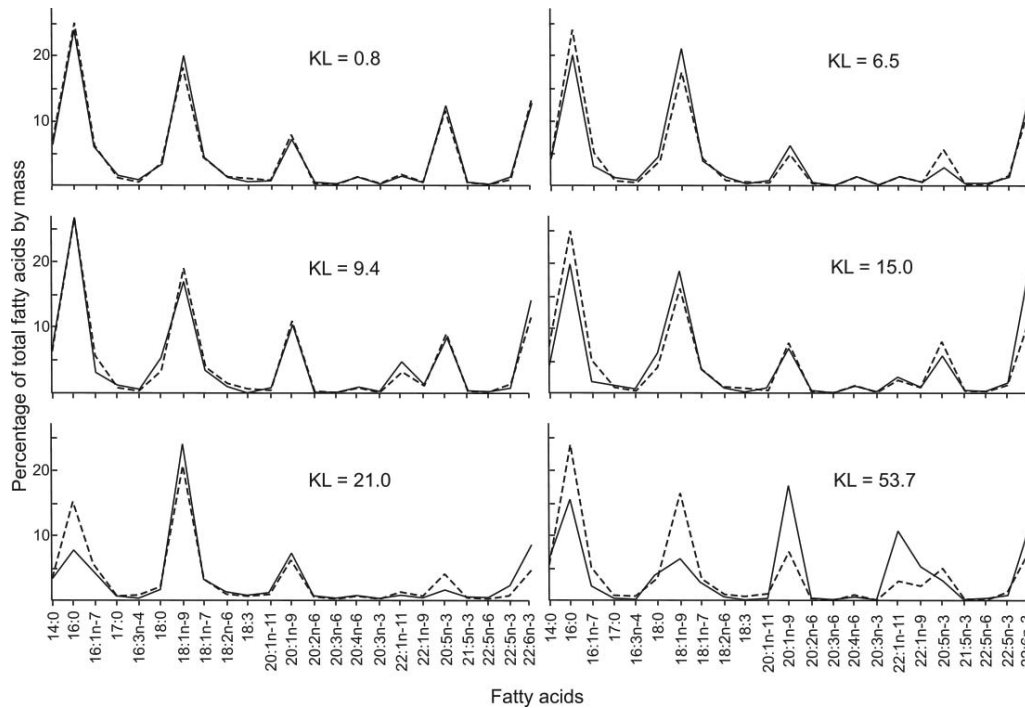


FIG. 1.—Examples of predicted fatty acid profiles (dotted line) by quantitative fatty acid signature analysis (QFASA) versus true fatty acid profiles (solid line) of New Zealand sea lions (*Phocarctos hookeri*), with different Kullback-Liebler (KL) values. KL is the mathematical distance minimized by the QFASA model.

