The distribution of fatty acids and presence of environmental contaminants in the blubber of the New Zealand sea lion (*Phocarctos hookeri*)

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ABSTRACT

The conservation of marine mammals relies on the knowledge of species ecology in order to assess the impacts of anthropogenic activites and make appropriate species management decisions. Blubber biopsies are a relatively non-invasive sampling protocol to provide ecological information; two particular uses are for dietary analysis via fatty acid signature analysis (FASA) and investigating the uptake of organochlorine (OC) and polychlorinated biphenyl (PCB) environmental contaminants. Blubber composition and structure may vary according to body site and depth due to its dynamic functioning. This may result in the vertical stratification and heterogenous distribution of blubber FAs, OCs and PCBs between body sites, giving variable interpretations of diet and contaminant levels depending on biopsy sample site and depth. The aim of this thesis is to determine the FA distribution of blubber from two body sample sites (dorsal pelvic and ventral thoracic) currently used for FASA of the New Zealand sea lion, *Phocarctos hookeri* (NZSL) and to determine the level of OCs and PCBs in the blubber of healthy NZSLs for comparison to diseased NZSLs recorded in the 1997/98 epidemic. Blubber samples were collected from 29 by-caught NZSLs incidentally captured by the squid fishery around the Auckland Islands (50º42'S, 166º5'E) during the years 2005 to 2007 (not all NZSLs were able to be analysed for each chapter). Full blubber cores from both sample sites were collected from 18 NZSLs. Both sites showed a relative homogeneity of FA profiles, indicating the similar deposition and mobilisation of FAs at the two sample sites. To determine if FA stratification occurred, full blubber cores from both sample sites of 20 NZSLs were divided into inner and outer halves. Both sites displayed the same pattern of vertical stratification or biochemical layering of FAs between the two divisions, indicating that stratification of FAs occurs in the blubber of the NZSL. A range of OCs and PCBs were then determined in full thoracic blubber cores of seven NZSLs. The levels were higher than those previously recorded in NZSLs affected by disease during a 1997/98 epidemic. The ΣPCB in NZSLs was 0.034-0.192 mg/kg lipids, below the suggested threshold of 17 mg/kg for adverse health affects in marine mammals. From the results of this study I can support current blubber biopsy sampling techniques for FASA in NZSL. Obtaining full blubber cores from either the thoracic or pelvic sample site will give a comparable interpretation of diet. The low levels of blubber OCs and PCBs suggest a minor role of contaminants acting as a

possible causative agent toward disease outbreaks in the NZSL. This research provides important information for developing correct and consistent blubber sampling techniques for NZSL and other pinniped species. This will ensure more accurate interpretations of ecological information obtained from blubber biopsies and therefore improved species management and conservation decisions that may be based on such research.

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Introduction

Fig. 1. An adult female New Zealand sea lion (*Phocarctos hookeri*) on the beach, Auckland Islands - *Photo by Laureline Meynier*

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1. General Introduction

Over the last few decades, the conservation of marine mammals has become increasingly important due to the mounting pressures of direct and indirect anthropogenic impacts, particularly fishery operations and environmental pollution. Knowledge of species ecology, biology and physiology plays an integral part of assessing the impacts of such human activities and implementing appropriate species management and conservation strategies.

Studying marine mammals, however, is a difficult task due to their aquatic existence and may also be problematic in terms of disturbance caused by the research activity (Reynolds, 2005). Ideally, research methods should be designed so as to avoid direct contact with live animals, and should minimise disturbance as much as possible. Some aspects of foraging behaviour can be investigated using 'remote' techniques such as satellite telemetry, but detailed determination of diet in marine mammals often involves more invasive or disruptive techniques. While conventional methods such as direct observations and necropsy of stranded or by-caught animals provide important information, the blubber or subcutaneous lipid store of marine mammals has also been recognised as a potential reservoir of information to study the diet and general ecology of threatened marine mammal populations, through biopsy sampling.

1.2. The New Zealand sea lion

The New Zealand sea lion, *Phocarctos hookeri* (NZSL) is one of five extant sea lion species (family *Otariidae*) and is the sole member of the genus *Phocarctos*. The species is New Zealand's only endemic pinniped and is classed as 'vulnerable' by the International Union for the Conservation of Nature (IUCN) (Reijnders et al., 1993) and 'threatened' under New Zealand's threat classification system (Hitchmough et al., 2007). These classifications are based principally on the species' highly localised range, with the population being confined largely to the sub-Antarctic Auckland Islands (50º42'S, 166º5'E). Over 86% of the annual pup production occurs within just three adjacent breeding grounds on the Auckland Islands (Childerhouse & Gales, 1998; Gales & Fletcher, 1999; Gales, 2002). Campbell Island (52º30'S, 169ºE), 400 km southeast of the Auckland Islands is the only significant external breeding site (McNally et al., 2001; Chilvers, 2008).

