# Feeding ecology of the New Zealand sea lion (*Phocarctos hookeri*)

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# Abstract

## Feeding ecology of the New Zealand sea lion (*Phocarctos hookeri*)

The New Zealand (NZ) sea lion *Phocarctos hookeri* is the only pinniped endemic to NZ with a population of approximately 12,000 individuals. Its breeding range is currently restricted to NZ sub-Antarctic islands, and it has failed to recolonise its pristine distribution around the NZ main islands despite its protection since 1881. The current hypothesis is that the population growth of this pinniped is limited by the distribution of suitable prey on the Auckland Islands (50°30'S, 166°E) shelf, and by the direct and indirect pressure exerted by the arrow squid *Nototodarus sloani* fishery. However, this hypothesis has not been fully tested to date as there has been limited information on the diet of the NZ sea lion and their potential prey. The objective of this thesis is to analyse the diet of NZ sea lions over several years with particular emphasis on the most reproductively important segment of the population: lactating females.

This thesis provides the first quantification by percentage mass of the diet of NZ sea lion using a combination of stomach content analysis, qualitative fatty acid (FA) analysis, and quantitative FA signature analysis (QFASA). Stomach contents and blubber FAs were analysed from 121 individuals incidentally caught (by-caught) in the southern arrow squid fishery from the years 1997 to 2006. The blubber FAs of 78 free-ranging lactating females captured at Enderby Island, Auckland Islands, were also examined during January and February of 2000 to 2005.

Data obtained from both stomach analysis and QFASA indicate that arrow squid, rattails Macrouridae, hoki *Macruronus novaezelandiae* and red cod *Pseudophycis bachus* are key prey species for NZ sea lions in the Auckland Islands region. Because these prey species live mostly at depths greater than 200 m, lactating females must undertake long foraging trips and dive regularly to greater depths than other sea lion species. Data from QFASA indicates that this foraging pattern is conducted over an extended period through the summer and autumn. The daily food requirement of a lactating female was estimated by a simple energetic model to be greater than 20% of its body mass. During years of low arrow squid recruitment such as 1999 and 2001, the

amounts of squid required by the NZ sea lion population may have been similar to the amount harvested by the fishery, suggesting that resource competition is likely to occur between the arrow squid fishery and NZ sea lions in years of low squid abundance.

Half of the fishing activity of the southern squid fishery occurs in the north of the Auckland Islands shelf where NZ sea lions forage, leading to incidental captures every year. This research emphasises that management of the NZ sea lion must not only consider the direct interactions with the arrow squid fishery, but also the likelihood of food resource competition between the fishery and NZ sea lions.

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Last, I shall dedicate this work to my parents who gave me their support to follow my dreams since my childhood, and to my fabulous knight for his encouragement, understanding, and affection.

# List of Abbreviations

| | |
|---|---|
| CC | Calibration coefficient |
| FA | Fatty acid |
| FAMES | Fatty acid methyl esters |
| Im | Immature |
| K-W | Kruskall Wallis (test) |
| LF | Lactating female |
| Ma | Male |
| MUFA | Monounsaturated fatty acid |
| M-W | Mann Whitney (test) |
| NLF | Non-lactating female |
| NZ | New Zealand |
| PUFA | Polyunsaturated fatty acid |
| QFASA | Quantitative fatty acid signature analysis |
| SAFA | Saturated fatty acid |
| TAG | Triacylglycerol |

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# 1 **CHAPTER 1**

# GENERAL INTRODUCTION AND LITERATURE REVIEW

**Photo:** Female New Zealand sea lions *Phocarctos hookeri* sleeping at Sandy Bay, Enderby Island, Auckland Islands

## 1.1 GENERAL INTRODUCTION

Understanding the feeding ecology of apex predators within marine ecosystems is crucial to measure their impact on lower trophic levels, *i.e.,* top-down control effects (Bowen 1997; *e.g.,* Boveng *et al.* 1998; Estes *et al.* 1998). Of particular interest to managers are the trophic interactions between protected apex predators such as marine mammals, and commercially exploited food resources. Information on such interactions requires research on marine mammals' diet and foraging energetics (*e.g.,* Furness 2002; Santos *et al.* 2004b; Butler *et al.* 2006; Cornick *et al.* 2006). Furthermore, the availability of prey and their quality will influence the foraging distribution of marine mammals and can affect their fitness (*i.e.,* bottom-up control effects). Thus studying the diet and subsequently the energetics of marine mammal populations that are declining can give a better understanding of the mechanisms causing negative effects on population growth and individual fitness (*e.g.,* Trites and Donnelly 2003; Winship and Trites 2003; Trites *et al.* 2007b).

The New Zealand (NZ) sea lion *Phocarctos hookeri* has a breeding range restricted to the NZ sub-Antarctic islands (Chilvers *et al.* 2007b). Despite its protection since 1881, this species has failed to recolonise its pristine distribution around the NZ main islands (Childerhouse and Gales 1998). To date, the causes impeding the growth of the NZ sea lion population remain unclear (*e.g.,* Chilvers *et al.* 2006; Chilvers 2008b). The current hypothesis is that the population growth is limited in part by bottom-up forces (*i.e.,* prey distribution and quality) and in another part by top-down effects (*i.e*., accidental capture by fisheries). The main objective of this thesis was to investigate the role of bottom-up forces by studying the diet of NZ sea lions.

The aim of this chapter is to introduce the thesis in a broader context, by 1) reviewing the biology and ecology of the NZ sea lion, and 2) reviewing the current methods used to study the diet of marine mammals. An outline of the thesis is presented at the end of this chapter.

## 1.2 NZ SEA LION BIOLOGY

### 1.2.1 Distribution

The NZ sea lion is endemic to NZ, with the present distribution of breeding sites limited to the NZ sub-Antarctic Islands (Gales and Fletcher 1999; Chilvers *et al.* 2007b). Prior to human colonisation, this species was distributed along the coasts of NZ main islands (Worthy 1994; Gill 1998), and had been significantly reduced in numbers and in range by subsistence and commercial sealing (Childerhouse and Gales 1998). Despite full protection in 1881, the NZ sea lions have failed to recolonise their former breeding range (Childerhouse and Gales 1998). More than 99% of the current breeding range is restricted to the Auckland Islands (50°30'S, 166°E) and Campbell Island (52°30'S, 169°E) (**Fig. 1-1**) (Chilvers *et al.* 2007b). The Auckland Islands alone host 86% of the annual pup production, distributed on three islands: Enderby (19%), Dundas (64%) and Figure of Eight (3%) (**Fig. 1-1**) (Chilvers *et al.* 2007b). Several females have been observed giving birth in the south east of the South Island of NZ each year, which is considered as a sign of re-colonisation of the species' former range (Childerhouse and Gales 1998; Gales and Fletcher 1999; McConkey *et al.* 2002). Female NZ sea lions display a high site fidelity and philopatry, while males breed in different locations and tend to disperse in areas distant from the Auckland Islands after the breeding season (Robertson *et al.* 2006; Chilvers and Wilkinson 2008). The highly restricted distribution of the NZ sea lion has led to the classification of the species as "vulnerable in decline" by the International Union for the Conservation of Nature (Gales 2008) and as "threatened" under the NZ Threat Classification System (Hitchmough *et al.* 2007).

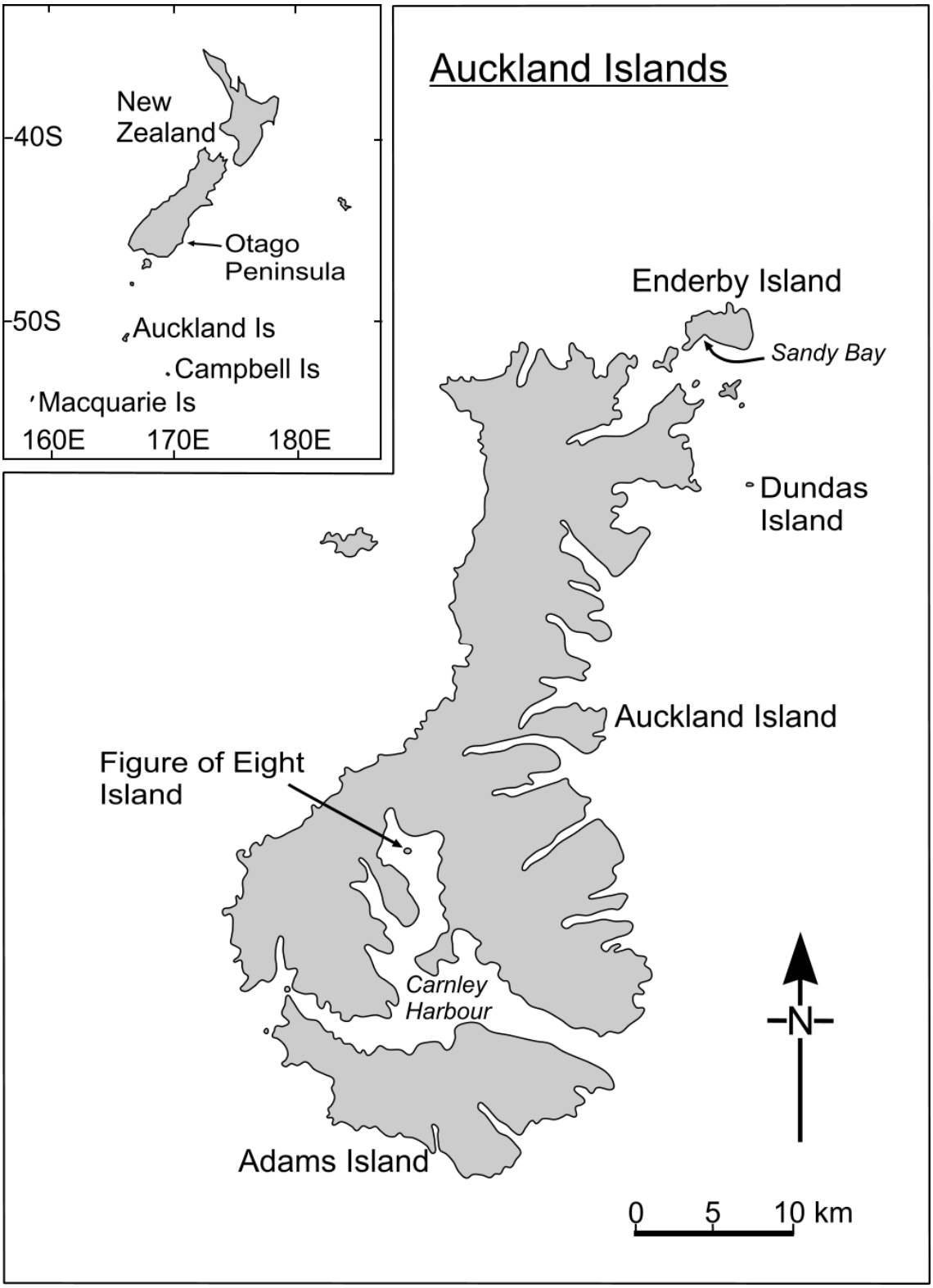


**Figure 1-1.** Auckland Islands showing the main breeding areas of New Zealand sea lions: Enderby Island, Dundas Island, and Figure of Eight Island. Inset: New Zealand's subantarctic. Map originally created by the Department of Conservation, Wellington.

### 1.2.2 Abundance and trends

Historic records suggest that the population of NZ sea lions has remained static since the mid-20th century (Taylor 1971; Childerhouse and Gales 1998). During the summers 1994/1995 and 1995/1996, the population size of NZ sea lions was estimated from the annual number of pups, and reached 11700 and 12500 individuals respectively (Gales and Fletcher 1999). Since then, pup production at the Auckland Islands has been assessed annually, and shows a significant decline in the past decade (**Fig. 1-2**) (Chilvers *et al.* 2007b).

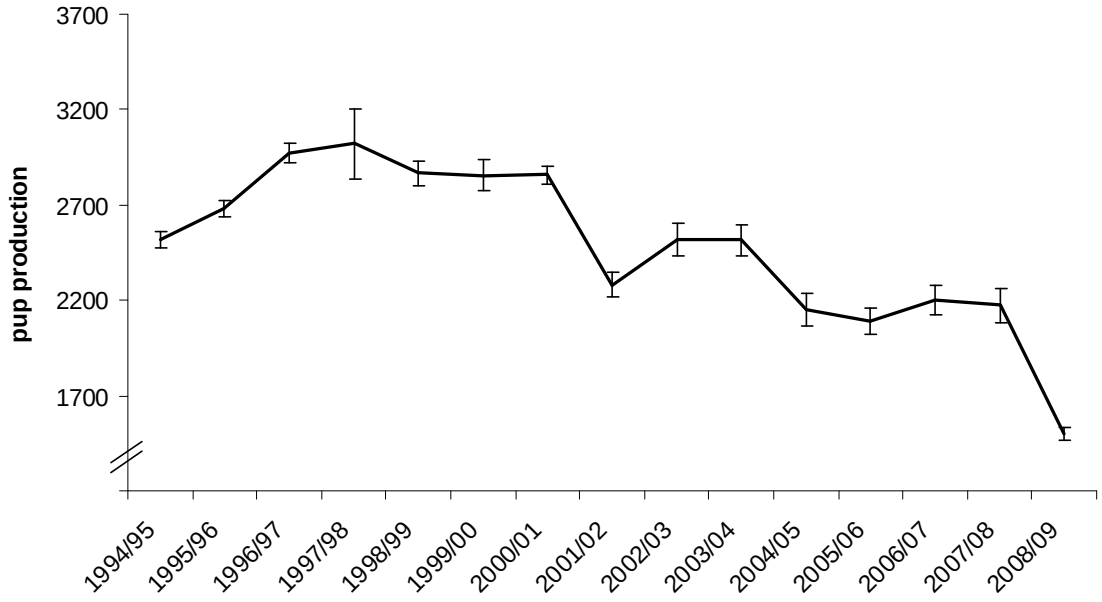


**Figure 1-2.** Annual pup production (mean ± 95% confidence intervals) of New Zealand sea lions at the Auckland Islands. Data from Chilvers *et al.* (2007b) for 1994/95 to 2003/04, and from B.L. Chilvers unpubl. data for the last three seasons.

During the summer 1997/98, an unusual mortality event, associated with the bacteria *Campylobacter*, caused the death of 53% of the pups born that summer, and at least 75 adults (Baker 1999). Two further epizootics occurred in 2001/2002 and 2002/2003, and impacted pups only with a mortality of 32 and 21% respectively (Wilkinson *et al.* 2006). *Klebsiella pneumoniae* has been implicated in these last two events, which was the first report of this pathogen causing an epizootic (Wilkinson *et al.* 2006). Serum analysis revealed that adults were immune but maternal antibodies were not transferred to pups (Castinel *et al.* 2008). The origin of this pathogen is still unknown but it has been hypothesised that adult males, which migrate between the Auckland Islands and

South Island (Robertson *et al.* 2006), could have been the vectors of *Klebsiella* from mainland into the population at the Auckland Islands (Wilkinson *et al.* 2006; Castinel *et al.* 2007b). These epizootics have reduced the number of adults being recruited in the breeding pool during the following years, and contributed to the decline of the pup production observed in the last decade (Wilkinson *et al.* 2006; Chilvers *et al.* 2007b).

### 1.2.3 Breeding biology and lactation

The breeding season of NZ sea lions starts in December each year when most of the pregnant females arrive at the rookeries already occupied by males (**Fig. 1-3**) (Gales and Fletcher 1999). Females form harems, attended by a dominant bull which can fast for several weeks until the end of the oestrous period (end of January). Pupping occurs soon after the arrival of females between mid-December and mid-January (**Fig. 1-3**) (Gales and Fletcher 1999; Chilvers *et al.* 2007b). The females make their first post-partum foraging trip to sea within two weeks of giving birth (Gales and Fletcher 1999; Chilvers *et al.* 2007a). During lactation (which lasts approximately 9 months), females alternate between feeding trips at sea for two to three days and nursing their pup ashore for one to two days (Cawthorn *et al.* 1985; Chilvers *et al.* 2005).

Although the breeding behaviour detailed above is relatively common among sea lions and fur seals, NZ sea lions show low reproduction and lactation performance compared to other otariid species. Indeed, it is estimated that only 67% of mature female NZ sea lions (> 3-4 years of age; Cawthorn *et al.* 1985; Duignan *et al.* 2003) have pups (Childerhouse 2008), whereas the reproductive rate is > 70% for Australian sea lions *Neophoca cinerea* (Higgins and Gass 1993) and California sea lions *Zalophus californianus* (Melin 2002), and between 60 and 75% for Steller sea lions *Eumetopias jubatus* (Pitcher and Calkins 1981; York 1994). Moreover, the milk is of low lipid content, with a mean of 19.9% recorded between 1997 and 2005 (Riet-Sapriza 2007). The lipid concentration in the milk of other otariid species was reported to be ≥ 25% (Higgins *et al.* 1988; Gales *et al.* 1996; Werner *et al.* 1996; Arnould and Hindell 1999).

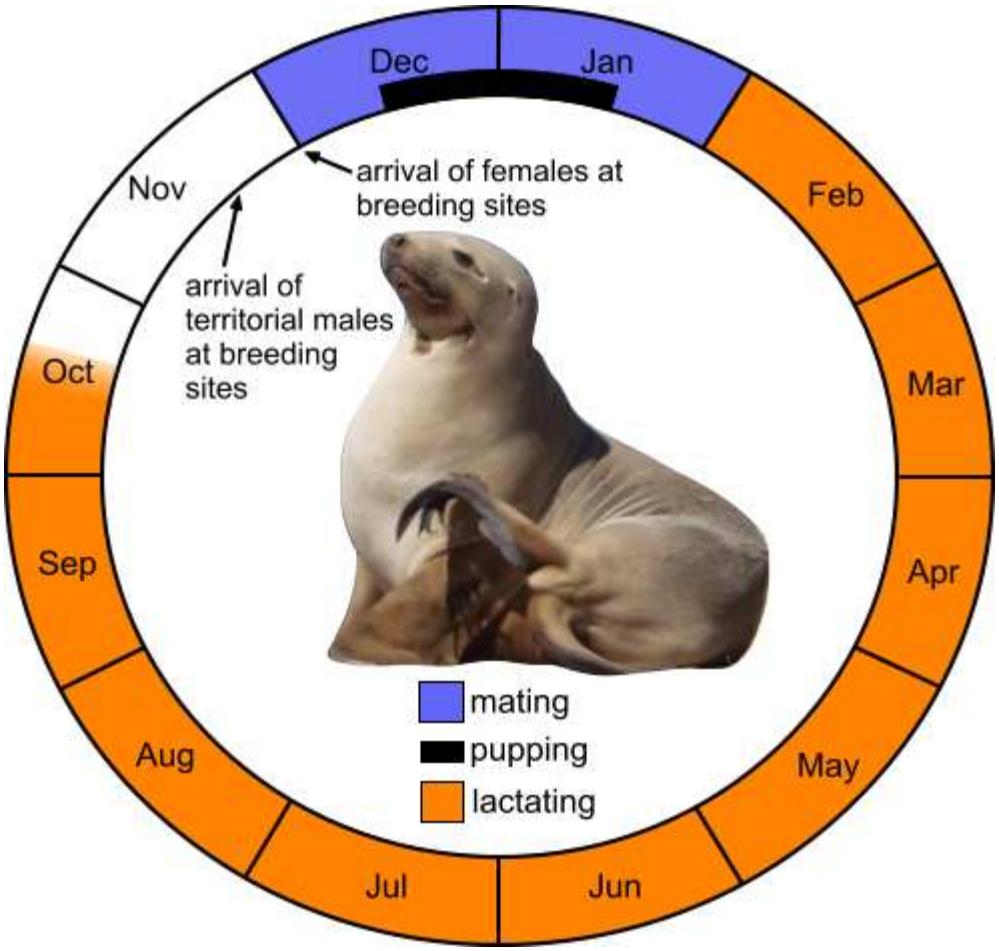


**Figure 1-3.** Life cycle of the New Zealand sea lion.

### 1.2.4 Management of the squid fishery at the Auckland Islands

Arrow squid *Nototodarus* spp has been commercially harvested since the late 1970s in NZ waters. A trawl fishery for arrow squid *Nototodarus sloani* developed in the 1980s around the Auckland Islands (50°30'S, 166°E), where arrow squid was considered a more attractive resource than in the main islands' waters because it could be caught with little finfish by-catch (Ministry of Fisheries 2007). This fishery operates on the Auckland Islands shelf between February and May each year (Gales 1995), which corresponds to the first months of the lactation period of the NZ sea lion. Early observations of NZ sea lion's feeding habits suggest that squid comprises a significant part of its diet (Cawthorn *et al.* 1985), leading to captures of sea lions in squid trawl nets. Since 1988, government observers have been placed on approximately 20% of the squid fleet every year to monitor the number of by-caught sea lions (Wilkinson *et al.* 2003). To minimise accidental captures, the NZ government set several management

controls on the southern squid fishery. A zone of no fishing was established in 1982 around the Auckland Islands out to 12 nautical miles, which became a marine mammal sanctuary in 1993 and a marine reserve in 2003 (Wilkinson *et al.* 2003; Chilvers 2008b). Despite the protected areas around the Auckland Islands, by-catch of NZ sea lions continued. This led the NZ government to impose a Fishing Related Mortality Limit (FRML) in 1993, providing a management tool to close the area to further fishing activity once this level of sea lion by-catch is reached (Wilkinson *et al.* 2003). In 1997, trawling nets were modified by installing a sea lion exclusion device (SLED) allowing sea lions to escape the net. Nonetheless, up to 118 sea lions a year were estimated to be caught by the southern arrow squid fishery since (Wilkinson *et al.* 2003; Baird 2005a, b). The main limitations of the current management of NZ sea lion by-catch are that the current marine reserve around the Auckland Islands does not protect the key foraging areas of NZ sea lions (Chilvers 2008b), and that SLEDs do not currently prevent sea lions from getting trapped in trawling nets (Baird 2005a, b; Smith and Baird 2007).

### 1.2.5 Foraging and feeding behaviour

During lactation, females alternate foraging at sea and nursing their pups on land. A foraging trip generally lasts two to three days during which females from Enderby Island forage in areas north of the colony to the edges of the Auckland Islands shelf (Chilvers *et al.* 2005). Lactating NZ sea lions have been reported to be the deepest- and longest-diving otariid (maximum of 597 m and 14.5 min) (Gales and Mattlin 1997; Chilvers *et al.* 2006). They dive continuously while at sea with a mean depth of 130 m during their foraging trips, and exhibit different diving patterns (benthic *versus* mesopelagic) depending on the region of the Auckland Islands shelf they exploit (Chilvers *et al.* 2006; Chilvers and Wilkinson 2009). Fidelity to a particular foraging location is high for females as they display the same foraging pattern within and between years (Chilvers *et al.* 2005; Chilvers 2008a).

When diving, lactating NZ sea lions exceed their calculated aerobic dive limits (cADL) in 68% of all dives (Chilvers *et al.* 2006), which is higher than reported in other otariids except in the Australian sea lion (Feldkamp *et al.* 1989; Boyd and Croxall 1996; Costa and Gales 2003). Despite a large oxygen storage capacity (Costa and Gales 2000), NZ sea lions operate at their physiological limits, and it has been hypothesised that the

Auckland Islands shelf represents a marginal foraging environment (Gales and Mattlin 1997; Costa and Gales 2000; Chilvers *et al.* 2006).

It must be stressed that all the previous studies on the foraging and diving behaviours of NZ sea lions were conducted on lactating females in the first weeks of the lactation period (austral summer), and it is currently not known if the results presented above are valid in winter or are shared by the other members of the population (*i.e.*, adult males, non-lactating females, juveniles).

The only information on the diet of NZ sea lions at the Auckland Islands to date is the work published by Childerhouse *et al.* (2001) who analysed faeces and regurgitates collected at Enderby Island (**Fig. 1-1**) between 1994 and 1997. They reported a variety of benthic, demersal and pelagic species known to inhabit waters > 300 m deep, which is consistent with the reported deep diving of NZ sea lions (Gales and Mattlin 1997; Chilvers *et al.* 2006).

### 1.2.6 Summary of NZ sea lion biology

Despite a distribution limited to remote locations, the NZ sea lion has been the subject of numerous studies this last decade (reviewed above), which permits a better understanding of the potential mechanisms impeding the growth of the population. The growth rate of pups, the milk fat content delivered to pups and the reproductive rate of females are lower than those reported in other otariids (Chilvers *et al.* 2007a; Riet-Sapriza 2007; Childerhouse 2008), while foraging and diving studies showed that lactating females dive beyond their aerobic dive limit at a higher rate than recorded in other species (Gales and Mattlin 1997; Chilvers *et al.* 2006). Furthermore, the breeding sites at the Auckland Islands have been affected by three epizootics during the last decade, which are still impacting the number of adults being recruited in the breeding population, and contribute to the decline of the pup production (Baker 1999; Wilkinson *et al.* 2006; Chilvers *et al.* 2007b).

It has been hypothesised that the low and declining number of the NZ sea lion population and its low reproductive success are due to the Auckland Islands shelf being a marginal environment (Gales and Mattlin 1997; Costa and Gales 2000; Chilvers *et al.* 2005, 2006), where the rookeries are far from the abundant and predictable resources that are deep off the shelf. Therefore, a detailed study of the diet of the NZ sea lion

would help to test this hypothesis. To date, only a qualitative estimate of the NZ sea lion's diet is available from the analyses of faeces and regurgitates collected between 1995 and 1997 (Childerhouse *et al.* 2001).

## 1.3 METHODS FOR DIETARY STUDIES ON MARINE MAMMALS

Studying the diet of marine mammals is particularly challenging as they spend part, if not their entire life, in the marine environment with most of the feeding events occuring below the surface. Therefore, researchers rely on indirect methods to study the diet of marine mammals, such as the analyses of stomach contents, faeces and regurgitates. These methods are named "traditional", in contrast to "new" methods such as DNA extraction from faeces, FA signatures of body lipids, or stable isotopes in various tissues, which have been increasingly used in the last two decades. None of these methods are perfect, and each has advantages and disadvantages which are discussed in the following sections. **Table 1-1** summarises the main characteristics for each method.
Other methods occasionally used in diet studies include the fitting of video cameras on animals or the direct observation of surface feeding. They are not detailed here as they give limited information on the diet.

**Table 1.1.** Pros and cons of the main methods used to examine the diet of marine mammals. The term "regression" means the relationship between hard part measurement and length or mass of individual prey. HP refers to hard parts; IS to isotopic signatures; TL to trophic levels; FA to fatty acids; and QFASA to quantitative fatty acid signature analysis.

| Methods | Impact on individual | Dietary time period | Cost | Identification of prey | Prey size estimate | Mass percentage (needed in food consumption models) | Requirements | Expected limitations |
|---|---|---|---|---|---|---|---|---|
| Faeces, hard remains | No | Short | Low | Yes (reference collection needed) | Yes (length regressions needed) | Yes (mass regressions needed) | - Reference collection<br>- regressions<br>- correction factors for loss and size reduction of HP | - individual characteristics generally unknown<br>- differential prey digestion and retention (prey without HP not represented, prey with fragile HP underestimated, large beaks underestimated) |
| Regurgitates, hard remains | No | Short | Low | Yes (reference collection needed) | Yes (length regressions needed) | Yes (mass regressions needed) | - Reference collection<br>- regressions | - individual characteristics generally unknown<br>- differential prey digestion and retention (overestimation of large HP) |
| Stomachs, hard remains | Extreme (from dead animals) | Short | Low | Yes (reference collection needed) | Yes (length regressions needed) | Yes (mass regressions needed) | - Reference collection<br>- regressions | - differential prey digestion and retention (prey with fragile HP underestimated, opposite with large HP or beaks)<br>- information from dead animals only, so representation of whole population uncertain |
| Faeces, DNA extraction | No | Short | High | Yes (genetic data on prey needed) | No | Possible? | - primers of prey | - prey species not in the range of tested primers are not identified |
| Stable isotopes | Moderate (capture or dart projector) | Short to long depending on the tissue | Moderate | No but possible estimation if IS of prey | No | No | - IS from lower TL | - limited in estimation of prey species<br>- interpretation of comparison between different environments and time-scales difficult without IS from lower TL |
| FA signatures | Moderate (capture or dart projector) | Short to long depending on the tissue | Moderate | Possible if QFASA (prey FA library needed) | No | Possible if QFASA (prey FA library needed) | - prey FA library and calibration coefficients for QFASA | - time frame not known precisely<br>- FA metabolism not known precisely and can be underestimated<br>- species not in prey FA library are not identified |

### 1.3.1 "Traditional" diet methods: faeces and stomach analyses

Analysis of prey hard parts from faecal samples is a common technique for estimating the diet of pinnipeds (*e.g.,* Prime and Hammond 1990; Thompson *et al.* 1991; Cherel *et al.* 1997; Casaux *et al.* 2003; Pierce and Santos 2003; Hume *et al.* 2004; Page *et al.* 2005a; Littnan *et al.* 2007; Trites *et al.* 2007a) since faeces are easy to collect on haul out sites, can be collected without disturbance on animals, and their analysis is inexpensive (Tollit *et al.* 2006). However, estimating the proportion and the size of prey from hard part remains is not straightforward, and includes well recognised biases such as differential rates of food passage and digestion (see earlier reviews, Pierce and Boyle 1991; Bowen 2000). Sagittal otoliths of fish and lower beaks of cephalopods are the most commonly used structures to identify such prey (Pierce and Boyle 1991). However, otoliths are subject to erosion by digestive juices and can be completely digested (Jobling and Breiby 1986; Murie and Lavigne 1986; Jobling 1987; Dellinger and Trillmich 1988). Thus fish consumed would not necessarily be recovered in faeces, especially if it is a species with small otoliths which digest faster than large ones (Pierce and Boyle 1991; Christiansen *et al.* 2005). In contrast, beaks are not affected by digestion (Harvey 1989; Gales and Cheal 1992; Tollit *et al.* 1997), but large beaks tend to accumulate in stomachs, as they do not pass through the pylorus (Bigg and Fawcett 1985; Yonezaki *et al.* 2003). Therefore, the proportion of fish with fragile otoliths and the proportion of cephalopods with large beaks are underestimated in scat analysis. Moreover, since mass quantification involves the back-calculation of prey size from measurements of otoliths and beaks, the partial digestion of otoliths prevents accurate estimation of the original fish size. Experimental feeding studies on captive seals (eared and true seals) aimed at reducing the biases from otolith loss and size reduction by the calculation of numerical correction factors and digestion rates (da Silva and Neilson 1985; Prime and Hammond 1987; Dellinger and Trillmich 1988; Harvey 1989; Gales and Cheal 1992; Cottrell *et al.* 1996; Fea and Harcourt 1997; Tollit *et al.* 1997; Bowen 2000; Orr and Harvey 2001; Cottrell and Trites 2002; Staniland 2002; Tollit *et al.* 2003; Tollit *et al.* 2004; Casper *et al.* 2006; Grellier and Hammond 2006; Tollit *et al.* 2007). In addition to the use of otoliths to identify and quantify fish intake, four feeding experiments on harbor seals (Cottrell *et al.* 1996) and on Steller sea lions (Cottrell and Trites 2002; Tollit *et al.* 2003; Tollit *et al.* 2004) considered a series of fish bones as diagnostic structures which reduced the probability of missing a fish species, and

improved fish recovery rates (Tollit *et al.* 2003) and size estimates (Tollit *et al.* 2004). A common finding over all the feeding studies is the high variability in otolith and bone recovery rates between individuals, even from the same species feeding on the same diet. Recovery rates vary with the size and shape of otoliths (Harvey 1989; Pierce *et al.* 1993; Tollit *et al.* 1997; Staniland 2002), whether or not they are encapsulated in skulls (Murie and Lavigne 1986), with the meal size (Marcus 1998) and composition (Dellinger and Trillmich 1988; Tollit *et al.* 2004), the seal activity (Helm 1984; Tollit *et al.* 2003), and the seal species (Helm 1984; Gales and Cheal 1992). Thus it is difficult to apply correction factors derived from captive experiments to wild species, which are more active and eat a more diverse food than captive animals.

There is still debate over how effectively scat samples can give a good estimation of prey proportions (see Casper *et al.* 2006), especially in otariids which tend to have lower recovery rates than phocids due to a longer digestive tract (Helm 1979). Several authors state that the analysis of faecal samples can properly estimate the occurrence and numerical proportion of prey providing large numbers of scats (generally > 100) are analysed (Dellinger and Trillmich 1988; Hammond and Rothery 1996; Sinclair and Zeppelin 2002; Trites and Joy 2005; Tollit *et al.* 2007). Nonetheless, Gales and Cheal (1992) and Casper *et al.* (2006) found very low fish recovery rates (< 10%) in feeding experiments with otariids. They questioned the validity of scat analysis in wild animals at least for the species studied (*Arctocephalus forsteri*, *A. tropicalus*, *Neophoca cinerea*), and stressed the need for using complementary diet methods.

Stomach analysis is hampered by biases similar to scat analysis (Pierce and Boyle 1991; Pierce *et al.* 2004a), and is widely used to study the diet of cetaceans, as the collection of faeces in the marine environment is difficult and logistically demanding. The majority of the studies on stomach contents of cetaceans rely on opportunistic sampling of dead animals from stranding or by-catch (accidentally captured in nets) events (*e.g.*, Ford *et al.* 1998; Pierce *et al.* 2004b; Santos *et al.* 2004b; De Pierrepont *et al.* 2005; Spitz *et al.* 2006; Pusineri *et al.* 2007; Meynier *et al.* 2008a; Meynier *et al.* 2008b; Mintzer *et al.* 2008). However, the diet of dead animals is not necessarily representative of the feeding of the population (Pierce and Boyle 1991; Pierce *et al.* 2004a). Strandings can be biased towards sick animals, whose diet is likely to be different from healthy individuals. Furthermore, the recent diet of animals incidentally captured in commercial

fisheries can be biased towards the targeted species of that fishery (Pierce and Boyle 1991; Pierce *et al.* 2004a). If an animal ingested food just before death, fresh material (named the fresh fraction) will be found in the stomachs among digested prey from previous feeding events (named digested fraction) (*e.g.*, Pusineri *et al.* 2007). If the fresh fraction is analysed alone, it can provide an unbiased estimate of actual intake because all items are ingested over a restricted period and are not subject to digestion. However, the fresh fraction from by-caught animals tends to be biased towards the targeted species of the fishery. In the digested fraction, beaks tend to accumulate (Bigg and Fawcett 1985; Yonezaki *et al.* 2003), although some are regurgitated as seen in pinnipeds (Fea and Harcourt 1997; Lalas 1997; Childerhouse *et al.* 2001; Hume *et al.* 2004) and dolphins (Silva-Jr. *et al.* 2004).

Due to the numerous biases associated with faeces and stomach analyses, there is a general consensus that these methods should be applied together with other methods, such as genetic analysis on scat material, stable isotope analysis or FA analysis (*e.g.*, Casper *et al.* 2006; Tollit *et al.* 2007), which overcome some of the problems encountered in hard part analysis (developed in next sections). Nonetheless, traditional techniques from stomachs and faeces are the only diet methods that provide an estimate of the prey size (**Table 1-1**). Although they do not require specialised equipment and are inexpensive, they require a strong experience and rigor from the analyst and an access to a comprehensive reference collection of diagnostic hard parts.

### 1.3.2 DNA extraction from scat samples

The application of DNA-based techniques to identify prey species from pinniped scat samples was initiated by the need to accurately assess the impact of pacific harbor seals (*Phoca vitulina richardsi*) on endangered salmonid populations in the north-east Pacific (Orr *et al.* 2004; Purcell *et al.* 2004; Kvitrud *et al.* 2005). Identification of salmonid species using conventional hard part analysis from scat samples is limited as salmonid otoliths are fragile, and salmonid bones cannot be differentiated between species (Purcell *et al.* 2004). Alternatively, genetic tools were successfully used to identify different salmonid species from bone remains in scats of pacific harbor seals (Orr *et al.* 2004; Purcell *et al.* 2004; Kvitrud *et al.* 2005). These DNA methods rely on the recognition of DNA sequences unique to prey, and requires the design of specific

markers (*e.g.*, Jarman *et al.* 2004). Parsons *et al.* (2005) and Matejusová *et al.* (2008) went further in the identification of salmonid species from phocid faeces by analysing the soft material of scat. These two studies not only showed that DNA techniques can help the identification of remains from prey with no species-specific structures (*e.g.*, salmonid bones), but also that these techniques can detect prey for which remains are soft and completely digested in the gut (*e.g.*, crustaceans, zooplankton) or can detect prey for which remains are retained in the stomach (squid beaks).

The identification of prey from the soft material of scat was further developed for otariids by feeding experiments with captive Steller sea lions *Eumetopias jubatus* (Deagle *et al.* 2005; Deagle and Tollit 2007) and captive fur seals *Arctocephalus forsteri* and *A. tropicalis* (Casper *et al.* 2007). The diet fed to captive animals comprised several species of fish and squid in different amounts, and the results based on hard part analysis and DNA-based analysis were compared (Deagle *et al.* 2005; Casper *et al.* 2007). The probability of detecting a prey was always higher with the DNA method (Deagle *et al.* 2005; Casper *et al.* 2007). Recently, Deagle and colleagues (2005, 2007) investigated the potential of DNA analysis in faeces to estimate the diet quantitatively by comparing proportions of prey species in the diet, and proportions of prey species DNA in faeces. DNA analysis identified the dominant species in the diet, but other fish species were not well quantified. The authors conclude that the denaturation of DNA varies according to the prey species, limiting the possibility of quantification without the application of correction factors. However, obtaining such factors will require extensive feeding experiments (Casper *et al.* 2007; Deagle and Tollit 2007).

The methods used in the identification of DNA from scat material are multiple, from the application of conventional polymerase chain reaction (PCR) amplifications followed by restriction fragment length polymorphism (RFLP) (Purcell *et al.* 2004; Parsons *et al.* 2005) or denaturing gradient gel electrophoresis (DGGE) (Deagle *et al.* 2005), to the application of real-time PCRs (Casper *et al.* 2007; Deagle and Tollit 2007; Matejusová *et al.* 2008), which offer a greater potential in prey quantification. To date, the methodology is still in development.

### 1.3.3 Stable isotopes

Diet information provided from faeces and stomach contents is limited to what the animal ate in the days preceding sampling. Biochemical methods such as stable isotopes and fatty acid (FA) analyses allow the inference of a broader diet picture by analysing tissues which retain assimilated nutrients for up to years (*e.g.*, stable isotopes in bones) and overcome the biases related to hard part recovery and digestion. Stable isotope analysis can give an insight into the trophic relations and sources of feeding of consumers since there is a predictable relationship between the isotopic composition of a predator and its prey. The isotopes generally used are those of nitrogen ($^{14}N$ and $^{15}N$) and carbon ($^{12}C$ and $^{13}C$), and they are expressed as δ values, *i.e.*, parts per thousand differences from a standard. $\delta^{15}N$ and $\delta^{13}C$ refer to the ratio of the heavy isotope to the more abundant light one, *i.e.*, $^{15}N/^{14}N$ and $^{13}C/^{12}C$, compared to the same ratio in the standard. The reference ratios (standards) used are calibrated to PeeDee belemnite (PDB) for $^{13}C$ and atmospheric nitrogen for $^{15}N$ (Ehleringer and Rundel 1989). Samples containing more of the heavier isotope compared to the standard are enriched, whereas those with more of the lighter isotope are depleted. Marine organisms are generally depleted in $^{13}C$ relative to PDB and thus have negative $\delta^{13}C$ values, whereas they have positive $\delta^{15}N$ values owing to a relative enrichment in $^{15}N$ in marine organisms compared to the atmospheric nitrogen.

$\delta^{15}N$ of a consumer is typically enriched by 3-4‰ relative to its diet (DeNiro and Epstein 1981; Minagawa and Wada 1984; Peterson and Fry 1987; Hobson *et al.* 1996), hence $\delta^{15}N$ serves as an indicator of the trophic level. In contrast, $\delta^{13}C$ varies little along the food chain, and is mainly used to determine the sources of primary productivity at the base of the trophic web (Fry and Sherr 1984; Peterson and Fry 1987). However, $\delta^{13}C$ varies between benthic and pelagic ecosystems (more enriched in benthic prey; Hobson *et al.* 1996; Hobson *et al.* 1997), and with latitude (more depleted towards higher latitude; Rau *et al.* 1982). Therefore, $\delta^{15}N$ and $\delta^{13}C$ provide a two-dimensional estimate of the dietary niche occupied by a consumer relative to other consumers in an ecosystem. Stable isotopes have been widely used in marine mammals to infer dietary variation between different species sharing the same habitat (*e.g.*, Hobson *et al.* 1997; Lesage *et al.* 2001; Das *et al.* 2003; Zhao *et al.* 2004; Dehn *et al.* 2007), different environments (*e.g.*, Kurle and Worthy 2002; Lewis *et al.* 2006; Niño-Torres *et al.* 2006;

Mendes *et al.* 2007), sexes (*e.g.*, Niño-Torres *et al.* 2006; Tucker *et al.* 2007), stages of development (*e.g.*, Hobson and Sease 1998; Knoff *et al.* 2008; York *et al.* 2008) and different time scales (season, year, decades) (*e.g.*, Walker *et al.* 1999; Kurle and Worthy 2001; Hobson *et al.* 2004; Hall-Aspland *et al.* 2005; Cherel *et al.* 2007; Newsome *et al.* 2007; Sinisalo *et al.* 2008).

Tissues used in stable isotope studies are diverse and their choice will depend on the time-scale studied. Indeed, the turnover of each tissue varies widely from several days to the entire lifetime of the animal, thus integrating isotopic signatures over different time periods (Dalerum and Angerbjörn 2005). Tissues generally used include plasma and serum (turnover of days), skin, red blood cells and muscle (turnover of months), hair and vibrissae (turnover of a year to several years) and teeth and bones (turnover of years to the entire life) (Welle 1999). Technically, all types of tissues are suitable for stable isotope analysis, but samples such as hair, skin, blood or vibrissae which require minimal impact on the animal, are preferred in studies of wild marine mammals (Hobson *et al.* 1997; Kurle and Worthy 2001; Lesage *et al.* 2001; Zhao *et al.* 2004; Hall-Aspland *et al.* 2005; Cherel *et al.* 2007). Research on stable isotopes from cetaceans mainly involves dead carcasses from stranding or by-catch in which skin, muscle, teeth, or internal organs have been analysed (Das *et al.* 2003; Niño-Torres *et al.* 2006; Mendes *et al.* 2007; Knoff *et al.* 2008).

The use of multiple tissues of different turnovers in stable isotope studies has proven to be useful to infer the trophic position over multiple temporal scales (review in Dalerum and Angerbjörn 2005; Hobson *et al.* 1997; Kurle and Worthy 2002; Das *et al.* 2003; Sinisalo *et al.* 2008). However, one must take into account that the tissues have a specific fractionation relative to the diet (Hobson *et al.* 1996; Kurle 2002), and it is important to assess the isotopic variation between the tissues of the same animal before the inference of trophic ecology can be made. Such isotopic variation between tissues has been assessed in a captive experiment on several species of phocids (Hobson *et al.* 1996). Hobson *et al.* (1996) found $\delta^{15}N$ fractionation factors between phocid tissues and diet of +1.7‰ (blood), +2.3‰ (skin, nail), +2.8‰ (whiskers), and +3.0‰ (hair), and $\delta^{13}C$ fractionation factors of +1.7‰ (whole blood), +2.8‰ (skin, nail, hair) and +3.2‰ (whiskers). The difference in $^{15}N$ enrichment is attributed to the different types of protein and amino acid composition among tissues, since amino acids show a large

variation in their $\delta^{15}N$ isotopic signature (Macko *et al.* 1987; Fantle *et al.* 1999; Welle 1999). Similarly, variation in $^{13}C$ enrichment is due to the differential amount of proteins and lipids among tissues, as lipids are depleted in $^{13}C$ by 6‰ compared to proteins (Tieszen *et al.* 1983). To reduce $\delta^{13}C$ variability between tissues due to the lipid amount, several studies strongly advise to extract lipids before isotope analyses (Hobson *et al.* 1996; Kurle 2002; Zhao *et al.* 2006). Hobson *et al.* (1996) not only showed a differential fractionation between tissues in pinnipeds with metabolically active tissues (whole blood) having lower fractionations than inactive tissues (hair, whiskers), but also that $\delta^{15}N$ and $\delta^{13}C$ fractionations were consistent among phocid species, age groups and sex. However, it is not clear if these fractionation factors can be accurately applied on wild marine mammals other than phocids, as a captive study on northern fur seals *Callorhinus ursinus* (Kurle 2002) showed higher $\delta^{15}N$ fractionations between blood components and diet (+4.1‰ to +5.2‰) than reported in phocids. Nonetheless, the isotopic variation between tissues is lower than the primary fractionation between diet and tissues, thus analysing a suite of tissues is still useful for studying the feeding pattern of wild animals over different time periods (Kurle and Worthy 2002; Dalerum and Angerbjörn 2005)

Another means of examining temporal variation in trophic position using stable isotopes is to compare successive sections of a tissue with a progressive growth pattern such as teeth in pinnipeds and odontocetes (toothed cetaceans) or baleen plates in mysticetes (baleen cetaceans) (review in Dalerum and Angerbjörn 2005; *e.g.,* Schell *et al.* 1989; Hobson and Sease 1998; Walker and Macko 1999; Mendes *et al.* 2007). The use of chronological sections of dentine allows the investigation of the dietary history of an animal, that be related to its age. Marine mammals are generally accessible to research during a particular time of their life cycle (*e.g.,* during breeding and molting for pinnipeds, or during migration to coastal areas for cetaceans), thus the investigation of dietary history can provide a better understanding of the ecology of marine mammals in periods when they are difficult to access. For instance, Schell *et al.* (1989) and Mendes *et al.* (2007) used carbon isotopic signatures from the baleen of bowhead whales *Balaena mysticetus* and from teeth of sperm whales *Physeter macrocephalus* respectively, to confirm the latitudinal migration of these cetaceans during certain periods of their life.

Furthermore, examination of teeth has proven to be informative of the length of the lactation period in marine mammals (Hobson and Sease 1998; Newsome *et al.* 2006; Knoff *et al.* 2008; York *et al.* 2008). Hobson and Sease (1998) found higher $\delta^{15}N$ and lower $\delta^{13}C$ in the first annulus of Steller sea lions *Eumetopias jubatus* tooth dentine relative to the dentine deposited after the first year, associating this isotopic variation to the weaning of young sea lions. Indeed, nursing neonates are feeding at higher trophic level than their mothers since they are consuming proteins from the mother's tissues, resulting in higher $\delta^{15}N$ than the post-weaning period. Low values of $\delta^{13}C$ in tissues of nursing young are associated with the composition of milk, which is depleted in $\delta^{13}C$ due to its high fat content (Tieszen *et al.* 1983). Recent studies have used this technique to detect the weaning period of California sea lions *Zalophus californianus* and northern fur seals (Newsome *et al.* 2006), bottlenose dolphins *Tursiops truncatus* (Knoff *et al.* 2008) and Steller sea lions throughout the last decades (York *et al.* 2008).

Variation in isotopic signatures through time does not mean necessarily a different diet, as variation can arise from a baseline change in isotopic ratios of the primary production (*e.g.*, seasonal upwelling) (Newsome *et al.* 2007). Thus it is important to understand the isotopic fluctuations in lower trophic levels before comparing isotopic levels of tissues sampled at different time periods and locations (Post 2002). For a similar trophic level, benthic organisms have generally higher $\delta^{15}N$ values than that of pelagic prey (Davenport and Bax 2002; Tucker *et al.* 2007). Thus, information on isotopic levels of potential prey may help the interpretation of comparative studies, and give a qualitative indication of the diet (Kurle and Worthy 2001; Lesage *et al.* 2001; Lewis *et al.* 2006; Niño-Torres *et al.* 2006; Dehn *et al.* 2007; Newsome *et al.* 2007; Tucker *et al.* 2007). $\delta^{15}N$ does not only vary with areas and seasons (Post 2002), but is also influenced by the feeding level, the diet quality and the physiological state of the animal (Hobson *et al.* 1993; Kurle and Worthy 2001, 2002; Gaye-Siessegger *et al.* 2003; Fuller *et al.* 2005; Zhao *et al.* 2006). For instance, in nutritionally stressed animals, proteins are broken down in the muscle and $^{14}N$ is mobilised. Since it is not replaced by dietary $^{14}N$, there is an increase of $\delta^{15}N$ in tissues (Hobson *et al.* 1993; Kurle and Worthy 2001; Fuller *et al.* 2005; Zhao *et al.* 2006). Pregnancy may have the opposite effect by lowering levels of $\delta^{15}N$ in tissues, although the mechanisms behind this decrease are not fully understood (Fuller *et al.* 2004). In view of the different factors affecting the levels of $\delta^{15}N$ in animal

tissues, the application of other dietary methods to complement isotopic results are necessary.