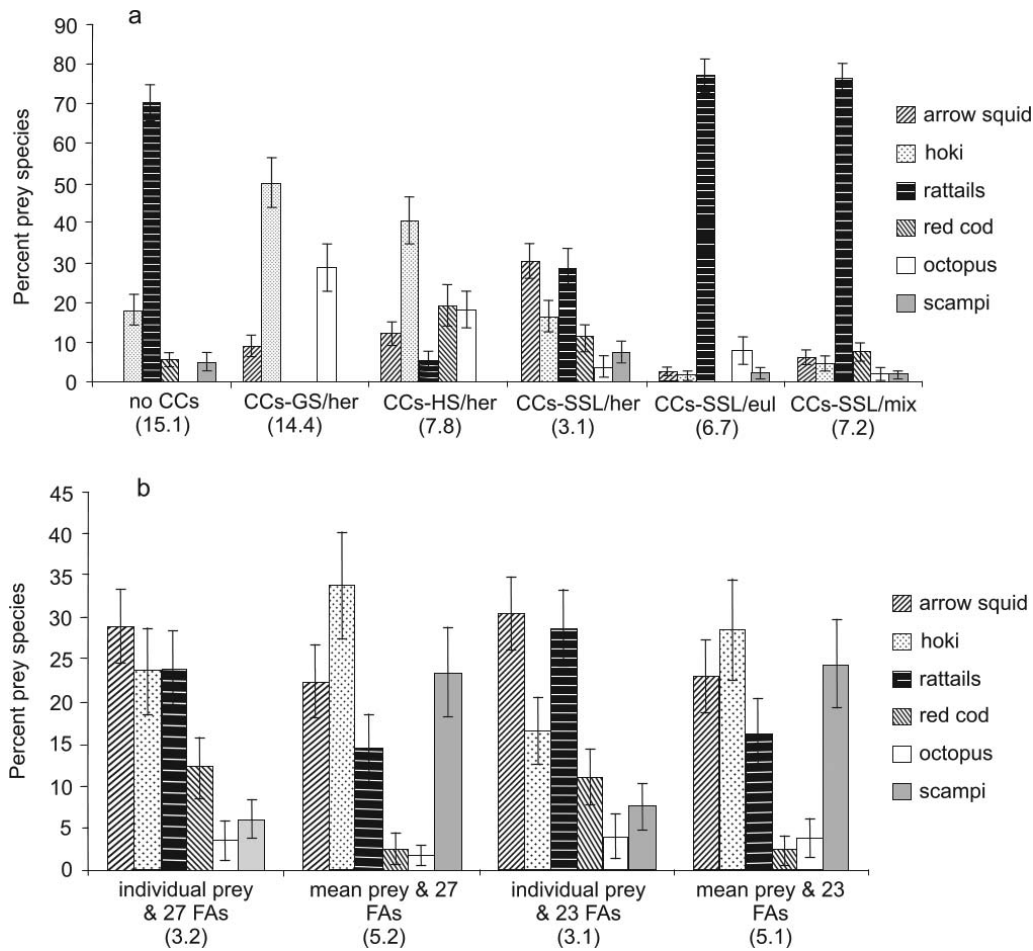
were applied. For simulations using CCs-SSL/her (Fig. 2b), diet estimates were similar between the simulations using different sets of FAs (27 versus 23 FAs;  $Z = -1.885$  to  $-0.022$ ,  $P = 0.059$ – $0.983$ ) but showed significant differences between the simulations using mean prey and individual prey ( $Z = -6.813$  to  $-4.183$ ,  $P < 0.001$ ), except for octopus ( $Z = -0.670$  to  $-0.233$ ,  $P = 0.503$ – $0.815$ ).

In summary, the simulations showing the lowest KL values were with CCs-SSL/her, in which the set of FAs had no significant influence on the diet estimates. We chose the CCs-SSL/her, 23 FAs, and individual prey or mean prey as the best-fit QFASA parameters and used these parameters in further model runs to estimate the diet of NZ sea lions. In the next sections, we named the best-fit simulations individual prey and mean prey only, but this implies that 23 FAs and CCs-SSL/her were the other parameters used.

*Diet estimates of bycaught NZ sea lions using QFASA.*—Overall, arrow squid, hoki, and rattails made up the bulk of the diet estimated by QFASA for both best-fit simulations (individual prey and mean prey), with  $\sim 70\%$  mean total mass, and were present in more than half the sea lion samples (Table 2). With the individual prey simulation, arrow squid and rattails contributed the most to the mass of estimated prey followed by hoki and red cod (Table 2). With the mean prey simulation, hoki made up the largest contribution, followed by scampi, arrow squid, and rattails (Table 2). Opalfish, octopus, and spiny dogfish were considered minor prey by both simulations with a median mass of 0. Only main prey were tested for difference among groups.

The estimated diet of Ms showed some significant differences from that of females in the contribution of arrow squid, red cod, and scampi (Fig. 3). In the mean prey simulation Ms ate less arrow squid than NLFs ( $H_{2,82} = 8.95$ ,  $P = 0.011$ ;  $U = 799.0$ ,  $n_1 = 31$ ,  $n_2 = 23$ ,  $P = 0.0037$ ). However, the diet of Ms consisted of more red cod than the diet of NLFs in the individual prey simulation ( $H_{2,82} = 6.08$ ,  $P = 0.048$ ;  $U = 498.0$ ,  $n_1 = 31$ ,  $n_2 = 23$ ,  $P = 0.019$ , near the limit of the Bonferroni-adjusted  $\alpha$  of 0.017), or more scampi than the diet of both LFs and NLFs in the mean prey simulation ( $H_{2,82} = 14.80$ ,  $P = 0.001$ ;  $M > LF$ , with  $U = 600.5$ ,  $n_1 = 31$ ,  $n_2 = 28$ ,  $P < 0.001$ ;  $M > NLF$  with  $U = 485.0$ ,  $n_1 = 31$ ,  $n_2 = 23$ ,  $P = 0.010$ ; Figs. 3a and 3b). Bycaught LFs and NLFs did not show any significant difference in their estimated diet ( $U = 657.0$ – $805.0$ ,  $n_1 = 28$ ,  $n_2 = 23$ ,  $P > 0.05$ ; Figs. 3a and 3b). Consequently, all females were grouped together to look at diet differences among years.