Historically, the NZSL inhabited coastal areas throughout mainland New Zealand, but was persecuted during commercial sealing (Childerhouse & Gales, 1998). The current population is estimated to be approximately 11,000-15,000 individuals, one of the smallest for an otariid species (Gales & Fletcher, 1999; Campbell et al., 2006b) and is thought to be in decline (Chilvers, 2008). Data shows that pup production has decreased by approximately 30% during the last eight years (Chilvers et al., 2007). The immediate cause of recruitment recession is not clear, but it may be due to a decline in the number of breeding adults which have been limited or affected by a combination of interactions with the Auckland Islands arrow squid fishery (*Nototodarus sloanii*) and recent infectious disease outbreaks (Chilvers, 2008).

The Auckland Island arrow squid fishery is a lucrative economic resource for New Zealand, operating on the Auckland Islands shelf since the 1970's (Wilkinson et al., 2003). The industry may contribute to poor population growth and NZSL decline both through the accidental by-catch of sea lions and possible competition for a primary food source (Chilvers, 2008; Meynier, 2008). The impacts of by-catch on population persistence are uncertain with several statistical models suggesting various levels of effects on population growth and recovery (Woodley & Lavigne, 1993; Breen et al., 2003). It is also unknown whether the fishery poses a threat or stressor to the NZSL population through competition for a primary food source. Recent data has suggested that squid comprises a consistent proportion of the NZSL diet, however the diet of the species is not yet fully understood (Childerhouse et al., 2001; Meynier et al., 2008). In order to help understand the potential impacts of the fishery as a competitor for a food source, research is currently being undertaken to comprehensively investigate the diet of the NZSL. This dietary analysis is based on the fatty acid signature analysis (FASA) of blubber collected via two different sampling protocols. In the first group, live NZSLs on the Auckland Islands are captured, anaesthetised and restrained in ventral recumbency. This makes the dorsal pelvic area the most accessible sampling site. The second group comprises by-caught NZSLs, which are placed in dorsal recumbency for necropsy, making the thorax the most accessible region for blubber sampling. It is not known whether these two blubber sample sites are comparable in terms of fatty acid (FA) profiles, and thus result in a similar interpretation of diet. It is

also not clear whether the blubber of the NZSL displays vertical stratification of FAs. If the blubber is stratified then this may have implications for the depth of blubber required for FASA. For accurate interpretation of the NZSL diet through blubber FASA it is important to determine the similarity between the two blubber sample sites and to determine whether stratification occurs at both sites.

Disease may also be contributing to poor recruitment and population recovery of the NZSL. During the last decade, three infectious bacterial disease outbreaks have occurred during the breeding seasons of 1997/98, 2001/02 and 2002/03 (Duignan et al., 2003; Wilkinson et al., 2006). In 1997/98, there was death of around 50% of pups and an unknown number of adults, thought to be caused by a *Campylobacter* bacterium (Baker, 1999). In 2001/02 and 2003/03, *Klebsiella pneumoniae* infection contributed to an increase of the natural rate of pup mortality by around 10% but did not appear to affect adults (Wilkinson et al., 2006; Castinel et al., 2007). These epidemics and the loss of adults from the *Campylobacter* outbreak may affect the long-term population persistence due to loss of pups, that are vital for future breeding season recruitment (Chilvers et al., 2007). The NZSL population may be of especially high risk to infectious disease due to their gregarious nature and restricted range.

In other pinniped species, environmental contaminants have been linked to immune suppression and infectious disease outbreaks (Aguilar & Borrell, 1994a; Ross et al., 1995; Ross, 2002; Kajiwara et al., 2008b) and it is plausible that contaminants may play a role as a stressor contributing to recent disease outbreaks in the NZSL. Following the epidemic of 1998, a small study investigated the levels of organochlorine (OCs) pesticides and polychlorinated biphenyls (PCBs) in the blubber of three affected sea lions. The levels were relatively low, suggesting environmental contaminants to play an unlikely role in facilitating recent disease outbreaks of the NZSL (Baker, 1999). However, there has been no investigation into the presence of OCs and PCBs in the blubber of healthy NZSLs.

1.3. Blubber in marine mammal research

The blubber of marine mammals is a multi-functional dynamic tissue that plays a fundamental role in their physical adaptation to the marine environment (Pabst et al., 1999; Iverson, 2002; Kershaw & Flier, 2004; Berta et al., 2006). It is a subcutaneous mammalian adipose tissue that serves as the primary energy store and regulates body temperature in the face of demanding cold environments (Ryg et al., 1988). It is lipid rich, composed primarily of FAs that are obtained largely from diet (Iverson, 2002) and effectively allows marine mammals to fast or reduce energy intake during their life cycle (Ryg et al., 1990; Slip et al., 1992; Rosen & Renouf, 1997; Mellish et al., 2007; Thordarson et al., 2007). Blubber is a vascular tissue, allowing for thermal regulation by retaining or dissipating heat into the environment (Heath & Ridgway, 1999; Willis et al., 2005), while thickness and lipid content may further determine insulative properties (Ryg et al., 1988; Koopman et al., 1996). It contains collagen and elastic fibers and plays a key role in hydrodynamics (Koopman, 1998; Pabst et al., 1999; Hamilton et al., 2004a) while additionally aiding in buoyancy (Ryg et al., 1988; Pond & Ramsay, 1992; Iverson, 2002). Marine mammals consist of several phylogenetic groups that have varying physiological forms and lifestyle adaptations. The topographical distribution of blubber may therefore vary accordingly and reflect functional properties and environmental adaptations (Pond & Ramsay, 1992; Phinney et al., 1994; Koopman, 1998; Iverson, 2002; Berta et al., 2006; Mellish et al., 2007).