### 1.3.4 FA signature analysis

Although FA analysis is categorised as a "new method" to infer the diet, the first attempt at comparing FAs from the blubber of a marine mammal and its prey occurred in the 1960s. Ackman *et al.* (1963) compared FAs in seal blubber oil with FAs in menhaden and herring oils, and found some similarities between FA compositions. Since, several experimental studies showed that the FA composition of depot fat is influenced by dietary FA composition (Xu *et al.* 1993; Cha and Jones 1996; Kirsch *et al.* 1998; Kirsch *et al.* 2000). Consequently, FA analysis has been increasingly popular in dietary studies on marine mammals in the last twenty years, as it can overcome biases related to hard part analysis (*e.g.*, Iverson *et al.* 1997a; Iverson *et al.* 1997b; Brown *et al.* 1999; Dahl *et al.* 2000; Walton *et al.* 2000; Lea *et al.* 2002a; Bradshaw *et al.* 2003; Olsen and Grahl-Nielsen 2003; Grahl-Nielsen *et al.* 2005; Staniland and Pond 2005; Thiemann and Iverson 2007; Budge *et al.* 2008; Tucker *et al.* 2008). The underlying principle of this method is the assumption that long-chain FAs in prey species are conservatively deposited into the adipose tissue of a monogastric predator, thus providing an integrated record of dietary intake over time.

FAs consist mostly of an even-numbered chain of carbons with a carboxyl terminus at one end, and a methyl terminus at the other (**Fig. 1-4**). The number of carbons in the marine environment ranges from 12 to 24 carbons. The common forms of FAs are the triacylglycerols (TAGs), the wax esters (WEs) and the phospholipids (PLs) (Ackman *et al.* 1968). TAGs consist of three FAs esterified to a glycerol backbone and represent the main form of energy storage in adipose tissue (Patton 1975). WEs consist of a FA esterified to a fatty alcohol, and are also related to energy storage for some marine species (*e.g.*, copepods, orange roughy *Haplostethus atlanticus*, myctophids, beaked whales and sperm whales) (Grigor *et al.* 1990; Phleger *et al.* 1997; Dalsgaard *et al.* 2003; Koopman 2007). PLs consist of two FAs esterified to a glycerol which also contain a polar derivative of phosphatidic acid, and compose the structure of all cell membranes (Sargent 1976). They are less influenced by the diet than TAGs and Wes (Dalsgaard *et al.* 2003).

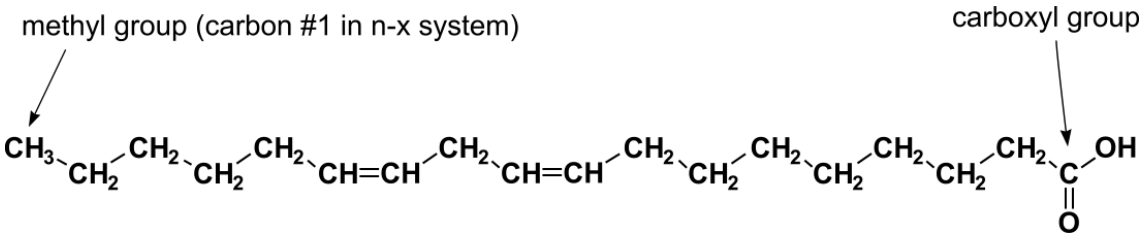


**Figure 1-4.** Chemical structure of the linoleic acid, noted 18:2n-6 (*i.e.*, 18 carbons, two double bonds, and the first double bond positioned at the 6$^{th}$ carbon from the terminal methyl group).

### *1.3.4.1 Metabolism of ingested FAs*

Uptake of dietary FAs by adipose tissue in a monogastric predator involves several steps: absorption in the small intestine, incorporation into chylomicron TAG, hydrolysis of TAG by the adipose tissue lipoprotein lipase, uptake of FAs and esterification within adipocytes. The fact that dietary FAs are not degraded during this process makes these molecules potential food markers. Early works by Iverson and colleagues (Iverson 1993; Iverson *et al.* 1995) found that dietary lipids were deposited with minimal modification in seal's blubber and milk. Experimental studies conducted since have shown that lipid deposition involves complicated processes of deposition from dietary lipids, differential metabolism, and biosynthesis *de novo* (*e.g.*, Raclot and Groscolas 1993; Raclot and Groscolas 1995; Kirsch *et al.* 2000; Summers *et al.* 2000; Budge *et al.* 2004; Iverson *et al.* 2004; Cooper *et al.* 2005; Cooper *et al.* 2006). For part of the research community led by Iverson's group, these processes can be understood and quantified by experimental studies ("deposited in a predictable manner"; Iverson *et al.* 2004) in order to apply FA analysis as a dietary method in marine mammals. For others led by Ghral-Nielsen's group, these processes cannot be quantified as they are influenced by too many factors, thus limiting the application of FAs in dietary studies (see comments and reply in Grahl-Nielsen *et al.* 2003, 2004; Thiemann *et al.* 2004a and Grahl-Nielsen 2009; Thiemann *et al.* 2009).

Depot FAs are generally classified in three categories according to their origin: 1) FAs which are readily biosynthesised by the animal (*e.g.*, 16:0, 16:1, 18:0, 18:1); 2) FAs which can be biosynthesised by the animal but are believed to come mostly from dietary origin (*e.g.*, 14:0, 20:1, some 22:1); and 3) FAs which originate only from the diet (*e.g.*, 22:1n11 and all PUFA >18 carbons with n-3 and n-6) (Holman 1986; Iverson 1993; Iverson *et al.* 2004; Cooper *et al.* 2006). This classification is based from known

metabolism of FAs in vertebrate endotherms, which is believed to be similar between species (Nelson 1992; *e.g.*, Linares and Henderson 1991; Nilsson *et al.* 1996). Recently, there has been an effort to understand FA metabolism in pinnipeds by radio-labeling techniques on captive animals (Budge *et al.* 2004; Cooper *et al.* 2006).

Most of the energy storage in marine mammals is situated in the subcutaneous fat (*i.e.*, blubber) (Iverson 2002), and can be biopsied in free-ranging marine mammals after capture or dart projectile sampling. Therefore, it is the tissue of choice for most dietary studies using FA analysis (*e.g.*, Iverson *et al.* 2004; Beck *et al.* 2005; Herman *et al.* 2005; Ruchonnet *et al.* 2006; Beck *et al.* 2007b). The potential of blubber FAs to investigate a change in the diet has been recognised in wild marine mammals by comparing similar species in different environments (*e.g.*, marine *versus* freshwater seals; Käkelä *et al.* 1993; Smith *et al.* 1996; Grahl-Nielsen *et al.* 2005) and by captive feeding studies (Kirsch *et al.* 2000; Iverson *et al.* 2004; Cooper *et al.* 2005). For example, Kirsch *et al.* (2000) investigated the blubber FA composition of captive juvenile harp seals *Phoca groenlandica* after a diet switch from herring *Clupea harengus* to pollock *Pollachius virens* (sampling at day 0 when switch occurred, at day 14 and day 30). The blubber FAs changed significantly and gradually over weeks reflecting the change in diet from herring to pollock. The authors also highlighted the fact that despite a noticeable diet change in blubber FAs, blubber FAs did not match that of the prey due to metabolic changes in the predator tissue. The degree of metabolic changes occurring in the blubber of marine mammals was investigated by comparing the FA composition of milk and of nursing young (hooded seals *Cystophora cristata*, Iverson *et al.* 1995; grey seals *Halichoerus grypus*, Grahl-Nielsen *et al.* 2000; white whales *Delphinapterus leucas*, Birkeland *et al.* 2005). Nursing young were chosen as an ideal model to investigate the transfer of dietary FAs to the blubber as the FA composition of their diet (*i.e.*, milk) can be easily sampled. Iverson *et al.* (1995) found that the FA composition of hooded seal pups resemble that of the milk ingested, concluding that there was little modification of FAs when deposited in the blubber. These results were challenged by two studies on grey seals (Grahl-Nielsen *et al.* 2000) and on white whales (Birkeland *et al.* 2005) that showed different compositions between milk and pup blubber, highlighting the importance of differential metabolism of FAs in depot lipids.

Blubber is not a uniform tissue along the body and vertically. Stratification of blubber has been reported in cetaceans (Lockyer *et al.* 1984; Koopman *et al.* 1996; Koopman 1998; Hooker *et al.* 2001; Olsen and Grahl-Nielsen 2003; Samuel and Worthy 2004; Ruchonnet *et al.* 2006; Smith and Worthy 2006; Koopman 2007; Budge *et al.* 2008; Montie *et al.* 2008), phocids (Käkelä and Hyvärinen 1996; Best *et al.* 2003; Andersen *et al.* 2004; Grahl-Nielsen *et al.* 2005; Wheatley *et al.* 2007), and otariids (Arnould *et al.* 2005). Despite different FA patterns of stratification among species, there is a common feature shared by all species studied, with lower levels of monounsaturated FAs and higher levels of saturated FAs in the inner layer of the blubber (close to the muscle) relative to that in the outer layer (close to the skin). The inner layer has been shown to be metabolically more active than the outer layer (*e.g.*, Lockyer *et al.* 1984; Montie *et al.* 2008), and to have a FA composition closest to that of the diet (*e.g.*, Olsen and Grahl-Nielsen 2003; Andersen *et al.* 2004; Grahl-Nielsen *et al.* 2005). Therefore, sampling the whole blubber core is crucial in species for which stratification exists, and can be challenging when dart techniques are used in large whales (Koopman 2007).

Moreover, lipids are not deposited in the blubber and metabolised uniformly along the body, as some body regions are more metabolically active than others (Koopman *et al.* 1996; Koopman 1998; Arnould *et al.* 2005; Mellish *et al.* 2007). For instance, Koopman (2001) distinguished the fat depot sites in the body trunk from the structural lipids in the caudal peduncle in harbour porpoises *Phocoena phocoena*. In otariids, differential deposition along the body occurs, but metabolic body sites are not well defined (Mellish (Arnould *et al.* 2005; Mellish *et al.* 2007), whereas phocids seem to present a more uniform fat distribution (Ryg *et al.* 1988; Mellish *et al.* 2007). Thus, it is critical to sample blubber tissue at a depot body site to be able to trace dietary records from blubber FAs.

Finally, rates of FA mobilisation can vary according to the nutritional and reproductive states of the animal (Käkelä *et al.* 1993; Raclot and Groscolas 1995; Kirsch *et al.* 2000; Andersen *et al.* 2004; Wheatley *et al.* 2007; Montie *et al.* 2008). Deposition of dietary FAs in adipose tissue occurs when the animal is in positive energy balance, whereas this process is limited when animals are in poor body condition (Kirsch *et al.* 2000). Furthermore, lactation can readily affect the FA composition of blubber independently of the diet, and this has been shown in phocids which fast generally during the entire

lactation period (Ackman and Jangaard 1964; Bryden and Stokes 1969; Iverson *et al.* 1995; Wheatley *et al.* 2007). FAs such as 20:5n-3 are selectively mobilised from blubber for the production of milk in the mammary gland. Thus, blubber FAs from lactating females encountering a long period of fasting may not be appropriate for diet inference (Wheatley *et al.* 2007). To my knowledge, the degree of FA mobilisation for milk production has not been investigated in otariids, but it is probably of less importance than in phocids since lactating otariids feed regularly through lactation (*i.e.*, income breeders) and fasting is limited to the perinatal period (period between parturition and first foraging trip).

Since pinnipeds lactate on land, milk is also a common "tissue" sampled for FA studies on this taxon (*e.g.*, Iverson 1993; Iverson *et al.* 1997a; Brown *et al.* 1999; Lang *et al.* 2005; Staniland and Pond 2005). It has the advantage over blubber of being less invasive during sampling, but its FA composition involves biochemical processes more complex than that of blubber. Indeed, milk fat in marine mammals originates from dietary FAs and blubber FAs selectively mobilised (Iverson 1993), and the role played by each FA source is not known. In a captive feeding study on grey seals, Grahl-Nielsen (2000) found that milk was enriched in saturated FAs, n-3 polyunsaturated FAs, and 20:1, and depleted in 14:1, 16:1 and 18:1 relative to the blubber FAs of the mothers. In another captive feeding study on Antarctic fur seals, Staniland and Pond (2004) investigated the suitability of milk to detect a dietary change. The authors found that milk could reflect a radical change in diet (fish-dominated diet in captive seals *versus* krill-dominated diet in wild seals) despite the presence of FAs selectively mobilised from blubber into the milk. However, the same authors in a study on free-ranging Antarctic fur seals (Staniland and Pond 2005) found no correlation between the dietary predictions from faecal samples and from milk FAs, and concluded that more feeding experiments were needed to understand the factors influencing milk production before the full potential of this technique can be used.

#### *1.3.4.2 Application of FA method*

There are two main ways to use FAs in dietary studies. The first and most common is to simply compare FA signatures over different time scales, locations or groups of individuals to investigate diet variation. The second is to infer the proportions of prey

by mass by comparing FA signatures of the predator and of the potential prey. Another technique reported by Budge *et al.* (2006) in their review is to infer qualitatively the diet or part of the diet by the presence in the predator's tissue of FA markers specific to a prey or prey taxon. The use of FA markers has been used mostly in fish studies (review in Dalsgaard *et al.* 2003), but their use in marine mammals is limited as no FA is specific to a particular marine mammal prey (Budge *et al.* 2006). Therefore, the use of FA markers has not been developed in my review.

The assumption behind the comparison of individual FA signatures is that different FA signatures mean different diets. FA analysis has been used this way to assess geographical (*e.g.*, Walton *et al.* 2000; Thiemann and Iverson 2007), temporal (*e.g.*, Iverson *et al.* 1997a; Lea *et al.* 2002a; Walton and Pomeroy 2003; Samuel and Worthy 2004; Beck *et al.* 2007b), sex (*e.g.*, Samuel and Worthy 2004; Beck *et al.* 2005; Smith and Worthy 2006) and ontogenetic (Beck *et al.* 2007b) variation in the diet of marine mammals. However, the influence of differential lipid metabolism on FA variation among individuals is often overlooked in the discussions of scientific papers. FA differences between individuals sampled at different locations or at different seasons will be mostly driven by differences in the diet if individuals have the same body condition and reproductive status (see metabolism of FAs in the above section). In contrast, lipid metabolism is likely to play a significant role in FA variation between females and males, or juveniles and adults because of their different energetic requirements. For instance, Beck *et al.* (2005) attributed FA variation between grey seals of different sex and age solely to diet. In a previous study, the same authors highlighted the difference of energy storage and expenditure between male and female grey seals (Beck *et al.* 2003). This difference in reproductive strategy between sexes can cause variation in diet but also variation in lipid metabolism, a last option not mentioned by Beck *et al.* (2005).

Due to the uncertainty of the importance of differential lipid metabolism in predator's FAs, and in order to ease the interpretation of dietary patterns, it is good practice to use this method in combination with other dietary techniques (*e.g.*, Iverson *et al.* 1997a; Brown *et al.* 1999; Lea *et al.* 2002a; Bradshaw *et al.* 2003; Grahl-Nielsen *et al.* 2005; Hall-Aspland *et al.* 2005; Herman *et al.* 2005; Staniland and Pond 2005; Dehn *et al.* 2007; Tucker *et al.* 2008).

The most ambitious way to use FA analysis is to estimate proportions of prey by the comparison of prey and predator FAs *via* a mathematical model. This method is named quantitative FA signature analysis (QFASA), and was initiated by Iverson *et al.* (2004). QFASA requires a FA profile library of all potential prey, and calibration coefficients for individual FAs to account for predator lipid metabolism. 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 the closest to match 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 (percentage mass).

Before the application of QFASA, one must understand the variation in FA profiles within a potential prey species (*e.g.,* Budge *et al.* 2002; Iverson *et al.* 2002), and investigate the variation in FA profiles among the prey species in the library. If there is an overlap between two species, it is advised to group them to avoid misclassification by the model. Sampling all potential prey for a given predator means a preliminary understanding of key species in an ecosystem, and of the predator's foraging habit. Therefore, the potential prey library is generally built from diet information provided by traditional methods.

Another prerequisite of QFASA is the account of lipid metabolism and deposition in the predator's adipose tissue, which is expressed by the calibration coefficients in the model. Calibration coefficients are certainly the most challenging parameter to obtain for QFASA, as they are calculated from captive animals fed on a controlled diet for several months. Even if the pattern of deposition is similar among marine species for which long-term diet studies were carried out (Iverson *et al.* 2004; Tollit *et al.* 2006; Iverson *et al.* 2007; Nordstrom *et al.* 2008), the calibration coefficient for a particular FA seems to depend on the predator's taxum (*i.e.,* otariidae, phocidae) or species considered (Iverson *et al.* 2004; Tollit *et al.* 2006), or even on the meals eaten by the same predator (D. Tollit, unpubl. data). This is an important drawback limiting the use of QFASA to species or gender for which calibration coefficients are available. QFASA is at its early stages of development, and a better understanding of the factors affecting lipid metabolism is clearly necessary before the full potential of this method can be applied. Since the first presentation of the model in 2004, QFASA has been used to estimate the prey proportions of free-ranging marine predators such as seabirds (Iverson *et al.* 2007), polar bears *Ursus maritimus* (Iverson *et al.* 2006) and grey seals (Beck *et al.* 2007a).

### 1.3.5 Summary of dietary methods

Pros and cons of each method are summarised in **Table 1-1**. None of these methods are ideal, and a combination of several methods must be encouraged. Although traditional techniques such as hard part analysis from stomachs and faeces are often regarded as strongly biased, they are the only methods to provide a size estimate of the prey, and are often needed by biochemical methods to help choose the potential prey to sample and/or to help the interpretation of isotopic and FA patterns in the predator's tissues. In fact, most of the studies on stable isotopes and FAs are compared with hard part analyses to support their conclusions. Within the new methods, DNA extraction from scat samples provides a diet picture limited to several days, whereas stable isotopes and FAs can reflect diet over long time scales, which are more relevant in terms of ecological feeding behaviour. In contrast to stable isotopes, FA analysis has the potential to qualitatively and "quantitatively" (proportions by mass) estimate the diet. Therefore, I chose FA analysis to analyse the long-term diet of NZ sea lions. Stomach samples were available from animals captured accidentally by fisheries, and were analysed to complement the results of the FA analysis. Whole prey were obtained to create a library of prey FAs, and QFASA was tested on NZ sea lions to estimate the importance of prey species in the long-term diet. The laboratory techniques used to analyse FAs vary and were reviewed in **Appendix 1** along with the difficulties encountered during the laboratory analyses, how they were dealt with, and the details of the protocol I used.

## 1.4 OUTLINE OF THE THESIS

This thesis is composed of seven chapters: Chapter 1 is the general introduction and literature review, chapters 2 to 6 are the research chapters, and Chapter 7 is the general discussion. Each research chapter is independent and written in a publication format. Chapters 2, 3 and 4 are published; Chapter 5 is in review and Chapter 6 is to be submitted. The reference with authorship is given in the front page of each chapter. Although I was assisted by my co-authors (supervisors), I designed the research, analysed the data and wrote the chapters. Since Chapters 3 to 6 are using FA analysis, there is some inevitable repetition in the introduction and method sections of these chapters.

In **Chapter 2**, I investigate the short-term diet of by-caught (incidentally captured) NZ sea lions by analysing their stomach contents. Diet variation between sex, maturity and locations were also assessed. Published as *Meynier, L., Mackenzie, D.D.S., Duignan, P.J., Chilvers, B.L., and Morel, P.C.H. 2009. Variability in the diet of New Zealand sea lion (Phocarctos hookeri) at the Auckland Islands. Marine Mammal Science, in press*

In **Chapter 3**, I investigate the long-term diet variability between by-caught female and male NZ sea lions and between years of capture, by using FA analysis on blubber tissue. Published as *Meynier, L., Morel, P.C.H., Chilvers, B.L., Mackenzie, D.D.S., MacGibbon, A., Duignan, P.J. 2008. Temporal and sex differences in the blubber fatty acid profiles of the New Zealand sea lion Phocarctos hookeri. Marine Ecology Progress Series, 366:271-279.*

In **Chapter 4**, I analyse the proximate composition (water, lipid and protein contents), energy content, and FA profiles of potential prey of NZ sea lions, and investigate the FA variation between prey species. This will allow the creation of a FA prey library necessary for QFASA on NZ sea lions. Published as *Meynier, L., Morel, P.C.H., Mackenzie, D.D.S., MacGibbon, A., Chilvers, B.L., Duignan, P.J. 2008. Proximate composition, energy content, and fatty acid composition of marine species from the Campbell plateau, New Zealand. New Zealand Journal of Marine and Freshwater Research, 42:425-437.*

In **Chapter 5**, I test the QFASA on by-caught NZ sea lions by performing a sensitivity analysis on several parameters needed for this method. After the identification of optimal parameters for NZ sea lions, the mass proportions of prey were calculated for by-caught animals.

In **Chapter 6**, I investigate the long-term diet of free-ranging lactating NZ sea lions by analysing the FAs from biopsied blubber. QFASA was also used and diet estimations were compared with results from by-caught NZ sea lions.

In **Chapter 7**, I summarise the results of the research chapters and the main interpretations in terms of the feeding behaviour and the management of NZ sea lions. I also discuss future research that should be considered for the NZ sea lion.

# 2 CHAPTER 2

# VARIABILITY IN THE DIET OF NEW ZEALAND SEA LION AT THE AUCKLAND ISLANDS, NEW ZEALAND


**Photo:** whole arrow squids (*Nototodarus sloani*) retrieved from a single stomach of a by-caught New Zealand sea lion (*Phocarctos hookeri*).

**Chapter reference:**

Meynier, L., Mackenzie, D.D.S., Duignan, P.J., Chilvers, B.L., and Morel, P.C.H. 2009. Variability in the diet of New Zealand sea lion (*Phocarctos hookeri*) at the Auckland Islands. Marine Mammal Science, *in press*

## Abstract

Stomach contents of 121 New Zealand (NZ) sea lions (*Phocarctos hookeri*) caught by the arrow squid *Nototodarus sloani* fishery were examined during the summer/autumn 1997-2006 around the Auckland Islands (50°30'S, 166°E). Dietary variation was assessed among juveniles, lactating females, non-lactating females and males, and between areas on the Auckland Islands shelf. The digested fraction of the contents consisted mostly of opalfish *Hemerocoetes* spp (50.1% by number [N], 4.7% by mass [M]), rattail *Coelorinchus* spp (12.0%N, 2.4%M), arrow squid (14.1%N, 17.9%M), octopus *Enteroctopus zealandicus* (2.1%N, 27.8%M) and red cod *Pseudophycis bachus* (3.8%N, 4.3%M). Opalfish was found in greater proportions in the stomachs of females (lactating: 58.1%N; non-lactating: 62.4%N) and juveniles (56.9%N) than males (14.5%N). Juveniles caught smaller opalfish and rattail than adults did. Over all classes, sea lions ate larger prey in the East than in the North of the Auckland Islands shelf. The common prey –arrow squid and rattail– constitute an abundant resource at the edges of the Auckland Islands shelf, where lactating NZ sea lions forage. Although these key areas are far from the rookeries and impacted by the squid fishery, they may provide the only reliable resource able to support the cost of benthic foraging behaviour in the deepest diver of all otariids.

## 2.1 INTRODUCTION

The New Zealand (NZ) sea lion (*Phocarctos hookeri*) is one of the world's rarest and most highly localised pinnipeds, classified as "Threatened" under the NZ threatened classification system, and the NZ Marine Mammals Protection Act 1978 (Hitchmough *et al.* 2007). The population size is estimated to be between 10,000 to 13,000 animals (Campbell *et al.* 2006) and has undergone a decline in pup production in recent years (Chilvers *et al.* 2007b). In addition to its low abundance, NZ sea lions have a restricted breeding range with 86% of the pup production at the Auckland Islands (50°30'S 166°E) (Campbell *et al.* 2006; Chilvers *et al.* 2007b). In the past eight years, this species has been affected by three disease epidemics, which resulted in the mortality of half the pups born in 1998, and about a third of the pups in both 2002 and 2003 (Wilkinson *et al.* 2006). These events highlight the vulnerability of this restricted population, and they are still impacting on the recruitment of mature females (Chilvers *et al.* 2007b).

Concern has arisen during the past decade over the interactions between NZ sea lions and the arrow squid (*Nototodarus sloani*) trawl fishery. This fishery operates on the Auckland Islands shelf between February and May each year (Gales 1995), which corresponds to the first months of the sea lion lactation period. Early observations of the NZ sea lion's feeding habits suggested that squid comprised a significant part of its diet (Cawthorn *et al.* 1985), leading to potential captures (by-catch) of sea lions in squid trawl nets. Since 1988, government observers have been placed on approximately 20% of the squid fleet every year (Wilkinson *et al.* 2003). The total number of by-caught sea lions is estimated by extrapolation of the number reported by the observers up to the entire fleet. It was calculated that up to 140 NZ sea lions were caught each year in fishing nets (Wilkinson *et al.* 2003; Baird 2005a, b).

Management efforts to date have focused on direct interactions, *i.e.*, by-catch, but not on the possible resource competition between the fishery and NZ sea lions. Knowledge of feeding habits of the NZ sea lion is essential to determine its trophic interaction with fisheries, and its role in the Auckland Islands ecosystem. To date, only Childerhouse *et al.* (2001) have investigated the diet of sea lions at the Auckland Islands from the analyses of faeces and regurgitates between 1994 and 1997. Two other diet studies from scat samples were carried out at sites visited by male NZ sea lions, Macquarie Island (54.5°S 159°E) (McMahon *et al.* 1999) and Otago Peninsula (South Island NZ) (Lalas 1997). In these three locations, sea lions fed on a wide variety of prey, mainly fish

species. Scat analyses are, however, known to be subject to biases such as differential retention/erosion rates of hard remains (Jobling and Breiby 1986; Jobling 1987; Harvey 1989; Tollit *et al.* 1997; Bowen 2000; Staniland 2002). Stomach analyses are hampered by biases similar to scat analyses, but to a lesser extent, as hard remains do not pass through the whole intestine. Moreover, the contents of each stomach can be directly related to the sex, age and reproductive status of the animal.

In this study, the diet of the NZ sea lion was assessed by analysing the stomach contents of animals by-caught between 1997 and 2006 in the squid trawl fishery. The data collected provided the first quantification by percentage mass of the diet of NZ sea lions. I also provide information on variation in diet among age and sex classes and between different locations on the Auckland Islands shelf was investigated.

## 2.2 MATERIALS AND METHODS

### 2.2.1 Sample collection

Since 1997, NZ sea lions incidentally captured by the squid fishery operating off the Auckland Islands (**Fig. 2-1**) have been frozen and sent to Massey University for necropsy. The whole stomach was removed and stored in a freezer at –20°C until further analysis. The sex/maturity status (immature [Im], lactating female [LF], non-lactating female [NLF] and male [Ma]) was recorded for each individual. Females without development of the mammary gland, and in the absence of *corpus luteum* or *corpus albicans* in the ovaries, were categorised as immature (Duignan *et al.* 2003). The maturity of the males was determined by histological examination of the testes: Immature males had a lack of lumen in their seminiferous tubules, a high proportion of interstitial cells, and no spermatids or spermatozoa (Duignan *et al.* 2003). Immature females and males were pooled. Age of the animals was estimated using incremental growth layers in the dentine of canine teeth (Duignan *et al.* 2003). The average body mass of Im LF, NLF and Ma was calculated since it is known to influence dive duration (Costa 1993), and thus may assist the understanding of the feeding behaviour.

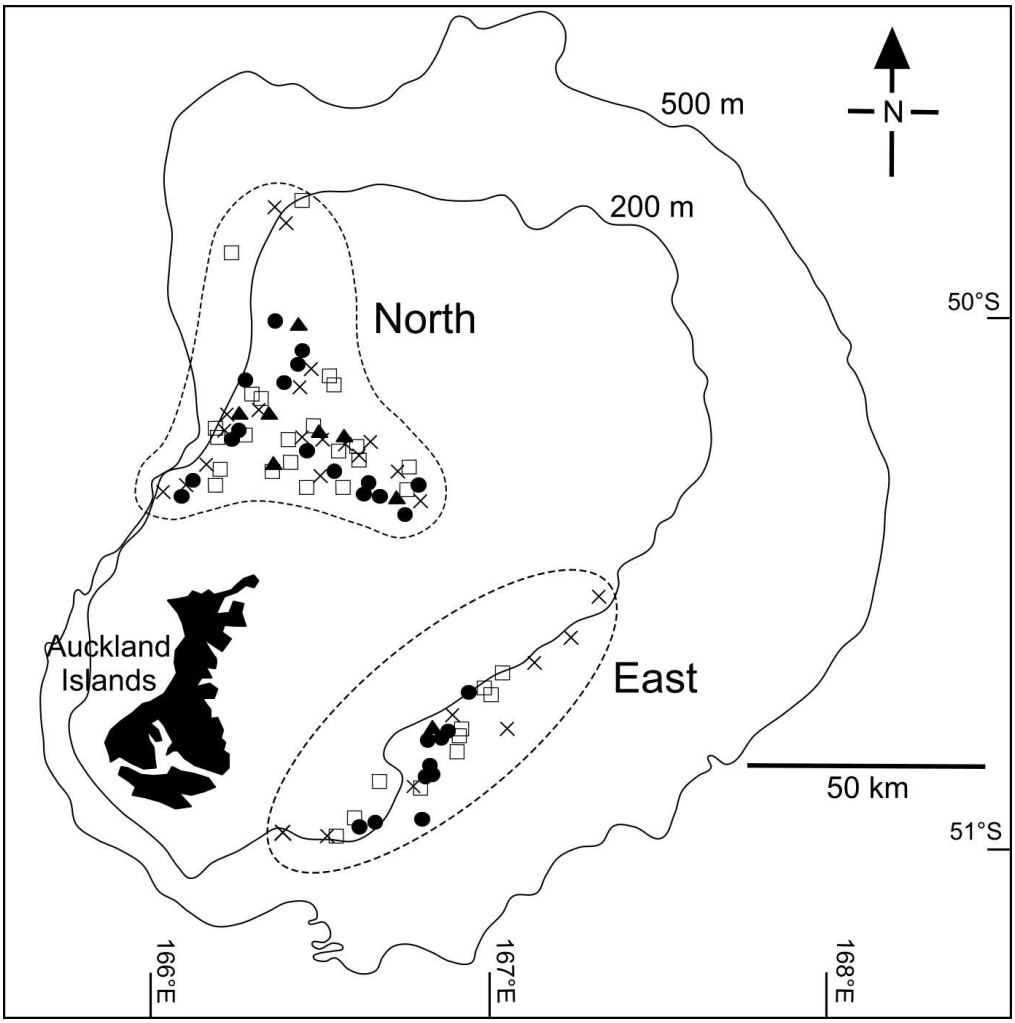


**Figure 2-1.** Location of the captures of New Zealand sea lions by the squid fishery between 1997 and 2006. Triangles are immature sea lions, solid circles are lactating females, crosses are non-lactating females and empty squares are males. Bathymetric contours are shown in black lines. The Auckland Islands shelf is delimited by the 200 m boundary. The two studied areas "North" and "East" are represented by dotted lines. Map generated from NABIS, Ministry of Fisheries, N.Z.

### 2.2.2 Stomach analysis

Stomach contents were thawed and washed through a 0.25 mm mesh sieve. Prey remains consisted mainly of whole or partially digested fish and squid, fish otoliths and bones, cephalopod beaks and eye lenses. Whole prey were identified by their morphologic traits using published guides (Nesis 1987 for cephalopods; Paulin *et al.* 1989 for fish) by technicians of the National Institute of Water and Atmospheric research (NIWA), Wellington, NZ. Fish otoliths, diagnostic bones (dentaries, premaxillaries, maxillaries, post-temporals and hyomandibulars) and cephalopod beaks

were identified using a reference collection of fish otoliths, fish bones and cephalopod beaks held at Massey University and published guides (Clarke 1986; Smale *et al.* 1995). Within the same genus, some otoliths and beaks show no or little specific difference. In such cases, the hard parts were attributed to the most abundant species occurring in the studied area. If more than one species belonging to the same genus was present in the area, the genus was noted 'spp'. This is the case for jack mackerels *Trachurus* spp (*T. declivis* or *murphyi*), warehous *Seriolella* spp (*S. brama*, *caerulea* or *punctata*), rattails *Coelorinchus* spp (*C. aspercephalus, bollonsi* or *fasciatus*), lanternfish *Diaphus* spp (*danae*, *hudsoni* or *meadi*) and opalfish *Hemerocoetes* spp (*monopterygius, artus* or *morelandi*). The sieved remains were stored in 70% ethanol except for bones and otoliths, which were kept dry.

All the animals sampled were by-caught from the arrow squid fishery and hence from a region characterised by abundant squid. This may lead to an overestimation of the squid contribution to the diet. Indeed, almost all stomachs contained fresh squid, which were ingested just before death. To minimise the overestimation of the squid contribution in the content, I distinguished a fresh and a digested fraction (Pusineri *et al.* 2007). The fresh fraction included whole prey and hard remains with some flesh attached (*e.g.*, skulls and vertebral columns) that were ingested shortly prior to death of the animal. The digested fraction consisted of remains without associated flesh (*e.g.*, free otoliths, bones and beaks), which were most likely part of meals ingested from one to several days before death (based on gut passage times estimated from feeding experiments with otariids; Bigg and Fawcett 1985; Dellinger and Trillmich 1988; Staniland 2002; Tollit *et al.* 2003). The digested fraction was assumed to be a better representation of the background diet of sea lions before they fed on squid in or close to the nets. Only this fraction was considered in the statistical analyses.

All diagnostic hard parts (otoliths, some bones, and beaks) were counted for each content, and the number of a taxon was determined as follows: The number of fish was estimated by the number of otoliths. If less than ten per taxon were present, left and right otoliths were sorted, and the highest number was taken. If more than ten, the total number was simply divided by two. The number of cephalopods was estimated by the number of upper or lower beaks, whichever was higher (Pierce and Boyle 1991).

Prey sizes and masses were estimated by measuring otolith length, or otolith width when the tip was broken, lower beak rostral length (LRL for squid) or lower beak hood length (LHL for octopods and sepiolids), and by using regressions from the literature or

my own reference collection (**Table 2-1**). When a species was represented by > 30 otoliths or beaks in a stomach, 30 to 40 were randomly picked and measured. In this case, a weighting factor (ratio of the total prey to the measured prey) was multiplied with each measure (Santos *et al.* 2004a). Only otoliths with no sign of erosion were measured in order to minimise the underestimation of size and mass.

The relative importance of each prey was estimated as the percentage of occurrence (number of stomachs in which the taxon was observed), the proportion of the total prey number in the sample set, and the proportion of the total reconstructed mass (product of the number of prey and the average body mass).

### 2.2.3 Statistical analysis

Variation among areas (North and East, **Fig. 2-1**), and sex classes (combined sex/maturity factor: LF, NLF, Im and Ma) was investigated on the number and median length of the common prey from the digested fraction. Prey were considered common when the occurrence was > 30%, and occasional when the occurrence was < 10% in the sample set.

Data in a stomach analysis consist of many zero values for each prey distribution, which makes the application of parametric tests such as ANOVA difficult. The Scheirer-Ray-Hare (SHR) test is a non-parametric equivalent of a two-way ANOVA (Dytham 1999), and was used to reveal significant differences on the number of common prey within areas and sex classes. *Post-hoc* comparisons were performed using Tukey tests. The median lengths of common prey were tested using a Kruskal-Wallis and *post-hoc* Mann-Whitney tests for sex classes, and using a Mann-Whitney test for areas. I set the statistical significance $\alpha$ at 0.10 to take into account the low statistical power of my analyses (low sample size). Tests were performed with the MINITAB package (MINITAB Release 14.1, MINITAB Inc. 2003), and all arithmetic means are followed by the standard deviation (SD).

**Table 2-1**. Regression equations used to estimate the length and mass of prey from otolith/beak measurements. HL is the hood length in mm, LRL is the lower rostral length in mm, DML is the dorsal mantle length in mm, M is the reconstructed biomass in g, OL is the otolith length in mm, OD is the longest otolith diameter in mm, SL is the standard length and TL is the total length. For these last two measurements, the unit is specified in the equations. *n* is the number of measurements used to construct the equations, *r* is the correlation coefficient. * No regression was available within the same family and a regression of a species from a related family has been used.

| Prey species | species used for length estimate | Estimated Length | *n* | *r* | Source | species used for mass estimate | Estimated Mass | *n* | *r* | Source |
|---|---|---|---|---|---|---|---|---|---|---|
| FISH | | | | | | | | | | |
| *Argentina elongata* | *A. silus* | $TL_{cm} = 3.87\ OL$ | 15 | 0.95 | (Leopold *et al.* 2002) | *A. silus* | $M = (0.16\ TL_{cm})^{3.46}$ | 7 | 0.99 | (Leopold *et al.* 2002) |
| *Brama brama* | same | $\ln TL_{mm} = 5.2987 + 0.5586 \ln OD$ | 43 | 0.787 | (Smale *et al.* 1995) | same | $\ln M = 3.5947 + 2.1934 \ln OD$ | 43 | 0.772 | (Smale *et al.* 1995) |
| *Trachurus* spp | same | $TL_{mm} = 17.82\ OL^{1.325}$ | 65 | | (Fea *et al.* 1999) | same | $M = 0.034\ OL^{4.285}$ | 44 | | (Fea *et al.* 1999) |
| *Seriolella brama* | *Seriolella* spp | $TL_{mm} = 40.475\ OL^{1.043}$ | 10 | 0.79 | L. Meynier, unpubl. data | *Seriolella punctata* | $M = 0.0193\ TL_{cm}^{3}$ | | | (Froese and Pauly 2007) |
| *Seriolella caerulea* | *Seriolella* spp | $TL_{mm} = 40.475\ OL^{1.043}$ | 10 | 0.79 | L. Meynier, unpubl. data | *Seriolella punctata* | $M = 0.0193\ TL_{cm}^{3}$ | | | (Froese and Pauly 2007) |
| *Seriolella punctata* | *Seriolella* spp | $TL_{mm} = 40.475\ OL^{1.043}$ | 10 | 0.79 | L. Meynier, unpubl. data | same | $M = 0.0193\ TL_{cm}^{3}$ | | | (Froese and Pauly 2007) |
| *Seriolella* spp | same | $TL_{mm} = 40.475\ OL^{1.043}$ | 10 | 0.79 | L. Meynier, unpubl. data | *Seriolella punctata* | $M = 0.0193\ TL_{cm}^{3}$ | | | (Froese and Pauly 2007) |
| *Congiopodus coriaceus* | *C. spinifer* | $\ln TL_{mm} = 3.799 + 1.1698 \ln OD$ | 46 | 0.923 | (Smale *et al.* 1995) | *C. spinifer* | $\ln M = -0.0974 + 3.5938 \ln OD$ | 45 | 0.919 | (Smale *et al.* 1995) |
| *Gnathophis habenatus* | *Bassanago albescens* | $\ln TL_{mm} = 4.1576 + 1.0912 \ln OD$ | 43 | 0.919 | (Smale *et al.* 1995) | *Bassanago albescens* | $\ln M = -2.1867 + 3.9603 \ln OD$ | 43 | 0.916 | (Smale *et al.* 1995) |
| *Emmelichthys nitidus* | same | $\ln TL_{mm} = 3.2242 + 1.2005 \ln OD$ | 40 | 0.983 | (Smale *et al.* 1995) | same | $\ln M = -2.4456 + 3.8311 \ln OD$ | 42 | 0.983 | (Smale *et al.* 1995) |
| *Micromesistius australis* | *M. poutassou* | $TL_{cm} = 5.65 + 2.66\ OL$ | 101 | 0.99 | (Leopold *et al.* 2002) | same | $M = 0.004688\ TL_{cm}^{3.0931}$ | 441 | 0.98 | (O'Driscoll and Bagley 2001) |
| *Thyrsites atun* | same | $\ln SL_{mm} = 3.9602 + 1.1198 \ln OD$ | 53 | 0.98 | (Smale *et al.* 1995) | same | $\ln M = -1.7389 + 3.9189 \ln OD$ | 61 | 0.994 | (Smale *et al.* 1995) |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *Gonorynchus gonorynchus* | same | ln $TL_{mm}$ = 4.4773 + 1.0094 ln OD | 25 | 0.988 | (Smale *et al.* 1995) | same | ln M = 0.7456 + 3.3375 ln OD | 25 | 0.988 | (Smale *et al.* 1995) |
| *Coelorinchus* spp | *C. aspercephalus* | $TL_{cm}$ = 1.488 $OL^{1.271}$ | | | (Holborow 1999) | *C. aspercephalus* | M = 0.011 $OL^{3.946}$ | | | (Holborow 1999) |
| *Lepidorhynchus denticulatus* | same | $TL_{cm}$ = 2.216 $OL^{1.153}$ | | | (Holborow 1999) | same | M = 0.01 $OL^{3.697}$ | | | (Holborow 1999) |
| *Macruronus novaezelandiae* | same | $TL_{mm}$ = 16.31 $OL^{1.238}$ | | | (Fea *et al.* 1999) | same | M = 0.004771 $TL_{cm}^{2.8796}$ | | | (O'Driscoll and Bagley 2001) |
| *Merluccius australis* | *Macruronus novaezelandiae* | $TL_{mm}$ = 16.31 $OL^{1.238}$ | | | (Fea *et al.* 1999) | same | M = 0.004771 $TL_{cm}^{2.8796}$ | | | (O'Driscoll and Bagley 2001) |
| *Austrophycis marginata* | *Pseudophycis bachus* | $TL_{mm}$ = 7.267 $OL^{1.625}$ | | | (Fea *et al.* 1999) | *Pseudophycis bachus* | M = 0.003 $OL^{4.979}$ | | | (Fea *et al.* 1999) |
| *Pseudophycis bachus* | same | $TL_{mm}$ = 7.267 $OL^{1.625}$ | | | (Fea *et al.* 1999) | same | M = 0.003 $OL^{4.979}$ | | | (Fea *et al.* 1999) |
| *Parapercis colias* | *Halidesmus scapularis** | ln $TL_{mm}$ = 4.2174 + 1.3009 ln OD | 93 | 0.883 | (Smale *et al.* 1995) | same | M = 0.01 $TL_{cm}^{3.1}$ | 93 | 0.875 | (Froese and Pauly 2007) |
| *Diaphus danae/hudsoni/meadi* | *D. hudsoni* | ln $SL_{mm}$ = 2.7179 + 1.0077 ln OD | 24 | 0.959 | (Smale *et al.* 1995) | *D. hudsoni* | ln M = -2.1402 + 2.487 ln OD | 23 | 0.937 | (Smale *et al.* 1995) |
| *Lampanyctodes hectori* | same | $SL_{mm}$ = 21.88 $OL^{1.239}$ | | | (Fea *et al.* 1999) | same | M = 0.123 $OL^{3.838}$ | | | (Fea *et al.* 1999) |
| *Myctophum/hygophum* | *Lampanyctodes hectori* | $SL_{mm}$ = 21.88 $OL^{1.239}$ | | | (Fea *et al.* 1999) | *Lampanyctodes hectori* | M = 0.123 $OL^{3.838}$ | | | (Fea *et al.* 1999) |
| *Scopelosaurus* spp | *Saurida undosquamis** | ln $TL_{mm}$ = 3.0124 + 1.1711 ln OD | 18 | 0.969 | (Smale *et al.* 1995) | *S. adleri* | M = 0.0009 $TL_{cm}^{3.4867}$ | 19 | 0.964 | (Froese and Pauly 2007) |
| *Genypterus blacodes* | *G. capensis* | ln $TL_{mm}$ = 2.393 + 1.563 ln OD | 67 | 0.984 | (Smale *et al.* 1995) | *G. capensis* | ln M = -6.4094 + 5.2076 ln OD | 65 | 0.984 | (Smale *et al.* 1995) |
| *Hemerocoetes artus* | *Hemerocoetes spp* | $SL_{mm}$ = 34.297 + 32.553 OL | 20 | 0.46 | L. Meynier, unpubl. data | *Hemerocoetes spp* | M = 3 $10^{-8}$ $SL_{mm}^{3.9565}$ | 20 | 0.9 | L. Meynier, unpubl. data |
| *Hemerocoetes monopterygius* | *Hemerocoetes spp* | $SL_{mm}$ = 34.297 + 32.553 OL | 20 | 0.46 | L. Meynier, unpubl. data | *Hemerocoetes spp* | M = 3 $10^{-8}$ $SL_{mm}^{3.9565}$ | 20 | 0.9 | L. Meynier, unpubl. data |
| *Hemerocoetes* spp | same | $SL_{mm}$ = 34.297 + 32.553 OL | 20 | 0.46 | L. Meynier, unpubl. data | same | M = 3 $10^{-8}$ $SL_{mm}^{3.9565}$ | 20 | 0.9 | L. Meynier, unpubl. data |

**Table 2-1**. Continued

| Prey species | species used for length estimate | Estimated Length | *n* | *r* | Source | species used for mass estimate | Estimated Mass | *n* | *r* | Source |
|---|---|---|---|---|---|---|---|---|---|---|
| FISH (*continued*) | | | | | | | | | | |
| *Neophrynichthys latus* | same | $TL_{mm}$ = -73.201 + 81.024 OL | | | S. Childerhouse, unpubl. data | *Helicolenus dactylopterus** | ln M = -3.2748 + 3.8463 ln OD | 171 | 0.982 | (Smale *et al.* 1995) |
| *Helicolenus percoides* | *H. dactylopterus* | ln $SL_{mm}$ = 2.6947 + 1.2357 ln OD | 195 | 0.977 | (Smale *et al.* 1995) | *H. dactylopterus* | ln M = -3.2748 + 3.8463 ln OD | 171 | 0.982 | (Smale *et al.* 1995) |
| SpA Scorpaenidae | *H. dactylopterus* | ln $SL_{mm}$ = 2.6947 + 1.2357 ln OD | 195 | 0.977 | (Smale *et al.* 1995) | *H. dactylopterus* | ln M = -3.2748 + 3.8463 ln OD | 171 | 0.982 | (Smale *et al.* 1995) |
| CEPHALOPODS | | | | | | | | | | |
| *Enteroctopus zealandicus* | *Octopus vulgaris* | DML = 5.39 + 24.9 HL | | | (Clarke 1986) | *Octopus vulgaris* | ln M = 1.82 + 3.03 ln HL | | | (Clarke 1986) |
| Octopoteuthidae | *Octopoteuthis* sp | DML = -0.4 + 17.33 LRL | | | (Clarke 1986) | *Octopoteuthis* sp | ln M = 0.166 + 2.31 ln LRL | | | (Clarke 1986) |
| *Nototodarus sloani* | *Nototodarus* spp (east coast) | ln DML = 4.18 + 0.788 ln LRL | | | (Clarke 1986) | *Nototodarus* spp (east coast) | ln M = 1.79 + 2.41 ln LRL | | | (Clarke 1986) |
| *Moroteuthis ingens* | same | DML = 39.61 LRL – 13.58 | 137 | 0.92 | (Jackson 1995) | same | ln M = -0.068 + 3.5 ln LRL | | | (Clarke 1986) |
| Sepiolidae | *Sepiolla* spp | DML = 5.39 + 24.9 HL | | | (Clarke 1986) | *Sepiolla* spp | ln M = 0.4 + 0.35 ln HL | | | (Clarke 1986) |

## 2.3 RESULTS

### 2.3.1 Overall diet

A total of 121 stomach contents of NZ sea lions by-caught between February and May from 1997 to 2006 were examined. Mature sea lions of both sexes were well represented in each area (**Table 2-2**). In total, mature males represented a third of the sample set, and mature females were more numerous with half of them lactating.

**Table 2-2**. Distribution of the number of stomachs of New Zealand sea lions analysed according to the by-catch location and whether they were immature (Im), lactating females (LF), non-lactating females (NLF), or males (Ma) together with their average body mass (BM ± SD). The first number in each category represents the total number of stomachs analyzed. The second number in parentheses is the number of stomachs containing some digested material (digested fraction). The difference between the two numbers is the number of stomachs containing fresh prey only.

| Category | North | East | Unknown location | **Total** | BM (kg) |
|---|---|---|---|---|---|
| Im | 8 (7) | 3 (1) | 1 (1) | 12 (9) | 91 ± 13 |
| LF | 21 (20) | 12 (12) | 1 (1) | 34 (33) | 108 ± 14 |
| NLF | 23 (23) | 8 (8) | 4 (3) | 35 (34) | 104 ± 16 |
| Ma | 22 (20) | 11 (9) | 7 (7) | 40 (36) | 167 ± 60 |
| **Total** | 74 (70) | 34 (30) | 13 (12) | 121 (112) | |

Overall, 35 different prey taxa were found with fish comprising the bulk of the diet (**Table 2-3**). Cephalopods ranked second and crustaceans occurred only sporadically. In total, 6004 diagnostic hard remains were recovered from stomach contents, which corresponded to an estimated 3627 individual prey of which 2309 were fish, 1311 squid, six crustaceans and one bird. Only nine out of 35 taxa had a frequency of occurrence > 10%. The common prey species were arrow squid (86.9% by Occurrence [O], 33.4% by Number [N], 43.0% by Mass [M]), rattail *Coelorinchus spp* (55.7%O, 10.0%N, 1.8%M), opalfish *Hemerocoetes spp* (48.4%O, 35.5%N, 2.4%M), octopus *Enteroctopus zealandicus* (38.5%O, 3.1%N, 14.4%M) and red cod *Pseudophycis bachus* (32.0%O, 3.1%N, 3.2%M). Hoki *Macruronus novaezealandiae* and hake *Merluccius australis* contributed significantly to the total mass (17.8%M) but they were present in only 16% of the stomachs.