Within females, hoki contributed more than 8% (median mass) of the diet for the first 5 years, but only  $\leq 1\%$  for the combined years of 2005–2006 for both simulations individual prey ( $H_{5,51} = 16.75$ ,  $P = 0.005$ ; year 2005–2006 < year 2004, with  $U = 125.0$ ,  $n_1 = 12$ ,  $n_2 = 8$ ,  $P = 0.002$ ; year 2005–2006 < year 2001, with  $U = 123.0$ ,  $n_1 = 12$ ,  $n_2 = 8$ ,  $P = 0.003$ ) and mean prey ( $H_{5,51} = 24.36$ ,  $P < 0.001$ ; year 2005–2006 < all previous years, with  $U = 91.0$ – $139.0$ ,  $n_1 = 12$ ,  $n_2 = 6$ – $9$ ,  $P = 0.002$ – $0.005$ ; Figs. 4a and 4b). Percentages of rattails showed some significant variation among years in the mean prey simulation only, with a reverse trend than that of hoki (median



**FIG. 2.**—Predicted percentages by mass ( $\bar{x} \pm 95\% CI$ ) of prey species by quantitative fatty acid signature analysis (QFASA) for 82 bycaught New Zealand sea lions (*Phocartos hookeri*) using a) the parameters 23 FAs and individual prey with different calibration coefficients (CCs) and b) CCs from Steller sea lions fed on herring (CCs-SSL/her) with the set of FAs varying and the account of prey species variability or not. CCs-GS/her and CCs-HS/her = CCs from gray seals (GS) and harp seals (HS) fed on herring; CCs-SSL/eul and CCs-SSL/mix = CCs from Steller sea lions fed on eulachon and a mixed diet, respectively. Kullback–Liebler (KL) values for each simulation are noted in parentheses. Prey with an estimated contribution <1% (opalfish and dogfish) were deleted for clarity.

of 28% mass in 2005–2006,  $\leq 9\%$  mass in previous years), but only the year 2004 showed a significant difference with 2005–2006 ( $H_{5,51} = 16.86, P = 0.005; U = 42.0, n_1 = 8, n_2 = 12, P = 0.002$ ; Fig. 4b). Proportions of scampi in the diet were tested only in the mean prey simulation and were different among years ( $H_{5,51} = 14.37, P = 0.013$ ; Fig. 4b). However, none of the post hoc tests were significant based on the Bonferroni adjusted  $\alpha$ -values. No statistical difference among years was found in the diet of Ms ( $H_{4,30} = 1.18\text{--}8.99, P > 0.05$ ). Among prey species estimated in this study, arrow squid, hoki, red cod, and scampi are commercially harvested. Estimated catches of hoki in 2005 and 2006 in the subantarctic were the lowest reported since 1989, whereas the amount of arrow squid harvested each year from 2004 to 2006 from the Auckland Islands region (named SQU6T) was higher than the amount harvested in the previous 3 years (Ministry of Fisheries 2007). These reported catches are consistent with the trends of mass percentages estimated by QFASA for hoki ( $r_s = 0.771, n = 6, P = 0.043$  with the simulation individual prey;  $r_s = 0.709, n = 6, P = 0.074$  with the simulation mean prey) and for arrow squid

( $r_s = 0.855, n = 6, P = 0.014$  with the simulation individual prey;  $r_s = 0.811, n = 6, P = 0.027$  with the simulation mean prey; Fig. 5). Scampi was not represented because the catches of this species were limited by the Total Allowable Commercial Catch (306 tonnes for the Auckland Islands region SCI6A—Ministry of Fisheries 2007), and therefore were not representative of the variation in availability.