Blubber biopsies may be considered a good sampling procedure for threatened species, as the methodology is a minimally invasive process (Fossi & Marsili, 1997; Fossi et al., 1997). Biopsies can also be obtained from both sexes, all age cohorts (Kirsch et al., 2000; Hooker et al., 2001; Cristina Fossi et al., 2003; Falk-Petersen et al., 2004; Iverson et al., 2004; Beck et al., 2005) and animals at sea by remote-sampling (Hooker et al., 2001; Cristina Fossi et al., 2003; Hoberecht et al., 2006), giving a wide source of species information.

1.4. Blubber for dietary analysis

Knowledge of diet is a key aspect to understanding species ecology and deciphering food web relationships. Such knowledge is particularly important for estimating the impacts of fisheries on marine mammal populations. Investigating the diet of marine mammals is difficult, as by its very nature a marine existence inhibits direct observation of feeding. Consequently there has traditionally been a reliance on indirect methods of dietary analysis in pinnipeds, including the recovery of ingested prey hard parts from scat samples recovered from rookeries (Gales & Cheal, 1992; McMahon et al., 1999; Sinclair & Zeppelin, 2002) and analysis of stomach contents of by-caught or stranded animals (Santos et al., 2001; Dehn et al., 2007). These methods provide important information but also have several biases associated with variable gut passage rates (Tollit et al., 1997; Marcus et al., 1998; Hammill et al., 2005), resistance of prey hard parts to digestion (Dellinger & Trillmich, 1987; Gales & Cheal, 1992; Cottrell et al., 1996; Bowen, 2000; Staniland, 2002; Arim & Naya, 2003; Tollit et al., 2003; Ridoux et al., 2007) and restriction to more recent meals.

A technique recently applied to marine mammals to overcome the difficulties of traditional dietary analysis is FASA of adipose tissues. This utilises the FA constituents of blubber (Iverson et al., 1997b; Dahl et al., 2000; Hooker et al., 2001; Bradshaw et al., 2003; Andersen et al., 2004) or milk (Iverson et al., 1997a; Smith et al., 1997; Brown et al., 1999) as indicators of diet. This method is based on the principle that long chain prey FAs are incorporated into predator adipose tissues in a relatively unaltered state, providing a record of dietary intake over a period of time (Iverson, 1993; Smith et al., 1997; Kirsch et al., 2000; Iverson, 2002). Studies have also shown that FASA of blubber can also be used to indicate intra- and inter-specific variability in diet (Iverson et al., 1997b; Walton et al., 2000; Lea et al., 2002; Moller et al., 2003; Walton & Pomeroy, 2003; Beck et al., 2005; Krahn et al., 2007; Thiemann et al., 2007).

In order to be indicative of diet, blubber FA profiles must be obtained from energy storage depots. The location of these depots varies between species (Pond & Ramsay, 1992; Koopman, 1998; Budge et al., 2006). To date, FASA blubber analysis techniques have been

applied mainly to cetaceans and phocid seals. In cetaceans, the anterior thorax region is found to be the most dynamic region of blubber energy storage (Samuel & Worthy, 2004; Rauchonnet et al., 2006) while the caudal peduncle area is more structural and is involved primarily in locomotion (Koopman et al., 1996; Koopman, 1998; Hamilton et al., 2004a; Struntz et al., 2004). As a result, it is recommended that blubber for dietary analysis should be collected from the anterior thorax (Aguilar & Borrell, 1991a; Dahl et al., 2000; Walton et al., 2007). In phocid seals, the main body trunk has a relatively consistent lipid distribution (Beck, Ryg et al., 1988; 1993; Mellish et al., 2007) and uniform mobilisation of lipid stores from around the body during energy deficits (Slip et al., 1992; Mellish et al., 2007). When sampling free-ranging pinnipeds, animals are usually anaesthetised and restrained in ventral recumbency (Iverson et al., 1997b; Kirsch et al., 2000; Walton et al., 2000; Best et al., 2003; Beck et al., 2005). This limits blubber sample areas, making the posterior dorsal flank area/mid pelvic region the most accessible region and thus the standardised site for obtaining phocid blubber biopsies (Iverson et al., 1997b; Grahl-Nielsen et al., 2000; Kirsch et al., 2000; Walton et al., 2000; Best et al., 2003; Bradshaw et al., 2003; Walton & Pomeroy, 2003). In otariid seals there has been a lack of investigation into blubber distribution or lipid deposition and mobilisation, and therefore no standardised techniques for blubber sampling have been developed. A literature search found only one otariid study, which investigated variation in FA profiles at three body sites in the Cape fur seal (*Arctocephalus pusillus pusillus*) (Arnould et al., 2005). This study found anatomical variability of body site FA profiles.