**Table 2-3.** Composition of the New Zealand sea lion's diet expressed as: % occurrence (%O; percentage of stomachs in which the taxon was observed), % number (%N; percentage of the total prey number across all samples) and % reconstructed mass (%M; percentage of the number of prey times the average body mass) of the total content, the digested fraction only and the fresh fraction only. Bold numbers represent the principal contributions. Common and occasional prey have an occurrence > 30% and < 10% respectively.

| FRACTION (nb of stomachs) | TOTAL (121) | | | DIGESTED (112) | | | FRESH (101) | | |
|---|---|---|---|---|---|---|---|---|---|
| Diet picture | | | | 1 to 2$^{+}$ days before death | | | Just before death | | |
| **FISH** | %O | %N | %M | %O | %N | %M | %O | %N | %M |
| **Argentinidae** *Argentina elongata* (silverside) | 3.3 | 0.3 | 0.1 | 2.7 | 0.3 | 0.2 | 1.0 | 0.3 | 0.1 |
| **Bramidae** *Brama brama* (sea bream) | 0.8 | < 0.05 | 0.2 | 0.9 | < 0.05 | 0.4 | | | |
| **Carangidae** *Trachurus* spp. (jack mackerels) | 8.2 | 0.7 | 3.0 | 7.1 | 0.7 | 2.6 | 5.9 | 0.6 | 1.6 |
| **Centrolophidae** *Seriolella* spp. (warehou) | 4.1 | 0.2 | 1.0 | 2.7 | 0.1 | 1.2 | 2.0 | 0.3 | 1.1 |
| **Congiopodidae** *Congiopodus coriaceus* (pigfish) | 11.5 | 0.7 | 0.1 | 9.7 | 0.6 | 0.1 | 4.0 | 0.8 | 0.1 |
| **Congridae** *Gnatophis habenatus* (silver conger) | 1.6 | 0.1 | 0.1 | 1.8 | 0.1 | < 0.05 | | | |
| **Emmelichthydae** *Emmelichthys nitidus* (redbait) | 0.8 | < 0.05 | < 0.05 | 0.9 | < 0.05 | < 0.05 | | | |
| **Gadidae** *Micromesistius australis* (S$^{t}$ blue whiting) | 2.5 | 0.1 | < 0.05 | 1.8 | 0.1 | < 0.05 | 1.0 | 0.1 | < 0.05 |
| **Gempilidae** *Thyrsites atun* (barracouta) | 7.4 | 0.5 | 4.6 | 5.3 | 0.5 | 5.8 | 5.0 | 0.4 | 3.0 |
| **Gonorynchidae** *Gonorynchus gonorynchus* (sand fish) | 0.8 | < 0.05 | < 0.05 | | | | 1.0 | 0.1 | 0.1 |
| **Macrouridae** *Coelorinchus* spp (rattails) | **55.7** | **10.0** | 1.8 | **54.0** | **12.0** | 2.4 | 10.9 | 4.3 | 0.7 |
| *Lepidorhynchus denticulatus* (javelin fish) | 9.0 | 3.3 | 0.4 | 7.1 | 4.3 | 0.7 | 4.0 | 1.8 | 0.1 |
| Unidentified Macrouridae | 1.6 | 0.3 | 0.6 | 1.8 | 0.1 | 0.9 | | | |
| Total Macrouridae | 59.0 | 13.6 | 2.8 | 57.5 | 16.4 | 4.0 | 11.9 | 6.1 | 1.0 |
| **Merlucciidae** *Macruronus novaezelandiae* (hoki) | 11.5 | 2.2 | **9.6** | 12.4 | 3.1 | **15.5** | 1.0 | 0.5 | 2.4 |
| *Merluccius australis* (hake) | 4.1 | 0.3 | 7.4 | 3.5 | 0.3 | 3.7 | 2.0 | 0.2 | 4.0 |
| Unidentified Merlucciidae | 1.6 | < 0.05 | 0.8 | 1.8 | 0.1 | 2.6 | | | |
| Total Merlucciidae | 16.4 | 2.5 | 17.8 | 15.9 | 3.5 | 21.8 | 3.0 | 0.7 | 6.4 |
| **Moridae** *Austrophycis marginata* (dwarf cod) | 6.6 | 0.9 | < 0.05 | 7.1 | 1.1 | 0.1 | | | |
| *Pseudophycis bachus* (red cod) | **32.0** | 3.1 | 3.2 | **30.1** | 3.8 | 4.3 | 5.9 | 0.7 | 0.9 |
| Total Moridae | 36.1 | 4.0 | 3.2 | 36.3 | 4.9 | 4.4 | 5.9 | 0.7 | 0.9 |
| **Mugiloididae** *Parapercis colias* (blue cod) | 0.8 | < 0.05 | < 0.05 | 0.9 | < 0.05 | < 0.05 | | | |
| **Myctophidae** *Diaphus* spp | 0.8 | < 0.05 | < 0.05 | | | | 1.0 | 0.1 | < 0.05 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Lampanyctodes hectori* | 4.1 | 0.7 | < 0.05 | 4.4 | 0.9 | < 0.05 | | | |
| *Myctophum* sp | 0.8 | 0.1 | < 0.05 | 0.9 | 0.1 | < 0.05 | | | |
| Total Myctophidae (lanternfish) | 5.7 | 0.8 | < 0.05 | 5.3 | 1.0 | < 0.05 | 1.0 | 0.1 | < 0.05 |
| **Notosudidae** *Scopelusaurus* sp | 0.8 | < 0.05 | < 0.05 | 0.9 | < 0.05 | < 0.05 | | | |
| **Ophidiidae** *Genypterus blacodes* (ling) | 13.1 | 0.9 | 4.4 | 14.2 | 1.5 | 8.8 | | | |
| **Percophidae** *Hemerocoetes* spp (opalfish) | **48.4** | **35.5** | 2.4 | **50.4** | **50.1** | 4.7 | 5.0 | 5.2 | 0.3 |
| **Psychrolutidae** *Neophrynichthys latus* (dark toadfish) | 10.7 | 1.0 | < 0.05 | 9.7 | 1.2 | < 0.05 | 3.0 | 0.4 | < 0.05 |
| **Scorpaenidae** *Helicolenus percoides* (scarpee) | 0.8 | < 0.05 | 0.1 | | | | 1.0 | 0.1 | 0.2 |
| Unknown species A | 7.4 | 0.7 | < 0.05 | 8.0 | 1.0 | < 0.05 | | | |
| Total Scorpaenidae | 8.2 | 0.7 | 0.1 | 8.0 | 1.0 | < 0.05 | 1.0 | 0.1 | 0.2 |
| **Rajidae** *Raja nasuta* | 3.3 | 0.3 | 0.2 | | | | 2.0 | 0.2 | 0.3 |
| Unidentified fish | 10.7 | 0.5 | 0.2 | 6.2 | 0.4 | - | 5.0 | 0.5 | - |
| **CEPHALOPODS** | | | | | | | | | |
| **Octopodidae** *Enteroctopus zealandicus* (Octopus) | **38.5** | 3.1 | **14.4** | **28.3** | 2.1 | **27.8** | 15.8 | 4.5 | 6.0 |
| **Octopoteuthidae**? Unknown species | 0.8 | < 0.05 | < 0.05 | 0.9 | < 0.05 | < 0.05 | | | |
| **Ommastrephidae** *Nototodarus sloani* (arrow squid) | **86.9** | **33.4** | **43.0** | **33.6** | **14.1** | **17.9** | **82.2** | **78.1** | **78.5** |
| **Onychoteuthidae** *Moroteuthis ingens* (Wary squid) | 3.3 | 0.9 | 1.9 | 3.5 | 1.1 | 2.9 | | | |
| **Sepiolidae** Unknown species | 2.5 | 0.1 | < 0.05 | | | | 1.0 | 0.1 | < 0.05 |
| Unidentified squid | 0.8 | < 0.05 | - | 0.9 | < 0.05 | - | | | |
| **CRUSTACEANS** (swimming crabs) unknown genus | 4.9 | 0.2 | - | 0.9 | < 0.05 | - | 5.9 | 0.5 | - |
| **Palinuridae** *Jasus* sp (crayfish) | 2.5 | 0.1 | - | 0.9 | < 0.05 | - | 2.0 | 0.2 | - |
| **BIRD** **Procellariidae** *Pachyptila* sp (prion) | 0.8 | < 0.05 | < 0.05 | | | | 1.0 | 0.1 | 0.1 |
| Total number of taxa/species | | 35 | | | 29 | | | 23 | |
| Total of prey | | 3627 | | | 2732 | | | 895 | |

The same prey species were common in both the total content and the digested fraction, however they were not in the same order of importance (**Table 2-3**). In the analysis of the digested fraction, rattail was the most commonly eaten (54.0%O), followed by opalfish (50.4%O) and arrow squid (33.6%O). Opalfish represented half of the diet by

number but was still low based on mass (4.7%M) due to a small individual mass (**Table 2-4**, 20 ± 13 g). In contrast, octopus was the first prey by mass (27.8%M) due to a high individual mass (1788 ± 2322 g). Red cod, hoki and hake occurred in the same proportions in the digested fraction as in the total content (**Table 2-3**). The majority of the fresh fraction was composed of arrow squid (82.2%O, 78.1%N, 78.5%M).

The estimated prey lengths from hard remains found in the stomach contents ranged from 1.6 cm (Sepiolids) to 119.5 cm (hake), but common prey were less than 30 cm long (**Table 2-4**). The intraspecific length variation was also important with coefficients of variation higher than 20% for all common prey but opalfish. The largest prey were fish that made a minor contribution to the diet such as hake, hoki, barracouta and ling.

**Table 2-4**. Length and mass ($x$ ± SD) of New Zealand sea lion's common prey (in bold) and minor prey species with a commercial value. CV is the coefficient of variation. Length is the total length for fish and dorsal mantle length for cephalopods, except for barracouta and opalfish for which standard length was calculated. Lengths and masses were back-calculated from measurements of specific hard parts using regressions in Table 2-1.

| | | ---------- Length (cm) ---------- | | | Mass (g) |
|---|---|---|---|---|---|
| Prey species | *n* | $x$ ± SD | CV (%) | Range | $x$ ± SD |
| FISH | | | | | |
| *Trachurus* spp (jack mackerels) | 27 | 40.9 ± 3.9 | 10 | 31.9-50.8 | 991 ± 307 |
| *Micromesistius australis* (S$^{th}$ blue whiting) | 40 | 30.7 ± 5.9 | 19 | 17.0-41.2 | 116 ± 98 |
| *Thyrsites atun* (barracouta) | 22 | 75.1 ± 10.4 | 14 | 41.2-84.8 | 2070 ± 1005 |
| ***Coelorinchus* spp (rattails)** | 520 | 19.4 ± 6.2 | 32 | 5.1-43.8 | 48 ± 52 |
| *Macruronus novaezelandiae* (hoki) | 97 | 71.9 ± 11.2 | 16 | 50.2-97.4 | 1034 ± 460 |
| *Merluccius australis* (hake) | 17 | 92.3 ± 11.2 | 12 | 77.7-119.5 | 6117 ± 1275 |
| ***Pseudophycis bachus* (red cod)** | 195 | 25.0 ± 10.4 | 42 | 4.9-54.7 | 263 ± 306 |
| *Genypterus blacodes* (ling) | 57 | 61.2 ± 19.3 | 32 | 23.0-95.2 | 1193 ± 1040 |
| ***Hemerocoetes* spp (opalfish)** | 1027 | 12.4 ± 1.6 | 13 | 9.3-25.0 | 20 ± 13 |
| CEPHALOPODS | | | | | |
| ***Enteroctopus zealandicus* (Octopus)** | 110 | 13.5 ± 9.0 | 67 | 2.5-37.1 | 1788 ± 2322 |
| ***Nototodarus sloani* (arrow squid)** | 963 | 23.0 ± 5.4 | 24 | 6.6-36.5 | 347 ± 189 |
| Smallest prey = Sepiolids | 3 | 1.7 ± 0.1 | 1 | 1.6-1.7 | 2 |
| Largest prey = Hake | 17 | 92.3 ± 11.2 | 12 | 77.7-119.5 | 6117 ± 1275 |

### 2.3.2 Dietary variation (digested fraction)

SHR tests on the numbers of common prey did not reveal any significant interactions between the factors sex/maturity and area, thus these factors can be treated independently. No significant difference existed between the North and the East of the shelf (SHR test, $P$ values > 0.10). Opalfish was the only common prey showing a significant difference between sex classes (SHR test, $F_{3, 99} = 2.33$, $P = 0.10$), though this

was at the limit of the statistical significance. Tukey tests revealed that fewer opalfish were retrieved from the stomachs of males (14.5%N) than those of lactating (58.1%N) and non-lactating females (62.4%N) and immatures (56.9%N). The number of different taxa eaten by an individual sea lion was similar among sex classes and averaged 3 ± 2.

The median length of rattail was significantly smaller in the stomachs of immature sea lions (14.2 ± 3.1 cm) than in the stomachs of lactating females (18.8 ± 5.3 cm) and adult males (21.0 ± 6.1 cm) (Kruskal-Wallis test; $H_{3,55}$ = 9.54, $P$ = 0.023; Mann-Whitney tests, $P < 0.10$). Similarly, immature sea lions fed on opalfish of smaller size (11.7 ± 0.6 cm) than did lactating females (13.0 ± 1.3 cm) and males (13.4 ± 1.6 cm) (Kruskal-Wallis test; $H_{3,54}$ = 8.49, $P$ = 0.041; Mann-Whitney tests, $P < 0.10$). The median lengths of rattail, red cod and arrow squid were significantly larger in the eastern area than in the northern area (Mann-Whitney tests; $P$ = 0.001 for rattail; $P$ = 0.032 for red cod; $P$ = 0.009 for arrow squid). Indeed, the length distributions of rattail and red cod (**Fig. 2-2a** and **b**) showed that most of the largest fish eaten were from the East. This difference in size distribution between the two areas was well marked for the arrow squid (**Fig. 2-2 c**) with a peak at 29-30 cm in the East and a peak at 20-21 cm in the North.

## 2.4 DISCUSSION

Common prey of by-caught sea lions during the early lactation period were opalfish, rattail, arrow squid, octopus and red cod, which are benthic species, or living at depth > 200 m. Diet variation between by-caught males and females concerned only opalfish, whereas lactating and non-lactating females showed no difference in their diet. By-caught immatures of 3-4 years old fed on the same diet as adults, but targeted smaller opalfish and rattail. These results are consistent with the benthic foraging behaviour described previously in diving studies (Gales and Mattlin 1997; Chilvers *et al.* 2006). Nonetheless, ontogenic variation in this study may have been underestimated due to the nature of the sample set (by-catch) and the small number of individuals analysed in each sex class.

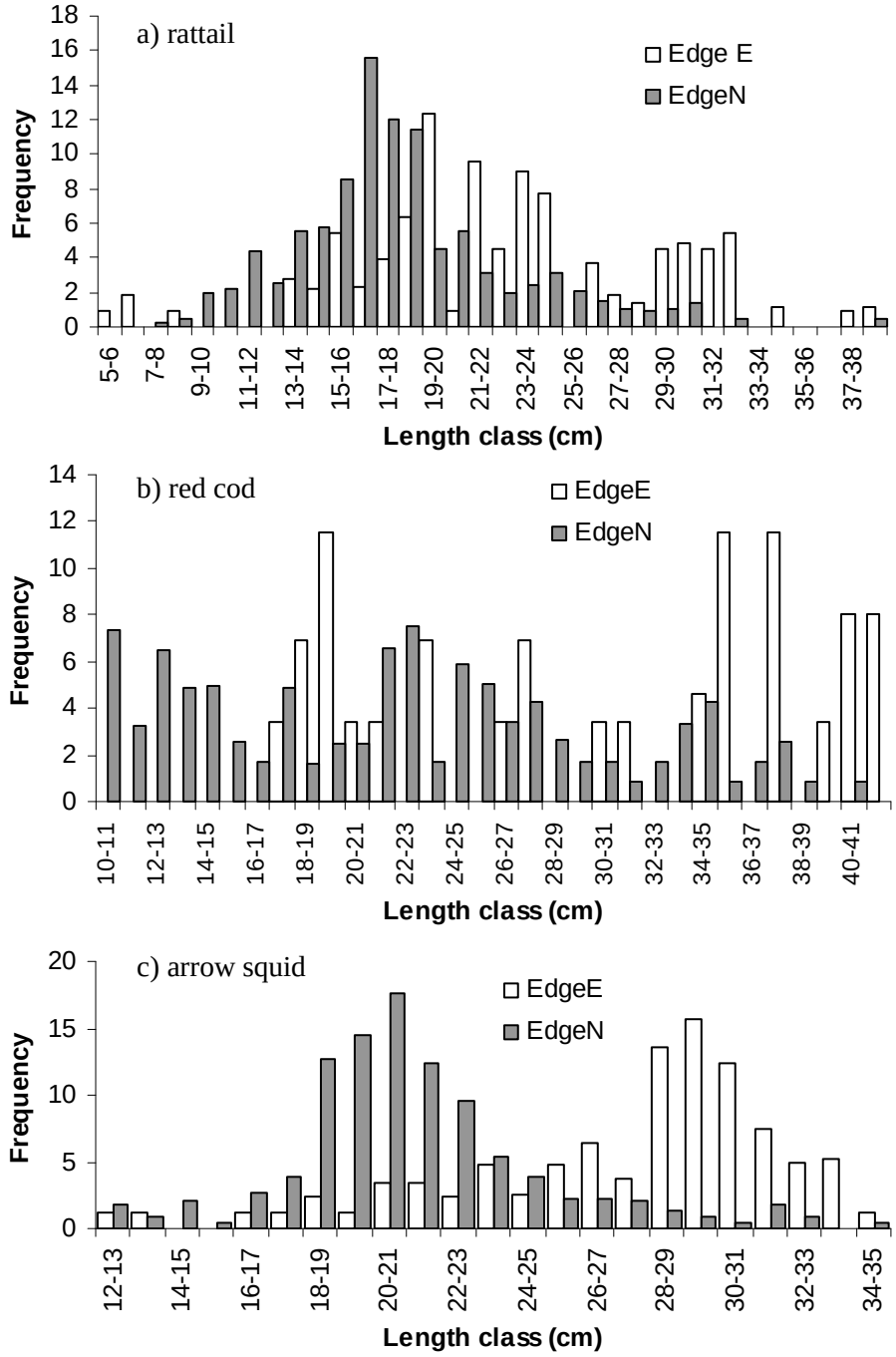


**Figure 2-2**. Length distribution of a) rattail, b) red cod and c) arrow squid estimated from hard parts found in stomach contents of New Zealand sea lions, according to the area of capture. EdgeN is the North area and EdgeE is the East area on the Auckland Islands shelf (see Fig. 2-1).

### 2.4.1 Limitations of the study

Total stomach contents were divided into fresh and digested fractions. The fresh fraction includes all items ingested over a similar period, and probably during the same "meal". It provides an unbiased estimate of actual intake although over a restricted period (Pierce *et al.* 2004a; *e.g.*, Pusineri *et al.* 2007). However, in the present study, the composition of the fresh fraction is obviously biased in that the individuals sampled were caught in fishing nets targeting squid. The comparison between the fresh (78.1%N, 78.5%M) and the digested fraction (14.1%N, 17.9%M) confirmed that total stomach contents overestimated the percentage of arrow squid in the diet (**Table 2-3**). The digested fraction is also subject to biases, related to differential digestion rates and hard part accumulation. Firstly, fish otoliths may be partially or completely digested in stomachs of pinnipeds (Murie and Lavigne 1985; Dellinger and Trillmich 1988) and fish with small otoliths tend to be underestimated. To reduce this bias, an "all-structure" approach was used (Tollit *et al.* 2003), in which both otoliths and diagnostic bones were identified. Secondly, cephalopod beaks are not affected by digestion and tend to accumulate in the stomach (Bigg and Fawcett 1985). Individuals of all classes have been seen regurgitating beaks on rookeries (B. L. Chilvers, unpubl. data), which may reduce the accumulation of cephalopod beaks. Consequently, I believe that the biases encountered in the digested fraction were minimised in this study, and that this fraction better represented the diet of NZ sea lions than the whole stomach content or the fresh fraction.

Estimates of fish length and mass are based on otolith measurements. Thus, the measurement of eroded otoliths leads to an underestimation of these parameters. Correction factors were derived from scat analysis of captive grey seals (*Halichoerus grypus*, Tollit *et al.* 1997) and Steller sea lions (*Eumetopias jubatus*, Tollit *et al.* 2004) during feeding trials, but involve different prey than in this study. Instead, I minimised the bias due to erosion by measuring only otoliths that didn't show any sign of erosion. Also, some species-specific regressions were applied on other species in a related family (**Table 2-1**). Therefore, length and mass reconstructions should be interpreted with caution.

### 2.4.2 Feeding ecology of the NZ sea lion

Although the diet of by-caught NZ sea lions during the early lactation period of 1997 to 2006 was diverse, only five prey –opalfish, rattail, arrow squid, octopus and red cod– were considered common (**Table 2-3**). These prey also comprised a significant part of the diet estimated from scats and regurgitates between 1994 and 1997 (Childerhouse *et al.* 2001). Both opalfish and octopus are benthic species occurring on the shelf, living on sandy beds and in rock holes respectively (Paul 2000). Arrow squid occurs in the water column over the shelf but also at the edge of the plateau and offshore, where they form large aggregations down to 300 m (Jackson *et al.* 2000). The edge is also the habitat of rattails which live near the bottom at depths > 200 m (Paul 2000). Benthic foraging is consistent with previous studies on the diving behaviour of female NZ sea lions (Gales and Mattlin 1997; Chilvers *et al.* 2006), where most of the dives were in waters > 150 m, consecutively at similar depths, and with a U-shaped dive, which is typical of diving to the sea bottom. Most of the uncommon prey are demersal or benthic species, although some are from the pelagic region such as barracouta and jack mackerel. NZ sea lions may opportunistically catch these prey at the beginning or the end of a dive. This is consistent with the conclusions of a study conducted at Otago (South Island, NZ) on male NZ sea lions (Lalas 1997), where the diet consisted of a wide range of benthic species with several pelagic species. The examination of prey sizes (**Table 2-4**) emphasises this opportunistic behaviour: All but one of the common prey displayed a coefficient of variation higher than 20%, showing that NZ sea lions don't target a particular length of prey, but instead are likely to eat what is available.

During summer, lactating female NZ sea lions forage over and at the edge of the shelf (Chilvers *et al.* 2005), where they dive continuously to depths > 150 m (Chilvers *et al.* 2006). The energy cost of deep diving is greater than transiting between locations (Costa and Gales 2000). Consequently, these benthic divers need a sufficient energetic income at each dive to balance their energetic budget, and are likely to forage on areas with predictable and concentrated resources. The edges of the Auckland Islands shelf appears to be a preferred foraging region for lactating females where they concentrate most of their time during a foraging trip (Chilvers *et al.* 2005). Although it is far from the rookeries (> 50 km), it may represent an energetic risk worth taking based on foraging returns from greater density of prey than would be available on the shelf. Indeed, arrow squid and rattails, two of the common prey, are schooling species forming large aggregations at depths > 200 m (Jackson *et al.* 2000; Paul 2000). In

contrast, opalfish and octopus live at the bottom of the shelf (Paul 2000) and are not in schools. Therefore, it appears that by foraging at the edge of the Auckland Islands shelf in depths > 200 m, NZ sea lions have access to concentrated food resources where great quantities can be consumed in a short time period, increasing their energetic investment per dive.

### 2.4.3 Ontogenic variation in diet

Dietary differences between adult females and males are most likely to be driven by different physiological constraints, metabolic requirements and reproductive strategies (Costa 1993). In this study, though body mass variation of males was important (**Table 2-2**), the average mass was a third heavier than that of females. Body mass influences dive capability in such a way that heavier animals are able to dive longer than lighter ones (Costa 1993). Furthermore, foraging trips of males are not constrained in duration by pup nursing, thus they may undertake trips of longer-duration than females and access different resources. The only difference found in this study between the diet of males and females was the amount of opalfish (demersal species occurring on the shelf, Paul 2000), which was significantly greater in the diet of females (approximately 60%N *vs.* 14.5%N for males). Males are able to dive deeper because of their greater body size, and may focus their food search in waters > 200 m, where opalfish are less abundant. This hypothesis could only be validated by the comparison of satellite-TDR data on males and females, but to date foraging studies have focused on lactating individuals only (Gales and Mattlin 1997; Chilvers *et al.* 2005, 2006). The lack of strong difference between the diet of female and male sea lions in this study may originate from the method used and the sample set: First, stomach contents give a diet picture over a foraging trip only, and may not be representative of the "routine" diet of females and males during the early lactation period. Second, most of the males in the present sample set were non-territorial animals during the breeding season. Territorial bulls are likely to show more dietary differences with females than the non-territorial animals, because they tend to disperse further than the study area after the breeding season (Robertson *et al.* 2006).

By-caught lactating and non-lactating females did not show any difference in their diet. Non-lactating females are not constrained to return to the breeding site to nurse when foraging, thus they can increase their time at sea and forage on a different resource.

However, lactating females are seen to have high site fidelity to certain foraging zones, which is though to represent long-term learnt foraging behaviour (Chilvers 2008a). Therefore it is expected that these foraging habits would be similar whether they were rearing a pup or not.

The by-catch location and the present dietary results indicated that by-caught immature NZ sea lions are able to feed on the same prey and at the same locations as mature animals. Their diet consists of bottom-dwelling (octopus, opalfish) and deepwater species (arrow squid, rattail), implying that they are able to forage on the bottom of the shelf and at depths > 200 m. Similarly to females, immature sea lions fed on a greater percentage of opalfish than did adult males. Moreover, the length of opalfish and rattail were significantly smaller than that of adults. The predation of smaller prey by juvenile otariids compared to adults has been reported before (*e.g.,* Page *et al.* 2005a), and is an expected result given their lower mass (**Table 2-2**). Nevertheless, it must be stressed that the class "immatures" in this study comprises animals from three to four year old, which have a mass close to that of females (**Table 2-2**), being able to forage on deep prey at depths > 200 m. This may explain their capacity of capturing red cod, arrow squid and octopus of the same size as adults. Yearlings or two year old juveniles, not represented here, are likely to forage in shallower areas closer to the rookeries. This hypothesis would be consistent with studies conducted on the diving patterns of other similar aged otariid species (*e.g.,* Baylis *et al.* 2005, *Arctocephalus forsteri*; Fowler *et al.* 2007, *Neophoca cinerea*; Spence-Bailey *et al.* 2007, *Arctocephalus pusillus doriferus).*

### 2.4.4 Geographical variation in diet

Individuals were grouped according to their by-catch location, either North or East of the Auckland Islands (**Fig. 2-1**). The foraging trips of lactating females are consistent within a year. One individual travels to the same location at the edge of the shelf and come back to the rookeries after several days (Chilvers *et al*. 2005). Therefore, if I extrapolate this behaviour to the other classes of the population, stomach contents of animals caught in each location represented the prey encountered on the way and/or return between the colony and either the North or the East. The diet from stomach analyses showed no difference between the two locations, suggesting no difference in prey distribution between the North and East of the Auckland Islands shelf. However,

sea lions caught in the East ate larger rattails, red cod and arrow squid than those caught in the North (**Fig. 2-2**). It is difficult to verify if these prey stocks in the Auckland Islands show size differences between areas as their biology is poorly known. The only length distribution available in the region is from the arrow squid fishery, which catches larger squid in the southern than in the northern part of the Auckland Islands shelf (Gibson 1995). This is consistent with my results showing that squid eaten by sea lions in the eastern zone, which is also the south of the shelf (**Fig. 2-1**), were 10 cm larger than those from the North (**Fig. 2-2**). The East and the North of the shelf are exploited by sea lions from different breeding colonies of the Auckland Islands (B.L. Chilvers, unpubl. data), and a stock of larger prey may be energetically advantageous for animals foraging in the eastern area.

### 2.4.5 Interactions with fisheries

Of the common prey species found in the stomachs of by-caught NZ sea lions, only arrow squid and red cod have commercial value. Some of the minor prey are also harvested by fisheries, such as hoki, hake, barracouta, ling, southern blue whiting and jack mackerel (**Table 2-3**) (Ministry of Fisheries 2007). These fish along with red cod and arrow squid accounted for 24.1%N and 58.6%M of the total prey in the digested fraction of the stomach's contents (**Table 2-3**), although arrow squid is the only prey with a commercial exploitation in the region of the Auckland Islands (51°S, 166°E). These islands are the southern limit of the NZ sea lion's historical breeding range (Childerhouse and Gales 1998), yet host 86% of the pup production (Chilvers *et al.* 2007b). This environment has been considered a marginal habitat for NZ sea lion females, which dive deeper than any other otariid, and operate at their physiological limits when foraging (Gales and Mattlin 1997; Costa and Gales 2000; Chilvers *et al.* 2005, 2006). Therefore, the recovery of this species depends on the development of new colonies around NZ main islands. A fragile recolonisation is visible in the South Island of NZ (Childerhouse and Gales 1998; Wilkinson *et al.* 2003), but this region is also an important ground for deepwater fisheries (hoki, hake, barracouta; Ministry of Fisheries 2007). The hoki fishery, which is the most important fishery in NZ, target fish of a size > 60 cm (Ministry of Fisheries 2007), similar to the size range exploited by NZ sea lions (**Table 2-4**). Moreover, the NZ sea lion appears to eat what is the most available in the benthos or deep waters, and is capable of eating a wide size range (**Table 2-4**). This

opportunistic behaviour, also reported in non by-caught animals (Lalas 1997; McMahon *et al.* 1999), may lead to direct and indirect interactions with fisheries occurring around the developing colonies in the future.

## 2.5 CONCLUSION

I investigated the diet of NZ sea lions and its variation from stomach contents of by-caught animals. The main prey species are benthic dwelling or deepwater fish and squid, which is consistent with the results of diving studies showing benthic foraging. Lactating, non-lactating females, males and juveniles of 3-4 years old commonly foraged on the same prey but the lack of differences in prey numbers in the diet between the different categories may be an effect of the small sample set. Future studies using techniques relying on biopsies and not on dead animals, such as fatty acid analysis, are needed to further investigate the dietary differences between classes of the population. However, the present study highlights the importance of the edges of the Auckland Islands shelf, where common prey of NZ sea lions such as schooling fish and squid aggregate. Though these key areas are far from the rookeries and are impacted by the squid fishery, they may provide the only predictable and abundant resource needed to cover the cost of benthic foraging for the deepest diver of all otariids.

## Acknowledgements

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# 3 CHAPTER 3

## TEMPORAL AND SEX DIFFERENCES IN THE BLUBBER FATTY ACID PROFILES OF THE NEW ZEALAND SEA LION

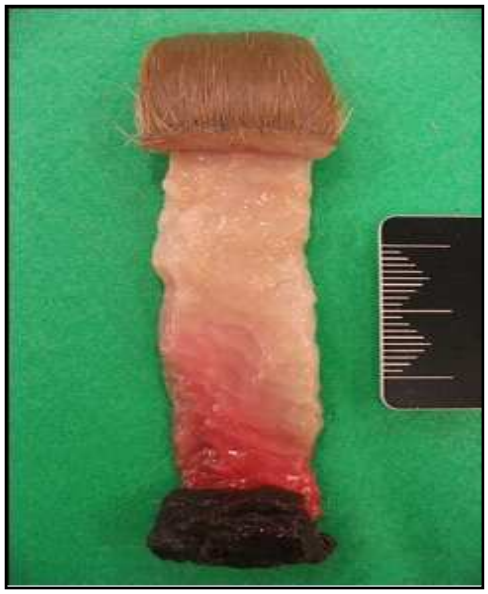

**Photo on right:** whole blubber core sampled from a by-caught New Zealand sea lion (*Phocarctos hookeri*)

**Chapter reference:**

Meynier, L., Morel, P.C.H., Chilvers, B.L., Mackenzie, D.D.S., MacGibbon, A., Duignan, P.J. 2008. Temporal and sex differences in the blubber fatty acid profiles of the New Zealand sea lion *Phocarctos hookeri*. Marine Ecology Progress Series, 366:271-279.

## Abstract

The fatty acid (FA) composition of the blubber of 82 New Zealand (NZ) sea lions caught as squid fishery by-catch was determined between the years 2000 and 2006 on the Auckland Islands (50°30'S, 166°E) shelf. A combination of univariate and multivariate analyses showed significant variation in the FA composition between sexes and years. Blubber FA compositions of some males differed significantly from that of females, whereas blubber FA compositions of lactating (LF) and non-lactating females (NLF) were similar. Significant annual FA variation was revealed between the pooled years 2005/2006 and the previous years and between 2000 and 2004. Part of these differences can be attributed to different diets. Indeed, FA variation between the sexes suggests that males feed on deeper species than females, which is consistent with the current knowledge on the different diving behaviours between male and female otariids. Concerning annual variation, NZ sea lions are generalist predators, thus their diet is expected to follow the trends of prey stock availability. Nonetheless, FA metabolism is likely to cause some of the FA variation observed between sexes and years, since the deposition and mobilisation of FAs would vary according to the nutritional and reproductive states of the individuals.

## 3.1 INTRODUCTION

Pinnipeds are amphibious mammals balancing their time between land, for breeding, nursing, resting and moulting, and sea, where they forage. However, different species use different strategies for breeding and foraging. Otariids are 'income breeders', with lactation periods of several months to several years during which lactating females (LFs) alternate between nursing pups on land and foraging at sea (Costa 1991). They are considered 'central place foragers', optimising the time and energy costs of foraging with the need to return frequently to the colony to feed their dependent pups (Orians and Pearson 1979). Thus, LFs would be expected to forage as close to the rookery as possible provided that enough energy can be obtained from prey to compensate for the energetic cost of the round trip (Orians and Pearson 1979). In contrast, male otariids and non-lactating females (NLFs), without the constraints of a dependent offspring, would be expected to forage in the most productive regions, which may be further from the colonies than the foraging grounds of LFs. Furthermore, male otariids are often twice the mass of females, giving them the ability to dive longer (Costa 1991), but have higher metabolic requirements, which they may satisfy by consuming more of the same food eaten by females or eating a different diet with a higher calorific content. It is possible that these differences in reproduction investment and body size between sexes could give rise to sex-specific foraging strategies and consequently differences in the composition of the diet between the sexes and between females of different reproductive status.

Little is known about how gender influences foraging behaviour or diet of otariids. Indeed, most foraging studies on otariids focused on LFs because they are a critical component of the population and easily accessible while lactating. For six species where male foraging patterns have been studied, they tend to have longer foraging trips and deeper dives than the female counterparts (*Arctocephalus gazella*, Green *et al.* 1997; Boyd *et al.* 1998; Staniland and Robinson 2008; *Otaria flavescens*, Campagna *et al.* 2001; *Eumetopias jubatus*, Raum-Suryan *et al.* 2004; *Arctocephalus forsteri*, Page *et al.* 2005b; *Arctocephalus pusillus doriferus*, Kirkwood *et al.* 2006; *Zalophus californianus*, Weise *et al.* 2006). Dietary studies comparing gender are even more scarce (Koen Alonso *et al.* 2000; Page *et al.* 2005a; Beck *et al.* 2007b), probably because the most common method for studying the diet of pinnipeds is the analysis of faeces collected on

haul-outs or rookeries, where the identity of the animal or its gender are generally unknown.

New Zealand (NZ) sea lions *Phocarctos hookeri* have a restricted breeding range, with 86% of the pups being born at the Auckland Islands (50°30'S, 166°E) (Chilvers *et al.* 2007b). To date, LF are the only segment of the population for which foraging and diving behaviours are known and this only over the summer (Chilvers *et al.* 2005, 2006). Stomach contents of NZ sea lions by-caught by the squid fishery at the edge of the Auckland Islands shelf indicate that females and males feed on the same prey with minor differences in the proportions (Chapter 2), but stomach data provides only a snapshot of the most recent meals, which may underestimate sex differences in the diet, if they exist.

In contrast to analyses of stomach contents and faeces, fatty acids (FAs) of adipose tissue have the potential to reflect the dietary intake over ecologically significant periods, *i.e.*, several weeks to months depending on the tissue turnover (*e.g.*, Kirsch *et al.* 1998; Kirsch *et al.* 2000). The underlying principle is the assumption that long-chain FAs in prey species are conservatively deposited into the adipose tissue of the predator, thereby providing biochemical signatures with which prey species can be identified. Although advantages over traditional methods have been pointed out, the inference of diet from FA profiles of an animal is not straightforward. Indeed, the FA composition in the blubber is the result of complicated processes of deposition from dietary lipids, differential metabolism and biosynthesis *de novo*. Moreover, stratification of FAs along blubber cores has been observed (*e.g.*, Arnould *et al.* 2005; Montie *et al.* 2008), and rates of mobilisation can vary according to the nutritional and reproductive states of the animal (*e.g.*, Andersen *et al.* 2004; Wheatley *et al.* 2007; Montie *et al.* 2008). However, despite the multiple origins of FA variation in adipose tissue, FA analysis has been used extensively to investigate the diets of pinniped species (*e.g.*, Käkelä and Hyvärinen 1998; Brown *et al.* 1999; Walton *et al.* 2000; Lea *et al.* 2002a; Staniland and Pond 2005; Beck *et al.* 2007b).

The aim of the present study was to investigate the potential of analysing the blubber FA profiles of NZ sea lions by-caught by the squid fishery during the beginning of the lactation period (February to May) as a means of assessing dietary differences between sexes and years. It was hypothesised that the post-breeding diet of LFs would differ from the other groups, given that foraging trips of LFs are restricted in time and distance by the need to nurse their pups.

## 3.2 MATERIALS AND METHODS

### 3.2.1 Tissue collection

Since 1997, NZ sea lions captured accidentally in the squid fishery (operating from February to May each year) have been frozen onboard and sent frozen to Massey University, NZ, for necropsy. During each necropsy, a full-depth 60 $mm^2$ piece of blubber (including skin and some muscle) is cut from the pectoral area and stored in a plastic bag in a freezer at –20°C. All blubber samples in the present study were taken from the mid-sternal region to be comparable, as FA profiles can vary with the location around the body (Arnould *et al.* 2005). Moreover, stratification of FAs can occur in the blubber of pinnipeds (*e.g.,* Best *et al.* 2003; Arnould *et al.* 2005; Wheatley *et al.* 2007), thus the complete blubber core was analysed. During necropsy, females were categorised as either NLF or LF by the examination of the mammary gland for development and presence of secretion. The individuals included in this study were all sexually mature as determined by visual examination of the ovaries and histological examination of the testes (details in Duignan *et al.* 2003).

### 3.2.2 Laboratory methods

Lipids from blubber were extracted following Folch *et al.* (1957), using a chloroform:methanol:water mixture. Approximately 0.5 g of blubber (whole core) was sub-sampled from the bulk sample and homogenised in 15 ml of chloroform:methanol (2:1, vol:vol) containing 0.01% butylated hydroxytoluene (BHT) as an antioxidant. The extract was filtered and washed with 1% sodium chloride (saline) to a final ratio of 8:4:3 chloroform:methanol:saline (v:v:v). The organic phase was then dehydrated over anhydrous sodium sulphate. Finally, the lipid extract was dried in a rotary evaporator at 38°C and weighed.

FA methyl esters (FAMEs) were prepared directly from 30 mg of the pure extracted lipid using 1.5 ml of toluene and 1.5 ml of 10% boron trifluoride in methanol (methylating reagent). Each extract was capped under nitrogen and heated at 50°C for

14 to 19 h (overnight). Esters were then extracted into hexane and stored over anhydrous sodium sulphate at –20°C before chromatographic analysis.

Analysis of FAMEs was carried out using temperature-programmed gas–liquid chromatography performed with a Shimadzu Gas Chromatograph GC-17A (Shimadzu Scientific Instruments) equipped with a flame ionisation detector and fitted with a 30 m × 0.25 mm i.d. column (50% cyanopropyl polysiloxane, 0.25 μm film thickness; J&W DB-23). Helium was the carrier gas. FAMEs (1 μl) were injected manually in split mode (1:50) at an injection port temperature of 250°C. The detector temperature was set at 270°C. The temperature of the oven was programmed to stay at 140°C for 4 min, rise to 190°C at 25°C $min^{-1}$, held for 5 min, then to 236°C at 2°C $min^{-1}$.

FA components were identified by comparison of retention time data to authentic (Nu-Chek GLC standard 68D, Supelco 37 FAME mix, Matreya menhaden oil) and laboratory standards (cod liver oil). Cod liver oil was used in every series of runs to determine accurate retention times. Nu-Check 68D was injected regularly to check the quantitation of each FA. Peak areas were measured by a computerised integration system attached to the gas chromatograph (CLASS-VP version 7.3, Shimadzu Scientific Instruments). Each chromatogram was checked to ensure correct identification. The identification of some minor peaks was uncertain, and these were not included in the final normalisation. 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. Theoretical response factors calculated according to Ackman and Sipos (1964) were used for the quantitation of FAs expressed in mass percentages.

### 3.2.3 Statistical methods

FAs were expressed as a percentage mass of total FAs and they were ln-transformed as advised by Budge *et al.* (2006) for parametric statistical analyses. A transformed FA i equalled ln (xi $18:0^{-1}$) where xi is the FA, i expressed as percent of total FAs, and 18:0 is the percentage of stearic acid of total FAs, used as a reference FA. A combination of principal component analysis (PCA), discriminant function analysis (DFA) and general linear model (GLM) was used to examine inter-annual and sex differences in the FA profiles of NZ sea lions (MINITAB Release 14.1, MINITAB 2003 and SPSS for Windows Release 15.0, SPSS 2006). These multivariate methods have been commonly used in numerous studies on FA analysis (*e.g.*, Grahl-Nielsen and Mjaavatten 1991;

Walton *et al.* 2000; Staniland and Pond 2005) and give complementary results. A first GLM was applied to the transformed FA compositions with year and sex as independent variables. A second GLM was used on the principal components (PCs). The Tukey test was used for *post-hoc* multiple comparisons. Finally, the DFA requires that the number of samples (sea lion blubber) per group exceeds the number of variables (FAs) to minimise the heterogeneity of covariance matrices and to avoid overfitting (Budge *et al.* 2006). Because the number of samples per year was too small to run a DFA, I limited this analysis to the sex category, and the number of FAs was reduced to satisfy the requirement stated above: the smallest group among sex categories was NLF with 23 individuals, therefore a set of 20 FAs was selected from the original set of 30 FAs, with the highest absolute PC loadings. After re-normalisation of the FA percentages, a linear DFA with cross-validation was performed.

All statistical tests have an α level of statistical significance of 0.05, and all averages were followed by the standard deviation (SD). Since the blubber thickness may have an influence on FA profiles, the average blubber thickness was calculated and tested for differences between each category by an ANOVA on log-transformed data.

## 3.3 RESULTS

### 3.3.1 Composition of the sample set

In total, 82 blubber samples were analysed from 51 female and 31 male sea lions by-caught between February and May annually from 2000 to 2006 (**Table 3-1**). Although mature, the majority of the males caught were not over 200 kg (average 167 ± 60 kg) and were considered to have been non-territorial animals during the breeding period (mid-December to mid-January). The sex categories were not all represented within the last two years, thus 2005 and 2006 were pooled for univariate and multivariate analyses. Overall thickness of sea lion blubber averaged 28 ± 11 mm and was similar among sex categories (**Table 3-1**). Between years, the blubber of sea lions caught in 2000 was significantly thicker than the blubber of animals caught in the combined years 2005/2006 (ANOVA, $F_{5,75} = 3.80$, $P = 0.004$; Tukey test between 2000 and 2005/2006, $P = 0.027$).

**Table 3-1**. Number of lactating female (LF), non-lactating female (NLF) and male (M) New Zealand sea lions analysed per year. The blubber thickness (mm) is expressed as the mean ± SD. Significant differences between categories were ANOVA-tested on log-transformed data (log values ± SD in parentheses). The years 2005 and 2006 were pooled for the ANOVA because of the limited number of individuals. * shows a significant difference between some years at $\alpha = 0.05$.

| Year | LF | NLF | M | **Total** | Blubber thickness x (log x) |
|---|---|---|---|---|---|
| 2000 | 6 | 2 | 8 | **16** | 32 ± 5 (1.50 ± 0.07)* |
| 2001 | 6 | 2 | 8 | **16** | 24 ± 5 (1.37 ± 0.10) |
| 2002 | 8 | 1 | 5 | **14** | 32 ± 10 (1.49 ± 0.14) |
| 2003 | 2 | 4 | 5 | **11** | 34 ± 20 (1.47 ± 0.28) |
| 2004 | 2 | 6 | 4 | **12** | 24 ± 6 (1.37 ± 0.10) |
| 2005 | 3 | | 1 | **4** | 22 ± 6 |
| 2006 | 1 | 8 | | **9** | (1.33 ± 0.10)* |
| **Total** | **28** | **23** | **31** | **82** | 28 ± 11 (1.42 ± 0.15) |
| Blubber thickness x (log x) | 29 ± 11 (1.43 ± 0.17) | 25 ± 12 (1.37 ± 0.15) | 30 ± 10 (1.45 ± 0.13) | 28 ± 11 (1.42 ± 0.15) | |

### 3.3.2 Overall blubber FA composition

Although 38 FAs were originally identified, only 30 FAs ranging from 14:0 to 22:6n-3 were used (**Table 3-2**), representing 99.4 ± 0.2% of the total. The 8 FAs removed were either short-chain FAs (<14 carbons), known to come primarily from endogenous biosynthesis (Budge *et al.* 2006), or FAs for which the identification was not certain.

Average FA composition of sea lion blubber is shown in **Table 3-2**. The FAs in greatest concentration in order of importance were 18:1n-9, 16:0, 22:6n-3, 20:1n-9, 16:1n-7, 14:0, 18:1n-7, 22:5n-3 and 20:5n-3, accounting for approximately 85% of the total FAs in the blubber. Monounsaturated and polyunsaturated FAs accounted for approximately 50% and 25% of the total, respectively.