**DISCUSSION**

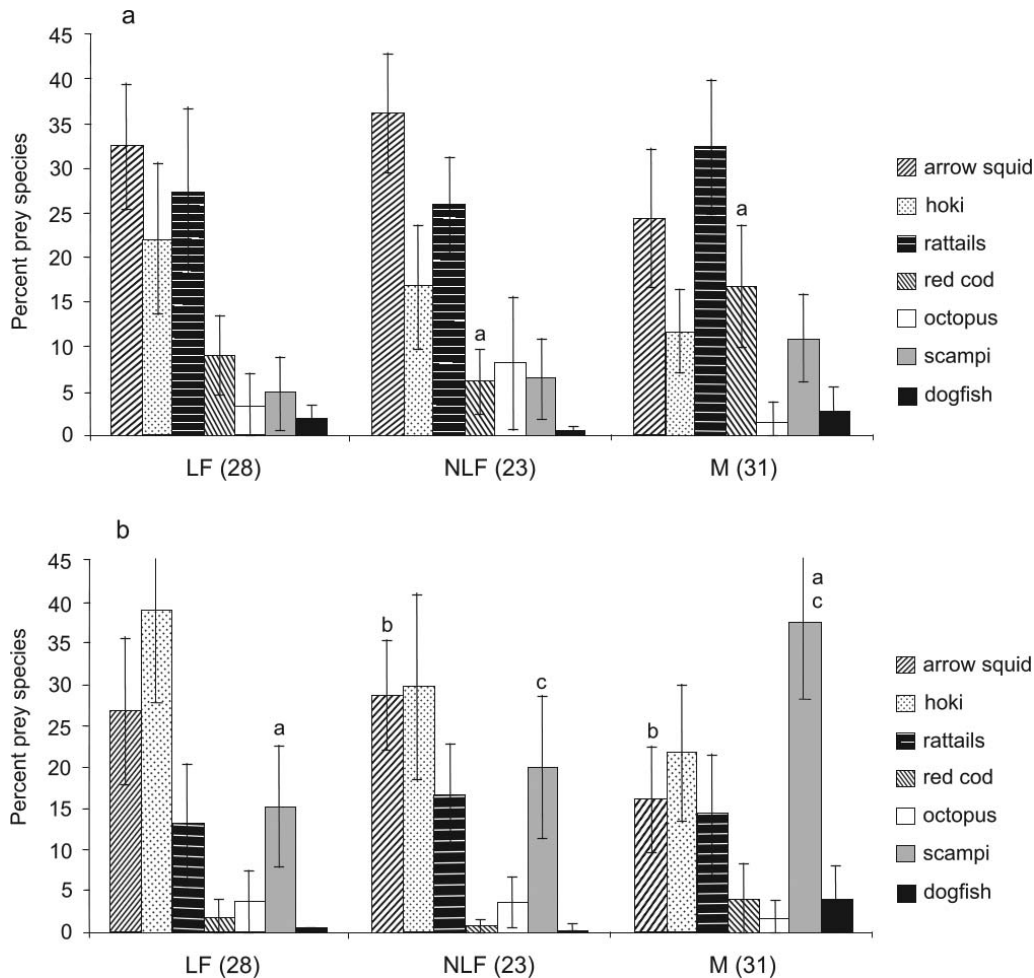
The purpose of this study was to estimate the long-term diet of NZ sea lions. Arrow squid, hoki, rattails, and red cod or scampi (depending on the QFASA simulation used) were estimated to be the major prey in the diet of NZ sea lions. Significant variation in diet estimates from both simulations was found between sexes and among years for females only. Diet estimates on bycaught animals presented here should be considered with caution, because the QFASA model is highly sensitive to the CCs, which at the present time are available for only 1 otariid species, the Steller sea lion. However, diet

**TABLE 2.**—Predicted proportions by mass of prey species by quantitative fatty acid signature analysis (QFASA) for 82 bycaught New Zealand sea lions (*Phocartos hookeri*) with the best-fit parameters from the optimization of the model (see text). O is occurrence (number of individuals for which the prey has a percentage mass >0). Values between the 25th and the 75th percentiles represent the interquartile range in which 50% of the data falls.

Prey	Simulation individual prey							Simulation mean prey						
	O	%O	$\bar{X}$	SD	Median	25th percentile	75th percentile	O	%O	$\bar{X}$	SD	Median	25th percentile	75th percentile
Arrow squid ( <i>Nototodarus sloani</i> )	77	94	30	20	28	14	46	78	95	23	20	18	6	35
Hoki ( <i>Macruronus novaezelandiae</i> )	74	90	17	18	10	2	27	72	88	29	28	27	3	48
Rattails (Macrouridae)	77	94	29	21	27	13	39	58	71	16	20	7	0	26
Red cod ( <i>Pseudophycis bachus</i> )	68	83	11	15	4	<1	18	15	18	2	8	0	0	0
Opalfish ( <i>Hemerocoetes</i> spp.)	4	5	<1	1	0	0	0	7	9	<1	1	0	0	0
Octopus ( <i>Enteroctopus zealandicus</i> )	28	34	4	14	0	0	<1	21	26	4	11	0	0	<1
Scampi ( <i>Metanephrops challengeri</i> )	50	61	8	12	1	0	8	68	83	24	25	19	1	43
Spiny dogfish ( <i>Squalus acanthias</i> )	37	45	2	5	0	0	1	10	39	2	7	0	0	<1