The depth of blubber sampled may also affect the interpretation of diet through FASA. In numerous cetaceans (Ackman et al., 1975a; Aguilar & Borrell, 1991b; Koopman et al., 1996; Olsen & Grahl-Nielsen, 2003; Krahn et al., 2004; Smith & Worthy, 2006; Budge et al., 2008), phocid seals (Kakela & Hyvarinen, 1993; Fredhiem et al., 1995; Kakela & Hyvarinen, 1996; Best et al., 2003; Andersen et al., 2004; Wheatley et al., 2007; Strandberg et al., 2008) and in one otariid species (Arnould et al., 2005) the vertical stratification or biochemical layering of FAs within the blubber has been documented. A general pattern of FA stratification can be a seen amongst marine mammals although the degree is species-specific (Fredhiem et al., 1995; Hooker et al., 2001; Krahn et al., 2004). This may be

influenced by environmental pressures (Kakela et al., 1993), sex and age of animals (Aguilar & Borrell, 1991b; Iverson, 2002; Samuel & Worthy, 2004; Arnould et al., 2005; Beck et al., 2005; Wheatley et al., 2007) and nutritional state (Iverson et al., 1997a; Best et al., 2003). The stratification of FAs has primarily been attributed to the different functions of the inner and outer blubber layers. The inner blubber layer is more metabolically active in terms of lipid deposition and mobilisation and more closely represents the diet (Olsen & Grahl-Nielsen, 2003; Andersen et al., 2004; Grahl-Nielsen et al., 2005; Wheatley et al., 2007; Strandberg et al., 2008), while the outer blubber layer is relatively inert with a more structural role (Lockyer et al., 1984; Koopman et al., 1996; Strandberg et al., 2008).

In order to appropriately use FASA of blubber for the interpretation of diet, it is therefore important to investigate the species-specific FA characteristics of blubber. This will avoid inconsistent interpretations of diet through any variation in the blubber sample sites and depths utilised.

1.5. Blubber for Environmental Contaminant Analysis

Persistent organic pollutants (POPs), particularly polychlorinated biphenyls (PCBs) and organochlorine (OCs) pesticides are highly lipophilic in nature, highly resistant to degradation and bioaccumulate readily within aquatic environments. Marine mammals may be highly susceptible to these contaminants as they are generally long-lived, feed at high trophic levels (Kannan et al., 2004) and have large lipid rich blubber stores (Schantz et al., 1993). Furthermore they seem to have a lower capacity than terrestrial mammals to metabolise contaminants (Schroder & P, 1998; Metcalfe et al., 2004). Marine mammals may therefore play important roles as bio-indicators of the state of contamination of the environment and general health of marine ecosystems (Mossner & Ballschmiter, 1997; Aguilar & Borrell, 2004).

The concentration of POPs in marine mammal blubber may vary with sex and maturity of animals (Aguilar & Borrell, 1990; Nakata et al., 1998; Krahn et al., 2001; Borrell et al., 2004; Stern et al., 2005). Mature males and juveniles usually have the highest levels of POPs (Kleivane et al., 1997; Bernt et al., 1999) as studies indicate that reproductive

females off-load burdens during gestation via trans-placental transfer and also during lactation through the mobilisation of blubber stores for milk production (Bacon et al., 1992; Ylitalo et al., 2001; Debier et al., 2003; Metcalfe et al., 2004; Borrell & Aguilar, 2005; Kajiwara et al., 2008a). The trophic level of the species may also affect the concentration levels of POPs within blubber. Those species feeding at higher trophic levels are shown to contain much higher concentrations of POPs than those at lower levels due to bioaccumulation within the food chain (O'Shea & Brownell, 1994; Mossner & Ballschmiter, 1997; Jones, 1998; Reijnders & Aguilar, 2002). Intra-specific variations in diet due to factors such as geographical location of consumption may also result in variable levels of POPs within populations (Krahn et al., 1997; Ylitalo et al., 2001; Metcalfe et al., 2004).

POPs may also be vertically stratified within blubber. Studies of cetaceans (Aguilar & Borrell, 1991a; Krahn et al., 2004) and phocid seals (Kleivane et al., 1997; Severinsen et al., 2000) have demonstrated higher concentrations of POPs toward the outer blubber layer than the inner. This is due to the dynamic functioning of blubber, with the inner layer being more metabolically active than the outer, resulting in an increase in concentration and accumulation of contaminants toward the outer more metabolically inert layer (Aguilar & Borrell, 1991a; Severinsen et al., 2000; Sormo et al., 2003).

Evaluating the biological effects of POPs on marine mammals is a difficult task with no defined cause-effect relationships. Circumstantial evidence has linked the presence of high levels of POPs to adverse affects on marine mammal health, particularly immunosuppression (Ross et al., 1995; De Swart et al., 1996; Ross et al., 1996; Van Loveren et al., 2000) leading to infectious disease outbreaks (Kannan et al., 1993; Aguilar & Borrell, 1994a; Simmonds & Mayer, 1997; Jepson et al., 1999). Studies also link POPs to numerous reproductive impairments such as reduced implantation rates (Reijnders, 1986), premature births, still births and increased neonatal mortality (DeLong et al., 1973; Schwacke et al., 2002). Other physiological impairments have included skeletal growth abnormalities (Bergman et al., 1992) and various forms of cancers (Martineau et al., 1994; Ylitalo et al., 2005).