**Table 3-2.** Fatty acid (FA) composition (mean mass percent ± SD) by sex category of blubber samples from 82 New Zealand sea lions. The values in parenthesis are the ln-transformations of the FA percentages [ln (FA% 18:0%$^{-1}$)] used in the general linear model (GLM). For the GLM, *post-hoc* comparisons were indicated when significant differences ($P < 0.05$, *P* values in bold) were present among groups: M = males, LF = lactating females, NLF = non-lactating females, 00 = year 2000 etc. The years 2005 and 2006 were pooled (coded 056). SAFAs are saturated FAs, MUFAs monounsaturated FAs and PUFAs polyunsaturated FAs. Origin represents the predominant origin of a FA: 'diet' when a FA came entirely or primarily from diet, 'both' when large contributions come from both endogenous biosynthesis and diet (Iverson *et al.* 2004). Boldface type FAs indicate the 20 FAs used in the first discriminant function analysis (DFA).* indicates the 10 FAs used in the second DFA.

| | FA composition mean % ± SD (ln transformed % ± SD) | | | *P*-values of GLM (on ln transformed %) | | | Origin |
|---|---|---|---|---|---|---|---|
| FAs | LF | NLF | M | Sex | Year | Sex * year | |
| SAFAs | 21.7 ± 3.1 | 22.0 ± 3.0 | 20.7 ± 2.9 | | | | |
| **14:0** | 5.1 ± 1.3 (0.6 ± 0.3) | 5.4 ± 1.5 (0.7 ± 0.4) | 3.8 ± 0.9 (0.2 ± 0.4) | <**0.001**; M ≠ LF, NLF | **0.009**; 056 ≠ others | 0.515 | both |
| **15:0*** | 0.4 ± 0.1 (-2.0 ± 0.3) | 0.4 ± 0.1 (-1.9 ± 0.2) | 0.5 ± 0.1 (-1.9 ± 0.2) | 0.364 | 0.115 | 0.504 | both |
| **16:0*** | 12.7 ± 1.8 (1.5 ± 0.2) | 13.0 ± 1.7 (1.6 ± 0.2) | 12.8 ± 1.9 (1.5 ± 0.2) | 0.119 | **0.024**; 056 ≠ others | 0.772 | both |
| 17:0 | 0.5 ± 0.2 (-1.7 ± 0.4) | 0.4 ± 0.2 (-2.0 ± 0.7) | 0.6 ± 0.1 (-1.7 ± 0.3) | 0.660 | <**0.001**; 056 ≠ others | 0.965 | diet |
| 18:0 | 2.8 ± 0.6 | 2.7 ± 0.5 | 3.0 ± 0.8 | - | - | - | |
| MUFAs | 52.9 ± 4.1 | 53.1 ± 2.6 | 54.8 ± 5.2 | | | | |
| **14:1** | 0.3 ± 0.1 (-2.4 ± 0.6) | 0.3 ± 0.1 (-2.5 ± 0.5) | 0.3 ± 0.1 (-2.6 ± 0.8) | 0.080 | **0.026**; 00 ≠ 04 | 0.656 | both |
| 15:1 | 0.1 ± 0.0 (-3.0 ± 1.7) | 0.2 ± 0.5 (-3.2 ± 1.2) | 0.1 ± 0.0 (-3.4 ± 1.2) | 0.873 | **0.008**; 04 ≠ 056 | 0.835 | both |
| **16:1n-7*** | 6.0 ± 1.5 (0.7 ± 0.4) | 7.0 ± 2.0 (1.0 ± 0.4) | 6.0 ± 1.7 (0.7 ± 0.5) | 0.085 | **0.031**; 056 ≠ 01,03,04 | 0.586 | both |
| **18:1n-9*** | 28.1 ± 3.8 (2.3 ± 0.3) | 28.6 ± 2.4 (2.4 ± 0.2) | 32.0 ± 4.7 (2.4 ± 0.3) | 0.740 | **0.003**; 00 ≠ 01,04 | 0.330 | both |
| **18:1n-7** | 3.9 ± 0.4 (0.4 ± 0.3) | 4.0 ± 0.4 (0.4 ± 0.2) | 4.4 ± 0.5 (0.4 ± 0.3) | 0.716 | **0.014**; 04 ≠ 00,03 | 0.402 | both |

**Table 3-2.** Continued

| | ---------- FA composition mean % ± SD ---------- (ln transformed % ± SD) | | | ---------- *P*-values of GLM ---------- (on ln transformed %) | | | Origin |
|---|---|---|---|---|---|---|---|
| FAs | LF | NLF | M | Sex | Year | Sex * year | |
| SAFAs | 21.7 ± 3.1 | 22.0 ± 3.0 | 20.7 ± 2.9 | | | | |
| **18:1n-5** | 0.4 ± 0.1 (-2.1 ± 0.3) | 0.4 ± 0.1 (-2.0 ± 0.3) | 0.3 ± 0.1 (-2.3 ± 0.5) | **0.004**; M ≠ NLF | **0.012**; 04 ≠ 00,056 | 0.583 | both |
| 20:1n-11 | 1.4 ± 0.3 (-0.7 ± 0.3) | 1.3 ± 0.3 (-0.7 ± 0.3) | 1.2 ± 0.3 (-0.9 ± 0.4) | **0.007**; M ≠ LF | **0.051**; 00 ≠ 04 | 0.751 | diet |
| **20:1n-9*** | 10.9 ± 2.4 (1.3 ± 0.3) | 9.9 ± 2.4 (1.3 ± 0.2) | 9.0 ± 1.8 (1.1 ± 0.3) | <**0.001**; M ≠ LF, NLF | **0.001**; 00 ≠ 04,056 | 0.392 | diet |
| **22:1n-11*** | 1.2 ± 0.6 (-0.9 ± 0.5) | 1.0 ± 0.5 (-1.0 ± 0.4) | 0.9 ± 0.4 (-1.3 ± 0.5) | <**0.001**; M ≠ LF | <**0.001**; 056 ≠ 00,01 | 0.668 | diet |
| **22:1n-9** | 0.6 ± 0.3 (-1.5 ± 0.3) | 0.5 ± 0.2 (-1.7 ± 0.3) | 0.6 ± 0.3 (-1.7 ± 0.4) | 0.088 | 0.259 | 0.999 | diet |
| PUFAs | 25.5 ± 3.4 | 24.9 ± 3.3 | 24.5 ± 4.5 | | | | |
| **18:2n-6** | 1.5 ± 0.2 (-0.6 ± 0.3) | 1.6 ± 0.2 (-0.5 ± 0.2) | 1.6 ± 0.2 (-0.6 ± 0.3) | 0.169 | **0.020**; 00 ≠ 04 | 0.180 | diet |
| **20:2n-6*** | 0.4 ± 0.1 (-2.0 ± 0.3) | 0.4 ± 0.1 (-1.9 ± 0.2) | 0.4 ± 0.1 (-2.1 ± 0.4) | 0.102 | 0.361 | 0.941 | diet |
| **16:3n-4*** | 0.3 ± 0.1 (-2.1 ± 0.4) | 0.4 ± 0.1 (-2.0 ± 0.4) | 0.5 ± 0.1 (-1.9 ± 0.4) | 0.106 | **0.010**; 00 ≠ 04 | 0.286 | diet |
| 18:3n-3 | 0.5 ± 0.2 (-1.7 ± 0.8) | 0.6 ± 0.2 (-1.5 ± 0.6) | 0.5 ± 0.2 (-1.9 ± 0.7) | 0.216 | 0.299 | 0.567 | diet |
| 20:3n-6 | 0.1 ± 0.0 (-2.9 ± 0.9) | 0.1 ± 0.0 (-2.9 ± 0.7) | 0.1 ± 0.0 (-2.8 ± 1.2) | 0.834 | 0.161 | 0.674 | diet |
| 20:3n-3 | 0.2 ± 0.0 (-2.6 ± 0.3) | 0.2 ± 0.0 (-2.5 ± 0.3) | 0.2 ± 0.1 (-2.8 ± 0.8) | 0.094 | 0.327 | 0.973 | diet |
| 18:4n-3 | 0.5 ± 0.2 (-1.8 ± 0.6) | 0.5 ± 0.2 (-1.7 ± 0.5) | 0.4 ± 0.3 (-2.1 ± 0.7) | **0.002**; M ≠ NLF | 0.069 | 0.378 | diet |
| 20:4n-6 | 0.7 ± 0.2 (-1.4 ± 0.3) | 0.7 ± 0.2 (-1.4 ± 0.3) | 0.9 ± 0.2 (-1.2 ± 0.3) | 0.378 | **0.005**; 01 ≠ 02,03 | 0.083 | diet |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **20:4n-3** | 1.2 ± 0.2 (-0.8 ± 0.3) | 1.3 ± 0.2 (-0.7 ± 0.3) | 1.0 ± 0.3 (-1.1 ± 0.5) | <**0.001**; M ≠ LF, NLF | **0.012**; 00 ≠ 04,056 | 0.083 | diet |
| 22:4n-6 | 0.1 ± 0.1 (-2.6 ± 1.0) | 0.1 ± 0.1 (-2.8 ± 0.7) | 0.2 ± 0.1 (-2.4 ± 0.9) | | | | diet |
| **20:5n-3** | 3.2 ± 1.0 (0.1 ± 0.4) | 3.3 ± 0.6 (0.2 ± 0.4) | 2.8 ± 1.4 (-0.2 ± 0.6) | <**0.001**; M ≠ LF, NLF | 0.084 | **0.013**; M056 ≠ others | diet |
| **21:5n-3** | 0.3 ± 0.1 (-2.3 ± 0.4) | 0.3 ± 0.1 (-2.2 ± 0.4) | 0.2 ± 0.1 (-2.6 ± 0.6) | **0.002**; M ≠ LF, NLF | 0.312 | 0.690 | diet |
| **22:5n-6** | 0.2 ± 0.1 (-2.5 ± 0.4) | 0.2 ± 0.1 (-2.7 ± 0.6) | 0.2 ± 0.1 (-2.6 ± 0.7) | <**0.001**; M ≠ LF, NLF | <**0.001**; 056 ≠ others | **0.001**; M056 ≠ others | diet |
| **22:5n-3*** | 3.7 ± 0.7 (0.3 ± 0.3) | 3.3 ± 0.8 (0.2 ± 0.3) | 3.2 ± 0.6 (0.1 ± 0.4) | **0.014**; M ≠ LF | 0.204 | 0.452 | both |
| **22:6n-3*** | 12.3 ± 2.0 (1.5 ± 0.3) | 11.9 ± 2.3 (1.5 ± 0.3) | 12.3 ± 2.5 (1.4 ± 0.3) | 0.121 | 0.115 | 0.199 | diet |
| n-3 | 22.0 ± 3.4 | 21.5 ± 3.2 | 20.6 ± 4.4 | | | | |
| n-6 | 3.1 ± 0.4 | 3.1 ± 0.3 | 3.4 ± 0.4 | | | | |

A PCA using a correlation matrix was run on ln-transformed data to assess the most important FAs explaining the variance between FA profiles. The first three PCs accounted for 65% of the total variation in blubber FAs. The FAs with the greatest influence on PC1 were 18:1n-5, 16:1n-7, 20:4n-3, 18:2n-6 and 22:6n3, with the last three coming primarily from the diet. High loadings in the other PCs were attributed to 22:1n-9, 22:1n-11, 15:0, 22:5n-6 and 16:3n-4 on PC2 and 18:4n-3, 14:0, 20:4n-6, 22:5n-6 and 17:0 on PC3, all of which are derived primarily from the diet with the exception of the three saturated FAs.

### 3.3.3 Temporal and sex differences in FA profiles

The GLM on FA percentages showed significant differences in the percentages of 11 FAs between sex categories and 17 FAs between years ($P < 0.05$, **Table 3-2**). There was an interaction between the two factors for only two FAs, 20:5n-3 and 22:5n-6, which involved the only male in years 2005/2006 being segregated from the other groups. The percentages of 14:0, 18:1n-5, 20:1s, 22:1n-11, 18:4n-3, 20:4n-3, 20:5n-3, 21:5n-3 and 22:5s in males differed significantly from those of LFs and NLFs. Most of the significant inter-annual variation involved a difference between the combined years 2005/2006 and some or all of the previous years (nine FAs) and a difference between the years 2000 and 2004 (nine FAs).

The GLM on PCs confirmed these results on a general scale (FA profile instead of individual FA, **Table 3-3**): Year and sex had a significant effect on each of the first three components and on the overall model, but the interactions between the two factors were not significant. *Post-hoc* Tukey tests on PCs showed that the combined years 2005/2006 were significantly different from the other years on PC2 and PC3 ($P < 0.05$), and the year 2000 was different from 2004 on PC1. Furthermore, males were different from all females for each PC.

A DFA was run on the 20 FAs with the highest PC loadings (noted in bold in **Table 3-2**). The classic analysis gave an overall percentage of correct classifications of 76%, while with cross-validation this result was lowered to 54%. This sizeable difference between the percentages was interpreted as too many predictors (FAs) in the analysis (Walton et *al.* 2000). Thus, I lowered the number of variables for a second DFA by choosing 10 FAs only (noted by an asterisk in **Table 3-2**), with the highest absolute coefficients on the discriminant functions generated by the first DFA. The new DFA

gave an overall percentage of correct classification of 66% with the classification matrix and 55% with cross-validation. The discriminant function plot showed that some male FA profiles differed from female profiles along the first discriminant function (**Fig. 3-1**) mainly because of differences in the proportions of 20:1n-9, 22:1n-11 and 16:3n-4. The percentage of correct classification for males was 68%. No difference was apparent between the two groups of females.

**Table 3-3.** General linear model (GLM) testing the influence of Year and Sex on the first three principal components (PC) representing the fatty acid (FA) composition of New Zealand sea lion blubber. Significant values are in bold for each component and the overall model.

| Factor | df | ---------- Principal components ---------- | | | | | | Overall model | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | PC1 (42%) | | PC2 (14%) | | PC3 (9%) | | MANOVA (Pillai's trace) | | |
| | | *F* | *P* | *F* | *P* | *F* | *P* | df | *F* | *P* |
| Year | 5 | 2.71 | **0.028** | 3.91 | **0.004** | 7.15 | **<0.001** | 15/192 | 4.52 | **<0.001** |
| Sex | 2 | 4.63 | **0.013** | 7.90 | **0.001** | 6.28 | **0.003** | 6/126 | 7.14 | **<0.001** |
| Year*sex | 10 | 1.28 | 0.261 | 0.19 | 0.997 | 0.90 | 0.539 | 30/192 | 1.17 | 0.884 |

## 3.4 DISCUSSION

The diet of the NZ sea lion and its variation has been studied through the analysis of stomach contents and faeces (Lalas 1997; McMahon *et al.* 1999; Childerhouse *et al.* 2001; Chapter 2), but these methods are subject to biases encountered with the recovery of prey hard parts. Since the 1960s, FAs have been used as food tracers in marine trophic webs, allowing dietary variation to be examined among marine individuals (*e.g.*, Ackman and Eaton 1966; Kirsch *et al.* 1998; Staniland and Pond 2005; Beck *et al.* 2007b). The present study is the first report of the FA composition of the NZ sea lion's blubber. FA compositions revealed some significant differences between females and males, and from the years 2000 to 2006, which may be attributed, at least in part, to differences in their diets. However, FA deposition and mobilisation in the blubber, and hence its FA composition, can vary according to the nutritional and reproductive states of the animal (Andersen *et al.* 2004; Wheatley *et al.* 2007; Montie *et al.* 2008). Thus, part of the FA differences between the sex categories is likely to be the result of differential metabolism.

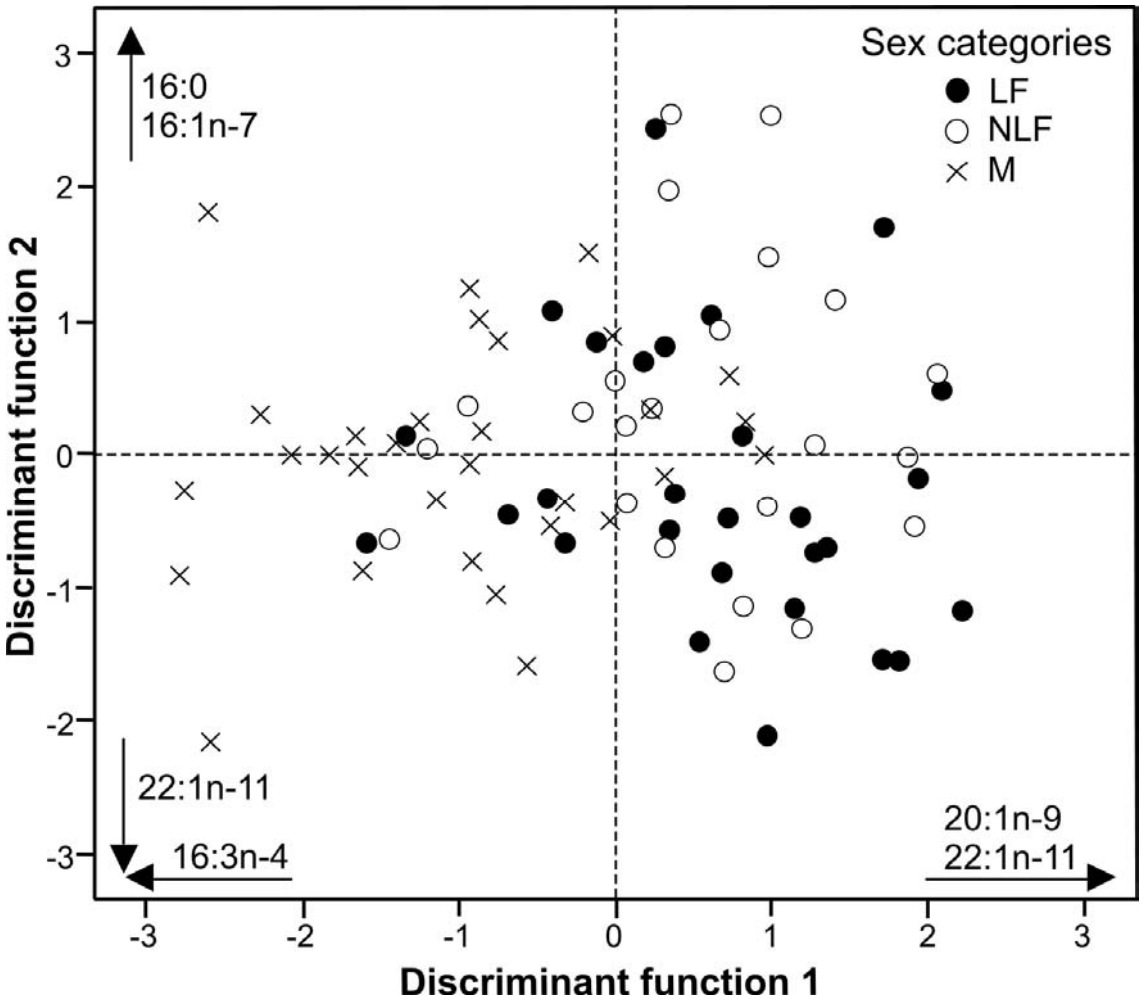


**Figure 3-1.** Plot of canonical discriminant functions. LF: lactating females; NLF: non-lactating females; M: males. This analysis included 10 FAs only (see details in 'Results'). The first and the second functions explained 93.8% and 6.2% of the variation among samples, respectively. The FAs with the most important positive or negative loadings on functions 1 and 2 are displayed along the axes

### 3.4.1 Limitations of the study

Blubber samples were stored at –20°C tightly wrapped but nonetheless under air for up to five years, which may have resulted in some oxidation of unsaturated FAs on the outside of the sample (Whiteley *et al.* 1992). Consequently lipids for analyses were extracted from a core taken from the centre of the original sample as outlined by Learmonth (2006) who reported no evidence of FA oxidation in the core of blubber of harbour porpoises stored at –20°C for 566 days.

Deposition of dietary lipids in the blubber will depend on the nutritional status of the animal: it is expected that a substantial amount of the FAs ingested above the metabolic requirements will be deposited in the blubber, whereas the extent to which deposition of

dietary FAs occurs during periods of negative energy balance is unclear. Thus, the blubber FA signatures are more likely to reflect dietary FAs in animals in positive energy balance (*e.g.*, Kirsch *et al.* 2000). LFs are thought to be the segment of the population with the highest metabolic constraints, and the lactating NZ sea lions, captured and weighed on land, had stabilised or were gaining mass at one month interval during the first months of the lactation period (B.L. Chilvers unpubl. data). Furthermore, the blubber thickness of the by-caught sea lions studied (**Table 3-1**) was comparable to captive adult female Steller sea lions *Eumetopias jubatus* kept on a maintenance diet (Mellish *et al.* 2007). Thus, I believed that sea lions caught were in positive energy balance and depositing dietary FAs.

Moreover, deposition and mobilisation of blubber lipids have been shown to vary with the body region in otariid seals (Arnould *et al.* 2005; Mellish *et al.* 2007), while phocids present a more uniform fat distribution (*e.g.*, Ryg *et al.* 1988; Mellish *et al.* 2007). The uniformity of fat distribution is believed to minimise heat loss to the environment by optimising insulation efficiency (Ryg *et al.* 1988). Otariids do not have extended periods of fasting like phocids, thus blubber fat may have a less important role in term of insulation (Mellish *et al.* 2007), resulting in heterogeneity of fat distribution with preferred depot sites along the body. Blubber samples were taken from the sternum region because the thickness of the blubber over the sternum is positively correlated with body mass (Massey University unpubl. data), suggesting that this region is a fat depot when sea lions are in positive energy balance.

### 3.4.2 Variation in FA profiles among sex categories

The interactions between sex and year were limited to two dietary FAs (20:5n-3 and 22:5n-6). Thus, the variation in FA profiles between the sex categories was consistent within each year, and each factor has been interpreted separately. LFs and NLFs did not show any significant difference in their FA composition (**Table 3-2**, **Fig. 3-1**). However, lactation is the most energetically demanding period of mammalian reproduction (Oftedal 1984), and marine mammals are no exception (Costa *et al.* 1986; Williams *et al.* 2007). Lactating marine mammals are thought to mobilise substantial quantities of lipids into milk, affecting their blubber FA profiles (*e.g.*, Wheatley *et al.* 2007, Montie *et al.* 2008). Similar FA profiles between LFs and NLFs show that blubber FAs in the sternum region were not mobilised to produce milk. Instead, sternal

blubber is likely to represent dietary lipids, and in this case, NLFs displayed the same diet as lactating conspecifics. Indeed, LFs are seen to have high site fidelity to foraging areas, which is thought to represent long-term learnt foraging behaviour (Chilvers 2008a); therefore, it is expected that this foraging fidelity would continue even when not rearing a pup.

Concerning variation between sexes, the GLMs (**Table 3-2**, **Table 3-3**) and the DFA (**Fig. 3-1**) demonstrated significant differences between FA profiles of females and males by-caught by the squid fishery at the Auckland Islands: percentages of individual FA in males were significantly different ($P < 0.05$) from those in LFs and NLFs in 11 out of 30 FAs (**Table 3-2**). Although some caution is necessary as the percentages are not independent values, these results were confirmed by a second GLM on the PCs (**Table 3-3**) and the DFA (**Fig. 3-1**). These variations result from the combination of different diet and different metabolism. Indeed, males caught by the fishery were generally heavier than females, which give them the ability to dive deeper at the edge or to stay longer at the bottom of the shelf than females, exploiting different resources or similar food in different proportions. The main FAs causing the separation between females and males in the DFA were 20:1n-9 and 22:1n-11 in higher proportions in females and 16:3n-4 in higher proportions in males (**Fig. 3-1**). FA compositions of several species of fish, cephalopods and crustaceans from the Auckland Islands shelf have been examined (Chapter 4) and indicate that the relative amount of 16:3n-4 is higher in deep-benthic species, while the reverse trend occurs for 20:1n-9 and 22:1n-11 in demersal fish. Therefore, if most of the FA variation is influenced by the diet, males would feed on more deep-benthic prey than the females do. This is consistent with foraging studies comparing female and male otariids, for which males displayed deeper dives than females (*e.g.*, Page *et al.* 2005b; Staniland and Robinson 2008). However, a previous study on the stomach contents of the same individuals studied here did not show such a trend: dietary differences between males and females were limited to the proportions of opalfish, a benthic prey living on the shelf, which was in higher proportion in the stomach contents of females (Chapter 2). But these differences may have been underestimated since stomach contents give a limited picture of the diet over several days only, which will not reflect long-term dietary differences between female and male NZ sea lions. Furthermore, part of the FA variation between sexes is likely to originate from differential metabolism. Indeed, a larger mass for males implies a higher maintenance metabolism (Costa 1991), which can result in a greater FA mobilisation in

the sternal blubber than in females. To date, there is no information on the differential use of fat depots between male and female otariids. Thus, it is difficult to draw any conclusion on the dietary differences between NZ sea lion males and females inferred from blubber FA profiles as long as no foraging and diving data are available for males. So far, LF are the only segment of the population for which foraging and diving behaviours are known (Chilvers *et al.* 2005, 2006). It must be stressed that males analysed here were mainly non-territorial during breeding and were caught in the same area as females, thus they were not representative of territorial males. Territorial males tend to disperse to distant regions after breeding (Robertson *et al.* 2006) and are rarely caught by the squid fishery around the Auckland Islands.

### 3.4.3 Year variation in FA profiles

Although the DFA was not performed on years due to a small sample size, both GLMs showed differences in the FA profiles of by-caught sea lions between years (**Tables 3-2** and **3-3**), especially between 2005/2006 and the previous years and between 2000 and 2004. Individual FA variation concerned 17 out of 30 FAs (**Table 3-2**). As discussed previously, the variation in blubber FA profiles between years may be caused by both differential metabolism and different diet. Differential FA metabolism between years could arise from animals in different body condition. The blubber thickness was significantly lower in the combined years 2005/2006 than in 2000 (**Table 3-1**); thus, the differences in FA profiles noticed between these years can be the result of different FA mobilization in the sternal blubber. However, significant variation in FA profiles was not limited to the differences between 2000 and the years 2005/2006; therefore, diet must play a significant role in the FA variation reported between 2005/2006 and the previous years and between 2000 and 2004. Inter-annual and seasonal variation in the diet of the NZ sea lion males has already been investigated through the analysis of faeces (Lalas 1997, McMahon *et al.* 1999, Childerhouse *et al.* 2001). Seasonal differences in the diet were found only at Otago Peninsula, South Island, NZ (Lalas 1997), and were attributed to changes in prey availability. Indeed, NZ sea lions are considered generalist predators, and the changes noticed in the present study between the combined years 2005/2006 and the previous years, and also between 2000 and 2004, may be interpreted by a variation in prey stocks availability on the Auckland Islands shelf. However, information on fish and squid populations and their variation around

the Auckland Islands are non-existent. Therefore, it is currently not possible to validate the hypothesis of a change in prey availability between 2000 and 2006.

## 3.5 CONCLUSION

In the present study, significant differences in the blubber FA compositions between male and female NZ sea lions, and between years from 2000 to 2006, were detected. These differences are likely to be the result of both metabolism and diet. Due to different metabolic requirements, an ability to dive deeper and a lack of investment in pup rearing, male NZ sea lions would utilise food resources differently, explaining some of the differences in FA profiles between the sexes. However, these differences were probably underestimated, as territorial males, with a significant higher mass than females, were not represented in the present study. FA metabolism in the blubber is still poorly understood, thus limiting the potential of FA signature analysis to infer diets of animals in different nutritional or reproductive states (*e.g.,* females *versus* males, LFs *versus* NLFs). However, this method can overcome some biases encountered in traditional dietary techniques. Thus, FA analysis must be considered as a complementary tool to stomach and faeces analyses, along with foraging telemetry studies to assess the feeding ecology of marine mammals.

## Acknowledgements

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# 4 CHAPTER 4

## PROXIMATE COMPOSITION, ENERGY CONTENT, AND FATTY ACID COMPOSITION OF MARINE SPECIES FROM THE CAMPBELL PLATEAU, NEW ZEALAND

**Photos from left to right and top to bottom:** hoki (*Macruronus novaezelandiae*), arrow squid (*Nototodarus sloani*), rattail (*Coelorhynchus arspercephalus*), red cod (*Pseudophycis bachus*). Copyright is owned by the New Zealand Seafood Industry which gives its agreement to copy the photos from Paul (2000).

**Chapter reference:**

Meynier L., Morel P.C.H., Mackenzie D.D.S., MacGibbon A., Chilvers, L., Duignan P.J. 2008. Proximate composition, energy content, and fatty acid composition of marine species from the Campbell plateau, New Zealand. New Zealand Journal of Marine and Freshwater Research, 42: 425-437.

## Abstract

The Campbell plateau is an important fishing ground for the major commercial New Zealand species. Yet, studies on trophic interactions between species and their nutritional values are limited. The objectives of this study were (1) to determine the proximate composition and energy contents of selected commercial and non-commercial marine species from the Campbell plateau, (2) to determine their fatty acid (FA) composition, and (3) to evaluate the degree to which species can be differentiated by their FA compositions. I analysed 10 *Macruronus novaezelandiae*, 11 *Lepidorhynchus denticulatus*, 10 *Pseudophycis bachus*, 10 *Hemerocoetes* spp., 2 *Squalus acanthias*, 10 *Nototodarus sloani*, 7 *Enteroctopus zealandicus*, and 6 *Metanephrops challengeri*. The variation of energy contents between fish species was not significant, but their lipid and protein contents varied significantly. FA signatures could distinguish the species analysed, and at a broader scale, the type of habitat. However, within-species variability is important among benthic species. In general, the diet inference from FA trophic markers was consistent with reported diets from stomach contents or reported FA compositions from related species analysed elsewhere.

## 4.1 INTRODUCTION

The Campbell plateau is an extensive submarine platform, in the subantarctic region south of New Zealand, with water depths ranging from 0-500 m on the rises to 1000 m at the plateau edge (**Fig. 4-1**). This region is an important fishing ground for the major commercial marine species in New Zealand, hoki (*Macruronus novaezelandiae*), arrow squid (*Nototodarus* spp.) and ling (*Genypterus blacodes*), but also for secondary harvested species such as hake (*Merluccius australis*), southern blue whiting (*Micromesistius australis*) and scampi (*Metanephrops challengeri*): In the fishing season 2005/2006, catches of hoki, arrow squid, ling, hake, southern blue whiting and scampi from the Campbell plateau were approximately 7000 t, 17000 t, 5000 t, 2500 t, 26000 t and 300 t respectively (Ministry of Fisheries 2007), leading to a total of 58000 t for these target species alone. This area is also of special interest to conservationists as it hosts large populations of resident seabirds and marine mammals, such as albatrosses (Gales 1998), penguins (Heather and Robertson 2005), fur seals (Crawley and Warneke 1979), and the New Zealand sea lion (Chilvers *et al.* 2007b).

Despite its commercial and ecological importance, the Campbell plateau has received limited attention in the literature in terms of ecosystem structure and interactions between marine species. Jacob *et al.* (1998) identified demersal species assemblages off southern New Zealand including the Campbell plateau, and Bradford-Grieve *et al.* (2003) described the functioning of this ecosystem through an ECOPATH model, but most of the data used to build the model were from other systems. With the exception of one study on the diet of the squid *Moroteuthis ingens* (Phillips *et al.* 2003), dietary studies in the region have been limited to important commercial fish: hoki, southern blue whiting and ling (Inada and Nakamura 1975; Mitchell 1984; Clark 1985). Traditionally, examination of the stomach contents has been used to examine the diet of marine specimens, but it only provides information on last meals, and differential prey digestion and erosion of hard parts can introduce biases (*e.g.*, Jobling and Breiby 1986; Pierce and Boyle 1991). Since the 1960s, fatty acid (FA) analysis has been developed as a complementary diet method to traditional analyses (*e.g.*, Sargent *et al.* 1987; Dalsgaard *et al.* 2003). The principle is that many dietary lipids are incorporated into the adipose tissue of the predator with little or no modification of their original structure (Sargent *et al.* 1987; Dalsgaard and St John 2004). Thus, the FA composition of the predator reflects the accumulation of dietary lipids over several weeks (Kirsch *et al.*

1998), and is unbiased from species-specific gut passage and digestion rates. In particular, polyunsaturated FAs (PUFAs) such as the arachidonic acid 20:4n-6, the eicosapentaenoic acid 20:5n-3, and the docosahexaenoic acid 22:6n-3, which are biosynthesised by primary producers (phytoplankton, macroalgae) in the marine environment (*e.g.,* del Rosario González-Baró and Pollero 1998), can be used as general indicators of food webs in marine ecosystems (*e.g.,* Sargent *et al.* 1987; Dalsgaard *et al.* 2003; Dalsgaard and St John 2004). However, the use of FAs as trophic markers has some limits: for instance, no single FA can be assigned to one particular species, and there is a "dilution effect" with increasing trophic level, which makes FA markers less evident in high trophic levels. Furthermore, diet is not the only factor affecting FA composition: selective retention, desaturation or elongation of particular FAs can occur depending on the phylogeny and environmental conditions of the animals (Dalsgaard (Dalsgaard *et al.* 2003; Dalsgaard and St John 2004).

Over the last two decades, the FA and lipid compositions of marine organisms have been used extensively to study the food web of the Southern Ocean (*e.g.,* Phleger *et al.* 1999; Hagen *et al.* 2000; Nelson *et al.* 2001). Also, FA and proximate compositions of marine species can give nutritional information of value to human nutritionists. In New Zealand, the nutritional studies on marine food are limited to species collected off the main islands of New Zealand (Hughes *et al.* 1980; Pickston *et al.* 1982; Vlieg 1982b, a, 1984a, b, c; Vlieg and Body 1988).

This study is the first stage of a larger project with the overall aim to create a library containing the FA compositions of potential prey of top predators occurring in the Campbell plateau. It was initiated to assess the diet of the New Zealand sea lion that breeds on the Auckland Islands and Campbell Island (Chilvers *et al.* 2007b). The specific objectives of the present research were (1) to determine the proximate composition and energy contents of selected commercial and non-commercial marine species from the Auckland Islands Rise on the Campbell plateau, (2) to determine their FA composition, and (3) to evaluate the degree to which species can be differentiated by their FA compositions. The results are discussed in terms of the life traits of the species and their trophic relationships.

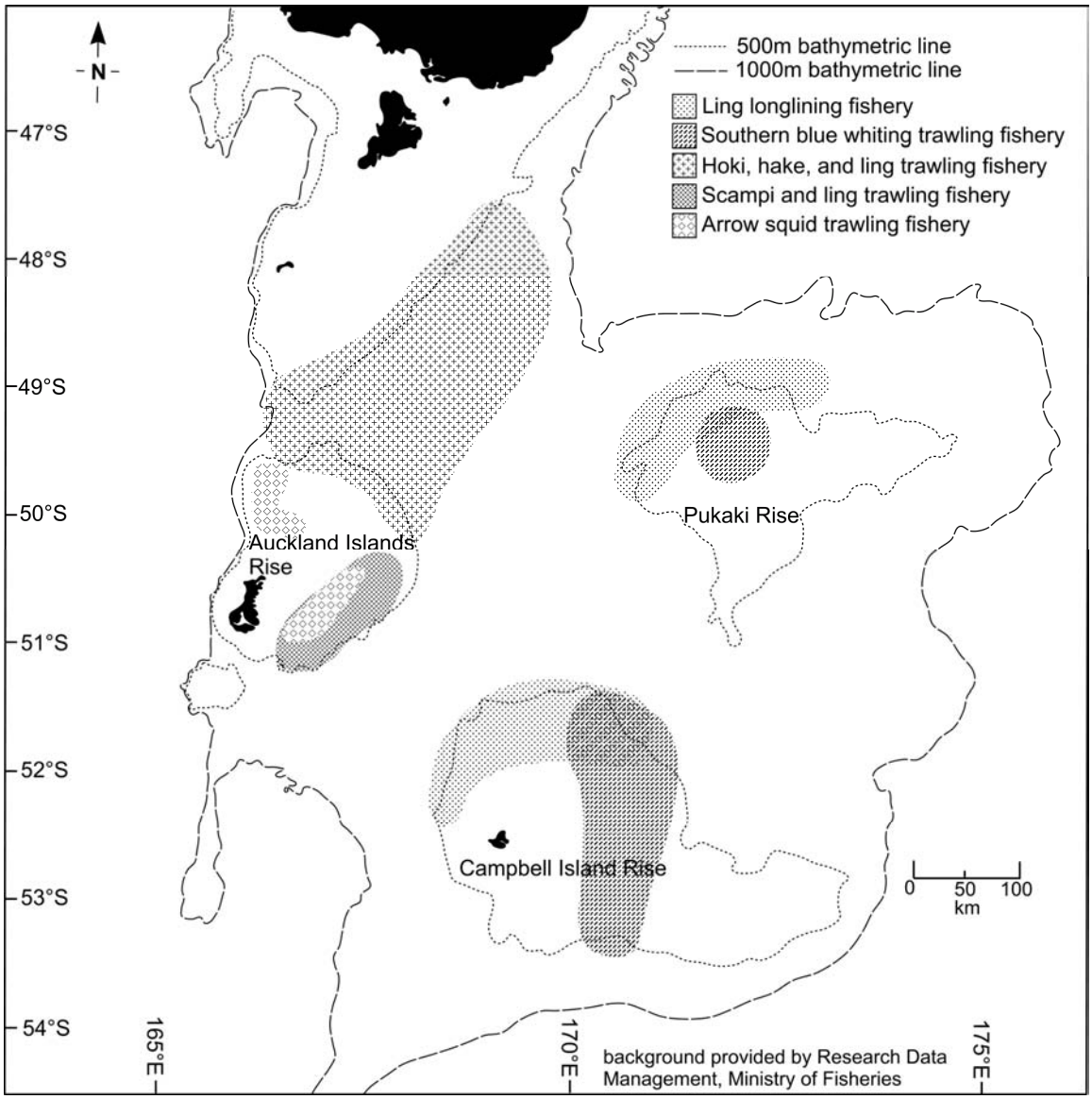


**Figure 4-1.** Map of the Campbell plateau with the different fishing areas as follows: ling longlining (Anderson *et al.* 2000); southern blue whiting, hake, and hoki trawling (Clark *et al.* 2000); ling trawling (Horn 2007); scampi distribution (O'Driscoll *et al.* 2003); and arrow squid trawling (Smith and Baird 2007).

## 4.2 MATERIALS AND METHODS

### 4.2.1 Sample collection

Fish, cephalopods and crustaceans were collected from various regions on the Auckland Islands Rise on the Campbell plateau (**Fig. 4-1**) during the austral summer and autumn of 2005 to 2007. Some specimens were collected by observers onboard commercial fishing trawlers of the squid and scampi fisheries, under contracts with the Ministry of Fisheries New Zealand. Others were collected by staff of the National Institute of Water and Atmospheric Research (NIWA) New Zealand, onboard the research vessel

*Tangaroa* in December 2005. At collection, specimens were sorted according to species, and stored frozen at –20°C in airtight plastic bags until analysed (within one year).

Fish, cephalopods and crustaceans were thawed, weighed and measured. Then each individual was cut into several pieces and ground using a kitchen blender until completely homogenised. For large fish, such as hoki, javelin fish and spiny dogfish, head bones, too hard to be ground, were removed. An aliquot of a third of the mixture was freeze-dried to a constant dry mass. Freeze-dried portions were stored at –20°C in sealed plastic bags pending proximate composition and FA analyses.

### 4.2.2 Proximate analysis

Duplicates of 1.5 g (or 3 g for scampi) were sampled from the freeze-dried portions for lipid and FA analysis. Fat content was expressed as an average of the duplicates. For some small prey such as scampi and opalfish, there was not enough material for two aliquots, thus only one sample per individual prey was analysed. Lipids were extracted following Folch *et al.* (1957): samples of 1.5 g (or 3 g) were homogenised in 30 mL (or 60 mL) respectively of 2:1 chloroform:methanol (v:v) containing 0.01% butylated hydroxytoluene (BHT) as an antioxidant. The extract was filtered and washed with 1% sodium chloride in water (saline) to a final ratio of 8:4:3 chloroform:methanol:saline (v:v:v). The organic phase was then dehydrated over anhydrous sodium sulphate powder. Finally, the lipid extract was dried in a rotary evaporator at 38°C to a constant mass. The crude protein content in the dried samples was estimated by multiplying the amount of nitrogen measured with a Leco nitrometer by 6.25 (AOAC Official Method 968.06; AOAC 2005). Energy values (kJ $g^{-1}$) of the dried sample were determined by adiabatic bomb calorimetry.

### 4.2.3 FA analysis

FA methyl esters were prepared directly from 30 mg of the extracted lipid using 1.5 mL of toluene and 1.5 mL of 10% boron trifluoride in methanol as the methylating reagent. Each extract was capped under nitrogen, and heated at 50°C overnight. FA methyl esters were then extracted into hexane and stored over anhydrous sodium sulphate at –20°C before chromatographic analysis.

Analysis of FA methyl esters was carried out using a temperature-programmed gas–liquid chromatography (Shimadzu Gas Chromatograph GC-17A, Shimadzu Corporation, Kyoto, Japan) equipped with a flame ionisation detector, and fitted with a 30 m × 0.25 mm i.d. column (50% cyanopropyl polysiloxane, 0.25 μm film thickness, DB-23, J&W, U.S.A.; or 70% cyanopropyl polysilphenylene-siloxane column, 0.25 μm film thickness, BPX70, SGE Analytical Science, Australia). Helium was the carrier gas. FA methyl esters (2 μL) were injected manually in split mode (1:50) at an injection port temperature of 250°C. The detector temperature was set at 270°C. The temperature of the oven was programmed to stay at 50°C for 2 min, rose to 180°C at 25°C $min^{-1}$, held for 5 min, rose again to 200°C at 2°C $min^{-1}$, then to 240°C at 2.1°C $min^{-1}$ (total of 42.2 min).

FA components were identified by comparison of retention time data to authentic (Nu-Chek GLC standard 68D, Supelco 37 FAME mix, Matreya menhaden oil) and laboratory standards (cod liver oil). Cod liver oil was used in every series of runs to determine accurate retention times. Nu-Check 68D standard was injected regularly to check the quantitation of each FA. Peak areas were measured by a computerised integration system attached to the gas chromatograph (CLASS-VP version 7.3, Shimadzu Scientific Instruments, Inc., Columbia, MD). Each chromatogram was checked to ensure correct identification. The identification of some minor peaks was uncertain and these were not included in the final normalisation. 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. I used theoretical response factors calculated according to Ackman and Sipos (1964) for the quantitation of FAs.

### 4.2.4 Statistical methods

Lipid, protein, and energy contents were originally estimated from lyophilised samples. They were transformed to wet mass values by considering the amount of water in the samples. Thus, lipid and protein contents refer to percentage wet mass, and energy content refers to kJ $g^{-1}$ wet mass. All statistical analyses were conducted using MINITAB package (MINITAB Release 14.1, MINITAB Inc. 2003) and SPSS package (SPSS for windows® Release 15.0, SPSS Inc. 2006). Kruskal-Wallis tests (K-W tests)

and *post hoc* Mann-Whitney tests (M-W tests) were performed to assess differences in the lipid, protein and energy values between species.

FAs were expressed as an average of the lipid duplicates, in percentage mass of total FAs. A principal component (PC) analysis based on a correlation matrix of the arcsine square root-transformed FA percentages was run to visualise the distribution of individual FA compositions, and to determine which FAs were most influential. In the PC plot, species were classified according to their pelagic or benthic habitat. Moreover, ANOVAs were run on the transformed percentages of each FA to assess significant FA differences among species. All statistical tests have an α level of statistical significance of 0.05 and all means are followed by standard deviations (SD).

## 4.3 RESULTS

In total, 43 fish from five species (bony fish hoki *Macruronus novaezelandiae*, javelin *Lepidorhynchus denticulatus*, red cod *Pseudophycis bachus*, opalfish *Hemerocoetes* spp, and the cartilaginous fish spiny dogfish *Squalus acanthias*), 17 cephalopods from two species (arrow squid *Nototodarus sloani* and octopus *Enteroctopus zealandicus*), and six scampi (*Metanephrops challengeri*) were analysed. Morphometric data and ecological characteristics are summarised in **Table 4-1**.

### 4.3.1 Proximate composition

The lipid content ranged from 1.1 ± 0.4% (scampi) to 12.2 ± 2.4% (spiny dogfish) (**Table 4-2**). Spiny dogfish had a significantly higher lipid content than the other species studied (K-W test, $H_{(7, 60)} = 34.89$, $P = 0.000$; M-W tests, $P < 0.05$) except hoki (M-W test, $P = 0.093$). Bony fish and arrow squid displayed similar lipid content (M-W tests, $P > 0.05$) with means from 4.0 ± 1.4% (red cod collected in April) to 6.8 ± 2.9% (hoki) (**Table 4-2**). Lipid values of octopus and scampi were less than 2.5% and significantly lower than those of the other species (M-W tests, $P < 0.05$).

The protein contents of octopus (13.6 ± 1.3%) and scampi (15.2 ± 1.7%) were also significantly lower (K-W test, $H_{(7, 60)} = 37.57$, $P = 0.000$; M-W tests, $P < 0.05$) than the bulk of the specimens (18.0 – 19.5%) (**Table 4-2**), and opalfish displayed the highest protein content (30.3 ± 6.3%).

**Table 4-1.** Morphometric data and ecological characteristics of marine species analysed from the region of the Auckland Islands Rise on the Campbell plateau (n = 66). The age was based on the length of the specimens. The length corresponds to the total length for fish and the dorsal mantle length for cephalopods. Age class, depth range, habitat and diet were from the literature: [a] (Ministry of Fisheries 2007); [b] (Kawakami 1976); [c] (Beentjes *et al.* 2002); [d] (Ayling and Cox 1982); [e] (O'Shea 1999); [f] (Blaber and Bulman 1987); [g] (Clark 1985); [h] Jeff Forman, pers. comm.; [i] (Coleman and Mobley 1984); [j] (Saito *et al.* 1974); [k] (Hanchet 1991).

| Name | Species | Date | n | Length (cm) | Mass (g) | Age class | Depth range (preferred depth) | Habitat | Diet |
|---|---|---|---|---|---|---|---|---|---|
| Hoki | *Macruronus novaezelandiae* | Dec-05 | 11 | 68.6 ± 8.8 | 1091 ± 383 | 3+ (not mature) [a] | 200-400+ (300) [c] | pelagic [f, g] | Pasiphea, amphipods, myctophidae [f, g] |
| Javelin | *Lepidorhynchus denticulatus* | Dec-05 | 10 | 46.4 ± 3.6 | 224 ± 63 | - | 200-400+ (300) [c] | pelagic [f, g] | Pasiphea, amphipods, cephalopods, euphausiids, myctophidae [f, g] |
| Red cod | *Pseudophycis bachus* | Apr-06 | 5 | 34.5 ± 3.1 | 476 ± 154 | 1+ (not mature) [a] | 50-300+ (120) [c] | benthic [h] | Galatheid, crangonid, crabs, flatfish, eels, dwarf cod [h] |
| | | Dec-05 | 5 | data lost | data lost | | | | |
| Opalfish | *Hemerocoetes* spp | Apr-06 | 10 | 16.8 ± 1.2 | 23 ± 6 | - | over shelf [d] | benthic [d] | - |
| Spiny dogfish | *Squalus acanthias* | Dec-05 | 2 | 61.2 ± 1.0 | 811 ± 11 | 6+ (mature if male, not if female) [a] | 50-300+ (100) [c] | pelagic [i] | Octopus, opalfish, crabs, galatheid, euphausiids, myctophids, squid [i, k] |
| Arrow squid | *Nototodarus sloani* | Mar-04 | 10 | 27.1 ± 4.4 | 466 ± 294 | 0+ [b] | 100-350+ (200) [c] | pelagic [j] | euphausiids, amphipods, crabs, myctophidae, saury, squid [j] |
| Octopus | *Enteroctopus zealandicus* | Apr-06 | 6 | 17.7 ± 3.5 | 2529 ± 1392 | - | littoral to 530m [e] | benthic [e] | - |
| | | Feb-07 | 1 | 20.5 | 3809 | | | | |
| Scampi | *Metanephrops challengeri* | Jan-07 | 6 | | 23 ± 9 | - | 200-500 [a] | benthic [a] | - |

The lowest energy contents were obtained for octopus and scampi (approximately 3.8 kJ $g^{-1}$), which were significantly different from the other species (K-W test, $H_{(7, 60)} = 34.89$, $P = 0.000$; M-W tests, $P < 0.05$). Fish and arrow squid had similar energy contents (M-W tests, $P > 0.05$) with means ranging from 6.3 ± 0.6 kJ $g^{-1}$ (arrow squid) to 8.5 ± 1.9 kJ $g^{-1}$ (opalfish) (**Table 4-2**).

**Table 4-2.** Mean ± SD of the proximate composition (% wet mass) and energy content (kJ $g^{-1}$ wet mass) of marine species from the region of the Auckland Islands Rise on the Campbell plateau. The scientific names and number analysed are given in Table 4-1.

| | ---------- Lipid -------- | | --------- Protein -------- | | ------ Moisture ------ | | ------- Energy ------ | |
|---|---|---|---|---|---|---|---|---|
| **Species** | % wet mass | Range | % wet mass | Range | % | Range | kJ $g^{-1}$ wet mass | Range |
| Hoki | 6.8 ± 2.9 | 2.8-10.6 | 19.5 ± 1.0 | 17.8-20.3 | 71.2 ± 2.9 | 67.8-75.6 | 7.1 ± 1.0 | 6.0-8.3 |
| Javelin | 6.1 ± 2.2 | 2.2-9.0 | 18.2 ± 1.7 | 15.0-21.6 | 71.7 ± 2.2 | 67.9-74.8 | 6.7 ± 0.8 | 5.3-7.8 |
| Red cod (April) | 4.0 ± 1.4 | 2.8-6.0 | 18.0 ± 1.0 | 17.3-19.3 | 69.1 ± 1.0 | 67.7-69.9 | 7.3 ± 0.5 | 6.9-7.5 |
| Opalfish | 4.2 ± 1.5 | 2.1-6.0 | 30.3 ± 6.3 | 19.8-37.1 | 53.3 ± 9.0 | 37.5-72.2 | 8.5 ± 1.9 | 5.2-10.6 |
| Spiny dogfish | 12.2 ± 2.4 | 10.4-13.9 | 19.3 ± 0.2 | 19.2-19.4 | 67.1 ± 2.3 | 65.5-68.7 | 8.3 ± 0.7 | 7.8-8.8 |
| Arrow squid | 5.6 ± 1.9 | 2.2-9.0 | 18.9 ± 1.2 | 16.7-20.1 | 72.7 ± 2.3 | 69.6-76.0 | 6.3 ± 0.6 | 5.3-7.0 |
| Octopus (April) | 1.8 ± 0.6 | 0.8-2.4 | 13.6 ± 1.3 | 12.2-15.3 | 80.2 ± 1.3 | 78.1-82.0 | 3.8 ± 0.4 | 3.2-4.4 |
| Scampi | 1.1 ± 0.4 | 0.6-2.0 | 15.2 ± 1.7 | 14.5-17.1 | 66.4 ± 4.4 | 60.8-73.4 | 3.8 ± 0.6 | 3.1-4.4 |

### 4.3.2 FA composition

Although 37 FAs were originally identified, only 30 ranging from 14:0 to 22:6n-3 were used (**Table 4-3**). The eight FAs removed were either short chain FAs (<14 carbons), known to come primarily from endogenous biosynthesis (Dalsgaard et al. 2003), or FAs (18:4n-3, 20:4n-3 and 22:2n-6) for which the identification was not certain. Some FA methyl esters tended to co-elute when injected through the BPX70 column. This is the case for 16:3n-4 with 17:1 and 21:5n-3 with 24:0, for which percentages were grouped for all samples.