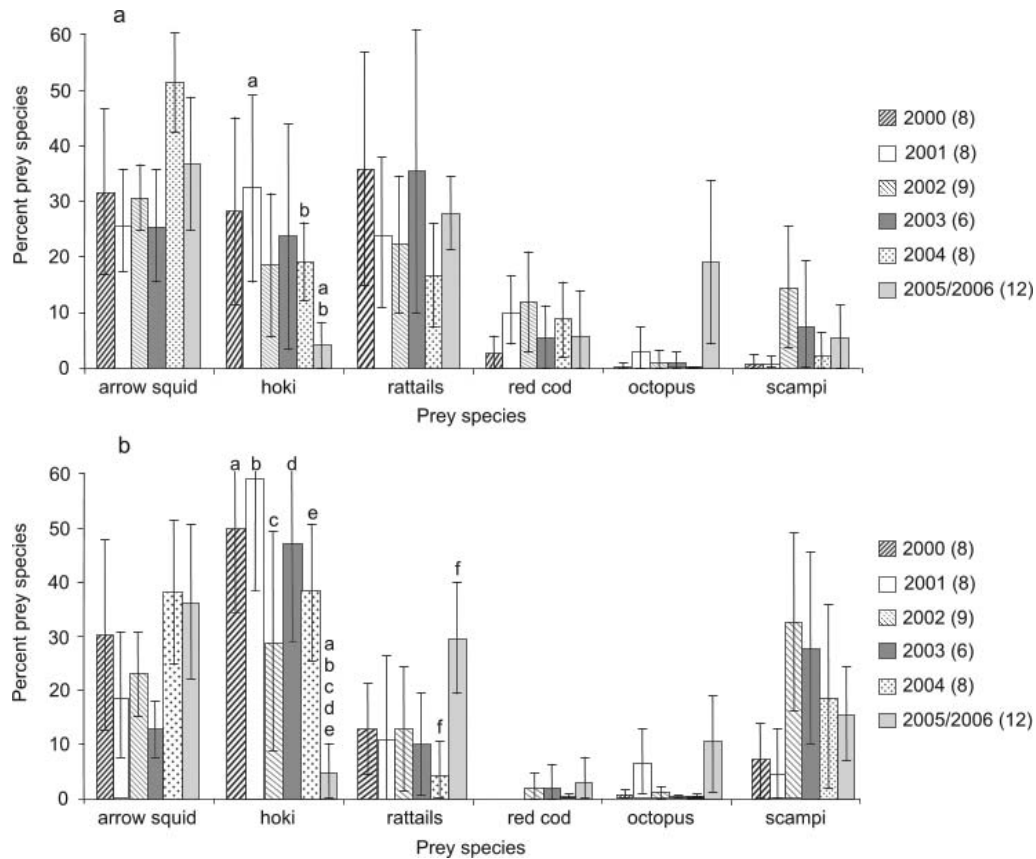
contributions of arrow squid and hoki, the major prey estimated by QFASA, showed a similar annual trend with the commercial catches of these species from 2000 to 2006, supportive of the present QFASA predictions.

The reliability of QFASA lies in the quality and accuracy of the data entered in the model. QFASA requires information on the FA profiles of the predator studied, the FA profiles and fat contents of all potential prey (prey FA library), and a measure



**FIG. 3.**—Percentages of prey species ( $\bar{X} \pm 95\% CI$ ) estimated by quantitative fatty acid signature analysis (QFASA) with the best-fit simulations a) individual prey and b) mean prey, for lactating female (LF), nonlactating female (NLF), and male (M) New Zealand sea lions (*Phocartos hookeri*). The number of samples for each category is shown in parentheses. Similar letters show significant differences between groups of sea lions.