Most investigations of contaminants in marine mammals have focused on northern hemisphere cetaceans (e.g. Hernández et al., 2000; Cristina Fossi et al., 2003; Hobbs et al., 2003; Aguilar & Borrell, 2004; Jepson et al., 2005) and phocid seal species (e.g. Bergman et al., 1992; Bernt et al., 1999; Hall et al., 1999; Cleemann et al., 2000; Kannan et al., 2004; Borrell et al., 2007). There has been little work published on southern hemisphere marine mammal species (Hutchinson & Simmonds, 1994; Kemper et al., 1994; Vetter et al., 2001; Aguilar et al., 2002).

1.6. Thesis Aim

The aim of this thesis is to determine the FA distribution of blubber from the two sample sites currently used for dietary analysis of the NZSL and to determine the levels of OCs and PCBs in the blubber of healthy NZSLs.

The sample group for this thesis comprised NZSLs that were incidentally captured by vessels operating on the Auckland Islands shelf squid fishery during the fishing season (February to May) of the years 2005 to 2007. Carcasses were frozen on board and transported to Massey University, Palmerston North, New Zealand for necropsy. This was under contract with the Conservation Service Providers administered by the New Zealand Department of Conservation and Ministry of Fisheries.

Due to limited amounts of blubber collected from NZSLs in previous by-catch years, not all animals could be utilised for each aspect of this study.

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~ CHAPTER 2 ~

Variation in blubber fatty acid profiles between two body sites in the New Zealand sea lion (*Phocarctos hookeri*)

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Abstract

Fatty acid signature analysis (FASA) of blubber is an increasingly popular tool for investigating the foraging ecology of marine mammals. This technique is based on the principle that fatty acids (FAs) from prey species become deposited in a relatively unaltered state, into the adipose tissue of monogastric predators. While numerous studies have documented a relatively homogenous distribution of blubber FAs in phocid seals and cetaceans, there has been little investigation into the blubber and FA distribution of otariid seals. The aim of this study was to investigate the homogeneity of blubber FA profiles from two body sample sites currently used for dietary analysis in the New Zealand sea lion (NZSL), *Phocarctos hookeri*. Full blubber cores from both pelvic and thoracic body sites were collected from 18 NZSLs incidentally caught by the squid fishery around the Auckland Islands (50º42'S, 166º5'E). The FAs from the two sites showed relative homogeneity with only two (20:5n-3 and 18:2n-6) present in variable amounts. This indicates there is similar deposition and mobilisation of FAs at the two sample sites. I conclude that blubber collected from either body site will be equally representative of the input taken by the predator.

Keywords: Blubber · fatty acid signature analysis · New Zealand sea lion · otariid

Abbreviations: Fatty acid (FA); Fatty acid signature analysis (FASA); Monounsaturated fatty acid (MUFA); New Zealand sea lion (NZSL); Polyunsaturated fatty acid (PUFA); Saturated fatty acid (SFA).

1. Introduction

Knowledge of diet and foraging ecology of top predatory marine mammals are principle elements for understanding marine ecosystem dynamics and specific predator-prey relationships. In turn, such information translates into improved conservation efforts for endangered marine mammal species through management strategies and estimations of anthropogenic impacts such as fisheries (Pauly et al., 1998; Trites, 2001).

Direct observation of feeding poses various challenges to biologists as many marine mammals operate on large spatial scales and at great ocean depths, hindering direct dietary analysis. As a result, there has been a reliance on indirect methods such as recovery of indigestible hard parts from scats collected on rookeries or from the stomachs of by-caught and stranded animals (Reid & Arnould, 1996; Merrick et al., 1997; Childerhouse et al., 2001; Santos et al., 2001; Trites, 2001; Sinclair & Zeppelin, 2002; Dehn et al., 2007). However, on-going research has increasingly suggested that such techniques may display bias associated with variable digestion rates of hard parts. In addition, these methods are limited to more recent meals (Dellinger & Trillmich, 1987; Gales & Cheal, 1992; Staniland, 2002; Arim & Naya, 2003; Yonezaki et al., 2003; Iverson et al., 2004).