**Table 4-3a.** FA composition (mean % mass of total FA ± SD) of fish species collected in the region of the Auckland Islands Rise on the Campbell plateau. *n* is the number of specimens analysed per species. SAFA is for saturated fatty acids, MUFA for monounsaturated fatty acids, PUFA for polyunsaturated fatty acids, ARA for arachidonic acid, EPA for eicosapentaenoic acid, and DHA for docosahexaenoic acid.

| | Fish | | | | |
|---|---|---|---|---|---|
| | **Hoki** | **Javelin** | **Red cod** | **Opalfish** | **Dogfish** |
| *n* | 11 | 10 | 10 | 10 | 2 |
| **SAFA** | | | | | |
| 14:0 | 5.73 ± 0.90 | 4.64 ± 1.07 | 9.04 ± 2.96 | 8.14 ± 2.50 | 4.12 ± 0.20 |
| 15:0 | 0.60 ± 0.08 | 0.64 ± 0.10 | 1.41 ± 0.49 | 0.95 ± 0.37 | 1.41 ± 0.03 |
| 16:0 | 24.53 ± 2.10 | 23.77 ± 2.18 | 31.83 ± 2.63 | 30.34 ± 4.49 | 28.23 ± 1.31 |
| 17:0 | 0.95 ± 0.22 | 0.58 ± 0.11 | 1.71 ± 0.64 | 0.96 ± 0.28 | 1.27 ± 0.40 |
| 18:0 | 3.34 ± 0.47 | 2.51 ± 0.39 | 6.14 ± 0.82 | 6.87 ± 1.18 | 5.23 ± 1.01 |
| 24:0 & 21:5n-3 | 0.20 ± 0.06 | 0.18 ± 0.05 | 0.18 ± 0.08 | 0.12 ± 0.15 | 0.18 ± 0.02 |
| **MUFA** | | | | | |
| 14:1n5 | 0.27 ± 0.07 | 0.22 ± 0.06 | 0.24 ± 0.06 | 0.27 ± 0.09 | 0.18 ± 0.11 |
| 15:1 | 0.11 ± 0.05 | 0.21 ± 0.06 | 0.22 ± 0.07 | 0.22 ± 0.09 | 0.33 ± 0.24 |
| 16:1n-7 | 5.87 ± 0.64 | 7.25 ± 0.80 | 4.96 ± 0.63 | 7.58 ± 1.35 | 5.59 ± 0.26 |
| 17:1 & 16:3n-4 | 0.42 ± 0.05 | 0.66 ± 0.17 | 0.47 ± 0.13 | 0.78 ± 0.31 | 1.05 ± 0.03 |
| 18:1n-9 | 19.34 ± 1.07 | 23.30 ± 4.20 | 13.40 ± 1.45 | 18.19 ± 1.24 | 18.97 ± 0.67 |
| 18:1n-7 | 3.64 ± 0.14 | 3.91 ± 0.68 | 3.27 ± 0.85 | 3.82 ± 0.61 | 4.18 ± 0.34 |
| 18:1n-5 | 0.22 ± 0.05 | 0.45 ± 0.06 | 0.38 ± 0.20 | 0.36 ± 0.08 | 0.31 ± 0.01 |
| 20:1n-11 | 0.38 ± 0.08 | 1.19 ± 0.36 | 0.45 ± 0.08 | 0.51 ± 0.17 | 1.56 ± 0.24 |
| 20:1n-9 | 9.37 ± 0.90 | 10.03 ± 1.52 | 5.41 ± 1.65 | 7.10 ± 2.10 | 5.92 ± 1.96 |
| 22:1n-11 | 2.70 ± 0.32 | 1.91 ± 0.58 | 1.16 ± 0.27 | 1.30 ± 0.34 | 2.60 ± 1.39 |
| 22:1n-9 | 0.88 ± 0.17 | 0.74 ± 0.29 | 0.51 ± 0.21 | 0.53 ± 0.12 | 2.98 ± 0.63 |
| **PUFA** | | | | | |
| 16:4n-1 | 0.08 ± 0.06 | 0.08 ± 0.03 | 0.07 ± 0.03 | 0.10 ± 0.08 | 0.10 ± 0.04 |
| 18:2n-6 | 1.32 ± 0.11 | 1.15 ± 0.10 | 1.08 ± 0.19 | 0.58 ± 0.32 | 1.10 ± 0.27 |
| 18:3 | 0.74 ± 0.05 | 0.70 ± 0.10 | 0.58 ± 0.10 | 0.39 ± 0.24 | 0.80 ± 0.06 |
| 20:2n-6 | 0.22 ± 0.04 | 0.21 ± 0.04 | 0.31 ± 0.06 | 0.14 ± 0.09 | 0.23 ± 0.02 |
| 20:3n-6 | 0.05 ± 0.04 | 0.07 ± 0.02 | 0.04 ± 0.00 | 0.03 ± 0.04 | 0.02 ± 0.02 |
| 20:3n-3 | 0.16 ± 0.03 | 0.20 ± 0.05 | 0.11 ± 0.03 | 0.05 ± 0.07 | 0.04 ± 0.00 |
| 20:4n-6 (ARA) | 0.49 ± 0.12 | 0.53 ± 0.09 | 0.73 ± 0.61 | 0.61 ± 0.92 | 1.03 ± 0.15 |
| 20:5n-3 (EPA) | 6.76 ± 1.16 | 6.36 ± 0.96 | 5.13 ± 1.27 | 3.73 ± 3.36 | 3.97 ± 0.08 |
| 22:5n-6 | 0.04 ± 0.03 | 0.08 ± 0.03 | 0.08 ± 0.09 | 0.05 ± 0.13 | 0.24 ± 0.06 |
| 22:5n-3 | 1.50 ± 0.30 | 0.93 ± 0.28 | 1.11 ± 0.63 | 0.43 ± 0.50 | 1.26 ± 0.34 |
| 22:6n-3 (DHA) | 10.08 ± 1.80 | 7.49 ± 1.67 | 9.98 ± 3.85 | 5.87 ± 5.17 | 7.11 ± 0.27 |
| Total SAFA | 35.33 ± 3.83 | 32.33 ± 3.90 | 50.32 ± 7.62 | 47.38 ± 8.97 | 40.44 ± 2.97 |
| Total MUFA | 43.21 ± 3.53 | 49.87 ± 8.77 | 30.47 ± 5.60 | 40.65 ± 6.51 | 43.66 ± 5.88 |
| Total PUFA | 21.45 ± 3.75 | 17.80 ± 3.38 | 19.22 ± 6.87 | 11.97 ± 10.93 | 15.90 ± 1.30 |

**Table 4-3b.** FA composition (mean % mass of total FA ± SD) of cephalopods and crustacean species collected in the region of the Auckland Islands Rise on the Campbell plateau. *n* is the number of specimens analysed per species. SAFA is for saturated fatty acids, MUFA for monounsaturated fatty acids, PUFA for polyunsaturated fatty acids, ARA for arachidonic acid, EPA for eicosapentaenoic acid, and DHA for docosahexaenoic acid.

| | **Cephalopods** | | **Crustacean** |
|---|---|---|---|
| | **Arrow squid** | **Octopus** | **Scampi** |
| *n* | 10 | 7 | 6 |
| **SAFA** | | | |
| 14:0 | 8.22 ± 1.44 | 7.67 ± 5.20 | 1.56 ± 0.21 |
| 15:0 | 0.74 ± 0.10 | 1.25 ± 0.62 | 1.08 ± 0.23 |
| 16:0 | 26.15 ± 2.40 | 29.38 ± 4.87 | 18.55 ± 1.13 |
| 17:0 | 1.76 ± 0.27 | 1.58 ± 0.39 | 0.79 ± 0.21 |
| 18:0 | 3.92 ± 0.27 | 7.31 ± 0.83 | 4.69 ± 0.76 |
| 24:0 & 21:5n-3 | 0.36 ± 0.09 | 0.22 ± 0.21 | 0.12 ± 0.05 |
| **MUFA** | | | |
| 14:1n5 | 0.29 ± 0.09 | 0.32 ± 0.22 | 0.09 ± 0.07 |
| 15:1 | 0.45 ± 0.08 | 0.66 ± 0.38 | 0.59 ± 0.44 |
| 16:1n-7 | 2.90 ± 0.37 | 6.17 ± 2.48 | 8.84 ± 3.58 |
| 17:1 & 16:3n-4 | 0.19 ± 0.04 | 0.98 ± 0.49 | 1.55 ± 0.23 |
| 18:1n-9 | 7.46 ± 0.77 | 10.98 ± 2.85 | 20.54 ± 4.54 |
| 18:1n-7 | 2.99 ± 0.28 | 4.70 ± 1.54 | 7.36 ± 1.32 |
| 18:1n-5 | 0.37 ± 0.05 | 0.43 ± 0.09 | 0.21 ± 0.09 |
| 20:1n-11 | 0.55 ± 0.18 | 0.77 ± 0.50 | 0.87 ± 0.51 |
| 20:1n-9 | 4.35 ± 1.04 | 5.83 ± 1.29 | 3.57 ± 1.43 |
| 22:1n-11 | 0.83 ± 0.31 | 0.58 ± 0.53 | 0.77 ± 0.49 |
| 22:1n-9 | 0.25 ± 0.07 | 1.16 ± 0.30 | 0.50 ± 0.24 |
| **PUFA** | | | |
| 16:4n-1 | 0.27 ± 0.08 | 0.05 ± 0.06 | 0.21 ± 0.07 |
| 18:2n-6 | 1.45 ± 0.22 | 0.35 ± 0.13 | 0.70 ± 0.10 |
| 18:3 | 1.39 ± 0.25 | 0.32 ± 0.15 | 0.59 ± 0.20 |
| 20:2n-6 | 0.26 ± 0.03 | 0.39 ± 0.28 | 0.81 ± 0.13 |
| 20:3n-6 | 0.02 ± 0.02 | 0 | 0 |
| 20:3n-3 | 0.29 ± 0.05 | 0.19 ± 0.12 | 0.16 ± 0.04 |
| 20:4n-6 (ARA) | 1.02 ± 0.18 | 2.88 ± 2.40 | 3.36 ± 1.40 |
| 20:5n-3 (EPA) | 13.30 ± 1.66 | 6.29 ± 3.92 | 10.79 ± 3.85 |
| 22:5n-6 | 0.17 ± 0.08 | 0.12 ± 0.08 | 0.23 ± 0.05 |
| 22:5n-3 | 0.68 ± 0.17 | 0.75 ± 0.55 | 0.51 ± 0.08 |
| 22:6n-3 (DHA) | 19.37 ± 3.07 | 8.66 ± 5.70 | 10.98 ± 3.34 |
| | | | |
| Total SAFA | 41.15 ± 4.57 | 47.41 ± 12.11 | 26.79 ± 2.60 |
| Total MUFA | 20.62 ± 3.28 | 32.60 ± 10.68 | 44.88 ± 12.94 |
| Total PUFA | 38.22 ± 5.80 | 19.99 ± 13.40 | 28.34 ± 9.26 |

Overall, the FAs in greatest percentages were 14:0, 16:0, 16:1n-7, 18:0, 18:1n-9, 18:1n-7, 20:1n-9, 20:5n-3 and 22:6n-3, accounting for approximately 88% of the total FAs (**Table 4-3**). Saturated FAs (SFAs) varied from 26.79 ± 2.60% (scampi) to 50.32 ± 7.62% (red cod), monounsaturated FAs (MUFAs) from 20.62 ± 3.28% (arrow squid) to 49.87 ± 8.77% (javelin fish), and PUFAs from 11.97 ± 10.93% (opalfish) to 38.22 ± 5.80% (arrow squid). A plot of the first three PCs representing 68% of the total variance was created from the PC analysis run on transformed percentages (**Fig. 4-2**). Overall, species were well distinguished from each other by their FA profiles, except the seven octopi which did not show any grouping. Individual FA percentages were all significantly different between species (ANOVAs, $DF = 7$ between groups and 65 in total, $P < 0.05$). FA compositions of pelagic (arrow squid, spiny dogfish, hoki and javelin fish) and benthic living species (octopus, opalfish, red cod and scampi) were well separated along PC1 (28% of total variance) and PC2 (21% of total variance) (**Fig. 4-2**). Pelagic species were mainly confined in the lower front region of the plot driven by high positive loadings of 20:5n-3, 22:6n-3 and 20:3n-3 on PC1, and high negative loadings of 14:0, 18:2n-6 and 22:1n-11 on PC2 (**Fig. 4-2**). FA profiles of arrow squid were distinguished from the other pelagic species on PC3 (19% of total variance), and displayed the highest levels of some of the main FAs 20:5n-3 and 22:6n-3 (**Table 4-3b**). Hoki and javelin fish were distributed closely (**Fig. 4-2**), both with high levels of 18:1n-9, 22:1n-11 and 20:1n-9 (**Table 4-3a**). The intra-specific variability among pelagic species was smaller than among benthic species, the latter showing a high dispersal of FA compositions (**Fig. 4-2**). Within the benthic species, only scampi individuals were well grouped on PC1 and PC2 at the top of the plot, and displayed high values of 20:5n-3, 22:6n-3, 20:4n-6, 18:1n-9 and 18:1n-7 (**Table 4-3b**). In contrast, benthic-living opalfish had the lowest levels of 20:5n-3 and 22:6n-3 recorded. The amount of 20:4n-6 is unique to scampi, being three times greater than in other species, except for some octopus (**Table 4-3b**). Finally, red cod caught in April showed distinct FA profiles from those caught in December (**Fig. 4-2**).

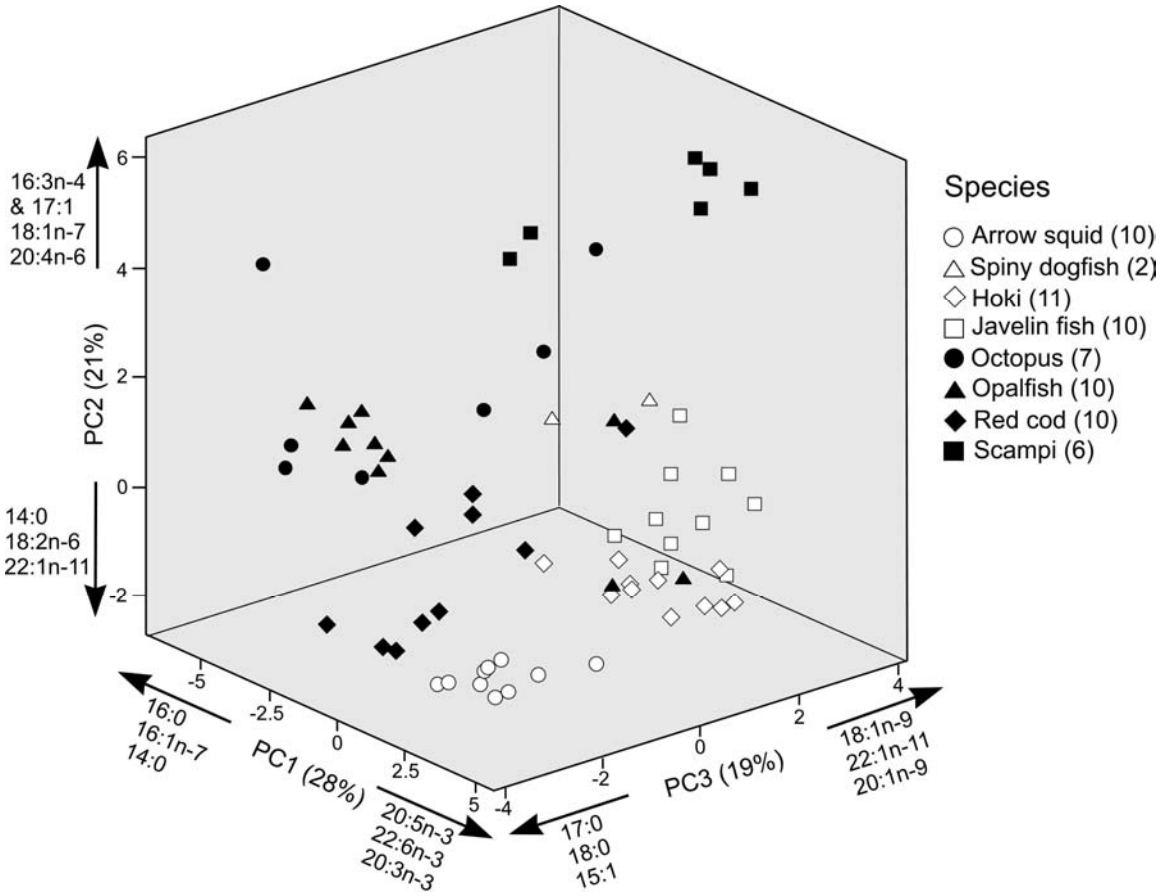


**Figure 4-2.** Plot of the first three principal components (PCs). The variance explained by each component is displayed in parentheses on each axis. Benthic species are represented by black symbols, and pelagic species by white symbols. The number of individuals analysed for each species is indicated in parentheses in the legend. Fatty acids with the highest positive and negative loadings are noted along each axis.

## 4.4 DISCUSSION

This study reported the proximate and FA compositions of marine species from the Auckland Islands Rise on the Campbell plateau, in the New Zealand sub Antarctic region. In general, my results demonstrated that the FA signatures could distinguish the marine species analysed, and on a broader scale, the type of habitat (**Fig. 4-2**). However, within-species variability is large among benthic species such as octopus, for which FA profiles overlap with other species. The digestive tracks of specimens were not removed, and this may have contributed to this high variability.

### 4.4.1 Proximate composition and energy density

Energy contents of the marine fish analysed did not show significant variation, although their lipid and protein contents varied significantly (**Table 4-2**). Within the

invertebrates, arrow squid had comparable proximate composition and energy content to most fish, whereas the lowest values were recorded in octopus and scampi individuals.

My results are consistent with the only other data recorded for the lipid and protein contents of arrow squid and red cod (Vlieg 1984a, c), although these specimens were collected further north in waters around the main islands of New Zealand. However, the present values for hoki were higher than previously reported for fish of similar lengths caught around the main islands of New Zealand (Vlieg 1984a), but comparable to those for hoki from waters off eastern Tasmania, Australia (Blaber and Bulman 1987). No comparable data are available in the literature for the invertebrates scampi and octopus, although similar results, including low content of lipid, have been reported for other species of octopus and benthic crustaceans (Gökodlu and Yerlikaya 2003; Rosa and Nunes 2003; Rosa *et al.* 2005; Pilar Sieiro *et al.* 2006; Ozogul *et al.* 2008). Graeve *et al.* (1997) explained the lower lipid values of benthic invertebrates in comparison with pelagic species by their "sluggish life" (wait for prey) that requires minimum energetic expenditure. In contrast, pelagic species accumulate lipids for energy reserve and for buoyancy (Sargent 1976). Red cod and opalfish, although considered benthic species, showed similar lipid levels to the pelagic species. This could be explained by a more dynamic feeding strategy than scampi and octopus.

Lipid content, and thus energy content and FA composition, are known to vary greatly according to prey size, season, and maturity stage (*e.g.*, Anthony *et al.* 2000; Budge *et al.* 2002; Iverson *et al.* 2002). Only few individuals per species were analysed here, therefore it must be kept in mind that these results represent individuals of a certain size and time of the year before making comparative studies.

### 4.4.2 FA composition in relation to diet

In marine ecosystems, PUFAs 20:4, 20:5, 22:5, and 22:6 originate in phytoplankton, and are conserved with little catabolism through the food chain (Sargent *et al.* 1987), and therefore are potential biomarkers for marine food webs. The relative conservation of FAs from one trophic level to another has been validated by experimental studies (*e.g.*, Fraser and Sargent 1989; Graeve *et al.* 1994b; Kirsch *et al.* 1998; Dalsgaard and St John 2004), and demonstrated the relevance of dietary FAs as qualitative markers of trophic interactions. FA profiles among individuals of the same species analysed herein were generally grouped together, and species distinguished from each other in the PC

plot (**Fig. 4-2**). The exception is the octopus showing a great variability in FA composition between specimens (**Fig. 4-2**).

Among the benthic species studied (red cod, opalfish, octopus and scampi), red cod is the only species for which diet information is available (J. Forman pers. comm.). Thus it is presently difficult to link the present results on FA composition with the feeding biology of the benthic species. However, it is known that opalfish and octopus are species which do not live in schools (Ayling and Cox 1982; O'Shea 1999), thus individuals are more likely to encounter different prey than schooling species, resulting in a higher variability in FA profiles. This is consistent with the high variation of the FA composition of octopi and some opalfish (**Fig. 4-2**). FA profiles of individual red cod showed variation with the time of capture (December *vs.* April; **Fig. 4-2**). Temporal variation in FA composition may be related to a different diet and/or rate of metabolism between seasons. Change in metabolism through time is generally due to different maturity stages or sizes (*e.g.,* Anthony *et al.* 2000; Budge *et al.* 2002; Iverson *et al.* 2002). Nevertheless, red cods analysed in this study were juvenile fish of similar sizes, thus changes in FA profiles between December and April must be mainly influenced by diet variation. This hypothesis cannot be validated to date as the only red cod specimens with diet information were collected in December-January only (**Table 4-1**, J. Forman pers. comm.). Among pelagic species, the two spiny dogfish displayed intermediate FA signatures between benthic individuals and other pelagic species (**Fig. 4-2**). Although these two specimens may not be representative of the species, these results are consistent with the reported diet of spiny dogfish in New Zealand waters, feeding on both benthic (octopus, opalfish, crabs) and pelagic species (galatheid, euphausiids, myctophids, and squids) (**Table 4-1**) (Hanchet 1991). There was a high degree of overlap in the FA profiles of hoki and javelin fish (**Fig. 4-2**), which is in agreement with previous data that these species have a similar diet (**Table 4-1)** (Clark 1985; Blaber and Bulman 1987). The average values of 20:1n-9 and 22:1n-11 were generally higher than in the other fish species studied (**Table 4-3a**). These FAs are the trophic markers of calanoid copepods (Sargent and Falk-Petersen 1988; Graeve *et al.* 1994a; Hagen *et al.* 1996), which are foraged by other zooplankton such as amphipods and euphausiids (Auel *et al.* 2002; Dalsgaard *et al.* 2003). Hoki and javelin fish eat amphipods and euphausiids (**Table 4-1**) (Clark 1985; Blaber and Bulman 1987), explaining the high values of 20:1n-9 and 22:1n-11 comparing to other species. However, arrow squid is also a known predator of zooplankton, yet did not show high values of these FAs.

Furthermore, FA profiles of arrow squid were well separated from those of javelin fish and hoki, with high concentrations of 20:5n-3 and 22:6n-3 (**Table 4-3b**, **Fig. 4-2**). This unexpected profile compared to other species sharing the same trophic niche, would suggest that factors other than diet influence the FA composition of the marine species studied, at least for the squid. 20:5n-3 and 22:6n-3 originate from phytoplankton (Sargent *et al.* 1987), and are generally found in important amounts in cephalopod species (Phillips *et al.* 2001; Rosa *et al.* 2005; Ozogul *et al.* 2008). Phillips *et al.* (2001) hypothesised that squid either selectively retains these FAs from the diet, or that this taxum is able to elongate n-3 precursors. Similarly, the benthic species scampi showed high levels of 20:5n-3 and 22:6n-3. Scampi has however a unique FA signature with a high proportion of 20:4n-6 (**Fig. 4-2**, **Table 4-3b**), which is consistent with reported levels of this FA in some other benthic invertebrates (Graeve *et al.* 1997; Hagen *et al.* 2000). Nonetheless, 20:4n-6 is only abundant in macro-algae (Graeve *et al.* 2002), whereas deep-systems, such as the scampi habitat, receive low quantities of 20:4n-6 from phytoplankton sedimentation (Ackman *et al.* 1968). Thus, for both arrow squid and scampi, diet alone does not explain the levels of some FAs, and it is suggested that these species may either selectively retain certain long-chain PUFAs, or elongate unsaturated 18-carbon FAs. To investigate this possibility, a further step would be to analyse the lipid classes of arrow squid and scampi. Indeed, triacylglycerols (TAGs), phospholipids (PLs), wax esters, and free FAs have different chemical properties, and their compositions are not influenced to the same degree by dietary FAs (Sargent 1976; Dalsgaard *et al.* 2003). TAGs, which are the main lipid class in most fish species (Clarke *et al.* 1984), are the common form of energy storage (Patton 1975), whereas PLs have a structural role in biomembranes (Sargent 1976). PLs were found to be the major lipid class in some species of cephalopods and crustaceans in which 20:5n-3, 22:6n-3 and 20:4n-6 were in high concentrations (Phillips *et al.* 2001; Rosa and Nunes 2003; Pilar Sieiro *et al.* 2006).

## 4.5 CONCLUSION

This chapter reports the proximate and FA compositions of marine species from the Auckland Islands Rise on the Campbell plateau, in the New Zealand sub Antarctic region. Despite the limitation of FA trophic markers for arrow squid and scampi, these findings provide a basis for greater understanding of trophic links between species of

great commercial importance (hoki) and of less value (javelin fish, spiny dogfish, red cod, octopus and opalfish). Moreover, this study can have several applications at higher trophic levels. The proximate composition and energy contents of the marine species studied gives an insight into their nutritional values in terms of food, and may be included in bioenergetics models of higher trophic predators. Also, the FA compositions reported here can be used to estimate the prey composition in the diet of predators such as marine mammals in a quantitative model (Iverson *et al.* 2004). Due to some overlap between the FA profiles of different species, the grouping of several species or splitting of one species in several groups may be necessary before the application of such a model. Further analyses on marine individuals from the Campbell plateau are required to understand the intraspecific variability of the FA composition.

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# 5 CHAPTER 5

## QUANTITATIVE FATTY ACID SIGNATURE ANALYSIS ON NEW ZEALAND SEA LIONS: SENSITIVITY ANALYSIS & DIET ESTIMATES

**Photo**: by-caught sub-adult male New Zealand sea lion (*Phocarctos hookeri*) before necropsy

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## Abstract

Quantitative fatty acid signature analysis (QFASA) was tested on New Zealand (NZ) sea lions (*Phocarctos hookeri*) incidentally caught in the NZ squid fishery to determine their long-term diet during the first half of the lactation period. The QFASA model used fatty acid (FA) profiles based on 82 blubber samples of sea lions (51 females and 31 males) by-caught between 2000 and 2006. First, QFASA was optimised by a series of simulations for which one model parameter (the set of calibration coefficients (CCs), the set of FAs and the consideration of prey individuals or groups) varied each time. Second, the diet of NZ sea lions was estimated using the optimal parameters and variation between sexes and years assessed. QFASA was highly sensitive to the set of CCs applied. The most important prey estimated using the optimal parameters (CCs from Steller sea lions *Eumetopias jubatus* fed on herring, 23 FAs, prey individuals) were arrow squid *Nototodarus sloani* (28% median mass), rattails Macrouridae (27% median mass), hoki *Macruronus novaezelandiae* (10% median mass), and red cod *Pseudophycis bachus* (4% median mass). The contribution of each prey in the diet was comparable to what was previously determined by stomach analysis with the exception of rattails which were found in low percentages by mass in the stomach contents. QFASA and stomach analysis represent different time frames of diet inference, which may explain the difference in the rattail contribution between the methods. Despite the uncertainty on the accuracy of the match between the optimal CCs used and the true FA metabolism of NZ sea lions, the consistency of the main prey estimated between QFASA and stomach analysis gave strength to the QFASA predictions. The most important estimated prey were demersal species living mainly at depths > 200 m that sea lions encounter on the slopes of the Auckland Islands shelf. The present study emphasised the importance of these areas for by-caught NZ sea lions over the first half of the lactation period.

## 5.1 INTRODUCTION

The New Zealand (NZ) sea lion (*Phocarctos hookeri*) has been classified as "vulnerable in decline" by the International Union for Conservation of Nature (IUCN) (Gales 2008) and "threatened" under the NZ Threat Classification System (Hitchmough *et al.* 2007). The population is limited to approximately 12000 individuals (Campbell *et al.* 2006), and it is one of the most localised pinniped with 86% of the entire pup production born at the Auckland Islands (50°30'S, 166°E) (Chilvers *et al.* 2007b). The stable or even decreasing population of the NZ sea lion (Chilvers *et al.* 2007b) is in marked contrast with the large growing population of the sympatric NZ fur seal (Harcourt 2001). The "success" of NZ fur seals *Arctocephalus forsteri* has been attributed to their epipelagic foraging strategy (Harcourt *et al.* 1995; Mattlin *et al.* 1998; Harcourt *et al.* 2001) shared with other otariids with large populations (*e.g.*, Boyd *et al.* 1994; Horning and Trillmich 1997; Georges *et al.* 2000; Weise 2006), whereas benthic feeders such as the NZ sea lion tend to have smaller or stable populations (*e.g.*, Thompson *et al.* 1998; Costa and Gales 2003; Chilvers *et al.* 2006). Female NZ sea lions make deep and long dives at depths averaging 130 m, and maximise their time at the benthos when diving (Costa and Gales 2000; Chilvers *et al.* 2006). This strategy is advantageous if the food resource on the seabed is predictable (Costa 1991). Therefore, data on the composition of the diet and prey availability are essential to better understand the energetics of the NZ sea lion, and may explain part of the low reproductive success of this marine mammal (Chilvers 2008b).

The diet of NZ sea lions at the Auckland Islands has been investigated through the analyses of scat samples, regurgitates and stomach contents (Childerhouse *et al.* 2001; Chapter 2). These methods rely on the recovery of prey hard parts, and are subject to biases, as prey may 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 non digestible parts (*e.g.*, squid beaks) (*e.g.*, Bigg and Fawcett 1985; Dellinger and Trillmich 1988; Bowen 2000; Staniland 2002; Tollit *et al.* 2007). Thus, dietary studies of marine mammals more and more include the combination of traditional techniques with biochemical ones such as fatty acid (FA) and stable isotope analyses, which overcome biases related to differential gut digestion (*e.g.*, Iverson *et al.* 1997a; Brown *et al.* 1999; Lea *et al.* 2002a; Bradshaw *et al.* 2003; Grahl-Nielsen *et al.* 2005; Hall-Aspland *et al.* 2005; Herman *et*

*al.* 2005; Staniland and Pond 2005; Dehn *et al.* 2007; Tucker *et al.* 2008). FAs are the main constituents of most lipids, and provide energy storage mainly situated in the subcutaneous fat (blubber) in marine mammals (Iverson 2002). Uptake of dietary FAs by adipose tissue involves several steps: absorption in the small intestine, incorporation into chylomicron triacylglycerol (TAG), hydrolysis of this TAG by the adipose tissue lipoprotein lipase, uptake of FAs and esterification within adipocytes. FA molecules are not degraded during this process, and the long-chain FAs are thought to be deposited in the adipose tissue with minimal modification and/or in a predictable manner (Summers *et al.* 2000; Iverson *et al.* 2004), potentially reflecting the diet over weeks to months (Kirsch *et al.* 2000; Iverson *et al.* 2004; Cooper *et al.* 2005; D. Tollit unpubl. data).

FA analysis has been extensively used to assess geographical, temporal, and ontogenetic variation in the diet of a variety of marine mammals (*e.g.,* Iverson *et al.* 1997a; Walton *et al.* 2000; Lea *et al.* 2002a; Walton and Pomeroy 2003; Samuel and Worthy 2004; Staniland and Pond 2004; Herman *et al.* 2005; Smith and Worthy 2006; Beck *et al.* 2007b; Thiemann and Iverson 2007; Walton *et al.* 2007). Inference of prey species ingested by the comparison of the raw FA profiles of prey and predator alone is not adequate, as the FA array is large and generally a FA is not specific to a particular prey. Furthermore, there is variation in the metabolism of individual FAs by the predator so that the proportion of FAs deposited in the adipose tissue of the predator will differ from that occurring in the prey. However, by using FA profiles in conjunction with a model taking into account FA metabolism (by including calibration coefficients (CCs)), it is believed possible to estimate the species composition. This method, named quantitative FA signature analysis (QFASA), was developed by Iverson *et al.* (2004) and since, has been used to infer the diet of several marine predators (Iverson *et al.* 2006; Beck *et al.* 2007a; Iverson *et al.* 2007). The CCs are determined empirically with captive animals fed on controlled long-term monotypic diets (*e.g.*, Iverson *et al.* 2004), and are unlikely to be determined specifically for NZ sea lions in the near future. Thus, CCs determined in other species of pinnipeds (Iverson *et al.* 2004; D. Tollit unpubl. data) are presently the only available CCs that can be used for the application of QFASA on NZ sea lions.

Given this limitation, the present study investigated whether QFASA was sufficiently robust to provide useful insights in the long-term diet of the NZ sea lion. My specific aims were (1) to evaluate the sensitivity of the QFASA model on blubber of by-caught NZ sea lions by using different CC sets available in the literature; (2) to optimise

QFASA parameters, and finally; (3) to estimate the diet of NZ sea lions. QFASA estimates by year and by sex are discussed in term of feeding ecology and biases related to differential FA metabolism between groups. Overall estimates are compared with stomach contents from the same individuals (Chapter 2).

## 5.2 MATERIALS AND METHODS

### 5.2.1 Sample collection

NZ sea lions captured accidentally in the NZ sub-Antarctic arrow squid (*Nototodarus sloani*) fishery (operating annually from February to May) were frozen onboard, and sent frozen to Massey University, NZ, for necropsy. During necropsy, a full-depth 60 $mm^2$ piece of blubber (including skin and some muscle) was cut from the mid-sternal region, stored in a plastic bag from which air was expelled, and placed at -20°C. Females were categorised as either non-lactating (NLF) or lactating (LF) by the examination of the mammary gland for development and presence of secretion. The individuals included in this study were all mature as determined by visual examination of the ovaries and histological examination of the testes (details in Duignan *et al.* 2003).

### 5.2.2 Lipid analysis

Lipids from blubber were extracted following Folch *et al.* (1957). Briefly, lipids were extracted in a mixture of 8:4:3 chloroform:methanol:saline water (v:v:v) containing 0.01% butylated hydroxytoluene (BHT) as an antioxidant. FA methyl esters were prepared using 10% boron trifluoride in methanol, and extracted into hexane. They were analysed by gas chromatography using a polar capillary column coated with 50% cyanopropyl polysiloxane (0.25 μm film thickness; J&W DB-23, Folsom, California). Further details of the laboratory protocol are given in Chapter 3.

### 5.2.3 QFASA model

The diets of individual sea lions were estimated using the model described by Iverson *et al.* (2004), and the FA library of prey from the Auckland Islands region (Chapter 4). 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 the closest to match 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 (percentage mass).

Therefore, in addition to the FA profile of the predator's adipose tissue, QFASA requires:

*1*. An optimisation model

*2*. CCs to take into account lipid metabolism occurring in the predator

and *3*. A FA library of potential prey and their lipid content

to estimate the diet of a predator.

#### *5.2.3.1 Optimisation model*

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

$$\hat{S}_y = \left[ \sum_{i=1}^{n} p_i S_i \right] \quad \text{(from Iverson } et\ al. \text{ 2004)} \qquad \text{[Eq. 1]}$$

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

$$KL = \sum_{j=1}^{m} (S_{y\,j} - \hat{S}_{y\,j}) \log \left( \frac{S_{y\,j}}{\hat{S}_{y\,j}} \right) \quad \text{(from Iverson } et\ al. \text{ 2004)} \quad \text{[Eq. 2]}$$

With $S_{y\,j}$, true value of FA *j* of predator *y*; $\hat{S}_{y\,j}$, predicted value of FA *j* of predator *y*; *m*, number of FAs. KL values given in the outputs are an indication of fit as the optimisation minimises this value. The optimisation uses a quasi-Newton algorithm with a BFGS (Broyden-Fletcher-Goldfarb-Shanno) formula, and was carried out with a package developed at Massey University (*Fatty acid solution*, R. Sherriff & P.C.H. Morel). Results given by this package were verified with the package Fascalc (Fascalc v 1.11, M.J.Walton, SMRU, University of St Andrews, UK).

#### *5.2.3.2 Calibration coefficients CCs*

CCs account for the differential deposition and synthesis of FA during lipid metabolism occurring in the predator's adipose tissue. CCs were determined from experiments in

which captive seals were fed on diets of know FA compositions. To date, they are available for several pinniped species (Iverson *et al.* 2004; Cooper *et al.* 2005; Tollit *et al.* 2006; D.Tollit unpubl. data), mink *Mustela vison* (Iverson *et al.* 2006) and common murre *Uria aalge* (Iverson *et al.* 2007). Before the optimisation starts, $S_y$ is divided by CCs for each FA *j* and renormalised to sum 100%.

#### *5.2.3.3 FA library of potential prey*

The prey library is composed of fish and cephalopods known to be significant prey of NZ sea lions in the Auckland Islands region by previous stomach content and faeces analyses (Childerhouse *et al.* 2001; Chapter 2). They include hoki *Macruronus novaezelandiae* (n = 11), javelin fish *Lepidorhynchus denticulatus* (n = 10, representing the rattail group, *i.e.*, Macrouridae), opalfish *Hemerocoetes* spp (n = 10), red cod *Pseudophycis bachus* (n = 10), arrow squid (n = 10) and octopus *Enteroctopus zealandicus* (n = 7). Scampi *Metanephrops challengeri* (a crustacean, n = 6) and spiny dogfish *Squalus acanthias* (a cartilaginous fish, n = 2) were added as potential prey, since they are abundant in the area where sea lions forage (Jacob *et al.* 1998; O'Driscoll *et al.* 2003). They were not reported as prey from traditional methods, probably because they are likely to digest quickly in the gut after ingestion, 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 previously analysed (Chapter 4).

### 5.2.4 Sensitivity analysis of QFASA

#### *5.2.4.1 Redefinition of prey groups*

The first part of the validation procedure for QFASA is to check that the model can correctly identify individual prey. For this, FA profiles of individual prey (Chapter 4) were entered as predators in the model, and were compared to mixtures of prey FA profiles computed by the model. Opalfish and octopus displayed a high diversity of FA profiles (Chapter 4), thus I expected some misclassification for these species by the QFASA model. Groups of prey were redefined by splitting octopus (seven individuals in total) into two groups, and by deleting three individuals in the opalfish group (ten in total) for which FA profiles were greatly different from that of the seven remaining

opalfish (Chapter 4). Proportions of prey species were predicted in a new model simulation with the mean of redefined prey groups.

#### *5.2.4.2 Optimisation of model parameters*

The optimisation involved the variation of three parameters: the FA set (1), the CC set (2), and the account of prey individual variability or not (3). (1) Two sets of FAs were used: The first set of 27 FAs includes all FAs in common between prey and blubber samples (list of FAs in Table 3-2 of Chapter 3 minus 18:4n-3, 20:4n-3 and 22:4n-6). FAs 15:0, 14:1, 15:1 and 18:1n-5 had a minor contribution to the total mass, and were removed to create a second 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). (2) 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 from grey seals *Halichoerus grypus* fed on herring *Clupea harengus* (Iverson *et al.* 2004; named CCs-GS/her), CCs from harp seals *Phoca 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* (D. Tollit, unpubl. data; named CCs-SSL/her), CCs from Steller sea lions fed on eulachon *Thaleichthys pacificus* (D. Tollit, unpubl. data; named CCs-SSL/eul), and CCs from Steller sea lions fed on a mixed diet (D. Tollit, unpubl. data; named CCs-SSL/mix). The mixed diet consisted of 64% mass herring, 15% eulachon, 14% squid and 7% rockfish (D. Tollit, unpubl. data). (3) For each set of FAs and CCs applied, prey individual variability was considered by taking each prey individual as a prey group to compute the different mixtures of prey FA profiles. Thus, instead of computing FA profiles from nine prey groups (eight different prey with octopus divided into two groups), the model computed FA profiles from 63 "groups" (total of individuals in the prey library).

The distributions of KL values for each simulation were compared (Wilcoxon signed ranks tests between two simulations, Friedman tests between three and more simulations). Since KL is the distance minimised by the model, the parameters with the lowest distribution of KL values were considered optimal and were kept to estimate the diet of NZ sea lions from blubber FA profiles.

#### *5.2.4.3 Diet estimates of by-caught NZ sea lions using QFASA*

From the sensitivity analysis carried out previously, the optimal scenario was used to determine diet estimates of NZ sea lions from QFASA on blubber FA profiles. Diet estimates were compared between LFs, NLFs and males (M), and between years. These groups were tested for significant variation of mass percentage by Kruskall-Wallis (K-W) tests. The QFASA estimate for all combined sea lions was compared with the mass percentages of the digested fraction of the stomachs (data in Chapter 2), believed to be a better representation of the short-term diet of by-caught NZ sea lions (Chapter 2).
All statistical tests have an α level of statistical significance of 0.05.

## 5.3 RESULTS

Eighty two whole blubber cores were analysed from 51 female and 31 male sea lions by-caught annually between February and May from 2000 to 2006 (**Table 5-1**). The sex categories were not all represented within the last two years, thus 2005 and 2006 were pooled for K-W tests.

**Table 5-1.** Number of lactating female (LF), non-lactating female (NLF), and male (M) New Zealand sea lions analysed per year.

| Year | Lactating females | Non-lactating females | Males | *Total* |
|---|---|---|---|---|
| 2000 | 6 | 2 | 8 | **16** |
| 2001 | 6 | 2 | 8 | **16** |
| 2002 | 8 | 1 | 5 | **14** |
| 2003 | 2 | 4 | 5 | **11** |
| 2004 | 2 | 6 | 4 | **12** |
| 2005 | 3 | | 1 | **4** |
| 2006 | 1 | 8 | | **9** |
| **Total** | **28** | **23** | **31** | **82** |

### 5.3.1 Sensitivity analysis of QFASA

#### *5.3.1.1 Redefinition of prey groups*

Most individual prey were correctly classified to the prey species they belong to: FA profiles of arrow squid, hoki, javelin fish, scampi and dogfish were well identified at > 90% (**Fig. 5-1a**). Red cod, opalfish and octopus had a correct classification percentage

of 85%, 74% and 51% respectively. After redefinition of groups of opalfish and octopus, only octopus and red cod fell into < 90% of correct classification: 11% of octopus 1 were misclassified as opalfish, and the same amount of red cod were misclassified as octopus 2 (**Fig. 5-1b**). Although the correct classification of red cod

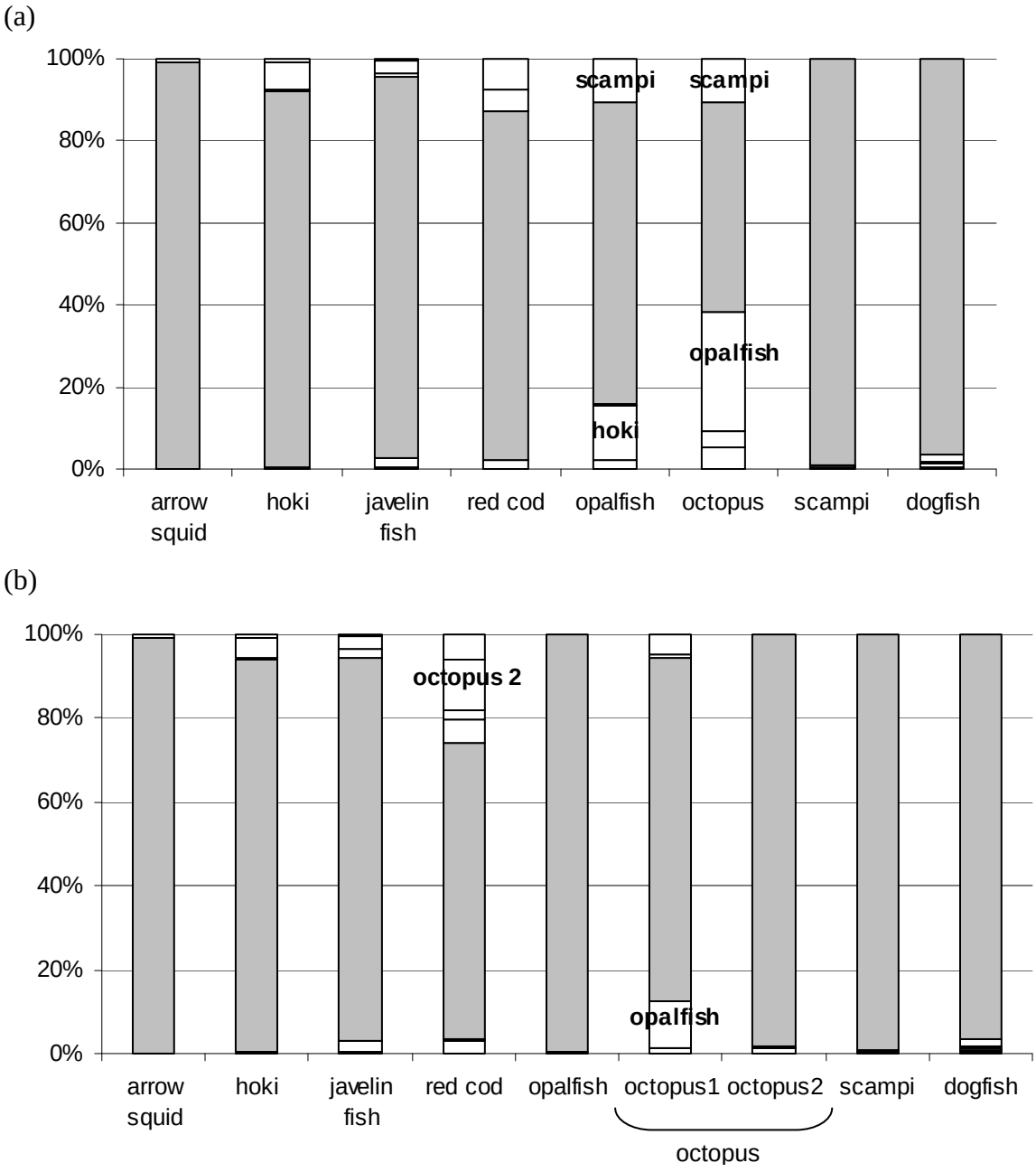


**Figure 5-1.** Mean predicted proportions of prey species by quantitative fatty acid signature analysis. Fatty acid profiles of prey individuals were entered as predators in the model and were compared to different mixtures of fatty acid profiles of (a) original mean prey species and (b) of redefined mean prey species (see results). The correct mean classification percentage is in grey. White parts represent the percentage of misclassification, with the prey species leading to the main misclassifications in bold. For instance, in histogram (a), approximately 20% of opalfish were misclassified as scampi and hoki.

was only 71% with the redefined prey groups instead of 85% with the original groups (**Fig. 5-1**), the overall correct classification had improved, therefore redefined prey groups were kept for the next steps of the sensitivity analysis when mean prey is applied.

#### *5.3.1.2 Optimisation of model parameters*

The distribution of KL values varied greatly between simulations with a minimal value of 0.8 (23 FAs, individual prey, CCs-SSL/her) and a maximal value of 53.7 (27 FAs, mean prey, CCs-GS/her) (**Table 5-2**). To visualise the meaning of these values, predicted and true FA profiles of some individual sea lions were represented in **Fig. 5-2** with different KL values. The predicted FA profile of the 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 sea lion with a KL value of 53.7 (**Fig. 5-2**). Simulations using 23 FAs gave median KL values that were up to 15% lower than those with 27 FAs (**Table 5-2**; Wilcoxon signed ranks tests, $P < 0.01$). Likewise, simulations using FA profiles of individual prey showed lower KL values than with those using mean profiles (Wilcoxon signed ranks tests, $P < 0.01$). KL values calculated using CCs-SSL/her were significantly lower than those using other CC scenarios, irrespective of the other parameters chosen (Friedman tests, $P < 0.01$; *post-hoc* Wilcoxon signed ranks tests, $P < 0.01$) (**Table 5-2**). The variation of KL values between the CCs scenarios was important, with a factor of four to five times between values (**Table 5-2**).

**Table 5-2.** Kullback-Liebler (KL) values (median and range) for the different simulations performed with quantitative fatty acid signature analysis on blubber fatty acid (FA) profiles of New Zealand sea lions. CCs-GS/her and CCs-HS/her = calibration coefficients (CCs) from grey seals (GS) and from harp seals (HS) fed on herring (Iverson *et al.* 2004); CCs-SSL/her, CCs-SSL/eul and CCs-SSL/mix = CCs from Steller sea lions fed on herring, eulachon and a mixed diet respectively (D. Tollit, unpubl. data). Superscript letters show the most important significant results from statistical tests: [a] KL values with 23 FAs and individual prey were significantly lower than values with 27 FAs and individual prey (Wilcoxon tests, $P < 0.01$). [b] KL values with 23 FAs and individual prey were significantly lower than values with 23 FAs and mean prey (Wilcoxon tests, $P < 0.01$). [c] KL values with CCs-SSL/her were significantly lower than values with other CC scenarios (Friedman tests, $P < 0.01$; Wilcoxon tests, $P < 0.01$).

| KL values with | | No CCs | CCs-GS/her | CCs-HS/her | CCs-SSL/her | CCs-SSL/eul | CCs-SSL/mix |
|---|---|---|---|---|---|---|---|
| 23 FAs | mean prey | 20.6 [10.1-42.4] | 19.3 [6.9-52.4] | 10.5 [3.9-30.1] | 5.1 [c] [1.4-15.0] | 9.8 [4.8-30.1] | 9.6 [3.8-29.0] |
| | individual prey | 15.1 [a b] [5.6-31.4] | 14.4 [a b] [4.4-44.6] | 7.8 [a b] [2.3-24.4] | 3.1 [a b c] [0.8-9.4] | 6.7 [a b] [4.0-15.7] | 7.2 [a b] [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 [c] [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 [c] [1.2-11.7] | 7.2 [4.3-17.8] | 7.3 [2.5-18.6] |

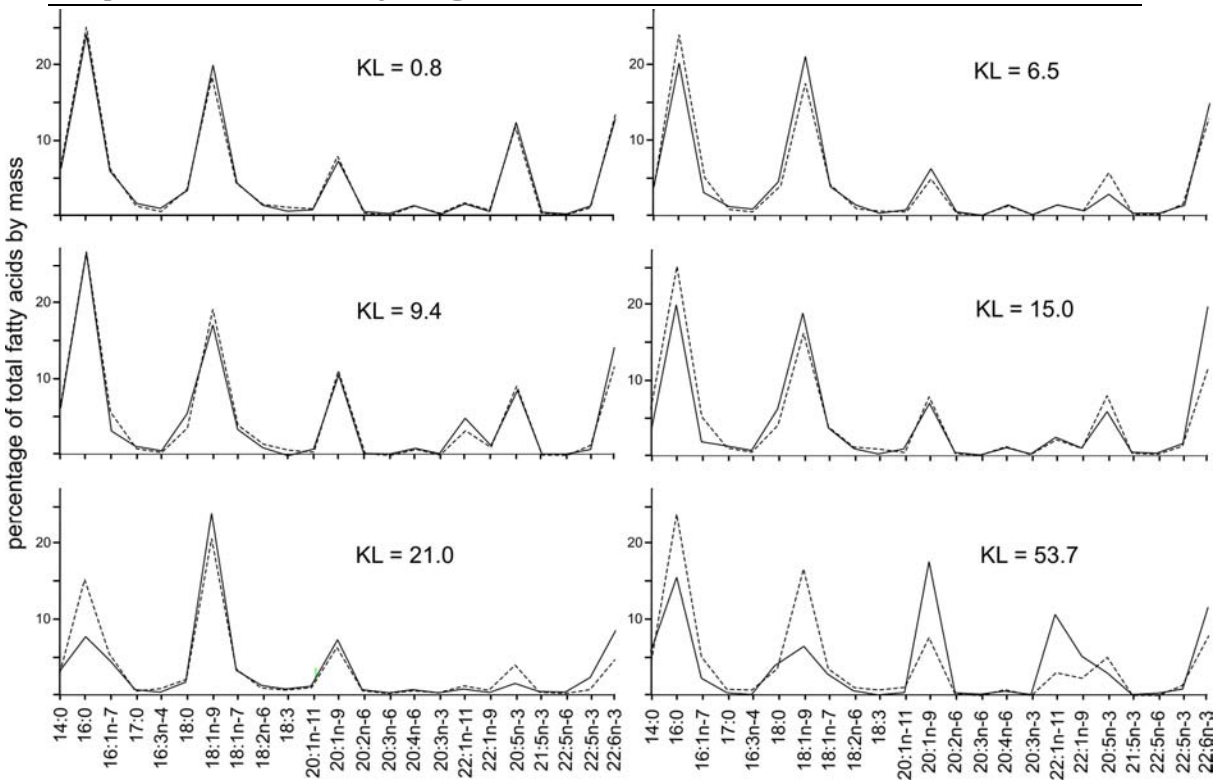


**Figure 5-2.** Examples of predicted fatty acid profiles (in dotted line) by quantitative fatty acid signature analysis *versus* true fatty acid profiles (in solid line) of New Zealand sea lions, with different Kullback-Liebler (KL) values. Kullback-Liebler is the mathematical distance minimised by the model

To observe the consequences of different CCs on diet estimated by QFASA, the percentages of prey from the simulations with 23 FAs, individual prey, and the different CC scenarios were presented in **Fig. 5-3**. When no lipid metabolism is taking into account (no CCs), the model predicted 70% by mass of rattails in the diet with a median KL value of 15.1 (**Fig. 5-3**, **Table 5-2**). The high dominance of rattails (> 70% by mass) was also found with the CCs from Steller sea lions fed on eulachon and mixed diet (**Fig. 5-3**), 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 (grey and harp seals) were applied.