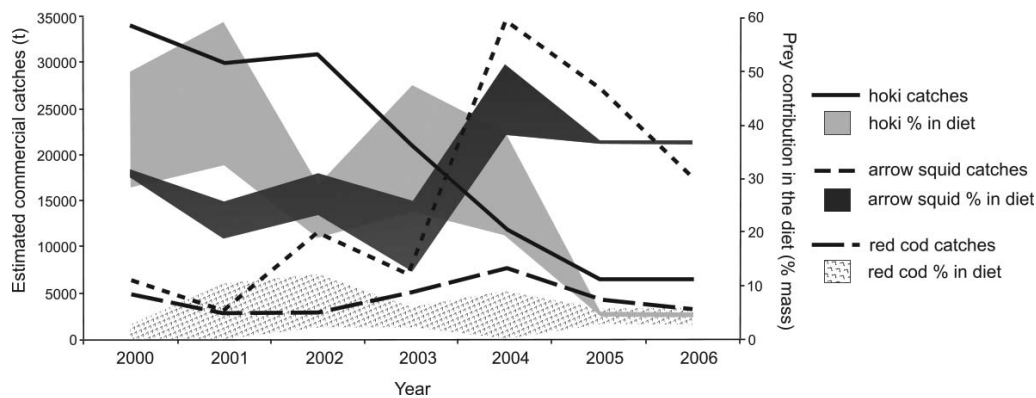




**FIG. 4.**—Between-year percentages of prey species ( $\bar{X} \pm 95\% \text{ CI}$ ) for female New Zealand sea lions (*Phocartos hookeri*) estimated by quantitative fatty acid signature analysis with the best-fit simulations a) individual prey and b) mean prey. Years 2005 and 2006 were pooled. The number of samples for each year is shown in parentheses. Similar letters show significant differences among years.

of the differential metabolism of FAs in the predator’s adipose tissue (expressed as CCs—Iverson et al. 2004). The prey FA library was composed of data from 6 species (arrow squid, hoki, javelin fish, red cod, opalfish, and octopus) identified as common in the diet of NZ sea lions from stomach analysis and 2 species (scampi and spiny dogfish) that are abundant in the Auckland Islands region (Jacob et al. 1998; O’Driscoll et al.

2003). However, it does not include all potential prey of the NZ sea lions: barracouta (*Thyrsites atun*), ling (*Genypterus blacodes*), jack mackerel (*Trachurus* spp.), warehou (*Seriolella* spp.), and the wary squid (*Moroteuthis ingens*) are minor prey in the stomach contents (Meynier et al. 2009) and were not included in the prey FA library because of a lack of material available for analysis. Also, the number of specimens



**FIG. 5.**—Estimated commercial catches (t, in tonnes) of hoki (*Macruronus novaezelandiae*), arrow squid (*Nototodarus sloani*), and red cod (*Pseudophycis bachus*) per year in comparison with the estimated proportions of these species in the diet of female New Zealand sea lions (*Phocartos hookeri*). Full areas represent the range of proportions in the diet between the simulations individual prey and mean prey. Catches of hoki were for the region HOK1 (subantarctic), catches of arrow squid for the region SQU6T (Auckland Islands), and catches of red cod for the region RCO3 (east of New Zealand’s South Island and subantarctic—Ministry of Fisheries 2007).

analyzed per prey species might not represent the variation of FA profiles among years and seasons. This study must be seen as a 1st step in using QFASA for NZ sea lions, and the inclusion of more prey individuals will improve future applications of QFASA on this marine mammal.

The FA profiles of the individual prey gave better optimizations (smaller KL medians) than the mean profiles of prey groups. Other studies using QFASA included the prey species variability by resampling the prey library using a bootstrap procedure (Beck et al. 2007a; Iverson et al. 2004), yet, in our study resampling mean FA profiles of prey did not show any significant differences in KL values compared with no resampling (L. Meynier, pers. obs.), probably because the number of individual prey available in each group was small.