Recently, there has been a shift in focus toward new methods addressing the chemical constituents of adipose tissues such as stable isotope (Kelly, 2000; Kurle, 2002; Dehn et al., 2007; Krahn et al., 2007) and fatty acid signature analysis (FASA) (Iverson et al., 1997b; Walton et al., 2000; Hooker et al., 2001; Lea et al., 2002; Bradshaw et al., 2003; Herman et al., 2005; Ridoux et al., 2007). FASA is based on the knowledge that ingested fatty acids (FAs) with carbon chain lengths greater than 14, are deposited in the adipose tissue of predators in a relatively unaltered state. This may indicate an accumulation of FAs from consumed prey species over a period of time. In pinnipeds, the use of milk for FASA is commonplace due to the relative simplicity of obtaining samples from animals on shore (Iverson, 1993; Iverson et al., 1997b; Brown et al., 1999; Debier, 1999; Staniland & Pond, 2004). Since milk production is limited to sexually mature females, analysis of milk FA signatures provides only a brief insight into the diet of adult females during the reproductive season. Conversely, FASA of blubber allows for the sampling of a range of

age cohorts for both sexes (Kirsch et al., 2000; Bradshaw et al., 2003; Grahl-Nielsen et al., 2003; Iverson et al., 2004; Arnould et al., 2005; Beck et al., 2005; Ridoux et al., 2007), providing a wider understanding of species foraging ecology. Blubber sampling of live animals is a minimally invasive procedure, involving punch or incision biopsies, which can be performed on hauled-out animals or via remote sampling at sea (Iverson et al., 1997b; Hoberecht et al., 2006). Blubber can also be collected at necropsy from freshly dead carcasses.

When captured for biopsy, pinnipeds are usually anesthetised and restrained in ventral recumbency. This position limits the possible areas of blubber collection, making the dorsal pelvic region most accessible. In contrast, pinnipeds undergoing necropsy are usually placed in dorsal recumbency, thus making the ventral surface of the animal (usually the thorax) more accessible for sampling (West et al., 1979a, 1979b; Fredhiem et al., 1995). For most pinniped species, it is not known if these two sites (dorsal pelvic and ventral sternal) have comparable lipid depots and FA signatures. Within phocid seals, it has been generally established that blubber has both a relatively uniform distribution and mobilisation rate along the main body trunk (Slip et al., 1992; Mellish et al., 2007; Beck & Smith, 1995). However a recent study of an otariid, the Cape fur seal (*Arctocephalus pusillus pusillus*) suggests a degree of anatomical variation along the body trunk (Arnould et al., 2005). It is therefore important to assess possible anatomical variations in blubber FA profiles to validate the use of specific body sample sites for the use of FASA in the interpretation of diet.

The New Zealand sea lion (NZSL), *Phocarctos hookeri*, is New Zealand's only endemic pinniped and is classified as a 'threatened' species (Hitchmough et al., 2007). The species has a highly restricted range, being confined primarily to the sub-Antarctic Auckland Islands (50º42'S, 166º5'E); home to 86% of the annual pup production within just three adjacent breeding grounds (Campbell et al., 2006a; Chilvers, 2008). Currently, a squid fishery operates annually from approximately February to May on the Auckland Islands shelf, resulting in the indirect capture of sea lions and possible competition for a primary food source (Wilkinson et al., 2003; Chilvers, 2008). To determine the trophic relationships

between sea lions and fisheries, research is currently being undertaken into the foraging ecology of the NZSL through the FASA of blubber (Meynier et al., 2008). This investigation relies on blubber collected from two different body sample sites (dorsal pelvic and ventral thoracic regions) from two sampling groups (live-sampling at the Auckland Islands and by-caught sea lions). Therefore, it is vital to establish whether or not the FA profiles at these two body sites are directly comparable. If the FA compositions are not homogenous, erroneous conclusions of diet could be made from inter-animal variations in FA profiles.

The purpose of this study was to investigate the difference between FA profiles from ventral thoracic and dorsal pelvic blubber in NZSLs in order to validate current dietary analysis techniques.

2. Materials and Methods

2.1. Sample collection

The NZSLs analysed were incidentally captured by vessels operating in the Auckland Islands shelf squid fishery during the fishing seasons (February to May) of 2005 and 2006. Carcasses were frozen on board and transported to Massey University, Palmerston North, New Zealand, for necropsy. This was under contract with the Conservation Service Providers administered by the New Zealand Department of Conservation and Ministry of Fisheries.

Blubber samples were collected from both the thoracic and pelvic areas (Fig. 1) of 18 by-caught NZSLs (4 males and 14 females) (Table 1).