In summary, the simulation showing the lowest KL values was with 23 FAs, individual prey and CCs-SSL/her. These parameters were considered optimal and were used in further simulations to estimate the diet of NZ sea lions by QFASA.

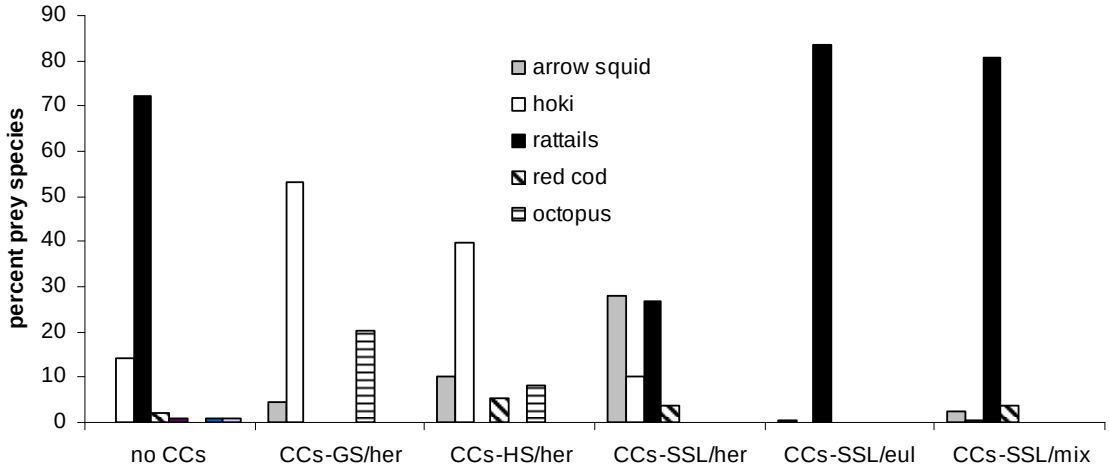


**Figure 5-3.** Predicted proportions by mass (median) of prey species by quantitative fatty acid signature analysis on 82 blubbers of New Zealand sea lions using the parameters "23 FAs" and "individual prey" (see text) with different calibration coefficients (CCs): CCs-GS/her and CCs-HS/her = CCs from grey seals (GS) and from harp seals (HS) fed on herring (Iverson *et al.* 2004); CCs-SSL/her, CCs-SSL/eul and CCs-SSL/mix = CCs from Steller sea lions fed on herring, eulachon and a mixed diet respectively (D. Tollit, unpubl. data). The prey opalfish, scampi and spiny dogfish were deleted because their estimated proportions were nil or only in low percentage.

### 5.3.2 Diet estimates of by-caught NZ sea lions using QFASA

Overall, arrow squid, hoki, rattails and red cod made up the bulk of the diet estimated by QFASA, with > 80% mean mass, and were present in more than half the samples (**Table 5-3**). Arrow squid was the most important prey (median of 28% mass) followed by rattails and hoki (median of 27 and 10% mass respectively). Scampi, octopus and spiny dogfish were considered minor prey by the model with mean mass < 10%, while opalfish was quasi-absent with a mass estimate for only four samples (**Table 5-3**).

The estimated diet of males was significantly different from that of females with a lower contribution of hoki in their estimated diet (K-W test, $H_{2,\ 82} = 7.96$, $P = 0.019$; M-W tests, $P < 0.05$), and a higher contribution of red cod (K-W test, $H_{2,\ 82} = 7.81$, $P = 0.020$; M-W tests, $P < 0.05$) (**Fig. 5-4**). Estimated diets of by-caught lactating and non-lactating females did not show any significant difference in their estimated diet (M-W test, $P > 0.05$) (**Fig. 5-4**). Consequently, all females were grouped together to look at diet differences between years.

**Table 5-3.** Predicted proportions by mass of prey species by quantitative fatty acid signature analysis for 82 by-caught New Zealand sea lions with the optimal parameters from the sensitivity analysis (23 FAs, calibration coefficients from Steller sea lions fed on herring, individual prey). O is occurrence (number of individuals for which the prey has a percentage mass > 0). Values between the 25$^{th}$ and the 75$^{th}$ percentiles represent the interquartile range in which 50% of the data falls.

| Prey | O | %O | mean | median | 25$^{th}$ percentile | 75$^{th}$ percentile |
|---|---|---|---|---|---|---|
| Arrow squid | 77 | 94 | 30 | 28 | 14 | 46 |
| Hoki | 74 | 90 | 17 | 10 | 2 | 27 |
| Rattails | 77 | 94 | 29 | 27 | 13 | 39 |
| Red cod | 68 | 83 | 11 | 4 | 0 | 18 |
| Opalfish | 4 | 5 | <1 | 0 | 0 | 0 |
| Octopus | 28 | 34 | 4 | 0 | 0 | <1 |
| Scampi | 50 | 61 | 8 | 1 | 0 | 8 |
| Spiny dogfish | 37 | 45 | 2 | 0 | 0 | 1 |

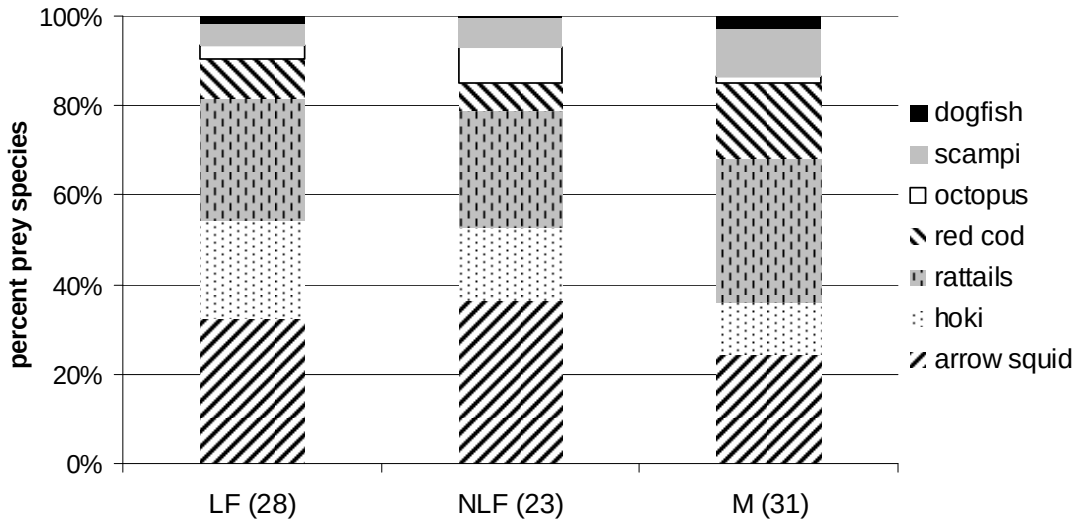


**Figure 5-4.** Mean percentages of prey species estimated by quantitative fatty acid signature analysis with the optimal parameters from the sensitivity analysis (23 FAs, calibration coefficients from Steller sea lions fed on herring, individual prey) for lactating female (LF), non-lactating female (NLF) and male (M) New Zealand sea lions. The number of samples available for each category is shown in parenthesis.

Within females, the estimated percentage of arrow squid in the last three years of study (2004 to 2006) was significantly higher (≥ 37% mass) than that in the previous years (mean mass of 26%; K-W test, $H_{5, 51} = 11.84$, $P = 0.037$; M-W tests, $P < 0.05$) (**Fig. 5-5a**). Hoki contributed more than 20% of the diet by mass for the first five years, but only 4% for the combined years of 2005-2006 (K-W test, $H_{5, 51} = 22.16$, $P < 0.001$). In contrast, the contribution of octopus was 19% by mass in 2005-2006, but less than 3% in previous years (**Fig. 5-5a**). The proportions of rattails and red cod in the diet of females did not differ significantly between years (K-W tests, $P > 0.05$). There was no statistical differences between years in the diet of males (**Fig. 5-5b**; K-W tests, $P > 0.05$).

The proportions of arrow squid, hoki and red cod in the diet were in comparable amounts when estimated by either stomach analysis or QFASA (**Fig. 5-6**). However, rattails were the second most important prey in the diet estimated by QFASA (24% mass), while there were only 5% of the digested fraction of the stomachs (**Fig. 5-6**). Octopus showed a reverse trend with the highest contribution in the stomach contents (33% mass) *versus* a small contribution estimated by QFASA (4% mass).

## 5.4 DISCUSSION

The purpose of this study was to assess the use of QFASA as a method for estimating the long-term diet of NZ sea lions. Diet estimates on by-caught animals presented here are to be considered with caution as the QFASA model is highly sensitive to the calibration coefficients, which at the present time are only available for one otariid species: the Steller sea lion. However, the fact that arrow squid, rattails and hoki were estimated major prey by QFASA in the long-term diet of NZ sea lions is consistent with previous knowledge on the diet and foraging behaviour of this species (Childerhouse *et al.* 2001; Chilvers *et al.* 2005; Chapter 2).

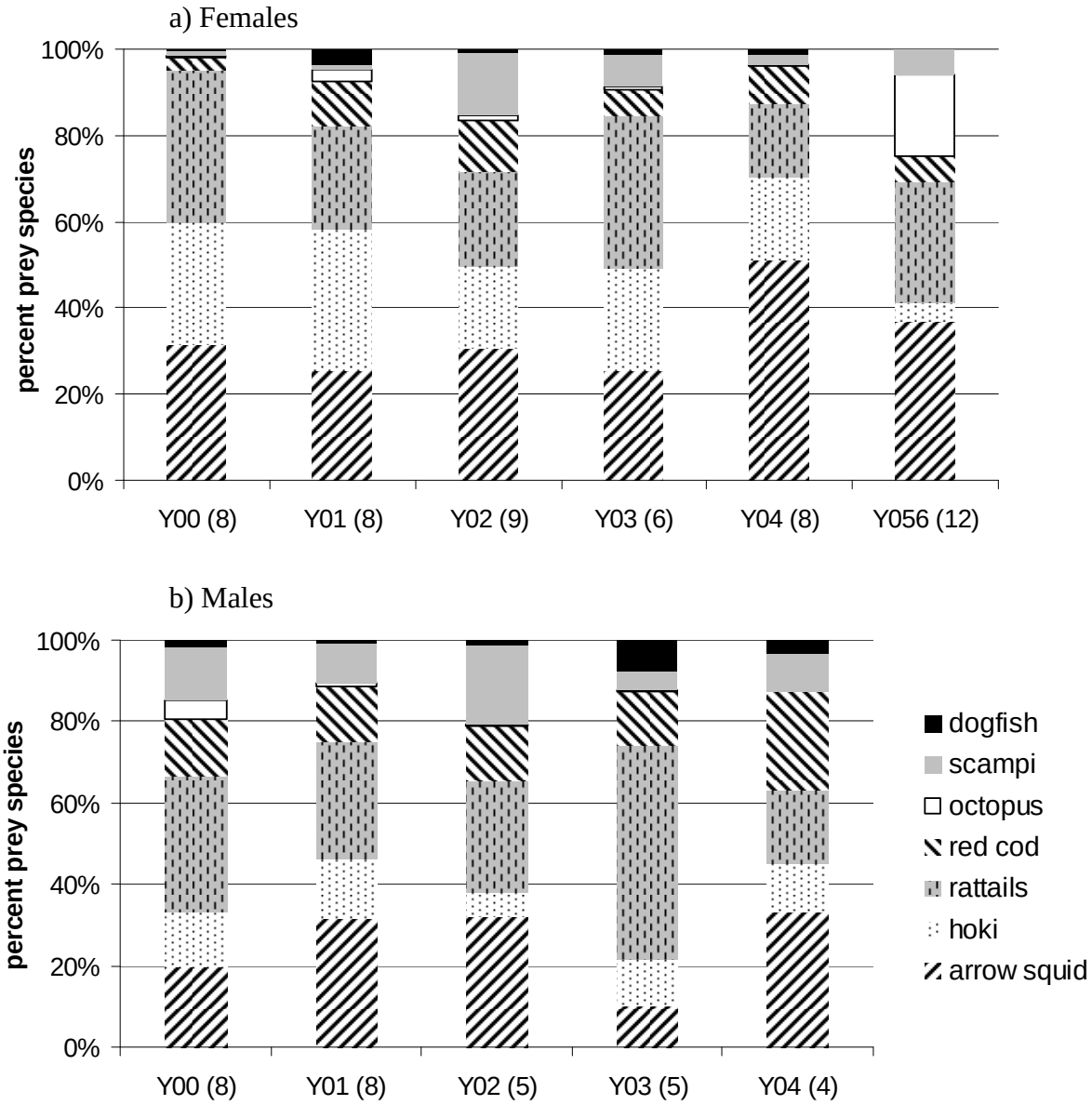


**Figure 5-5.** Between-year percentages of prey species estimated by quantitative fatty acid signature analysis with the optimal parameters from the sensitivity analysis (23 FAs, calibration coefficients from Steller sea lions fed on herring, individual prey) for female and male New Zealand sea lions. Years are noted Y00 for 2000, Y01 for 2001 etc…Years 2005 and 2006 were pooled (noted Y056). The number of samples available for each category is shown in parentheses.

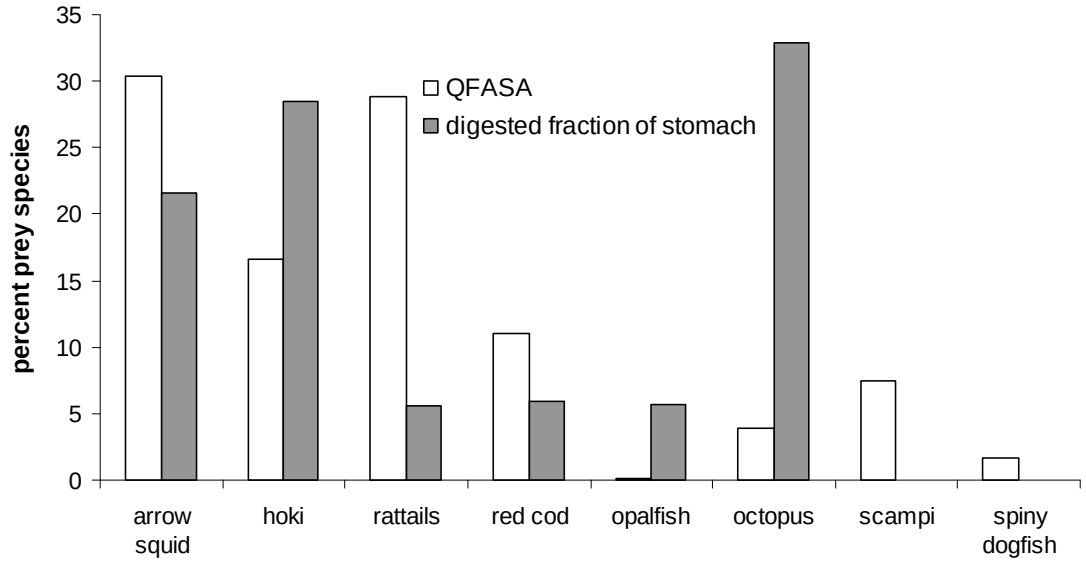


**Figure 5-6.** Diet estimates by percent mass of 82 by-caught New Zealand sea lions from quantitative fatty acid signature analysis (this study) and stomach analysis (digested fraction; Chapter 2).

### 5.4.1 Sensitivity of QFASA

The reliability of QFASA lies in the quality and accuracy of the data that are 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 an understanding on the differential deposition of FAs in the predator's adipose tissue (expressed as CCs) (Iverson *et al.* 2004).

Blubber FA signatures will reflect dietary FAs if the adipose tissue sampled is actively metabolising (Budge *et al.* 2006). The blubber analysed in this study, collected from the sternum region, is thought to be a fat depot because its thickness in this area is positively correlated with body mass (Massey University, unpubl. data).

The prey FA library is comprised of six species identified as common in the diet of NZ sea lions from stomach analysis (arrow squid, hoki, javelin fish, red cod, opalfish and octopus) (Chapter 2), and two species that are abundant in the Auckland Islands region (scampi and spiny dogfish) (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 (Chapter 2) and were not included in the prey FA library due to a lack of material available for analysis.

Nonetheless, they might be significant prey by mass in the long-term diet of sea lions because they are common species in the waters of the Auckland Islands region (Jacob *et al.* 1998; O'Driscoll *et al.* 2003). Thus, the addition of the species cited above in the prey FA library will significantly improve the accuracy of the diet estimation of NZ sea lions.

Once a prey library is acquired, a prerequisite of QFASA is the assessment of overlap between FA profiles of prey species, and of FA variability within the same prey. The regrouping of prey such as octopus (subdivision into two groups) and opalfish (removal of some individuals) was necessary in this study as these benthic species showed a great variation in their FA profiles (Chapter 4). This diminished the overall misclassification of prey species (**Fig. 5-1**) with all prey groups except red cod at a correct classification percentage of > 80%. However, the FA profiles of the individual prey gave better optimisations (smaller KL medians) than the mean profiles of prey groups and were preferred in this study for the estimation of the diet of NZ sea lions. Other studies using QFASA included the prey species variability by resampling the prey library using a bootstrap procedure (Iverson *et al.* 2004; Beck *et al.* 2007a). Yet, resampling mean FA profiles of prey did not give any significant differences in KL values with no resampling (L. Meynier unpubl. data), 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 deposition is similar among marine species for which long-term diet studies were carried out (Iverson *et al.* 2004; Iverson *et al.* 2006; Iverson *et al.* 2007; Nordstrom *et al.* 2008), the CC for a particular FA seems to depend on the predator's taxum (*i.e.*, otariidae, phocidae) or species considered (Iverson *et al.* 2004; Tollit *et al.* 2006), or even on the meals eaten by the same predator (D. Tollit, unpubl. data). This study showed that the model was very sensitive to different sets of CCs, with diet estimates switching from a high dominant species (> 70% mass) to several major species depending on the CCs used (**Fig. 5-3**). I did not expect to see such strong variation among CC sets from the Steller sea lion (herring, eulachon and mixed diet): Indeed, while 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 is perhaps due to the fact that this fish has a FA profile closer to the mean FA profile of NZ sea lion prey than eulachon (**Appendix 2**) or a mixed diet does (for which a single FA profile is hard to estimate). The lipid content of herring (9.9 ± 2.8%) is however higher than the common prey of the NZ sea lions (overall mean of 4.2 ± 2.5%; Chapter 4). Besides FA composition, the lipid content of food plays a role in the metabolism and deposition of FAs (e.g., Summers *et al.* 2000; Cooper *et al.* 2005). One may expect that CCs calculated from sea lions fed on a species with a close FA composition, and also a lipid content similar to that of NZ sea lion prey, would give better diet estimates. Such feeding experiments in the future would greatly improve the QFASA application on NZ sea lions.

### 5.4.2 Overall diet estimate and feeding ecology

Among the eight potential prey species that QFASA computed, arrow squid, rattails, hoki, and red cod were estimated to be the major prey in the diet of by-caught NZ sea lions during the first half of the lactating period (January to May) (**Table 5-3**). Significant variation in diet estimates was found between sexes (**Fig. 5-4**) and between years (**Fig. 5-5**). The diet estimate of all sea lions was compared with the stomach contents of the same animals analysed previously (Chapter 2). Since stomach analysis and QFASA are associated with different time frames of diet inference (several months versus several days), individual diet estimates from both methods were not compared, and variation between overall diet estimates was not tested statistically. Both methods estimated that arrow squid and hoki are major prey in the diet (**Fig. 5-6**), but gave different proportions of rattails (more with QFASA) and octopus (less with QFASA). An overestimation of the cephalopod contribution by the stomach analysis is expected because beaks tend to accumulate in the stomach (Bigg and Fawcett 1985; Yonezaki *et al.* 2003). This is consistent with the large contribution of octopus in stomach contents in comparison to the small amount estimated by QFASA (**Fig. 5-6**). Yet, this pattern does not appear for arrow squid, and this is probably due to the fact that arrow squid found in stomachs were mostly fresh (Chapter 2), thus not contributing to the digested fraction, which is used here for comparison. QFASA also considered as prey some taxa not present in the stomachs (scampi and spiny dogfish) although their contribution was low (**Fig. 5-6**).

Rattail fishes (Macrouridae) and hoki are demersal species, living in abundance on the shelf slope from 200 m deep to > 1000 m (Beentjes *et al.* 2002; MinistryFisheries 2007). Arrow squid is present from the surface to 500 m, but large amounts occur at depths > 250 m (Jackson *et al.* 2000). In Chapter 2, I suggested the importance of the edge of the Auckland Islands shelf as a feeding ground where arrow squid and rattails are found in abundance. The fact that arrow squid, rattails and hoki were estimated to be major prey by QFASA emphasises this previous result over a longer time frame, and are in agreement with the known foraging behaviour of lactating females at the beginning of the lactation period (Chilvers *et al.* 2005). Thus, the slopes of the Auckland Islands shelf are key foraging areas for NZ sea lions, not only after the breeding season (Chilvers *et al.* 2005), but probably for the first half of the nursing period. This area is likely to provide concentrated food where animals can optimise their energy investment per foraging trip.

### 5.4.3 Diet estimates of males and females

In Chapter 3, FA profiles of NZ sea lions were differentiated between sexes, while they were similar between LFs and NLFs. As QFASA used these FA profiles to compute diet estimates, it is logical to find similar trends in this study (**Fig. 5-4**). Also in Chapter 3, I 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 males. The monounsaturated 20 and 22 carbon-FAs are biomarkers of zooplankton, and were found in relative high amounts in hoki and javelin (a rattail species) (Chapter 4). Accordingly, the QFASA model estimated more hoki in the diet of by-caught females (**Fig. 5-4**). However, 16:3n-4 is correlated with the deep bottom-living scampi and some octopus (Chapter 4). I did not test statistically the variation of these species between the genders as their estimated occurrence was weak, but the expected trend of more scampi and octopus in the diet of males is not visible in **Fig. 5-4**. Besides, red cod is a benthic prey occurring both in shallow and deep areas and was found in higher contribution in the diet of males. Therefore, from the QFASA results, I could not conclude that male NZ sea lions forage preferentially in deeper areas than females do, as it has been hypothesised in Chapter 3.

The higher contribution of hoki in the diet of by-caught females could be energetically advantageous, since hoki available at the Auckland Islands during summer are large fish

of > 1kg with moderate energy content (6.0 to 8.3 kJ $g^{-1}$; Chapter 4). The other prey with a greater mass is the octopus but its lipid, protein, and energy contents are low (Chapter 4). I must stress that these gender differences prevail if most of the FA variation comes from diet. Part of the FA variation between sexes is likely to originate from differential metabolism and deposition of ingested FAs into adipose tissue. This was not considered in the QFASA model because the same CCs were applied to all individuals. Hence, it is difficult to conclude on the long-term diet of male and females NZ sea lions without foraging and diving data from males. To date, these data are only available for lactating NZ sea lions foraging around the Auckland Islands (Chilvers *et al.* 2005, 2006).

### 5.4.4 Between-year variation in the diet estimates

Within females, diet variation involved mostly differences between the combined years 2005-2006 and the previous years (**Fig. 5-5a**). Only one male was available in 2005-2006, thus it is not possible to check if this trend was shared by males. QFASA indicated a greater contribution of arrow squid and a lesser contribution of hoki in 2005-2006 (**Fig. 5-5a**). Differential metabolism between years, arising from animals in different body condition, might play a role in the temporal variation of FA profiles. However in Chapter 3, I remarked that the difference in body condition between individuals from different years was not enough to explain the FA variation, and concluded that diet must play a significant role. NZ sea lions are considered generalist predators (Lalas 1997; McMahon *et al.* 1999; Childerhouse *et al.* 2001; Chapter 2), 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 of hoki and arrow squid can be used as a proxi of variation in abundance. Estimated catches of hoki in 2005 and 2006 in the subantarctics were the lowest reported since 1989, whereas the amount of arrow squid harvested each year in 2004 to 2006 from the Auckland Islands region (named SQU6T) was higher than the amount harvested in the previous three years (Ministry of Fisheries 2007). These reported catches correlate well with the trends of mass percentages estimated by the QFASA model (**Fig. 5-7**), suggesting that diet plays a significant role in the FA variation between years.

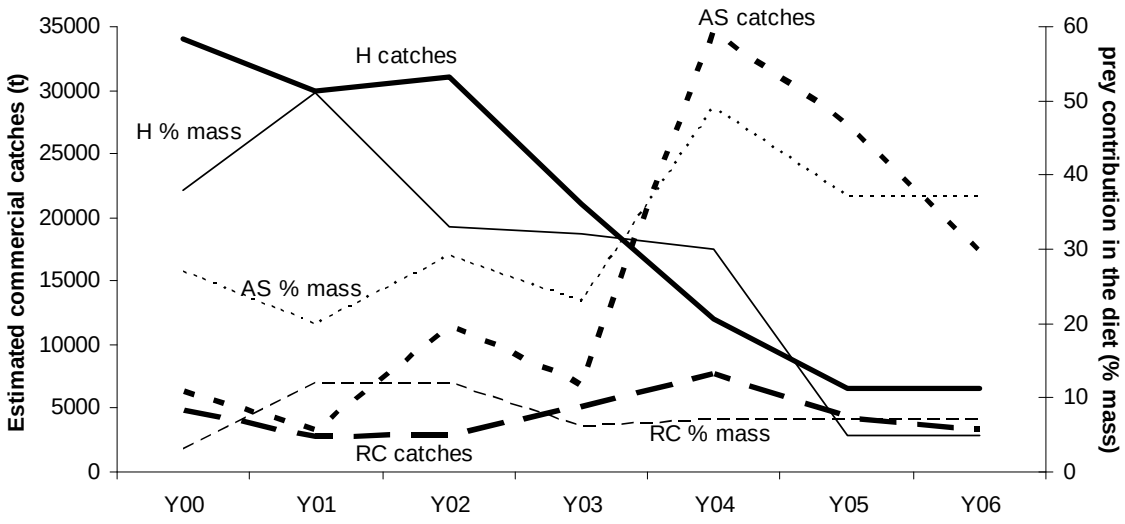


**Figure 5-7.** Estimated commercial catches of hoki *Macruronus novaezelandiae* (H), arrow squid *Nototodarus sloani* (AS), and red cod *Pseudophycis bacchus* (RC) per year in comparison with the estimated proportions of these species in the diet of New Zealand sea lions. Catches of hoki were for the region "HOK1 subantarctics", catches of arrow squid for the region "SQU6T" (Auckland Islands), and catches of red cod for "RCO3" (east of NZ south island and subantarctics).

## 5.5 CONCLUSION

QFASA has been used to provide long-term estimates of diet on several marine species (Iverson *et al.* 2006; Beck *et al.* 2007a; Iverson *et al.* 2007). Nevertheless, the necessity for CCs which take into account FA metabolism in the predator, presently limits the use of this method to a narrow range of species. This study tested QFASA on by-caught NZ sea lions, and showed the importance of suitable CCs in the estimation of the diet by QFASA. The predicted diets of males and females should be taken with care as QFASA did not take into account the possible differential FA metabolism between sexes. However, the overall predictions were partly in agreement with stomach contents, and the variation estimated between years was highly correlated with the trends of commercial catches, giving some confidence in the present QFASA predictions.

Arrow squid, hoki and rattails were estimated to have large contributions by mass in the long-term diet of NZ sea lions. They are all demersal species found mainly at depths > 200m, being harvested by sea lions on the slopes of the Auckland Islands shelf. This area was shown to be a foraging ground for lactating sea lions just after the breeding season (Chilvers *et al.* 2005). This study suggests that the slopes are visited regularly during the first half of the lactating period. Future studies with a better understanding of

FA metabolism on otariids and a greater prey library will improve the reliability of QFASA on sea lions.

## Acknowledgements

NZ sea lions were necropsied at Massey University under contracts with the Conservation Service Levies Providers, administrated by the Department of Conservation and the Ministry of Fisheries, NZ. I thank observers who collected the carcasses as well as people who have assisted with the necropsies over the past ten years. Special thanks to R. Galois (Ifremer, France), M. Walton (SMRU, UK) and M. Reynolds (Fonterra, NZ) for their advice on fatty acid analysis. Thanks to L. Donaldson who extracted the lipids of the samples collected in 2005 and 2006. For the QFASA model, I am grateful to M. Walton who provided the programme Fascalc, and to D. Tollit (UBC, Canada) who provided the calibration coefficients calculated for Steller sea lions. R. Sherriff (Brimble Sherriff Young Limited, NZ) programmed the optimisation model for Massey University. This project was sponsored by Lewis Fitch Research Fund, Massey University Research Fund, the Whale and Dolphin Adoption Project, the Department of Conservation and the NZ Ministry of Fisheries.

# 6 CHAPTER 6

## FORAGING DIVERSITY IN LACTATING NEW ZEALAND SEA LIONS: INSIGHT FROM QUALITATIVE AND QUANTITATIVE FATTY ACID SIGNATURE ANALYSIS

**Photo:** the “New Zealand sea lion team” weighing a female sea lion under anaesthesia at Sandy Bay, Enderby Island, Auckland Islands

## Abstract

Lactating NZ sea lions *Phocarctos hookeri* exhibit different foraging strategies at the Auckland Islands (50°41'S, 166°E) shelf, which may be the result of niche specialisation. The purpose of this study was to examine the fatty acid (FA) composition of lactating NZ sea lions, to estimate their diet by quantitative FA signature analysis (QFASA), and to determine if variation in FAs exists between foraging strategies and years. FAs were analysed from the blubber of 78 lactating NZ sea lions captured at Enderby Island, Auckland Islands, between 2000 and 2003 and in 2005, during the first month of the lactation period. FA profiles revealed distinct separation between years (ANOVAs on principal components, $P < 0.05$), but not between foraging patterns, thus this study does not support the hypothesis of a niche specialisation between the different foraging strategies displayed by lactating NZ sea lions. The long-term diet estimated by QFASA mainly consisted of rattails Macrouridae (30% median mass) and hoki *Macruronus novaezelandiae* (26% median mass), followed by red cod *Pseudophycis bachus* (11% median mass) and arrow squid *Nototodarus sloani* (49% median mass). My findings suggest that most of the energy intake during a foraging trip take place on the slopes where large fish gather in abundance at depths > 200m. Comparison of diet estimates from QFASA between the females studied herein and females by-caught by the squid fishery later in the lactation period suggest that females change their fish diet to a more squid-based diet in autumn when arrow squid gather in the area to spawn. Although QFASA is at its early development and uncertainties remain on the reliability of calibration coefficients (CCs) used, estimates of arrow squid proportions in the diet followed the same trend as the reported arrow squid catches since 2000.

## 6.1 INTRODUCTION

Female New Zealand (NZ) sea lions (*Phocarctos hookeri*) display a "central place foraging" strategy during lactation because they are restrained in foraging range by the need to regularly nurse their pup on land (Orians and Pearson 1979). If resources are limited or unpredictable in the foraging range of female otariids, individuals may develop feeding specialisations to reduce intra-specific competition for food and maximise energy intake (Polis 1984; Schoener 1986). NZ sea lions have a restricted breeding range with 86% of the pups being born at the Auckland Islands (50°41'S, 166°E) (Chilvers *et al.* 2007b). During the beginning of the lactation period (austral summer), lactating NZ sea lions exhibit different diving patterns (Chilvers *et al.* 2006) depending on the region of the Auckland Islands shelf they exploit during their foraging trips, which may be the consequence of feeding specializations (Chilvers and Wilkinson 2009). Chilvers and Wilkinson (2009) classified females as benthic divers and mesopelagic divers. Benthic divers travel northeast from the breeding site, and make consecutive dives at similar depths on the Auckland Islands shelf, while mesopelagic divers travel north/northwest from the breeding site and make most of their dives at deeper and variable depths at the edges of the shelf (**Fig. 6-1**). Since the shelf and the shelf edges host different prey communities, it is expected that benthic divers and mesopelagic divers would forage on different prey. Previous diet studies using stomach contents and scat samples showed that NZ sea lions feed on benthic neritic prey such as octopus (*Enteroctopus zealandicus*) and opalfish (*Hemerocoetes* spp.), and on deep-living oceanic species such as rattail (Macrouridae) and hoki (*Macruronus novaezelandiae*) (Childerhouse *et al.* 2001; Chapter 2). Therefore, I would expect that the benthic prey octopus and opalfish would contribute in higher proportions to the diet of benthic divers than to that of mesopelagic divers, while the demersal prey rattail and hoki would be in greater proportions in the diet of mesopelagic divers. In the present study, I propose to test this hypothesis by fatty acid (FA) analysis on females for which the foraging area on the Auckland Islands shelf and/or the diving pattern is known. Furthermore, the long-term diet of female NZ sea lions has already been investigated with quantitative fatty acid signature analysis (QFASA) on blubber from animals by-caught in the sub-Antarctic arrow squid (*Nototodarus sloani*) fishery (Chapter 5), but it is not known if the diet of by-caught lactating females is representative of the diet of lactating females at the population level, or if these by-caught individuals developed a

particular adaptive behaviour towards the fishing activity. Thus, this study is also an opportunity to compare the FA compositions and diet estimates of different pools of females (biopsied blubber of captured females on breeding sites *versus* blubber of by-caught females).

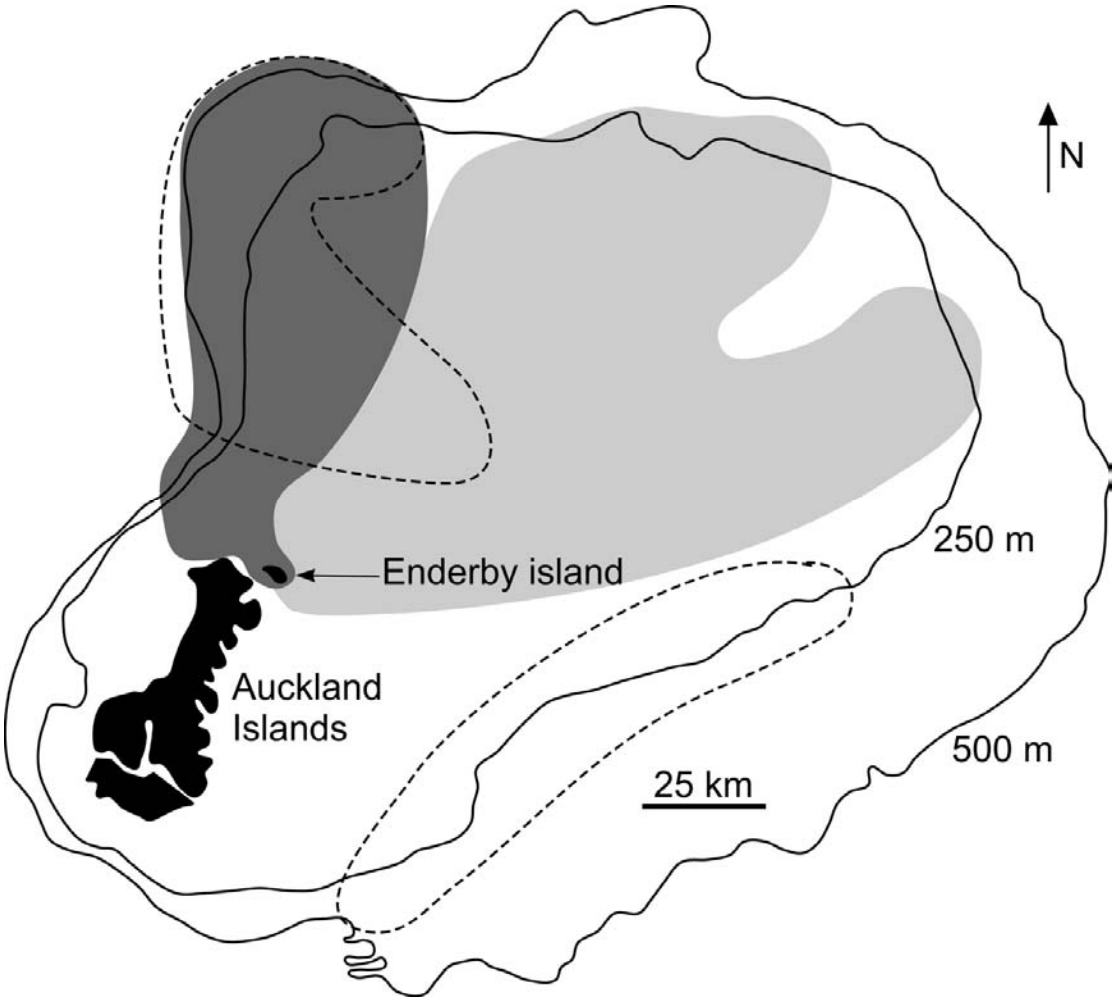


**Figure 6-1**. Foraging locations of lactating New Zealand sea lions according to their diving patterns. The regions of the Auckland Islands (50°41'S, 166°E) shelf visited by benthic and mesopelagic divers are in light grey and in dark grey respectively. These data are derived from Chilvers & Wilkinson (2009). The bathymetric contours are in solid lines, and areas of fishing activity (arrow squid trawl fishery) in dashed lines (Smith and Baird 2007).

FA analysis has been used extensively to assess diet variation between groups of individuals in various species of pinnipeds (*e.g.*, Iverson *et al.* 1997a; Brown and Pierce 1998; Käkelä and Hyvärinen 1998; Walton *et al.* 2000; Lea *et al.* 2002a; Bradshaw *et al.* 2003; Grahl-Nielsen *et al.* 2005; Staniland and Pond 2005; Beck *et al.* 2007b). FAs undergo predictable biochemical change when deposited in the predator's adipose tissue (*e.g.*, Summers *et al.* 2000; Iverson *et al.* 2004), and have the potential to reflect dietary intake over several weeks to months depending on the tissue turnover (*e.g.*, Kirsch *et al.* 1998; Kirsch *et al.* 2000; Iverson *et al.* 2004; Cooper *et al.* 2005). QFASA allows the estimation of the contribution by mass of potential prey in the diet of the predator by applying a model minimising a mathematical distance between the FA profiles of the predator and potential prey (Iverson *et al.* 2004; Iverson *et al.* 2006; Beck *et al.* 2007a; Iverson *et al.* 2007; Chapter 5).

In the present study, I first investigate the FA composition of the blubber of lactating NZ sea lions captured at the Auckland Islands, and assess the FA variation between years and between females displaying different diving patterns. Second, the overall diet and the diet between groups are estimated by QFASA. Third, the overall FA composition and diet estimates of females are compared with the FA profiles and diet estimates of by-caught females analysed previously (Chapter 3, Chapter 5).

## 6.2 MATERIALS AND METHODS

### 6.2.1 Sample collection

Lactating NZ sea lions were captured at Sandy Bay, Enderby Island, Auckland Islands (50°30'S, 166°17'E) (**Fig. 6-1**) in January of 2000 to 2003 and 2005. The Sandy Bay colony is the second largest breeding colony for NZ sea lions, with approximately 400 pups born per year (Chilvers *et al.* 2007b). Females were anaesthetised following standard procedures detailed in previous studies (Gales and Mattlin 1997; Costa and Gales 2000; Chilvers *et al.* 2005, 2006). Blubber was biopsied from females by scrubbing the posterior flank (dorso-lateral lumbar region) with surgical disinfectant and making a 10 mm incision in the skin using a sterile scalpel. A core was sampled through the full depth of the blubber layer excluding the skin, wrapped in aluminium foil, transferred into a 2 mL cryovial and stored at –196°C in liquid nitrogen. On return from the field, blubber samples were stored at –80°C until analysed.

Stratification has been shown to occur in the blubber of NZ sea lions (Donaldson et al. unpubl. data). Inner blubber is believed to be more metabolically active than the outer blubber, reflecting recent dietary FA intake (Olsen and Grahl-Nielsen 2003; Grahl-Nielsen *et al.* 2005). I chose to analyse the full blubber core in this study because it provides the long-term integration of dietary FAs (Iverson *et al.* 2004).

Previous studies assessed the foraging and diving behaviours of some of the females from which blubber samples were collected: Satellite tags and time-depth recorders were fitted to captured lactating females during the summers of 2003 and 2004, while satellite tags only were attached during the summers of 2001, 2002 and 2005 (Chilvers *et al.* 2005, 2006). Depending on the foraging location used, females exhibited different diving patterns and were categorised benthic divers or mesopelagic divers (Chilvers and Wilkinson 2009) (**Fig. 6-1**). For females tagged only with satellite devices, the diving pattern was deduced from the foraging location used. Moreover, the blubber samples analysed in a particular year could have been collected from a female which was fitted with satellite and/or depth devices in a different year. Because females are returning to the same foraging location from year to year (Chilvers 2008a), the diving pattern observed during a particular summer would be representative of the diving patterns displayed in the other years. For instance, some lactating females for which blubber was analysed in 2000 were attributed a diving pattern from the satellite and/or depth recorder information collected on these animals in 2001 and subsequent years.

### 6.2.2 Lipid analysis

Lipids from blubber were extracted following Folch et *al.* (1957). Briefly, lipids were extracted in a mixture of 8:4:3 chloroform:methanol:saline water (v:v:v) containing 0.01% butylated hydroxytoluene (BHT) as an antioxidant. FA methyl esters were prepared using 10% boron trifluoride in methanol, and extracted into hexane. They were analysed by gas chromatography using a polar capillary column coated with 50% cyanopropyl polysiloxane (0.25 μm film thickness; J&W DB-23, Folsom, California). Further details of the laboratory protocol are given in Chapter 3.

### 6.2.3 Statistical analysis

FAs were expressed as a percentage mass of total FAs. A combination of principal component analysis (PCA) and discriminant function analysis (DFA) was used on arcsine square-root transformed data to examine inter-annual differences in the FA profiles of lactating NZ sea lions (MINITAB Release 15.1, MINITAB Inc. 2007 and SPSS for windows® Release 16.0, SPSS Inc. 2007). An ANOVA was performed on the principal components (PCs) to assess the effect of year. DFA requires that the number of blubber samples per group of individuals exceeds the number of variables (FAs) to minimise the heterogeneity of covariance matrices and to avoid overfitting (Budge *et al.* 2006). Therefore, the number of FAs was reduced by deleting the ones with the lowest absolute loadings on the first three PCs. After re-normalisation of the FA percentages, a linear DFA with cross validation was performed for the factor "year". To avoid any confounding effect between factors "year" and "diving pattern", the FA differences between diving patterns were explored only within each year for which both diving profiles (benthic *versus* mesopelagic divers) were available. The small number of females of known diving pattern per year (**Table 6-1**) precludes the use of multivariate analyses or statistical tests. Last, FA profiles of biopsied females (this chapter) and of by-caught females analysed previously (Chapter 3) were compared on a PCA plot. Only females by-caught in the north area of the Auckland Islands shelf were included. Some by-caught females were not lactating but were grouped with the by-caught lactating females for the comparison with biopsied females, since it was found that the FA composition between by-caught females did not vary with the lactation state (Chapter 3).

Differences in FA profiles between different groups of individuals may be caused by a different diet, but also by different rates of metabolism due to different energy requirements. To explore this last possibility, I calculated a body condition index for each group (different years, benthic divers, mesopelagic divers, all biopsied females, by-caught lactating females and by-caught non-lactating females) by dividing the mass (kg) by the nose-tail length (cm) (Lunn and Boyd 1993; Arnould 1995), and tested for any difference using a Kruskal-Wallis (K-W) test with *post-hoc* Mann-Whitney (M-W) tests. The index was calculated for the 43 of the females biopsied in 2002, 2003 and 2005 (morphometric measurements are not available before 2002; B.L. Chilvers unpubl. data) and for the 73 NZ sea lions by-caught between 2000 and 2005 and measured during necropsy (Massey University unpubl. data).

**Table 6-1.** Number of biopsied lactating New Zealand sea lions analysed each year, including the number of females classified as benthic or mesopelagic divers (Chilvers and Wilkinson 2009) from information on foraging location (satellite tag ST; Chilvers *et al.* 2005) and/or on dive pattern (time-depth recorder TDR; Chilvers *et al.* 2006)

| Years | 2000 | 2001 | 2002 | 2003 | 2005 | Total |
|---|---|---|---|---|---|---|
| Females analysed | 20 | 12 | 15 | 20 | 11 | 78 |
| Including benthic divers | 4 | 2 | 3 | 0 | 7 | 16 |
| mesopelagic divers | 0 | 3 | 2 | 5 | 2 | 12 |

### 6.2.4 QFASA model

The diets of individual sea lions were estimated using QFASA described by Iverson *et al.* (2004), and a prey FA library from the Auckland Islands region (Chapter 4). 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 the closest to match 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 (percentage mass). Details of the QFASA model and the prey FA library are given in Chapter 5.

Optimal QFASA parameters determined earlier for by-caught NZ sea lions (Chapter 5) were used in this chapter: They were CCs calculated from experiments on Steller sea lions (*Eumetopias jubatus*) fed on pacific herring (*Clupea pallasii pallasii*) (D. Tollit unpubl. data), a set of 23 FAs (14:1, 15:0, 15:1, 18:1n-5, and 22:4n-6 were deleted from the original set; **Table 6-2**), and the account of prey individual variability (see Chapter 5 for details). Diet estimates were tested for significant variation between groups by K-W and *post-hoc* M-W tests. All statistical tests have an α level of statistical significance of 0.05.

## 6.3 RESULTS

Blubber samples were analysed from 78 lactating NZ sea lions captured in January of the years 2000 to 2003 and 2005 (**Table 6-1**). The blubber biopsied in 2004 was not analysed because of an accidental liquid nitrogen leak from the storage bottle that year, which left the samples at ambient temperature for an unknown period of time. Data on diving patterns were available either directly from diving studies or deduced from satellite telemetry (see methods) for four females in 2000, five in 2001, five in 2002, five in 2003, and nine in 2005 (**Table 6-1**).