Another prerequisite of QFASA is the accounting of lipid metabolism and deposition in the predator's adipose tissue, which is expressed by the CCs in the model. CCs are certainly the most challenging parameter to obtain for QFASA because they are calculated from captive animals fed on a controlled diet for several months. Even if the pattern of FA metabolism expressed by CCs is similar among marine species for which long-term diet studies were carried out (Iverson et al. 2004, 2006, 2007), the CC for a particular FA seems to depend on the family (i.e., Otariidae or Phocidae) or species (Iverson et al. 2004; Tollit et al. 2006) of the predator, or even on the type of meals eaten by the same predator (Tollit et al. 2007b). This study showed that the model was very sensitive to different sets of CCs, with diet estimates switching from a highly dominant species (>70% mass) to several major species depending on the CCs used. The different sets of CCs calculated from the Steller sea lion (herring, eulachon, and mixed diet) gave a large variation in diet estimates. Although CCs from Steller sea lions fed on herring showed the best simulations, diet estimates using CCs based on eulachon or mixed diets were comparable to the simulation without any consideration of lipid metabolism (no CCs). The better performance of CCs on herring perhaps is due to this fish having a FA profile closer than eulachon or a mixed diet to the mean FA profile of NZ sea lion prey (Tollit et al. 2007b). However, the lipid content of herring ( $9.9\% \pm 2.8\%$ ) is greater than that of the common prey of the NZ sea lions (overall mean of  $4.2\% \pm 2.5\%$ —Meynier et al. 2008b). Other than FA composition, the lipid content of food plays a role in the metabolism and deposition of FAs (Cooper et al. 2005; Summers et al. 2000). One might expect that CCs calculated from a sea lion species fed on a prey with a close FA composition, and also a lipid content similar to that of NZ sea lion prey, would give better diet estimates. Because of the current status of the population of the NZ sea lion ("vulnerable in decline"—International Union for Conservation of Nature 2009), it currently is not conceivable to have NZ sea lions in captivity for feeding experiments.

Despite these numerous uncertainties QFASA estimates of prey exploited commercially showed annual variation consistent with trends of commercial catches, providing confidence in the selected QFASA simulations to estimate the diet of this

generalist predator. In comparison to traditional diet methods, QFASA estimates are not affected by biases related to digestion and allow diet inference integrated over several weeks or months. However, when specific CCs are not available, comparison with other long-term diet methods, or with prey availability in the case of a generalist predator, or both, is needed.

Among the 8 potential prey species that QFASA computed using the best-fit model simulations (CCs-SSL/her, 23 FAs, individual prey or mean prey), arrow squid, hoki, rattails, and red cod or scampi were estimated to be the major prey in the diet of NZ sea lions bycaught during the 1st half of the lactating period (January–May). The contribution of each prey in the diet varied with the simulation used, with, by order of importance, arrow squid, rattails, hoki, and red cod for individual prey simulation; or hoki, scampi, arrow squid, and rattails for mean prey simulation.

Arrow squid and hoki have been described as significant prey by mass in the stomach contents of the same animals analyzed previously (Meynier et al. 2009). Because stomach analysis and QFASA are associated with different time frames of dietary intake (several days versus several months, respectively), individual diet estimates from both methods were not compared, and variation between overall diet estimates was not tested statistically. Scampi was estimated as the 2nd prey of importance after hoki in the mean prey simulation. Crustaceans already have been recorded in the diet of NZ sea lions at the Auckland Islands by stomach and scat analyses as a minor prey taxon (Childerhouse et al. 2001; Meynier et al. 2009), but the efficiency of traditional methods to estimate the contribution of crustaceans in the diet is limited, because this taxon has no hard part resistant to digestion. Scampi live on the benthos at depths ranging from 200 to 500 m (Ministry of Fisheries 2007). These depths are regularly visited by LF NZ sea lions, which are considered the deepest divers of all female otariids (Chilvers et al. 2006). Rattail fishes (Macrouridae) and hoki are demersal species, living in abundance on the shelf slope from 200 m to >1,000 m deep (Beentjes et al. 2002; Ministry of Fisheries 2007). Arrow squid are present from the surface to 500 m, but large densities occur at depths > 250 m (Jackson et al. 2000). Meynier et al. (2009) suggested the importance of the edge of the Auckland Islands shelf as a feeding ground where arrow squid and rattails are found in abundance. That arrow squid, rattails, and hoki were estimated as major prey by the best-fit QFASA simulations emphasizes this previous result over a longer time frame and is in agreement with the known foraging behavior of LFs at the beginning of the lactation period (Chilvers et al. 2005). Thus, the slopes of the Auckland Islands shelf are critical foraging areas for NZ sea lions, not only after the breeding season (January–February—Chilvers et al. 2005) but probably for the 1st half of the nursing period (January–May). The slopes are likely to be the closest areas from the breeding sites to provide enough concentrated food for sea lions to optimize their energy investment per foraging trip. This study is limited to animals caught in the arrow squid fishery, and whether the contribution

of arrow squid in the long-term diet of such animals is overestimated compared to the long-term diet of the rest of the population can be questioned. Arrow squid occur in abundance at the edges of the Auckland Islands shelf in austral summer and autumn (Jackson et al. 2000), and NZ sea lions are considered generalist predators (Childerhouse et al. 2001; Lalas 1997; McMahon et al. 1999; Meynier et al. 2009). Therefore, we believe that arrow squid are an important prey for all sea lions foraging at the edges of the shelf at depth >200 m.