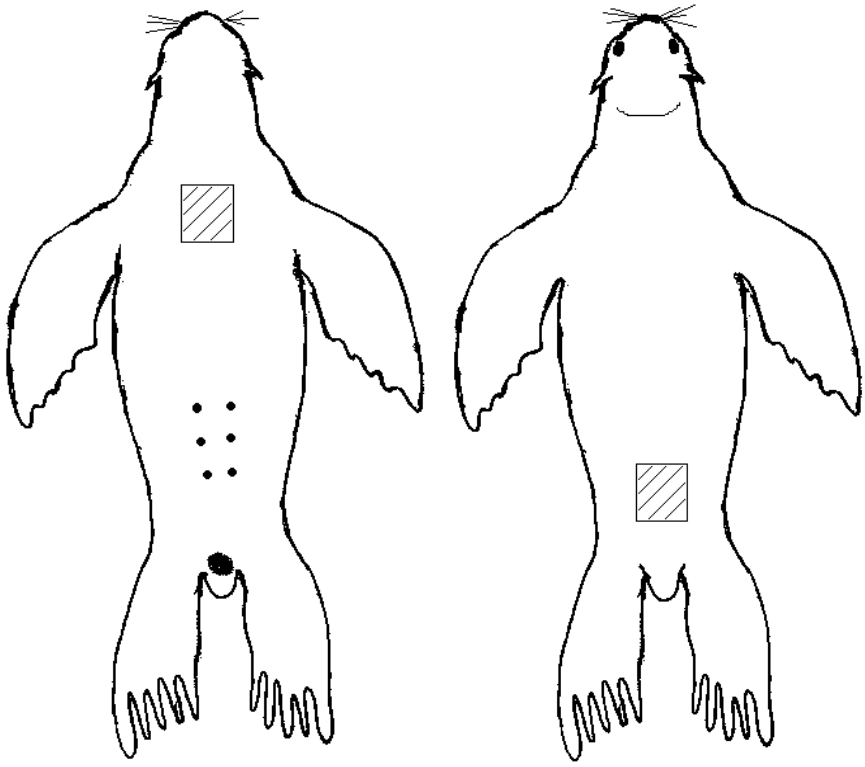

Fig. 1. Location of the blubber samples for the thoracic and pelvic areas from each New Zealand sea lion (*Phocarctos hookeri*) during necropsy as indicated by hatched square area.

Ten blubber samples were collected from the ventral thorax by cutting a 60 x 60 mm piece from the outer epidermis through to the underlying muscle (Fig. 2). Samples were then sealed in plastic bags and frozen at -20ºC (Table 1). The remaining 26 blubber samples were collected by a core biopsy instrument, cutting a 10 mm diameter core from the outer epidermis to the muscle on the ventral thoracic and the dorsal pelvic regions of the animal. Samples were then placed in 'Nunc cryotube vials' (Nunc, Thermofisher Scientific) and frozen at -80ºC (Table 1).

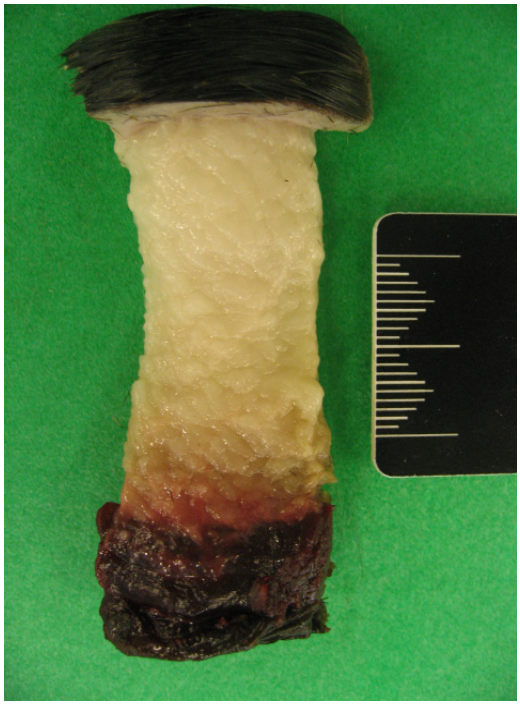

Fig. 2. Cross-section of thoracic blubber from a New Zealand sea lion (*Phocarctos hookeri*). The epidermis is shown above and blubber-muscle boundary below. The scale bar represents 2 cm.

Table 1. By-catch year, identification number (I.D. No.), sex and storage temperature of analysed blubber samples from New Zealand sea lions (*Phocarctos hookeri*).
* L indicates lactating females, F non-lactating females and M males.

Sea lion			Temperature of storage (°C)	
Year	**I.D. No.**	**Sex**	**Thoracic**	**Pelvic**
2005	505	L	-20	-80
2005	506	M	-20	-80
2005	508	L	-20	-80
2005	509	M	-20	-80
2005	514	M	-20	-80
2005	515	L	-20	-80
2005	516	L	-20	-80
2006	601	F	-20	-80
2006	602	L	-20	-80
2006	603	F	-20	-80
2006	605	M	-80	-80
2006	606	L	-80	-80
2006	607	F	-80	-80
2006	608	F	-80	-80
2006	609	F	-80	-80
2006	610	F	-80	-80
2006	611	F	-80	-80
2006	612	F	-80	-80

2.2. Lipid Extraction and Esterification

Lipids were extracted from blubber samples employing a modified Folch method (Folch et al., 1957; Iverson et al., 2001). Thawed blubber samples were extended to their full length and any attached skin or muscle removed. Any freeze burned areas indicated by a darkish

yellow colour were also discarded. A 0.5 g sub-sample was taken from the center of the blubber piece and homogenised in a 15 ml 2:1 chloroform/methanol solution using butylated hydroxytoluene (BHT) as an antioxidant. The extract was filtered and washed with 1% sodium chloride to a final ratio of chloroform:methanol:saline water of 8:4:3 (v;v;v). Fatty acid methyl esters (FAMES) were prepared from 30 g of the lipid extract using 1.5 ml of 10% boron trifluoride in methanol (methylating reagent) and 1.5 ml of toluene. Each extract was capped under nitrogen, and heated at 50°C overnight. FAMES were then extracted into hexane and stored over anhydrous sodium sulphate at -20°C before chromatography analysis.