**Table 6-2.** By-year fatty acid composition from 78 biopsied lactating New Zealand sea lions in mean mass percent ± SD. Fatty acids noted with a star are the ones selected for the discriminant function analysis. Fatty acids noted with a cross were deleted for quantitative fatty acid signature analysis.

| | Fatty acid composition (mean mass % ± SD) | | | | |
|---|---|---|---|---|---|
| Fatty acids | 2000 *n* = 20 | 2001 *n* = 12 | 2002 *n* = 15 | 2003 *n* = 20 | 2005 *n* = 11 |
| **SFA** | **22.08 ± 2.47** | **25.95 ± 2.78** | **23.15 ± 2.55** | **21.91 ± 2.86** | **25.71 ± 3.72** |
| 14:0* | 5.99 ± 1.40 | 8.29 ± 1.86 | 5.92 ± 1.83 | 5.29 ± 0.97 | 8.14 ± 2.47 |
| 15:0† | 0.50 ± 0.09 | 0.59 ± 0.12 | 0.51 ± 0.08 | 0.54 ± 0.12 | 0.59 ± 0.09 |
| 16:0 | 13.02 ± 1.29 | 14.46 ± 1.78 | 13.87 ± 1.06 | 13.34 ± 1.69 | 14.36 ± 2.13 |
| 17:0 | 0.36 ± 0.05 | 0.37 ± 0.08 | 0.36 ± 0.06 | 0.39 ± 0.08 | 0.37 ± 0.07 |
| 18:0 | 2.22 ± 0.32 | 2.25 ± 0.47 | 2.49 ± 0.49 | 2.43 ± 0.35 | 2.25 ± 0.43 |
| **MUFA** | **59.58 ± 2.67** | **56.91 ± 2.88** | **60.36 ± 3.59** | **59.22 ± 4.35** | **55.57 ± 2.61** |
| 14:1*† | 0.59 ± 0.19 | 0.72 ± 0.27 | 0.45 ± 0.19 | 0.43 ± 0.10 | 0.65 ± 0.23 |
| 15:1† | 0.17 ± 0.03 | 0.15 ± 0.05 | 0.13 ± 0.03 | 0.15 ± 0.05 | 0.17 ± 0.05 |
| 16:1n-7* | 8.48 ± 1.63 | 8.45 ± 1.53 | 7.27 ± 1.14 | 7.89 ± 0.90 | 9.22 ± 1.39 |
| 18:1n-9* | 33.79 ± 2.94 | 30.44 ± 3.80 | 33.38 ± 4.17 | 33.76 ± 3.57 | 29.43 ± 3.06 |
| 18:1n-7* | 4.34 ± 0.39 | 3.93 ± 0.43 | 4.33 ± 0.23 | 4.65 ± 0.31 | 3.98 ± 0.43 |
| 18:1n-5*† | 0.41 ± 0.08 | 0.42 ± 0.10 | 0.32 ± 0.07 | 0.30 ± 0.05 | 0.41 ± 0.05 |
| 20:1n-11 | 1.80 ± 0.31 | 1.73 ± 0.28 | 1.92 ± 0.29 | 1.67 ± 0.22 | 1.87 ± 0.30 |
| 20:1n-9 | 8.07 ± 2.11 | 9.01 ± 1.95 | 10.42 ± 1.49 | 8.61 ± 1.66 | 7.83 ± 1.38 |
| 22:1n-11 | 1.64 ± 0.34 | 1.77 ± 0.45 | 1.77 ± 0.29 | 1.44 ± 0.22 | 1.73 ± 0.28 |
| 22:1n-9 | 0.27 ± 0.11 | 0.29 ± 0.08 | 0.38 ± 0.10 | 0.32 ± 0.09 | 0.28 ± 0.10 |
| **PUFA** | **18.38 ± 2.25** | **17.14 ± 2.23** | **16.49 ± 2.07** | **18.78 ± 3.78** | **18.73 ± 2.56** |
| 16:3n-4* | 0.14 ± 0.05 | 0.19 ± 0.07 | 0.15 ± 0.05 | 0.15 ± 0.05 | 0.24 ± 0.05 |
| 18:2n-6 | 1.92 ± 0.19 | 1.62 ± 0.16 | 1.66 ± 0.23 | 1.68 ± 0.31 | 1.82 ± 0.18 |
| 18:3* | 0.89 ± 0.14 | 0.78 ± 0.14 | 0.70 ± 0.12 | 0.74 ± 0.15 | 0.93 ± 0.10 |
| 20:2n-6 | 0.38 ± 0.07 | 0.33 ± 0.06 | 0.33 ± 0.09 | 0.35 ± 0.08 | 0.36 ± 0.06 |
| 20:3n-6 | 0.11 ± 0.02 | 0.08 ± 0.03 | 0.10 ± 0.02 | 0.1 ± 0.02 | 0.10 ± 0.02 |
| 20:3n-3 | 0.18 ± 0.03 | 0.17 ± 0.04 | 0.16 ± 0.04 | 0.16 ± 0.03 | 0.17 ± 0.06 |
| 20:4n-6† | 0.69 ± 0.19 | 0.50 ± 0.10 | 0.56 ± 0.07 | 0.81 ± 0.26 | 0.59 ± 0.19 |
| 20:5n-3* | 1.86 ± 0.56 | 1.71 ± 0.46 | 1.48 ± 0.33 | 1.78 ± 0.60 | 2.17 ± 0.57 |
| 21:5n-3* | 0.21 ± 0.07 | 0.18 ± 0.07 | 0.14 ± 0.04 | 0.17 ± 0.06 | 0.25 ± 0.05 |
| 22:4n-6 | 0.15 ± 0.06 | 0.11 ± 0.05 | 0.11 ± 0.05 | 0.17 ± 0.10 | 0.11 ± 0.06 |
| 22:5n-6 | 0.22 ± 0.04 | 0.18 ± 0.05 | 0.17 ± 0.04 | 0.19 ± 0.03 | 0.19 ± 0.05 |
| 22:5n-3 | 2.73 ± 0.43 | 2.65 ± 0.50 | 2.71 ± 0.46 | 3.00 ± 0.84 | 2.84 ± 0.70 |
| 22:6n-3 | 8.87 ± 1.29 | 8.65 ± 1.25 | 8.23 ± 1.23 | 9.48 ± 2.22 | 8.96 ± 1.66 |

### 6.3.1 FA composition of biopsied lactating sea lions and variation

Although 38 FAs were originally identified, only 28 FAs were used ranging from 14:0 to 22:6n-3 (**Table 6-2**). The 10 FAs removed were either short chain FAs (<14 carbons), known to come primarily from endogenous biosynthesis (Budge *et al.* 2006), or FAs for which the identification was not certain. Together the FAs in greatest concentration were 14:0, 16:0, 16:1n-7, 18:1n-9, 18:1n-7, 20:1n-9 and 22:6n-3, which accounted for approximately 83% of the total FAs in the blubber (**Table 6-2**). Monounsaturated FAs accounted for more than half of the total mass. Saturated FAs ranked second in importance with approximately 24%, and polyunsaturated FAs were the least represented group with approximately 18% (**Table 6-2**).

A PCA using a correlation matrix was run on arcsine square-root transformed data to identify the FAs that were the most important in explaining the variance between FA profiles. The factor year was not presented on a PC plot, because the number of years (5) would make the graphic interpretation difficult. However, an ANOVA revealed that year had a significant effect on all three PCs (PC1: $F_{4, 77} = 8.83$, $P < 0.001$; PC2: $F_{4, 77} = 3.36$, $P = 0.014$; PC3: $F_{4,77} = 2.67$, $P = 0.038$). Females captured in 2002 and 2003 displayed different FA profiles from the females captured in other years on PC1 (Tukey tests, $P < 0.05$). This result was confirmed by a canonical discriminant plot (**Fig. 6-2**) on the 10 FAs with the highest PC loadings (noted by a star in **Table 6-2**). The years 2002 and 2003 were segregated from the other years along the first discriminant function by high loadings of 18-carbon MUFAs and 18:3 (**Fig. 6-2**). DF2 separated the year 2005, mainly due to a higher concentration of 16:3n-4 in 2005 than in the other years (**Fig. 6-2**, **Table 6-2**). There was no apparent difference in the FA profiles of benthic and mesopelagic divers when they were compared visually within the years 2001, 2002 and 2005 (**Fig. 6-3**). Body condition indices were similar between years (K-W test, $H_{2,42} = 3.75$, $P = 0.153$) and diving types (M-W test, $W = 72$, $P = 1.000$).

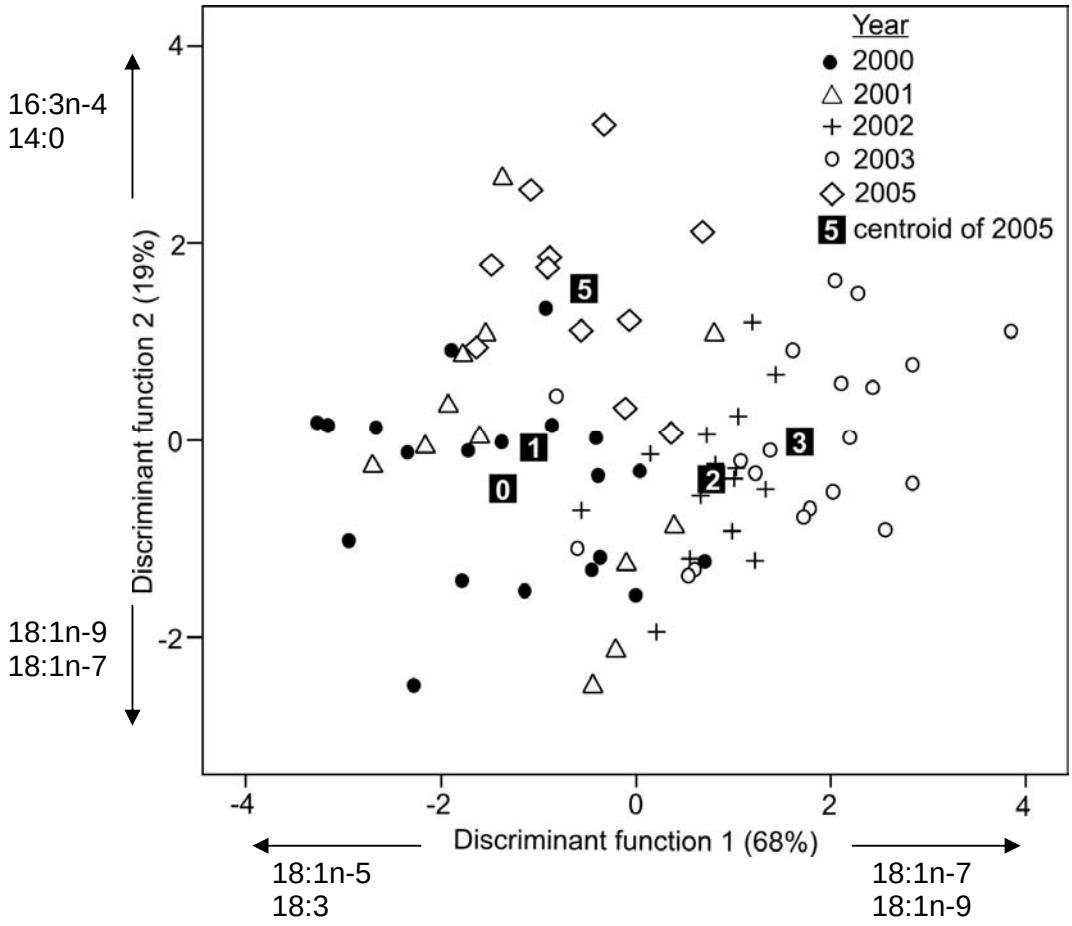


**Figure 6-2.** Plot of the first two canonical discriminant functions of fatty acid (FA) profiles from biopsied lactating New Zealand sea lions. This analysis included 10 FAs only (see details in Results section). The FAs with the most important positive or negative loadings on function 1 and function 2 are displayed along the axes.

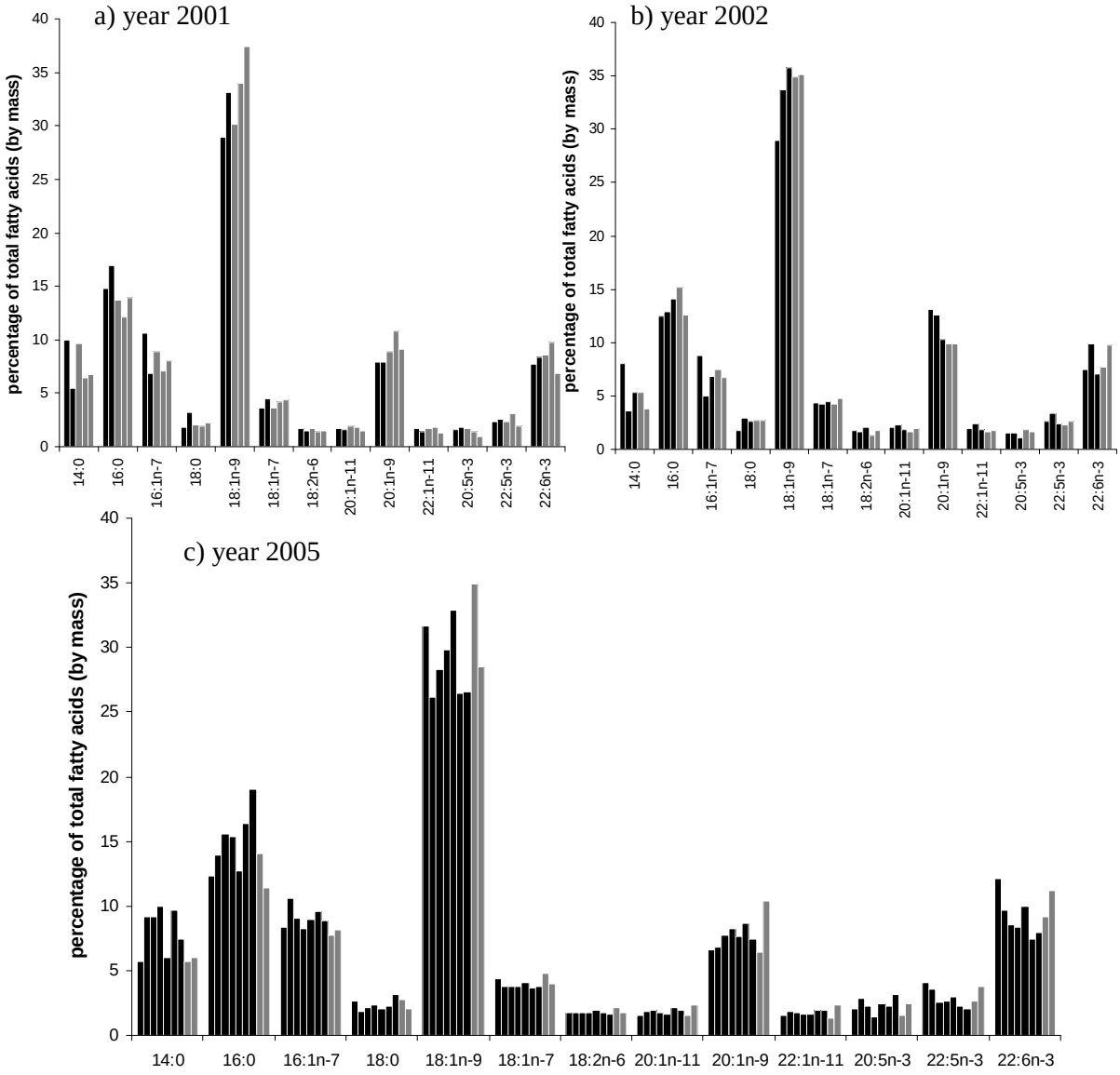


**Figure 6-3.** Comparison between fatty acid (FA) profiles of lactating New Zealand sea lions with a benthic diving pattern (black; each bar being the % for one female) and a mesopelagic diving pattern (grey) per year. Years 2000 and 2003 were not represented because only one type of diving pattern was present. Only the major FAs were represented (accounting for ≥ 95% total FAs by mass) for better clarity.

### 6.3.2 Biopsied lactating sea lions *versus* by-caught female sea lions

FA profiles from biopsied females were clearly segregated from that of by-caught female sea lions along PC1 explaining 27% of the variance (**Fig. 6-4**). They were attributed negative values on PC1 and were positively correlated with the FAs 16:1n-7, 14:1 and 15:0, and negatively correlated with 22:6n-3, 22:1n-9 and 22:5n-3. Although PC2 and PC3 explained similar amounts of variation, PC3 was not displayed because it was not informative on the separation of the groups of females.

Body condition indices were significantly different between the biopsied lactating females and the by-caught females (K-W test, $H_{2,\ 115} = 11.61$, $P = 0.003$). The median index was 0.64 for both biopsied and by-caught lactating females (M-W test, $P = 0.715$), while the median index of by-caught non-lactating females was slightly lower (0.59) but nonetheless significantly different (M-W test, $P = 0.002$).

### 6.3.3 Diet estimates of NZ sea lions using QFASA

Overall, rattails, hoki, red cod and arrow squid contributed > 80% mass of the diet as estimated by QFASA, and were considered prey in more than half the samples (**Table 6-3**). Rattails and hoki were the most important prey estimated (median mass of 30% and 26% respectively), and were represented in all but one individual. Opalfish, octopus and scampi were considered minor prey with an occurrence < 50%. Spiny dogfish was considered present in few percentages for only seven sea lions (**Table 6-3**).

QFASA results are derived from the FA profiles of sea lions, so that dietary estimates will be similar for individuals with similar FA profiles. FA profiles of biopsied lactating females displayed some variation between years but not between diving profiles, thus only the diet estimates between years have been investigated. The estimated percentage of rattails in the diet of females captured in 2005 was lower than in the diet of females captured in the other years (K-W test, $H_{4,77} = 10.12$, $P = 0.038$; M-W tests, $P < 0.05$; **Fig. 6-5**) except in 2001 (M-W test, $W = 188.0$, $P = 0.148$). In contrast, the estimated contribution of arrow squid in the diet in 2005 was significantly higher than in the previous years (K-W test, $H_{4,77} = 17.76$, $P = 0.001$; M-W tests, $P < 0.05$; **Fig. 6-5**) but not in 2000 (M-W test, $W = 287.0$, $P = 0.179$). The lowest contribution of arrow squid in the diet was estimated for females captured in 2002 (only 1%), which was lower than

the proportion estimated for the other years (M-W tests, $P < 0.05$) except 2003 (M-W test, $W = 218.0$, $P = 0.065$). The contributions of octopus and scampi were highly variable between years, with for instance 20% of scampi estimated in 2000 while it was absent from the simulations in 2002 (**Fig. 6-5**). However, the percentages of octopus and scampi were not tested for difference between years because these species were estimated in too few samples (**Table 6-3**).

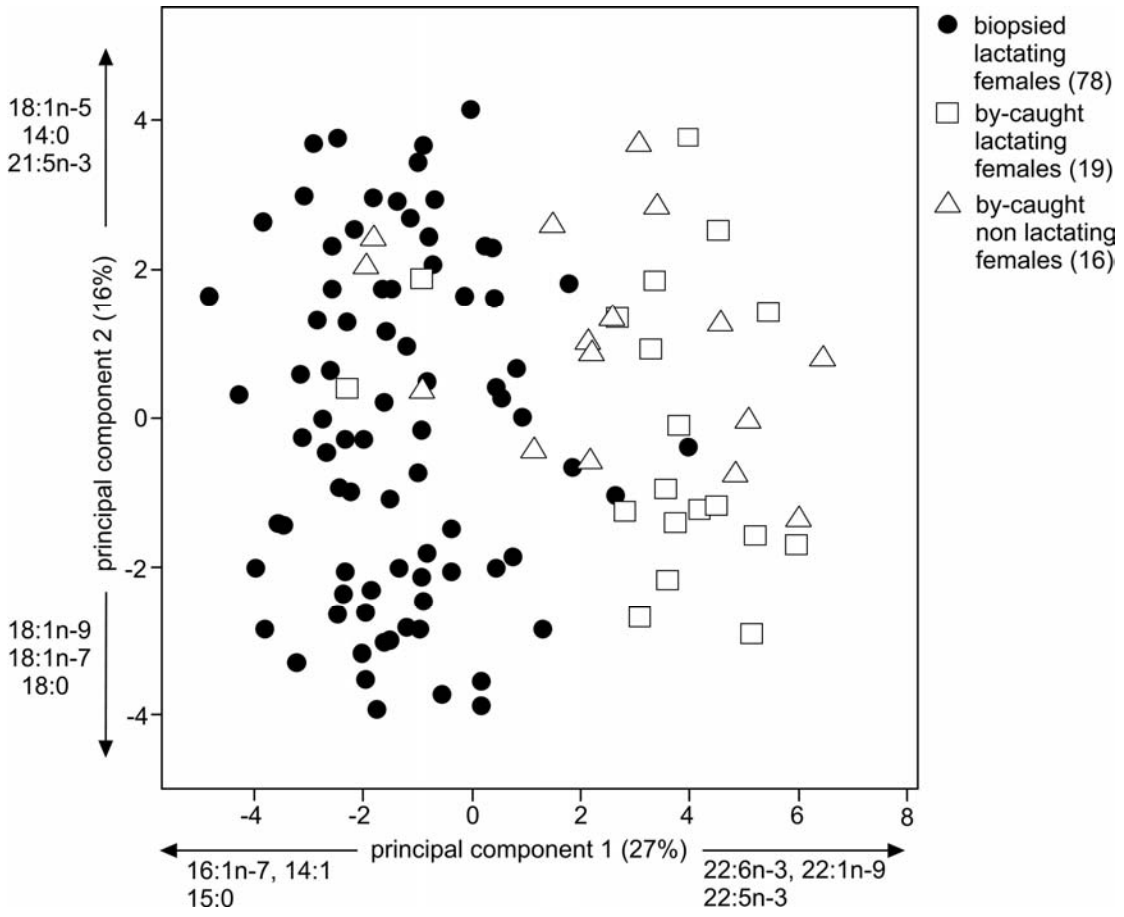


**Figure 6-4.** Plot of the first two principal components (PCs) of fatty acid (FA) profiles from female New Zealand sea lions. Biopsied lactating females (this study) are represented by full circles, and by-caught females (from Chapter 3) are represented by empty symbols. The variance explained by each PC is in parenthesis. The FAs with the highest positive and negative loadings are also displayed.

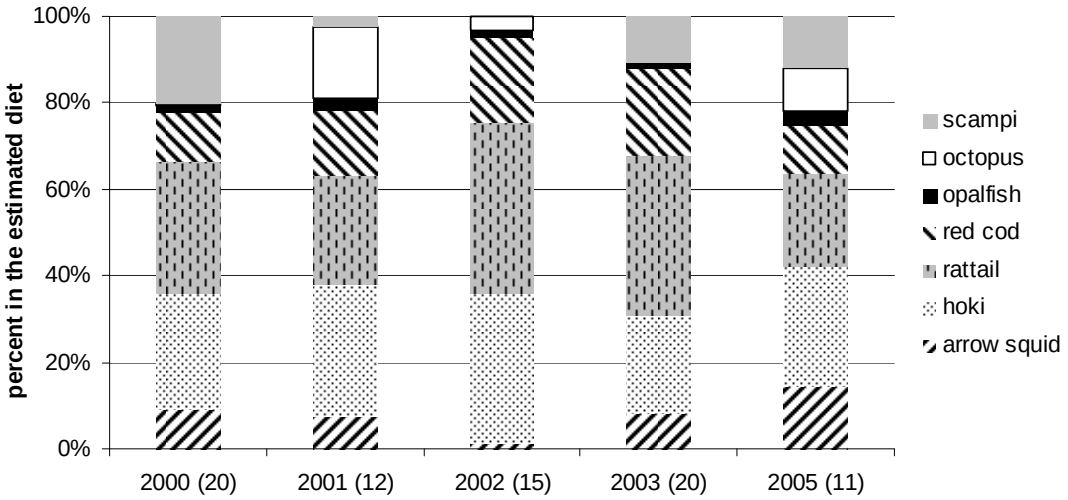


**Figure 6-5.** Mean estimated percentages of prey species by year from the quantitative fatty acid signature analysis for biopsied lactating New Zealand sea lions. The number of samples available for each category is displayed in parentheses.

**Table 6-3.** Predicted proportions (mean and median % mass) of prey species by quantitative fatty acid signature analysis for 78 biopsied lactating New Zealand sea lions. O is occurrence (number of individuals for which the prey has a percentage mass > 0); values between the 25$^{th}$ percentile and the 75$^{th}$ percentile represent the interquartile range in which 50% of the data are.

| Prey | O | %O | mean | median | 25$^{th}$ percentile | 75$^{th}$ percentile |
|---|---|---|---|---|---|---|
| Arrow squid | 52 | 67 | 8 | 4 | 0 | 12 |
| Hoki | 78 | 100 | 28 | 26 | 16 | 36 |
| Rattails | 77 | 99 | 32 | 30 | 16 | 44 |
| Red cod | 68 | 87 | 16 | 11 | 2 | 27 |
| Opalfish | 28 | 37 | 2 | 0 | 0 | 0 |
| Octopus | 17 | 23 | 5 | 0 | 0 | 0 |
| Scampi | 34 | 44 | 10 | 0 | 0 | 12 |
| Spiny dogfish | 7 | 10 | <1 | 0 | 0 | 0 |

## 6.4 DISCUSSION

In this study, the blubber samples from 78 lactating NZ sea lions captured in January of each year from 2000 to 2003 and 2005 have been analysed for FAs. The long-term diet estimated by QFASA mainly consisted of rattails and hoki followed by red cod and arrow squid. This is somewhat in contrast with the QFASA results from by-caught females (Chapter 5) for which arrow squid and rattails were considered the most important prey. Given that lactating females have been shown to display different diving strategies depending on the area of the Auckland Islands shelf exploited (Chilvers and Wilkinson 2009), it has been hypothesised that benthic and mesopelagic divers would have different diets that can be revealed in different FA profiles. This study did not show any variation in FA profiles between females with different diving strategies, however patterns of FAs did vary between years of capture.

### 6.4.1 Limits encountered with FA analysis and QFASA

Deposition of dietary lipids in the blubber will depend on the nutritional status of the animal. It is expected that a substantial amount of the FAs ingested above the metabolic requirements will be deposited in the blubber, but the extent to which dietary FAs are deposited during periods of negative energy balance is unclear. Thus, the blubber FA signatures are more likely to reflect dietary FAs in animals in positive energy balance (Kirsch *et al.* 2000). Lactating NZ sea lions, captured and weighed on land, had a stable body mass or were gaining mass during the one-month interval between January and February (first months of lactation period) (B.L. Chilvers, unpubl. data). Thus, I believed that lactating sea lions were in positive energy balance and depositing dietary FAs at the time of capture (January).

Blubber FA profiles have been shown to vary with the body region for Cape fur seals *Arctocephalus pusillus pusillus* (Arnould *et al.* 2005) suggesting preferred depot sites along the body of otariids. The dorso-lateral lumbar region for which blubber was sampled in this study is a common region for biopsying blubber in free-ranging pinnipeds (*e.g.,* Best *et al.* 2003; Andersen *et al.* 2004; Beck *et al.* 2007b; Wheatley *et al.* 2007). There is no particular reason for choosing this area in otariids other than practical access when the animal is restrained and sedated. Indeed, the only study on fat

depots in otariids concerns the assessment of variation in blubber thickness for only two captive Steller sea lions throughout the year (Mellish *et al.* 2007) for which the dorsal hip region (named D4 and D5 in the study) did not parallel the changes of mass. Needless to say, more data are necessary before conclusion can be drawn on the deposition of dietary FAs in this body region. Nevertheless, the FA signature of the whole blubber core at the dorso-lateral lumbar region has been shown to be similar to the FA signature of the whole blubber core at the ventral thoracic region (considered a fat depot, see Chapter 5) for 18 by-caught NZ sea lions (Donaldson *et al*. unpubl. data). Therefore, part of dietary FAs must be deposited in the dorso-lateral lumbar region when the animal is in positive energy balance.

QFASA requires information on the FA profiles and fat content of all potential prey and an understanding on the differential deposition of FAs in the predator's adipose tissue (Iverson *et al*. 2004). The list of species present in the prey library is not fully inclusive of all potential prey that sea lions can eat but covers the most common and important prey found in stomach contents of NZ sea lions (Chapter 4), and the most abundant species in the Auckland Islands area (Jacob *et al.* 1998; O'Driscoll *et al.* 2003). Concerning the lipid metabolism and deposition in the predator's tissue, the QFASA model takes into account that prey FA profiles do not match perfectly that of the predator by applying CCs for each FA (Iverson *et al*. 2004). Although having similar trends, CCs calculated from feeding experiments on captive animals vary with the animal taxon or species, and the diet they are fed (Iverson *et al.* 2004; Iverson *et al.* 2006; Tollit *et al.* 2006; Iverson *et al.* 2007; D. Tollit unpubl. data). The CC set chosen for this study (Steller sea lions fed on pacific herring; D. Tollit unpubl. data) gave the best goodness of fit for the model among the CC sets available (Chapter 5), but it is not known how close this optimal CC set accounts for the metabolism occurring in adult NZ sea lions. However, the diet of by-caught NZ sea lions as estimated by QFASA was comparable with data from stomach contents (Chapter 5), suggesting that the CCs do give appropriate estimates.

### 6.4.2 Foraging diversity are not reflected in FA profiles

Foraging diversity within a species is generally driven by factors such as sex and size dimorphism, body development, breeding behaviour, energy requirements, foraging experience, and reduction of resource competition (Schoener 1986; Bolnick *et al.* 2003).

Numerous examples are documented in the literature for pinniped species (*e.g.*, variation between sexes: Campagna *et al.* 2001; Raum-Suryan *et al.* 2004; Sterling and Ream 2004; Page *et al.* 2005; Kirkwood *et al.* 2006; Staniland and Robinson 2008; variation between age groups: Baylis *et al.* 2005; Field *et al.* 2005; Field *et al.* 2007; Fowler *et al.* 2007; Spence-Bailey *et al.* 2007).

Niche specialisation can also occur at a narrower scale within the same sex or age group, and has been shown to take place among otariids for lactating Antarctic fur seals (Lea *et al.* 2002b; Staniland *et al.* 2007), female Northern fur seals (Goebel *et al.* 1991; Zeppelin and Ream 2006), and recently for lactating NZ sea lions (Chilvers and Wilkinson 2009). Lactating NZ sea lions exploit diverse areas of the Auckland Islands shelf by taking different directions from the colony during their foraging trips (Chilvers and Wilkinson 2009). Some females forage northeast from the colony and dive at regular depths on the Auckland Islands shelf, mostly at 100-150 m (benthic divers). Others forage north/northwest from the colony and dive at variable depths on the edge of the shelf, mostly at depths > 200 m followed by dives at 50 m (mesopelagic divers; **Fig. 6-1**). The diet between the types of divers was expected to be different because the communities of prey found on the shelf and at the shelf edge would vary, but this hypothesis was not supported in this study. Indeed, no difference was noted between the blubber FA profiles of benthic and mesopelagic divers (**Fig. 6-3**) and the diet estimated by QFASA for both diving types was dominated by rattail and hoki, two species abundant at depths > 200 m. Several reasons can lead to this result. First, the number of benthic and mesopelagic divers per year is limited to few individuals, which may not be enough to detect differences in FA profiles. Second, some individuals may have been misclassified in the wrong type of diver if they don't show the same dive pattern every year. Indeed, blubber FAs were not analysed necessarily the same year as the diving study for an animal. Hence, it was hypothesised that the diving pattern of a female for a particular year was consistent during its whole life based on the high land-site and foraging-site fidelity displayed by lactating NZ sea lions (Chilvers *et al.* 2005; Chilvers 2008a). Third, lactating females make most of their diving efforts in different habitats (shelf *versus* shelf edge) but most of the energy required during a foraging trip may be provided at the shelf edge whatever the diving types. Indeed, if benthic divers harvest more prey per dive, larger prey and/or prey with a higher lipid content at the edge than they do on the shelf, the FAs of prey found at the edge will mainly contribute to the FA pool of sea lion blubber. This is likely to be true as the diet estimated by QFASA

consisted mainly of rattail, hoki, arrow squid and red cod (**Table 6-3**), the first three species being found in greater abundance at the edge than on the shelf (Jackson *et al.* 2000; Beentjes *et al.* 2002; MinistryFisheries 2007) and at a larger size than shelf species (Chapter 2). Furthermore, Chilvers & Wilkinson (2009) showed that benthic divers have more energy-demanding and longer foraging trips than mesopelagic divers do, whereas the present results suggest that benthic and mesopelagic divers have similar long-term energy intakes. Therefore, the foraging strategy displayed by benthic divers does not seem compensated by greater energy income. It might have been an advantageous strategy in the past, which persists across generations because of a strong site fidelity and philopatry in females and a possible vertical transmission of foraging tactics from NZ sea lion mother to pups (Chilvers 2008a; Chilvers and Wilkinson 2009). However, no historical records on prey availability exist to support this hypothesis.

### 6.4.3 Differences between biopsied and by-caught females

There were significant differences between the FA profiles of samples analysed in this study (biopsied blubber) and blubber from by-caught female NZ sea lions analysed previously (Chapter 5). These differences consisted mainly of lower proportions by mass of the 22 carbon-chain FAs 22:1n-9, 22:5n-3 and 22:6n-3 (**Fig. 6-4**) in the biopsied samples. QFASA on by-caught females estimated that arrow squid was the most important prey (median % mass of 28, Chapter 5), whereas the median % mass of arrow squid in this study was only 4% (**Table 6-3**). Before the results can be interpreted as a variation in diet, one must investigate whether these differences may be due to variation in FA metabolism and deposition between the sample sets. First, the blubber from by-caught animals was sampled from the thorax whereas biopsied samples were from the dorso-lateral lumbar region, but FA profiles of blubber cores sampled at these two sites do not differ significantly (from 18 individuals; Donaldson *et al.* unpubl. data). Second, females from the two sample sets may be in different body condition thus not depositing FAs at the same rate. This hypothesis is also rejected as the body condition calculated for lactating females in both sample sets is similar. Hence, diet must play a significant role in the differences in FA profiles between the two sample sets. To fully interpret these diet variations, one must understand the dietary time frame represented by the blubber of each sample set. Female NZ sea lions arrive at the breeding sites in

December, and pup soon after their arrival (Chilvers *et al.* 2007b). The peak of parturition for female NZ sea lions is at the end of December after which females fast, nursing their pup for approximately 9 days (perinatal period) before going at sea for their first foraging trip of the lactation period (Chilvers *et al.* 2007a). Energetic studies on Galapagos fur seals *Arctocephalus galapagoensis* and Antarctic fur seals *A. gazella* during the perinatal period revealed that females lost only 1.68% and 3.15% respectively of their initial mass after a seven day fast (Costa and Trillmich 1988). Although similar studies are not available for the NZ sea lion, it is likely that lactating NZ sea lions do not deplete all their energy stores during their perinatal fast of nine days; therefore the blubber collected at the end of January in this study would not represent only dietary FAs stored from the first foraging trip to the time of sampling, but also dietary FAs from the foraging period before parturition. The squid fishery starts in February of each year. By-caught females for which blubber was analysed were retrieved from squid nets between end of February and May (Chapters 3), thus blubber FAs from by-caught females would reflect the diet over a period slightly later in the lactation period. Therefore, the comparison between the blubber analysed in this study and the blubber from by-caught females suggests that LFs rely primarily on deep sea fish before the breeding season and during the first weeks of the lactation period, and switch to a mixed diet of squid and fish later in the lactation period, probably taking advantage of a greater abundance of arrow squid on the slope at the end of the summer (February; M. Cawthorn pers. comm.).

### 6.4.4 Between-year variation in FA profiles and long-term diet

Females captured in January 2002 and 2003 showed differences in their FA profiles with the other years of capture, mostly due to a greater proportion of monounsaturated 18 carbon chain FAs (**Fig. 6-2**, **Table 6-2**). The year 2005 also displayed some variation with the other years, mostly driven by a higher percentage of 16:3n-4 (**Fig. 6-2**, **Table 6-2**). Body condition of females was similar between the years 2002, 2003 and 2005 (K-W test, $P > 0.05$). Although the body condition in the years 2000 and 2001 is not known, the fact that FA differences exist between females with similar body condition (years 2002 and 2003 different from year 2005) suggest that differential metabolism have little influence relative to diet on the FA variation observed between years. Differences in FA profiles resulted in different diet estimates by QFASA (**Fig. 6-5**),

with a greater proportion of arrow squid and a smaller proportion of rattails for 2005, and a smaller proportion of arrow squid for 2002 and 2003. Temporal variation in the diet of NZ sea lions has been generally attributed to a change in prey availability (Lalas 1997; McMahon *et al.* 1999; Childerhouse *et al.* 2001; Chapter 2). The only information on the abundance of marine species in the NZ sub-Antarctics is from commercial catches of arrow squid and hoki (Ministry of Fisheries 2007). The year 2005 was considered a good harvest by the squid fishery in the Auckland Islands area whereas low catches were recorded in the years before 2004 (Ministry of Fisheries 2007). Despite the fact that blubber analysed reflect the diet over a period prior to the start of the fishery, the present diet estimates (greater proportion of arrow squid in the diet of the year 2005) are consistent with the main trends of arrow squid catches.

## 6.5 CONCLUSION

In this study, QFASA has been used to assess the diet of lactating NZ sea lions captured in the first week of the lactation period between 2000 and 2003 and in the year 2005. Lactating NZ sea lions seem to rely primarily on deep-sea fish such as rattails and hoki before parturition and during the first weeks of lactation, whatever the area of the Auckland Islands shelf they exploit. Females displaying a benthic diving pattern go further from the colony when foraging, and spend more energy per foraging trip than do females displaying a mesopelagic pattern (Chilvers and Wilkinson 2009). The present study did not support the hypothesis of feeding specialisations, and benthic divers do not appear to benefit from a more energy-rich diet. Previous foraging studies have identified the slopes of the Auckland Islands shelf as important foraging areas for lactating NZ sea lions (Chilvers *et al.* 2005; Chilvers and Wilkinson 2009). The present study suggests that most of the energy intake during a foraging trip occurs on the slopes where large fish gather in abundance at depths > 200 m (Beentjes *et al.* 2002; Ministry of Fisheries 2007). Comparison of diet estimates from QFASA between the females studied herein and females by-caught in the squid fishery later in the lactation period (autumn, Chapter 5) suggest that females change their fish diet to a more squid-based diet in austral autumn when arrow squids gather in the area (M. Cawthorn pers. comm.). This study also showed a variation in FA profiles between years, due mostly to a variation in arrow squid contribution to the diet estimated by QFASA. Although QFASA is at its early development and uncertainties remain on the reliability of CCs

used, estimates of arrow squid proportions in the diet followed the same trend as the reported arrow squid catches since 2000 (Ministry of Fisheries 2007).

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# 7 CHAPTER 7

# FEEDING ECOLOGY OF THE NEW ZEALAND SEA LION: GENERAL DISCUSSION

**Photo:** Group of New Zealand sea lions (*Phocarctos hookeri*) at Sandy Bay, Enderby Island, Auckland Islands. In front, a young male.

Adequate nutrition is essential to the sustainability of any species as it underpins key elements of population ecology such as reproductive success and recruitment. For those species that have evolved to live in marginal habitats, or now find themselves in such habitat due to environmental degradation, range restriction, or competition, diet is even more critical. The New Zealand (NZ) sea lion is one such species, with its breeding range restricted to the NZ sub-Antarctic. Knowledge of its feeding behaviour and nutritional requirements were, until now, poorly understood. This thesis makes a significant advance on the understanding of NZ sea lion feeding ecology, particularly for adult females in the critical early lactation period.

The diet of this species was investigated using stomach content analysis, qualitative fatty acid (FA) signature analysis, and quantitative FA signature analysis (QFASA) from the years 1997 to 2006. In Chapters 2, 3 and 5, I examined the short-term diet (stomach contents) and the long-term diet (FAs from blubber) of NZ sea lions incidentally caught in the southern arrow squid fishery around the Auckland Islands (50°30'S, 169°E). In Chapter 6, the blubber FAs of free-ranging lactating NZ sea lions captured at Enderby Island, Auckland Islands, were examined. The purpose of this general discussion is to integrate the main research outcomes of my dissertation, and place them in the context of the conservation management of the NZ sea lion.

**Limits of the dietary methods**

The literature review on dietary methods in Chapter 1 highlighted the advantages and disadvantages of the stomach content and FA analyses, which were the methods used in this study. Stomachs were collected from NZ sea lions caught incidentally in squid trawl nets (Chapter 2), therefore the arrow squid targeted by the fishery was believed to be over-represented in the stomach contents. To minimise the overestimation of arrow squid, I distinguished the fresh fraction (ingested shortly prior the death; 79% by mass of arrow squid) from the digested fraction (ingested from one to several days before the death; 18% by mass of arrow squid) in the stomach contents, and I considered only the digested fraction in the interpretation of the results. Although this fraction is likely to better represent the "background" diet of NZ sea lions over a foraging trip than the total stomach content, there is still a possible overestimation of the cephalopod component of the diet due to the accumulation of cephalopod beaks in the stomachs (Bigg and Fawcett 1985; Yonezaki *et al.* 2003), and a possible underestimation of species with fragile hard parts (Dellinger and Trillmich 1988; Pierce and Boyle 1991).

A significant advance in research on diets of free living marine mammals over the past decade was the development of techniques to estimate the proportions of ingested prey from FAs in adipose tissue (Iverson *et al.* 2004). I adopted this methodology to the analysis of NZ sea lion blubber in order to overcome the biases related to differential prey retention and digestion rates encountered in stomach analysis. Another advantage of FA analysis over traditional methods is that FAs in blubber have the potential to reflect the diet over ecologically significant periods (*i.e.*, weeks to months) (*e.g.*, Kirsch *et al.* 1998; Kirsch *et al.* 2000). Nevertheless, inferring diet variation between individuals from FA analysis is limited when individuals vary in body conditions and/or energetic expenditure, leading to differential rates of metabolism of ingested FAs and deposition into the adipose tissue. For instance, the FA variation observed between sexes in Chapter 3 is likely to originate from a combination of different diets and/or from different rates of FA metabolism. Therefore, at present, it is difficult to draw any conclusion on the dietary differences between male and female sea lions based on blubber FA profiles alone as long as foraging and diving data are not available for both sexes. To date, lactating females are the only segment of the population for which

foraging and diving behaviours are known (Gales and Mattlin 1997; Costa and Gales 2000; Chilvers *et al.* 2005, 2006).

QFASA requires the application of calibration coefficients (CCs) to account for the FA metabolism in the blubber of the predator. Yet, these coefficients are not available for the NZ sea lions as they require controlled feeding trails on captive animals and no NZ sea lions are in captivity. To date, the Steller sea lion *Eumetopias jubatus* is the only otariid species for which CCs have been calculated from feeding experiments (D.Tollit, unpubl. data). In Chapter 5, QFASA was tested on by-caught NZ sea lions using different CCs from Steller sea lions (D.Tollit, unpubl. data), and from phocid species (Iverson *et al.* 2004). This study showed the high sensitivity of QFASA for CCs and highlighted the importance of suitable CCs in the estimation of the diet. This was also stressed in a study on harbour seals, during feeding experiments in which the authors compared QFASA diet estimates produced with CCs from different species of phocids (Nordstrom *et al.* 2008). Despite the uncertainty of the accuracy of the match between the optimal CCs I used (from Steller sea lions fed on herring) and the true FA metabolism of NZ sea lions, the diet estimations by QFASA (mainly arrow squid, rattails and hoki; Chapter 5) are consistent with previous knowledge on the diet estimated from scat and stomach methods (Childerhouse *et al.* 2001; Chapter 2), foraging behaviour (Chilvers *et al.* 2005) and diving pattern (Chilvers *et al.* 2006) of this species. Moreover, the diet variation estimated by QFASA between years was consistent with the trends of the commercial catches of hoki and arrow squid since 2000 (Chapters 5 and 6), giving greater confidence in the diet predictions presented in this thesis.

QFASA is at its early stages of development and a better understanding of the factors affecting lipid metabolism for otariids is clearly necessary in the future. To my knowledge, published CCs on pinnipeds are restricted to two species of phocids and one species of otariid feeding on a pure herring diet (Iverson et al. 2004; Tollit et al. 2006). It is only recently that the effect of different meals on the blubber FA composition has been studied (D. Tollit, unpubl. data). The different sets of CCs calculated on Steller sea lions feeding different meals gave drastic differences in diet estimates (Chapter 5), highlighting the importance of CCs in the reliability of QFASA. Moreover, before applying QFASA on blubber FA profiles, one must consider that variation in FA

profiles between individuals of different sex, age or nutritional status may come from both different diets and different rates of FA metabolism. Therefore, at the present state of knowledge, I believe that the reliability of "qualitative" and "quantitative" FA analyses to detect dietary differences between groups of individuals ("qualitative"), or to estimate prey contributions in the diet ("quantitative"), must be tested by the comparison with diet estimates from other dietary methods.

**Feeding ecology**

Data obtained from both stomach analysis and QFASA indicate that arrow squid, rattails, hoki and red cod are key prey species for both by-caught NZ sea lions (Chapters 2 and 5), and biopsied NZ sea lions (Chapter 6). However, the order of prey contribution by mass varied according to the method used and the origin of the samples. For the by-caught sea lions, the most common prey found in stomach contents were arrow squid (17.9% by mass M), rattail (2.4%M), red cod (4.3%M), opalfish (4.7%M) and octopus (27.8%M) (Chapter 2). This method showed only a weak variation between sexes and maturity stages, with the only significant difference being a greater contribution of opalfish in the stomach contents of females. The ontogenetic diet variation is probably underestimated due to the nature of the sample set (absence of territorial males and juveniles < 3 years of age) and the fact that stomach contents give a diet picture limited to the last foraging trip (Chapter 2). An important finding in Chapter 2 is that by-caught juveniles of 3-4 years old feed on similar prey to adults, implying that they are able to forage at the slopes of the shelf and at depths >200 m, despite their diving capacity not being fully developed.

I applied QFASA to the blubber samples from the same by-caught individuals, and estimated that arrow squid (28%M) and rattails (27%M) were the most important prey over the months prior to their death (*i.e.*, first half of the lactation period, Chapter 5). Females and males showed different FA profiles (Chapter 3), which resulted in different diets predicted by QFASA (higher contribution of hoki in females than in males, higher contribution of red cod in males than in females; Chapter 5). However, these predictions prevail only if most of the variation in FA profiles between sexes comes from a different diet, and not from differential rates of FA metabolism and deposition (see

limits of the methods above). Lactating and non-lactating females showed similar stomach contents (Chapter 2) and FA profiles (Chapter 3), suggesting a fidelity to foraging habits displayed by females from a year to the next regardless of whether they were rearing a pup or not.

Rattails (30%M) and hoki (26%M) were the most important prey estimated by QFASA for biopsied lactating females during the pre-breeding period and the first month of lactation (30%M and 26%M respectively, Chapter 6). The comparison of diet estimates between by-caught and biopsied females (Chapter 6) suggests that females change their deep sea fish-based diet to a more squid-based diet at the end of summer when arrow squid gather in the area (M. Cawthorn pers. comm.). Alternatively, another possibility is that female sea lions are still depending on a fish diet in autumn and some individuals specialise in pursuing squid in trawling nets.

FA profiles of by-caught and biopsied NZ sea lions showed some variation between the years from 2000 to 2006 (Chapters 3 and 6). Because sea lions had similar body condition between most of the years, I attributed the FA variation to annual variation in diet. NZ sea lions are generalist predators (Lalas 1997; McMahon *et al.* 1999; Childerhouse *et al.* 2001; Chapter 2), thus their diet is expected to follow the trends of prey stock availability. The similar dietary pattern between the two types of sampling (by-caught *versus* biopsied) was the higher contribution of arrow squid estimated in the diet of animals sampled in 2005 (or pooled years 2005-2006) relative to the previous years (Chapters 5 and 6). These findings are consistent with the trend shown by reported commercial catches of arrow squid from the Auckland Islands region (Ministry of Fisheries). A parallel study on the milk composition of the same NZ sea lion population between the years 1997 and 2005 reported high lipid and low protein contents of the milk sampled in 2002 and 2003 relative to the other years, and explained this pattern by an underfeeding of females with good initial body condition during the first month of lactation (Riet-Sapriza 2007). Furthermore, low lipid and protein contents of the milk collected in 2001 were attributed to underfeeding of females in poor body condition during the first month of lactation. Therefore, it is possible that a low biomass of arrow squid around the Auckland Islands in the years previous to 2004 has limited the production of a milk of good quality. It is worth mentioning that arrow squid contains the highest values of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) among the common prey of the NZ sea lion (> 20% of total FAs; Chapter 4), and that

these FAs are associated with health benefits such as the prevention of autoimmune diseases (Sidhu 2003). Milk with high concentrations of EPA and DHA would likely benefit the pup and may increase its chance of survival in the face of infectious diseases such as the ones occurring in the population in 2002 and 2003 (Castinel *et al.* 2007a; Castinel *et al.* 2007b).

**Importance of the edges of the Auckland Islands shelf**

It has been suggested that the low population number of the NZ sea lion (approximately 12000; Campbell *et al.* 2006) and its low reproductive performance originate from unfavourable foraging conditions occurring on the Auckland Islands shelf, where abundant and/or predictable resources would be far from the rookeries and deep (Gales and Mattlin 1997; Costa and Gales 2000; Chilvers *et al.* 2005, 2006). My principal findings on the short-term and long-term diet of NZ sea lions during the first months of the lactation period (Chapters 2 and 5) appear to support this hypothesis. I have shown that the NZ sea lions predominatly feed on arrow squid and deep-sea living fish such as rattails and hoki. The later are found at depths >200 m, thus are accessible only at the edges of the shelf (Beentjes *et al.* 2002; Ministry of Fisheries 2007). A previous foraging study on lactating NZ sea lions highlighted the importance of the edges of the Auckland Islands shelf during foraging trips of females during the first month of lactation (Chilvers *et al.* 2005). My results suggest that the slopes are visited regularly not only during the first month of lactation (Chapter 6), but later in the lactating period (Chapter 5). Given the high fidelity for foraging grounds displayed by female NZ sea lions (Chilvers 2008a), it is likely that the edges of the Auckland Islands shelf remain key foraging locations until the end of the lactation period. Though these key areas are far from the rookeries and impacted by the squid fishery, they may provide the only predictable and abundant food resource required to cover the cost of lactation.