According to QFASA, NLFs ate more arrow squid than Ms did, and less red cod or scampi, depending on the simulation. The diet of LFs differed from that of Ms by a lesser contribution of scampi in the mean prey simulation. These sex differences prevail if most of the FA variation comes from diet. Part of the FA variation between sexes likely originates from differential rates of metabolism and deposition of ingested FAs into adipose tissue due to different energetic needs between Ms and females and between different reproductive statuses. This was not considered in the QFASA model because the same CCs were applied to all individuals. In a previous study FA profiles of NZ sea lions differentiated between sexes but were similar between LFs and NLFs (Meynier et al. 2008a). Because QFASA used these FA profiles to compute diet estimates, it is logical to find similar trends in this study. Meynier et al. (2008a) found that the most important FAs driving the segregation between sexes were 20:1n-9 and 22:1n-11 in higher proportions in females and 16:3n-4 in higher proportions in Ms. Arrow squid have low values of 16:3n-4 compared to other species in the prey library, and high values of 20:5n-3 (Meynier et al. 2008b), an FA in higher concentration in the blubber of females than that of Ms (Meynier et al. 2008a). Accordingly, the QFASA model estimated more arrow squid in the diet of bycaught NLFs; 16:3n-4 is positively correlated with scampi (Meynier et al. 2008b). In the simulation mean prey scampi contributed largely to the diet of Ms, with 38% of mean prey mass. This is consistent with Ms diving deeper than female otariids (Staniland and Robinson 2008; Weise 2006), although this trend has not been confirmed for NZ sea lions. However, because the role played by different rates of FA metabolism on the variation of FA profiles between sexes is not known, it is currently difficult to make conclusions about the long-term diet of M and female NZ sea lions without foraging and diving data from Ms. To date, foraging and diving studies have focused on LF NZ sea lions (Chilvers et al. 2005, 2006; Gales and Matlin 1997).

Within females, variation in the diet from 2000 to 2006 involved differences in the proportions of hoki and rattails in the diet, depending on the QFASA simulation performed. Only hoki showed variation for both individual prey and mean prey simulations, with a smaller contribution in 2005–2006, and rattails showed a larger contribution in 2005–2006 than in the previous year for the mean prey simulation. Both of these changes involved the years 2005–2006 and probably were not detected in Ms because only 1 M was sampled during these combined years. The trends of mass percentages of hoki and arrow squid estimated by QFASA are consistent with the reported commercial catches from 2000 to 2006.

The QFASA has been used to provide long-term estimates of diet of several marine species (Beck et al. 2007a; Iverson et al. 2006, 2007). Nevertheless, the necessity for CCs that take into account FA metabolism in the predator presently limits the use of this method to a narrow range of species. When specific CCs are not available, QFASA should be compared to other long-term dietary methods or prey availability to test the feasibility of its estimates for generalist predators. This study tested the sensitivity of QFASA parameters on diet estimates of bycaught NZ sea lions by using the CCs currently available for pinnipeds. Despite the uncertainty in the accuracy of the match between the selected CCs and the true FA metabolism of NZ sea lions at the time of sampling, annual variation of QFASA predictions was highly consistent with the trends of commercial catches, providing some confidence in the selected CCs and the estimated diet. However, the predicted diets of Ms and females should be interpreted with care, because QFASA do not take into account the possible differential rates of FA metabolism between sexes. Arrow squid, hoki, rattails, and possibly scampi were estimated to make large contributions by mass in the long-term diet of NZ sea lions. All are demersal species found mainly at depths >200 m, being harvested by NZ sea lions on the slopes of the Auckland Islands shelf. This area was shown to be a foraging ground for LF NZ sea lions just after the breeding season (Chilvers et al. 2005). Our study suggests that the slopes are visited regularly during the 1st half of the lactating period (January–May). Future research on the FA metabolism on otariids and using a greater prey library will improve the reliability of QFASA on NZ sea lions.

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