**Nutritional stress hypothesis and energetics**

In addition to the costs of exploiting a resource far from the colony and deep, the energetic income from the prey must be considered. Indeed, a low energetic diet might cause a nutritional stress, potentially causing an adverse impact on the reproductive performance of sea lions (Trites and Donnelly 2003). A similar hypothesis has been

proposed to explain the decline of the western stock of Steller sea lions, which feeds primarily on gadids, a group of prey considered to be of low energy density (Rosen and Trites 1999; Rosen and Trites 2000; Donnelly *et al.* 2003; Trites and Donnelly 2003; Winship and Trites 2003; Rosen and Trites 2005). The calorific values of gadids (3 to 8 kJ $g^{-1}$, Fritz and Hinckley 2005) are in the same range as those of NZ sea lions' main prey (5 to 8 kJ $g^{-1}$, Chapter 4). Therefore it is quite possible that the energy obtained through prey consumption does not meet the energetic demands of reproduction and lactation in NZ sea lions, causing a low reproductive rate and a low fat content in the milk relative to other otariid species (Riet-Sapriza 2007; Chilvers 2008b). However, the nutritional stress hypothesis has been criticised (see critical review in Fritz and Hinckley 2005; Atkinson *et al.* 2008) as it is difficult to make a direct link between energy of prey and the well-being of a population, without better knowledge of the physiological processes governing the assimilation of nutrients into marine mammal tissues. Furthermore, low prey energy density alone is not sufficient to cause nutritional stress if total prey intake is adequate to meet energetic needs (Donnelly *et al.* 2003; Rosen and Trites 2005). In fact, otariid populations relying on low energy prey can do fine. For instance, the population of southern sea lions *Otaria flavescens* in Patagonia is increasing (Dans *et al.* 2004) but the females prey on the lean prey octopus *Enteroctopus magalocyathus* and squid *Illex argentinus* (Werner and Campagna 1995; Koen Alonso *et al.* 2000), and the sea lion population in this area is increasing (Dans *et al.* 2004). In contrast to NZ sea lions however, the costs of foraging are lower for southern sea lions which dive to maximum depths of 30 m on the shallow Patagonian shelf (Werner and Campagna 1995; Campagna *et al.* 2001). Among the otariids displaying a benthic foraging behaviour such as Australian sea lion *Neophoca cinerea* (Costa and Gales 2003), Australian fur seal *Arctocephalus pusillus doriferus* (Arnould and Hindell 2001), southern sea lion (Werner and Campagna 1995), northern fur seal *Callorhinus ursinus* (Goebel *et al.* 1991) and NZ sea lion (Gales and Mattlin 1997; Chilvers *et al.* 2006), the NZ sea lion is the only species for which regular dives deeper than 100 m have been recorded during the foraging trips of lactating females on the shelf (Gales and Mattlin 1997; Chilvers *et al.* 2006). A preliminary study on the diving physiology of the NZ sea lion showed that despite their deep and long-duration diving ability, they do not have a greater diving capacity than other otariids thus are presumably foraging near their physiological maximum (Costa and Gales 2000). Therefore, it is probable that the reliance on low energy prey distributed in deep waters

adversely affects the reproductive performance of lactating NZ sea lions. More detailed studies on nutrient quality and sea lion physiology will be required to test this hypothesis further.

To test the hypothesis that the total prey intake during a foraging trip may not be sufficient to cover energetic needs due to the low energy contents of the prey, I calculated the daily food requirement of a lactating sea lion by using a simple bioenergetic model derived from Kleiber's predictive equation of energy requirements (Kleiber 1975), where the basal metabolism *BM* (kg $d^{-1}$) is defined as:

$$BM = 292.88 \times M^{0.75}$$

with 292.88 in kJ $d^{-1}$; and *M*, mass of the animal in kg. Here, I took an average mass of 110 kg which represent the most common mass class of lactating NZ sea lions (Childerhouse 2008). By accounting for the different energy contents of the prey and their proportions in the diet, the daily food requirement *FR* (kg $d^{-1}$) of a lactating NZ sea lion is as follows:

$$FR = \frac{5.8 \times BM}{\sum (0.95 \times P_i \times E_i)}$$

With $5.8 \times BM$, activity metabolic rate calculated as 5.8 times the basal metabolism for lactating NZ sea lions (Costa and Gales 2000); 0.95, mean assimilation efficiency from feeding experiments on monk seals *Monachus schauinslandi* and harp seals *Phoca groenlandica* (Lawson *et al.* 1997; Goodman-Lowe *et al.* 1999); $P_i$, proportion by mass of prey *i* in the diet; and $E_i$, energy content of prey *i* (kJ $kg^{-1}$ wet mass). The daily food requirement of a lactating female was estimated to be 25 kg $d^{-1}$ or 23% of its body mass with the prey proportions predicted by the stomach analysis, 24 kg $d^{-1}$ or 21% of its body mass with the prey proportions predicted by QFASA on by-caught lactating females, and 23 kg $d^{-1}$ or 21% of its body mass with the prey proportions predicted by QFASA on biopsied lactating females. These values are likely to be underestimated since females need to obtain more energy than required to sustain the fast on land when nursing their pup (1-2 days, Chilvers *et al.* 2005). Fiscus and Baines (1966) suggested that the maximum stomach capacity of a two-year old Steller sea lion was approximately 10% of its body mass. Therefore, a lactating NZ sea lion would theoretically need to fill its stomach three times daily to meet its food requirement. Based on the passage times of prey items through the gut of Steller sea lions (*e.g.*, Tollit

*et al.* 2003), it is likely that most of the prey are digested or passed through the intestine within 6 hours. Thus, lactating females would theoretically "have time" to meet their food requirements. However, the food requirements of lactating NZ sea lions calculated herein are well above the estimations for other sea lion species (approximately 10-11% body mass for Australian and California sea lions *Zalophus californianus*; Costa 1991; Winship *et al.* 2006), and are at the higher limit of the estimated food requirements for the declining population of Steller sea lions in western Alaska (Winship and Trites 2003). Consequently, the food requirements from low energy prey such as the ones available around the Auckland Islands may be at such a level that lactating females have to strike a delicate balance between meeting their energetic needs and returning to the rookery on time to nurse their pup. Nevertheless, lactating NZ sea lions captured twice in a month interval during the first months of the lactation period, had stabilised or were gaining mass (B.L. Chilvers unpubl. data), hence were in positive energy balance. If the energy of prey does not appear to be the cause of the low reproductive performance of female NZ sea lions, the quality of prey might be a factor. It has been mentioned earlier in this discussion that arrow squid has high levels of FAs necessary for important health functions. It is now necessary to assess other nutrients such as amino acids and minerals in order to further investigate the nutritional stress hypothesis in this species.

**Interactions with the arrow squid fishery and potential resource competition**

The aim of the conservation management strategy for the NZ sea lion is the reduction of fisheries by-catch, and is currently undertaken in three ways: maintaining the no-fishing zone of 12 mile surrounding the Auckland Islands, the setting of an annual by-catch limit, and the use of sea lion exclusion devices (SLEDs) in trawling nets (Wilkinson *et al.* 2003; Chilvers 2008b). Despite these management controls, the number of sea lions estimated to be caught varied from 14 to 118 every year (Chilvers 2008b). Lactating females from Enderby Island (Auckland Islands) travel north or north east to the edge of the shelf, where part of the fishery occurs (see Fig 6-1, Chilvers *et al.* 2005; Chilvers 2008b). Therefore, there is a high overlap in the regions harvested by sea lions and the fishery, which can lead to potential competition for arrow squid. To assess the resource competition, one must know the abundance of arrow squid available in the area. Unfortunately, no biomass estimate is available for arrow squid (Ministry of Fisheries 2007). However, a comparison of the quantity harvested by the lactating NZ sea lions

and the quantity fished at the Auckland Islands can give an indication of the impact of NZ sea lions on the squid stock. I re-used the bioenergetic equation presented earlier to estimate the daily requirement of arrow squid by lactating females by-caught in the squid fishery. I found a daily requirement of 7 kg $d^{-1}$ based on diet predictions from stomach contents, or 8 kg $d^{-1}$ based on diet predictions from QFASA. The arrow squid requirement $R_{AS}$ (kg per season) of all lactating females during a fishing season was calculated as:

$$R_{AS} = FR_{AS} \times nb\,inds \times nb\,days$$

with $FR_{AS}$, the daily requirement of arrow squid *AS*; *nb inds*, the number of individuals; and *nb days*, the number of days during which sea lions are foraging. The number of adult NZ sea lions was estimated at 7000 (Wilkinson *et al.* 2003). Approximately 67% of females are lactating (Childerhouse 2008), and assuming a sex ratio of 1:1, the number of lactating NZ sea lions is 2345. The fishing season is approximately 120 days (from the 1st of February to the 1st of June). Lactating females spend approximately two thirds of their time foraging at sea during lactation, thus the number of foraging days was set at 80. This gives an estimation of 1300 t to 1500 t of arrow squid required by lactating NZ sea lions during a fishing season, and equals 10 to 22% of the annual catches of arrow squid in the sub-Antarctic in 2002 and 2003, and up to 46% of the annual catches of arrow squid in the sub-Antarctic in 2001. Based on a study of milk quality in these sea lions, it was hypothesised that the population faced a food shortage in the summers 2001 and 2002. To the substantial amount of arrow squid required by lactating females, one must add the food requirements of males and juveniles of 3-4 years old, which also forage at the edges (Chapter 2). Therefore, the entire NZ sea lion population may require similar amounts of arrow squid as the amount harvested by the fishery in some years, suggesting that resource competition exists during years of low arrow squid recruitment.

**Future research on NZ sea lions**

- The food requirement equations used in this chapter were made simplistic because my aim was to estimate the approximate food requirements of sea lions. A more complicated energetic model has been developed for Steller sea lions (Winship *et al.* 2002) and could be adapted to NZ sea lions. Mass distribution of the population instead of an average mass, activity metabolic rates for males and

juveniles, and variable assimilation efficiencies depending on the lipid content of prey are examples of factors which could be added in the model.

- The examination of the nutritional quality of the main prey species (*i.e.*, amino acids, minerals, vitamins) will allow further insight on the nutritional stress hypothesis on NZ sea lions.
- Most of the research on the NZ sea lions at the Auckland Islands has focused on lactating females in summer (Gales and Mattlin 1997; Costa and Gales 2000; Chilvers *et al.* 2005, 2006) because females are the most critical component in the population and because of logistic restrictions (*i.e.*, remote locations difficult to access in winter). However, in terms of species management, it becomes crucial to understand the foraging and feeding behaviour of other components of the population (males and juveniles), and of lactating females in winter and spring at the end of the lactation period. Males and juveniles are the focus of a current foraging study at the Auckland Islands (B.L. Chilvers, pers. comm.). I strongly recommend sampling blubber from males and juveniles in the future in order to use QFASA on these animals.
- The collection of feeding information in winter and spring seems limited in the near future due to the high cost of sending a team to the Auckland Islands in winter. Nevertheless, stable isotope analysis can provide a means of inferring historic diets over months or years (Dalerum and Angerbjörn 2005) thus it may be possible to use this method to obtain diet information in winter. For instance, stable isotopes of hair collected from females captured in January would give an isotopic signature since the previous moult at the end of summer. The comparison of isotopic signatures between hair and another tissue with a turnover of several weeks only (*e.g.*, red blood cells) will allow any dietary changes between the summer (breeding season) and the months preceding the breeding season to be assessed.
- QFASA used a limited prey FA library (Chapter 4) and CCs from Steller sea lions. First, the prey FA library needs to be expanded to other potential prey species which are abundant in the Auckland Islands shelf, and the number of individuals within each prey species needs to be increased especially for species with a high FA variability such as opalfish and octopus. Secondly, better CCs for NZ sea lions could be obtained by carrying out feeding experiments on

otariids fed on species similar to NZ sea lions' prey in their lipid and FA compositions. Since it is unrealistic to keep a NZ sea lion in captivity due to the current critical status of the population, it is possible to use another species of sea lion, such as California sea lions held captive in several zoos in NZ.

**General conclusions**

- The diet of NZ sea lions was investigated using stomach content analysis, qualitative FA analysis, and quantitative FA signature analysis (QFASA) from the years 1997 to 2006. Stomach and FA analysis are complementary methods, both with advantages and disadvantages, and gave comparable results even if they infer the diet of NZ sea lions over different temporal scales.
- Data obtained from both stomach analysis and QFASA indicate that arrow squid, rattails, hoki and red cod are key prey species for NZ sea lions. My results suggest that the slopes of the Auckland Islands shelf are visited regularly not only during the first month of lactation, but later in the lactating period. Given the high fidelity for foraging grounds displayed by female NZ sea lions, it is likely that the edges of the Auckland Islands shelf will remain key foraging locations until the end of the lactation period.
- Variation in diet between sexes is likely to occur but the degree of segregation could not be assessed in my study due to the unknown role of different rates of metabolism in the FA variation between males and females. By-caught juveniles of 3-4 years old feed on similar prey as adults, implying that they are able to forage at the slopes of the shelf and at depths >200 m, despite having a diving capacity not fully developed. Lactating and non-lactating females showed similar stomach contents and FA profiles, suggesting a fidelity to foraging habits displayed by females from a year to the next whether they rear a pup or not. Finally, the comparison of diet estimates between by-caught and biopsied females suggests that females change from a deep sea fish-based to a more squid-based diet at the end of summer when arrow squid gather in the area.
- FA profiles of NZ sea lions showed some variation between the years from 2000 to 2006, with mostly a higher proportion of arrow squid in the diet of sea lions sampled in 2005 and 2006. The daily food requirement of a lactating female was estimated to be > 20% of its body mass. During years of low arrow squid

recruitment such as 1999 and 2001, the amounts of arrow squid required by the NZ sea lion population may have been similar to the amount harvested by the fishery, suggesting that resource competition occur between the arrow squid fishery and NZ sea lions in years of low arrow squid recruitment.

# APPENDIX 1

# Analysis of fatty acids and gas chromatography: development of the laboratory protocol

A recent review on the analysis of fatty acids (FAs) in marine ecosystems (Budge *et al.* 2006) stated that analysis of FA methyl esters (FAMEs) by gas chromatography is the most reliable and accessible method for routine quantification of FA composition. Gas chromatography allows the identification of a wide range of FAs (Christie 1989), which need to be converted first into a volatile derivative, usually methyl esters. Therefore, FA analysis commonly requires the following steps: (1) lipid extraction, (2) derivatisation (methylation), and (3) gas chromatography, which are developed in details in the following sections.

This appendix aims at describing and justifying the methodology chosen to analyse FAs in the "development of a protocol". The second section is the description of the protocol used during this project. Samples analysed here were either blubber from sea lions or prey (fish, cephalopod or crustacean). While the review on the methods involves different disciplines beyond the marine environment, the references generally referred to marine mammal tissues.

## DEVELOPMENT OF A PROTOCOL

### Lipid extraction

*Which method to choose?*

The first laboratory step in FA analysis is the extraction of lipids from animal tissue. By using a bipolar solvent system such as methanol:chloroform mixture, lipids are separated from other compounds. The most common extraction procedures are the Folch et al. (1957) technique and its simplified version, the Bligh and Dyer (1959) technique. Modified versions of these techniques were applied in numerous studies on marine mammals (*e.g.,* "Folch": Walton *et al.* 2000; Hooker *et al.* 2001; Staniland and

Pond 2004; Käkelä *et al.* 2005; "Bligh and Dyer": Iverson *et al.* 1997b; Brown *et al.* 1999; Lea *et al.* 2002a; Best *et al.* 2003).

Folch et al. (1957) first developed the chloroform:methanol:water phase system (8:4:3 v/v/v), which mixes polar (methanol) and non-polar (chloroform) solvents necessary to dissolve a wide range of lipids (and all the marine ones). First, lipids are extracted by homogenising the tissue with 2:1 chloroform:methanol (v:v) for a final dilution of 20-fold the volume of the tissue sample (*e.g.*, 1g of tissue diluted in 20 mL). Second, saline water is added to the system for a final ratio of 8:4:3 chloroform:methanol:water (v:v:v). A biphasic system is obtained with the lower phase containing most of the tissue lipids. Bligh and Dyer (1959) based their method on Folch et al. (1957) but used less solvent (1 part sample to 3 parts solvent instead of 20 for the Folch method). However, this method underestimates the lipid content of samples containing more than 2% of lipids and should not be used without modification for the quantification of lipids in marine animals (Iverson *et al.* 2001). In the present project, a modified version of the Folch method was used to analyse the samples.

*Oxidation of samples*

Lipids can oxidize in contact with air before storage and when frozen. Indeed, peroxidation of lipids occurred in some tissues at temperatures lower than –20ºC (Pizzocaro *et al.* 1980; Whiteley *et al.* 1992). Some of the blubber samples used in the present project were stored at –20ºC for up to 5 years before being processed. In addition, fish and squid specimens were homogenised at room temperature and freeze-dried before storage at –20ºC. Thus, the potential for oxidation occurring in these samples before analysis must be considered. To my knowledge, only one study on the effect of the blubber decomposition on FA composition has been reported (Learmonth 2006), in which there was little evidence of lipid oxidation after 566 days at –20ºC. It was hypothesised that this lack of oxidation was due to the presence of natural tissue antioxidants (Christie 2003). Also, the outer surface of the blubber samples, which oxidise more rapidly than the inside tissue, were removed before analysis in Learmonth (2006). Similarly in the present project, the tissue at the surface of the blubber sample was removed. However, the effect of long-term storage at –20ºC on FA composition of blubber and prey type tissues was not specifically tested here.

During the lipid analysis, butylated hydroxy toluene (BHT) was added to the chloroform:methanol 2:1 mixture to a percentage of 0.01% of the total solvent system. BHT acts as an antioxidant, does not interfere with the extraction process, and allows a safe storage of lipid extracts at –20ºC (Wren and Szczepanowska 1964; Christie 1989), before the FA methylation.

**Fatty acid methylation**

*Which method to choose?*

FAs are difficult to analyse by gas chromatography because of their poor peak shape and quantification. Their conversion to volatile derivatives is necessary to improve their solubility and thus ensuring better gas chromatographic peak shape and quantification (Christie 1989). Methyl esters are by far the favourite derivatives for gas chromatograph (GC) analysis of FAs (Christie 1993). The most common methylating catalysts are boron trifluoride ($BF_3$) and sulphuric acid ($H_2SO_4$). Heating is required for completion of the esterification reaction and FA methyl esters (FAMEs) are extracted with alkanes such as hexane, following by the injection into a GC. Although $BF_3$ and $H_2SO_4$ produce equivalent results (*e.g.*, Iverson *et al.* 1997a; Thiemann *et al.* 2004b), some laboratories experienced problems with the use of $BF_3$, such as moisture in the reagent avoiding complete esterification (Budge *et al.* 2006), and damage to the GC column (*e.g.*, Lepage and Roy 1984). Several precautions need to be applied with this reagent *i.e.*, the use of refrigerated fresh reagents only, and a low concentration in methanol to avoid its degradation (apparition of flakes, personal observation). Although it is now recommended to use its equivalent $H_2SO_4$ (Budge *et al.* 2006), $BF_3$ is still highly popular probably because it can be purchased from commercial suppliers and have a shelf life time of several months (Christie 1993), whereas $H_2SO_4$ needs to be prepared weekly (Budge *et al.* 2006). Another advantage of $BF_3$ is that it methylates both TAG and free FAs, these last ones resulting from the oxidation of the sample.

An alternative procedure for the formation of methyl esters is the direct transesterification, in which lipid extraction and methylation are done in one step. It is simpler and faster analysis, using less organic solvents, and showed to have comparable results to the traditional two-step procedure (Lepage and Roy 1984; Grahl-Nielsen and

Barnung 1985; Lui 1994; Guillou *et al.* 1996; Cantellops *et al.* 1999; Thiemann *et al.* 2004b; Indarti *et al.* 2005). It involves the treatment of a small tissue sample (< 25 mg) with generally a solution of HCl in methanol. Despite these advantages, its use in marine lipid studies is still limited. In marine mammal studies, only Grahl-Nielsen's group is using this technique routinely (*e.g.,* Andersen *et al.* 2004; Birkeland *et al.* 2005; Grahl-Nielsen *et al.* 2005). The fact that only a small sample of tissue can be used may be a problem for blubber, in which FA composition varies within the depth (Iverson 2002). Thus, a little sample cannot provide an accurate representation of the FA composition of the full depth or even a selected depth of the blubber core (Thiemann *et al.* 2004b). Direct esterification can still be appropriate for homogeneous samples such as milk and homogenised fish. However, it cannot give accurate estimates of the fat content of the prey types (Budge *et al.* 2006), which is needed in quantitative models of FA analysis (Iverson *et al.* 2004) and in nutritional studies of marine resources (*e.g.,* Nichols *et al.* 1994; Lea *et al.* 2002c; Phillips *et al.* 2002; Kitts *et al.* 2004)

The use of picolinyl derivatives, instead of methyl ester derivatives, has been used extensively to study the lipids of plants and marine invertebrates (*e.g.,* Plants: Van Boven *et al.* 2000; Wolff and Christie 2002; Tsydendambaev *et al.* 2004; marine invertebrates: Nechev *et al.* 2004; Kawashima 2005). However, their use in upper marine taxa is limited. They were recently subject to a discussion in Marine Mammal Science (Budge *et al.* 2007; Wetzel *et al.* 2007), following the review of Budge et al. (2006) on FA methods. Although this technique coupled with the use of a GC-MS has been shown to produce more accurate results than the more common methyl esters with a GC-FID (Wetzel and Reynolds III 2004), the separation of some monounsaturated FA isomers, important dietary tracers, is not easy, and not all laboratories have access to a GC-MS, which is more expensive than a GC-FID (Wetzel and Reynolds III 2004; Budge *et al.* 2007).

For this project, direct esterification was not suitable because 1) stratification occurs within the blubber of New Zealand sea lions (L. Donaldson et al., unpubl. data) thus a full blubber core needed to be analysed to study the long-term diet; 2) the fat content of the prey types needed to be accurately estimated for the application of quantitative

models. Moreover, the use of picolinyl esters was not considered, as there was no access to a GC-MS. Thus, FAs were methylated from a lipid extract and analysed through a GC-FID. $BF_3$ was the methylating reagent. It was purchased in small volumes at a time (100 mL) to use only a fresh reagent (less than one year old), and in low concentration (10% in methanol) to avoid degradation.

*Loss of fatty acids during methylation?*

Lipids are stored in the adipose tissues of marine mammals in different forms, the most common ones being triacylglycerols (TAGs) and wax esters (WEs) (Iverson 2002; Christie 2003). TAGs consist of three FAs esterified to a glycerol backbone. In the intestine of the predator, the FAs and the monoglycerol are released by the digestive enzymes, absorbed by the epithelial cells of the mucosa, and are re-formed into TAG (with the exception of carbon chain < 14). WEs consist of a FA esterified to a fatty alcohol and when digested, the FA is separated from the fatty alcohol. Then the fatty alcohol is oxidised to its corresponding FA (same number of carbons with same double bond positions). The resulting two FAs from the WE molecule are absorbed and formed into TAG (Budge *et al.* 2006). Thus, when analysing a prey containing a large amount of WEs, both the FAs and the fatty alcohols must be analysed to determine the FA composition of all the lipids ingested (Budge *et al.* 2006). However, the types of samples analysed in this project were blubber and prey type (fish, cephalopod or crustacean). TAGs make up the majority of the lipids in blubber (Iverson 2002; Christie 2003) and in the studied prey species (Vlieg and Body 1988). Consequently, the samples were not analysed for the fatty alcohols in WEs, believed to be in minor proportion in the lipid extracts analysed.

**Gas chromatograph analysis**

Methyl esters were analysed on a gas chromatograph GC-17A (Shimadzu Corporation, Kyoto, Japan) equipped with a flame ionisation detector (FID). It was connected to computerized integration system (CLASS-VP version 7.3, Shimadzu Scientific Instruments, Inc., Columbia, MD) to identify and quantify the FAMEs present in the samples. Samples were injected into two highly polar columns: a 50% cyanopropyl polysiloxane column (30 m × 0.25 mm i.d., 0.25 μm film thickness; DB-23 from J&W

Scientific Inc., U.S.A.), and a 70% cyanopropyl polysilphenylene-siloxane column (30 m × 0.25 mm i.d., 0.25 μm film thickness; BPX70 from SGE Analytical Science Pty Ltd, Australia). Blubbers from by-caught sea lions (Chapter 3) and some of prey type samples (Chapter 4) were injected through the DB-23 column whereas blubbers from the rest of prey type samples and biopsied females (Chapter 6) were injected through the BPX70 column. Results from control samples analysed on both columns were similar.

*Identification of FAMEs*

FAs were identified by comparing the retention times of FAMEs with authentic standards (NU-CHEK GLC standard 68D, SUPELCO 37 FAME mix, SUPELCO PUFA3 menhaden oil). The list of FAs present in each standard is shown in **Table A1-1**. Peaks not included in these standards were compared with the peaks of a well-documented sample, *i.e.,* cod liver oil (Christie 1989) (**Table A1-1**).

**Table A1-1.** Fatty acids (FAs) identified in different standards and in my experiments. SAFAs are saturated FAs, MUFAs are monounsaturated FAs, and PUFAs are polyunsaturated FAs. Identified FAs are noted by a star. The ones for which the identification is not certain are noted with a question mark.

| Fatty acids (FAs) | NU-CHEK 68D | SUPELCO 37 FAME Mix | SUPELCO PUFA 3 (menhaden) | Cod Liver Oil (Christie 1989) | My experiments |
|---|---|---|---|---|---|
| **SAFAs** | | | | | |
| 4:0 | | * | | | |
| 6:0 | | * | | | |
| 8:0 | | * | | | |
| 10:0 | | * | | | * |
| 11:0 | | * | | | |
| 12:0 | | * | | | * |
| 13:0 | | * | | | co-elute with BHT |
| 14:0 | * | * | * | * | * |
| 15:0 | | * | | * | * |
| 16:0 | * | * | * | * | * |
| 17:0 | | * | | * | * |
| 18:0 | * | * | * | * | * |
| 20:0 | * | * | | | ? |
| 21:0 | | * | | | |
| 22:0 | * | * | | | |
| 23:0 | | * | | | |

| | | | | | |
|---|---|---|---|---|---|
| 24:0 | * | * | | | ? co-elute with 24:1 |
| **MUFAs** | | | | | |
| 14:1n-5 | * | * | | * | * |
| 15:1 | | * | | | * |
| 16:1n-7 | * | * | * | * | * |
| 17:1 | | * | | * | ? |
| 18:1n-9 | * | * | * | * | * |
| 18:1n-7 | * | | | * | * |
| 18:1n-5 | | | | | * |
| 20:1n-11 | | * (20:1) | | * | * |
| 20:1n-9 | * | * (20:1) | * | * | * |
| 22:1n-11 | | | | * | * |
| 22:1n-9 | * | * | * | * | * |
| 24:1n-9 | * | * | | | ? co-elute with 24:0 |
| **PUFAs** | | | | | |
| 16:3n-4 | | | * | | * |
| 18:2n-6 | * | * | * | * | * |
| 18:3n-6 | * (18:3) | * | * | | ? |
| 18:3n-3 | * (18:3) | * | * | * | * |
| 18:4n-3 | | | * | * | * |
| 20:2n-6 | * | * | * | | * |
| 20:3n-6 | | * | * | | * |
| 20:3n-3 | * | * | * | | * |
| 20:4n-6 | * | * | * | * | * |
| 20:4n-3 | | | * | * | * |
| 20:5n-3 | * | * | * | * | * |
| 21:5n-3 | | | * | | * |
| 22:2n-6 | | * | | | ? |
| 22:4n-6 | | | | * | * |
| 22:5n-6 | | | | * | * |
| 22:5n-3 | | | * | * | * |
| 22:6n-3 | * | * | * | * | * |

Cod liver oil was used in every series of runs to determine accurate retention times (see example **Fig.A1-1**). The FA components were not verified by a GC-MS as this equipment was not available. However, even if doubts remain on the identification of peaks without information of their specific mass spectrum, it is not so much the absolute values of each blubber or prey FA that is important but the relative values between individuals. Because each sample is treated the same way, errors if they exist are consistent between individual FA compositions that I compared.

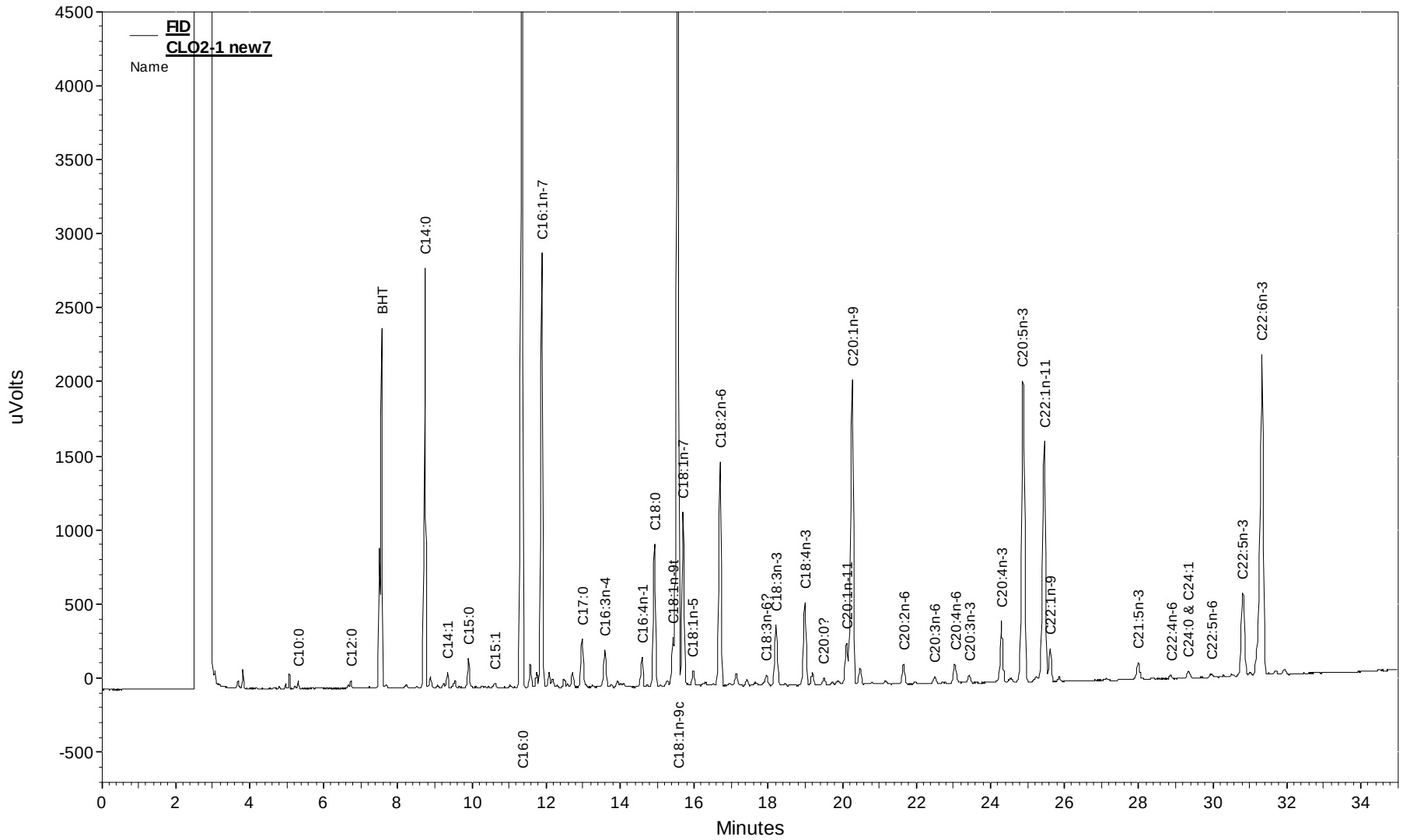



**Figure A1-1.** Chromatograph of cod liver oil FAMEs separated on a 50% cyanopropyl polysiloxane column (30 m × 0.25 mm; J&W DB-23, Folsom, California). The oven was maintained at 140ºC for 4min, then was raised at 25ºC/min to 190ºC, held for 5min, and was raised at 2ºC/min to 240ºC, where it remains for a further 4min (Total run = 40min). Helium was the carrier gas at a velocity of 20cm/s. The split ratio was 83:1.

*Validation of GC results*

Standard Nu-Chek 68D (Nu-Chek, U.S.A.) was run to validate the GC equipment and the conditions of injection such as the quality of the column, the quality of the manual injection, the oven's temperature programme and the interpretation of the chromatograph, before running the first samples. Nu-Chek provided the weight of each FA in percentage contribution of the total FA content (**Table A1-2**), which was compared to the % weight obtained with the GC, after taking into account FID correction factors (Ackman and Sipos 1964): Initially, Nu-Chek 68D was injected 10 times and the average FA composition was compared with actual values obtained by the manufacturer to calculate the calibration coefficients (CC, **Table A1-2**). Most of them equal one, *i.e.*, there was a good match between the actual % and what I found. The GC overestimated slightly the weight of the lightest FAs 14:0 and 16:0 (CC of 0.9) and underestimated slightly the weight of the heaviest FA 22:6n-3 (CC of 1.1). The variations to the actual values were reduced by changing the conditions of the injection (*e.g.*, way of injecting, volume injected, oven's temperature programme, carrier gas velocity), and the results shown in **Table A1-2** were with the optimum conditions. CCs were extrapolated to FAs not present in the standard but considered in my experiments: 0.9 was applied to all FAs that eluted between 14:0 and 16:0 (**Fig. A1-1**, **Table A1-2**), 1.1 was applied to FAs that eluted after 20:5n-3.

**Table A1-2.** Comparison of the standard Nu-Chek 68D between actual % FA weights provided by the manufacturer (Nu-Chek, U.S.A.) and % FA weights measured by the GC I used. My results were presented by the average (*x*) over 10 injections, and the confidence intervals (95%CI). CC are the calibration coefficients for each FA, which were multiplied to the % weights obtained from the GC.

| | ----------NU-CHEK 68D---------- | | | |
|---|---|---|---|---|
| Fatty acids (FAs) | Actual weights (%weight) | Weights from GC *x* (%weight) | 95%CI | CC |
| **SAFAs** | | | | |
| 14:0 | 6 | 6.7 | 6.6-6.8 | 0.9 |
| 15:0 | | | | 0.9 |
| 16:0 | 16 | 16.9 | 16.7-17.0 | 0.9 |
| 17:0 | | | | 1.0 |
| 18:0 | 8 | 8.0 | 8.0-8.1 | 1.0 |
| 20:0 | 1 | 1.0 | 0.9-1 | |
| 22.0 | 1 | 0.8 | 0.8-0.8 | |
| 24:0 | 1 | 0.8 | 0.7-0.8 | |
| **MUFAs** | | | | |
| 14:1n-5 | 1 | 1.1 | 1-1.1 | 0.9 |
| 15:1 | | | | 0.9 |
| 16:1n-7 | 5 | 5.3 | 5.2-5.3 | 1.0 |
| 18:1n-9 | 13 | 13.3 | 13.2-13.4 | 1.0 |
| 18:1n-7 | 4 | 3.9 | 3.9-4 | 1.0 |
| 18:1n-5 | | | | 1.0 |
| 20:1n-11 | | | | 1.0 |
| 20:1n-9 | 9 | 8.6 | 8.5-8.6 | 1.0 |
| 22:1n-11 | | | | 1.1 |
| 22:1n-9 | 3 | 2.6 | 2.5-2.7 | 1.1 |
| 24:1n-9 | 1 | 0.9 | 0.9-0.9 | |
| **PUFAs** | | | | |
| 16:3n-4 | | | | 1.0 |
| 18:2n-6 | 2 | 2.1 | 2-2.1 | 1.0 |
| 18:3n-3 | 2(18:3) | 2.0 | 2-2 | 1.0 |
| 18:4n-3 | | | | 1.0 |
| 20:2n-6 | 1 | 0.9 | 0.9-0.9 | 1.0 |
| 20:3n-6 | | | | 1.0 |
| 20:3n-3 | 1 | 0.9 | 0.9-0.9 | 1.0 |
| 20:4n-6 | 3 | 2.9 | 2.8-2.9 | 1.0 |
| 20:4n-3 | | | | 1.0 |
| 20:5n-3 | 10 | 9.8 | 9.7-9.9 | 1.0 |
| 21:5n-3 | | | | 1.1 |
| 22:4n-6 | | | | 1.1 |
| 22:5n-6 | | | | 1.1 |
| 22:5n-3 | | | | 1.1 |
| 22:6n-3 | 12 | 11.4 | 11.1-11.5 | 1.1 |

*Degradation of FAME extracts over time?*

FAMEs are volatile compounds. The degree of volatility depends on the number of carbons, the number of bonds and the location of the bond. In general, the less carbons and the more bonds a FA has, the more volatile it is. Because of this property, FAMEs could evaporate each time the tube containing them was open, affecting the composition of the sample. I tested the effect of time on the composition of the FAME extracts, by injecting the same extract (cod liver oil) at different intervals over time (**Fig. A1-2**). The sample was kept at –20ºC between injections. **Fig. A1-2** showed that the proportions of FAs of the same sample varied slightly over time with no apparent trend. However, the FAME extracts were injected regularly within two weeks of their preparation.

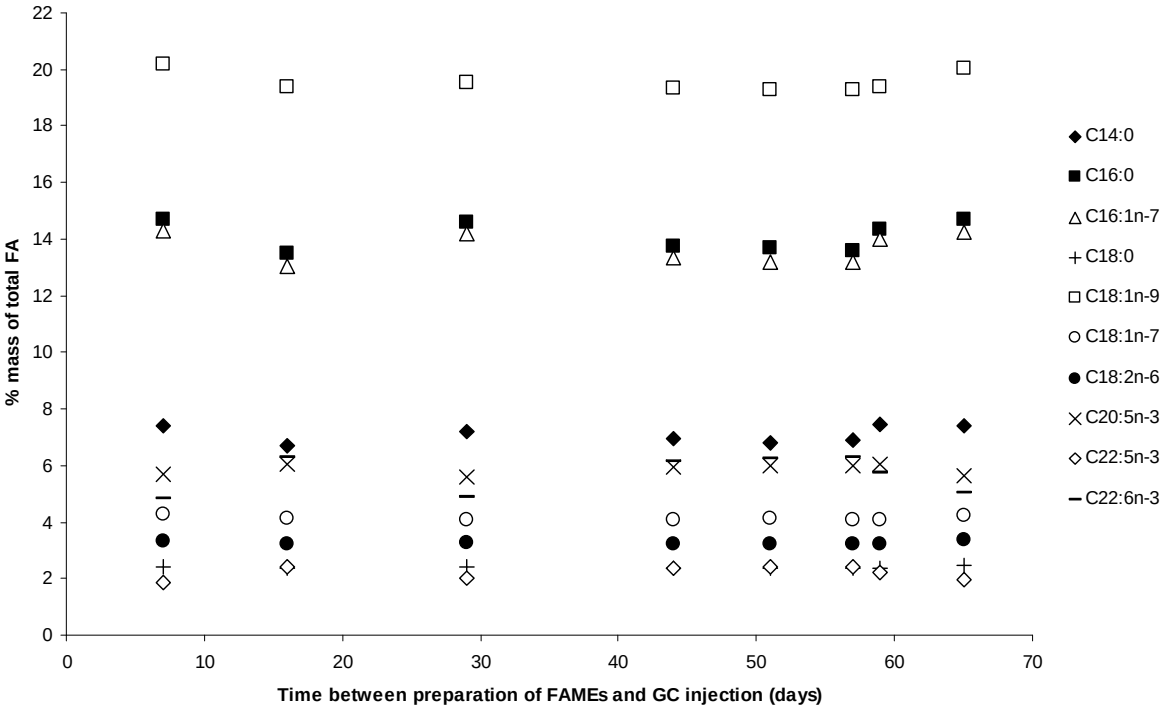



**Figure A1-2.** % mass of the main FAs from the same sample (cod liver oil) injected over time

# PROTOCOL ADOPTED

## Lipid extraction

Lipids from blubber were extracted following the Folch method (Folch *et al.* 1957), using a chloroform:methanol:water ($CHCl_3$:MeOH:$H_2O$) mixture. Approximately 0.5 g

of blubber (whole core) was sub-sampled from the bulk sample. The sample was then cut in small pieces to assist homogenisation. The pieces were accurately weighed, placed in a glass homogeniser (15 mL, Wheaton, U.S.A), and homogenised in 15 mL of chloroform:methanol (2:1, v:v) containing 0.01% butylated hydroxytoluene (BHT) as an antioxidant. The homogeniser was left for 10 min in ice to avoid evaporation of the solvents. The extract was filtered through a glass microfibre filter (GC/C, Whatman, U.K.), transferred to a glass centrifuge screw top tube (50 mL Pyrex, Cole-Parmer International, U.S.A), and washed with 1% sodium chloride (NaCl) to a final ratio of chloroform:methanol:water 8:4:3 (v/v/v). Centrifugation (5808R centrifuge, Eppendorf) was for 10 min at 1000G at 10ºC. The upper methanol:water layer was removed with a water suction pump. The lower chloroform layer was then dehydrated with anhydrous sodium sulphate ($Na_2SO_4$). The lipid extract with chloroform was transferred to a pre-weighed round bottom flask and the solvent was removed by rotary evaporation (Büchi rotovapor, Switzerland) using a water bath at approximately 38°C. The vacuum in the rotary evaporator prevents any oxidation of the lipid while in the water bath. The rotary evaporation was stopped when a constant weight was obtained (no solvent left). The weight of the lipid extracted from the sample was calculated by subtracting the weight of the empty flask from the final weight of the flask and lipids. After weighing, 3 mL of chloroform was added to the flask, and the content transferred to a glass screw top tube (13 mL Kimax tube, Kimble Glass, U.S.A). An aliquot of 30 mg was taken to dryness by rotary evaporation for methylation process. The remaining lipids were kept in chloroform and the tube was capped under nitrogen before storage at –20°C.

**Fatty acid methylation**

FA methyl esters (FAMEs) were prepared directly from 30 mg of the pure extracted lipid following Morrison & Smith (1964). The extracted lipid was dissolved in toluene (1.5 mL) and transferred into a glass centrifuge screw top tube (50 mL Pyrex, Cole-Parmer International, U.S.A), where 1.5 mL of 10% boron trifluoride in methanol (methylating reagent) was added. The tube was capped under nitrogen, and heated at 50°C in a water bath for 14 to 19 h (overnight).

At this point, the protocol detailed in Learmonth (2006) was followed: After heating, the extract was cooled to room temperature. Water (5 mL) containing 5% sodium chloride was added, and the extract was shaken and then left to settle until the layers

were separated. The FAMEs were then extracted into 5 mL of hexane, which was again shaken and left to settle. Once separated, the upper organic layer was removed by pipette into a glass screw top tube (24 mL Kimax tube, Kimble Glass, U.S.A). The process was then repeated. The organic layers were combined and washed with 4 mL of water containing 2% potassium bicarbonate ($NaHCO_3$) to stop potential saponification. Once the layers had clearly separated, the upper organic layer was transferred to a glass screw top tube (13 mL Kimax tube, Kimble Glass, U.S.A) containing approximately one-third anhydrous sodium sulphate to remove traces of water. The tube was stored at – 20°C prior to chromatographic analysis (no more than two weeks).

**Gas chromatograph analysis**

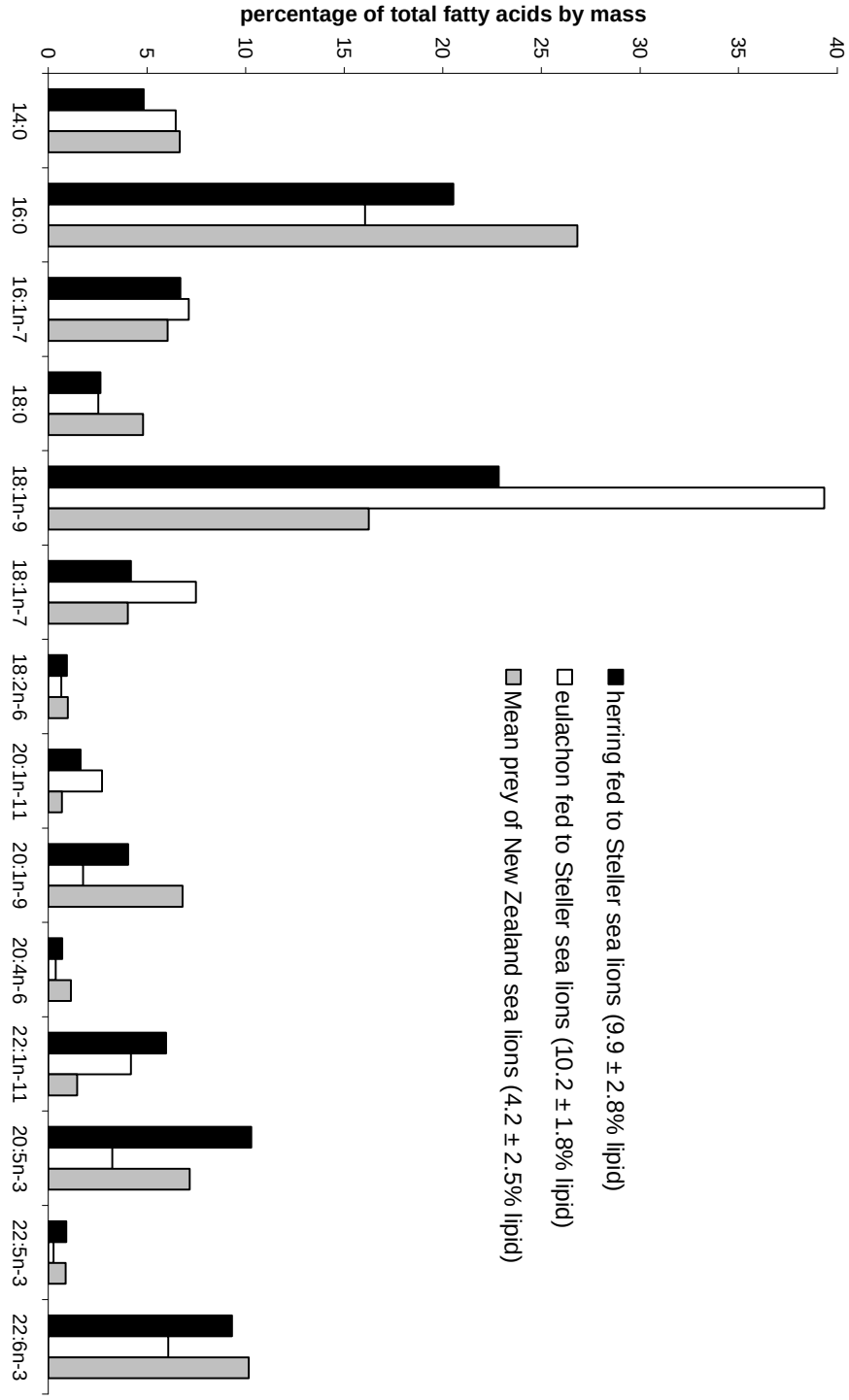
Analysis of FAMEs was carried out using temperature-programmed gas–liquid chromatography performed with a Shimadzu Gas Chromatograph GC-17A (Shimadzu Corporation, Kyoto, Japan) equipped with a flame ionisation detector (FID), and fitted with a 30 m × 0.25 mm i.d. column (50% cyanopropyl polysiloxane, 0.25 μm film thickness, DB-23, J&W, U.S.A.; or 70% cyanopropyl polysilphenylene-siloxane column, 0.25 μm film thickness, BPX70, SGE Analytical Science, Australia). Helium was the carrier gas with a flow rate of 1.3 mL/min. FAMEs (1 to 2 μL) were injected manually (5 μL fixed needle syringe, Hamilton Company, U.S.A.) in split mode (50:1) at an injection port temperature of 250°C. The detector temperature was set at 270°C. Two oven temperatures programmes were used depending of the sample. The first one started at 140°C for 4 min, rose to 190°C at 25°C $min^{-1}$, held for 5 min, then to 240°C at 2°C $min^{-1}$, and held for another 4 min (total of 40 min), and was preferred for blubber samples. The second one started at 50°C for 2 min, rose to 180°C at 25°C $min^{-1}$, held for 5 min, rose again to 200°C at 2°C $min^{-1}$, then to 240°C at 2.1°C $min^{-1}$ (total of 42.2 min), and was preferred for prey type samples.

FA components were identified by comparison of retention time data to authentic (Nu-Chek 68D, Supelco 37 FAME mix, Matreya menhaden oil) and laboratory standards (cod liver oil). Cod liver oil was used at every series of runs to determine accurate retention times. Nu-Chek 68D was injected every month to check the quantitation of each FA. Peak areas were measured by a computerised integration system attached to the GC (CLASS-VP version 7.3, Shimadzu Scientific Instruments, Inc., Columbia, MD). Each chromatogram was checked to ensure correct identification. Some minor

peaks were identified with uncertainty and were thus not included in the final normalisation. 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. Quantitation of FA was done using theoretical response factors calculated according to Ackman & Sipos (1964).

# APPENDIX 2

Fatty acid composition (14 main fatty acids) of pacific herring *Clupea pallasii pallasii* and eulachon *Thaleichthys pacificus* fed to Steller sea lions *Eumetopias jubatus* during long-term diet studies at the University of British Columbia (Vancouver, Canada). Compositions were provided by D. Tollit (unpubl. data). The mean FA composition of New Zealand sea lion *Phocarctos hookeri* prey is displayed for comparison (data from Chapter 4). The mean lipid content was provided in parenthesis for each group (herring and eulachon from D.Tollit, unpubl. data; prey of New Zealand sea lions from Chapter 4.



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