2.3. 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 and fitted with a 30 m × 0.25 mm i.d. column coated with 50% cyanopropyl polysiloxane (0.25 μm film thickness; J&W DB-23; Folsom, California). Helium was used as the carrier gas. FAMES (1 ul) were injected manually in split mode 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}, hold for 5 min, then finally rise to 236°C at 2°C min^{-1}.

2.4. Identification and quantitation of FAs

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-chek 68D was injected regularly to check the quanitification of each FA. Peak areas were measured by an electronic integrator attached to the gas chromatograph (CLASS-VP version 7.3, Shimadzu Scientific Instruments, Inc., Columbia, MD). Each chromatogram was manually checked to ensure the accurate identification of FA peaks and adjusted if incorrect. FAs were expressed as mass percent of total FAs and were designated by the shorthand IUPAC (International Union of Pure and

Applied Chemistry) nomenclature of carbon chain length: number of double bonds and location (n-x) of the double bond nearest to the terminal methyl group.

2.5. Data Analysis

A combination of multivariate and univariate statistical analysis was carried out to examine the variability of the thoracic and pelvic blubber FA profiles (Minitab Release 15.0 MINITAB Inc. 2007). The data was normalised by arcsine square-root transformation prior to parametric testing to reduce heterogeneity of data.

FAs were grouped into total saturated fatty acids (SFA), short chain monounsaturated fatty acids (SC-MUFA; chain length < 18 carbons), long chain monounsaturated fatty acids (LC-MUFA; chain length > 18 carbons) and polyunsaturated fatty acids (PUFA). Their percentages were tested along with individual FAs.

Multivariate analysis.

Due to the multivariate nature of FA analysis, principal component analysis (PCA) was used to view the data in two dimensions and to determine the initial relationship between the pelvic and thoracic FA profiles. A bi-plot of principal component 1 (PC1) and principal component 2 (PC2) was produced describing the variation amongst the samples.

Univariate analysis.

Paired-t tests were carried out to detect any differences between individual FAs and major FA groupings of pelvic and thoracic blubber sites.

3. Results

3.1. FA composition of NZSL blubber

Thirty-four FAs were consistently identified in all blubber samples. Individual FAs present at < 0.5% of total FA concentration were excluded from analysis, these were: 10:0, 12:0, 17:0, 20:0, 14:1, 18:1n-5, 20:2n-6, 20:3n-3, 20:3n-6, 21:5n-3, 22:4n-6, 22:5n-6. The total number of FAs included in the analysis was therefore reduced to 22 and accounted for approximately 97- 98% of total FAs (Table 2).

Overall the FA composition of the pelvic and thoracic blubber was very similar. The three most abundant FAs (proportion of total FAs) were 18:1n-9 (30.17 in thoracic, 30.64 in pelvic), 16:0 (14.65 in thoracic, 14.05 in pelvic) and 22:6n-3 (10.61 in thoracic, 10.98 in pelvic). These three accounted for approximately 55% of total FAs. No single FA was present within one blubber type and absent from the other (Table 2). In both the pelvic and thoracic FA profiles, MUFA comprised the greatest proportion of total FAs (approximately 50%), followed by SFA (approximately 24%) and closely PUFA (approximately 22%). The most prominent MUFAs were 18:1n-9 & 16:1n-7, SFAs were 16:0 and 14:0; and PUFAs 22:6n-3 and 20:5n-3 (Table 2).

Table 2. Fatty acid (FA) profiles (mean ± S.D, % mass total FAs) of blubber from the two body sites (pelvic and thoracic areas) sampled from 18 by-caught New Zealand sea lions (*Phocarctos hookeri*). P-values for paired-t tests are also shown; highlighted values indicate significance (P-value < 0.05).

FA	Thoracic		Pelvic		P-value
	Mean %	± S.D	Mean %	± S.D	
SFA					
14:0	6.53	1.51	6.17	2.04	0.385
15:0	0.44	0.09	0.42	0.09	0.255
16:0	14.65	2.22	14.05	2.22	0.412
18:0	2.57	0.75	2.59	0.62	0.777

MUFA					
15:1	0.29	0.61	0.13	0.03	0.169
16:1n-7	8.46	2.36	8.01	2.12	0.294
18:1n-9	30.17	3.87	30.64	3.05	0.331
18:1n-7	3.98	0.49	4.08	0.37	0.236
20:1n-11	1.16	0.34	1.28	0.34	0.198
20:1n-9	8.30	1.66	9.03	1.66	0.059
22:1n-11	0.68	0.41	0.64	0.24	0.990
22:1n-9	0.44	0.17	0.45	0.18	0.765
PUFA					
16:2n-4	0.53	0.12	0.55	0.11	0.605
16:3n-4	0.36	0.09	0.32	0.08	0.072
18:2n-6 (LL)	1.56	0.13	1.66	0.17	**0.004**
18:3n-3	0.66	0.11	0.66	0.14	0.981
18:4n-3	0.52	0.20	0.49	0.18	0.418
20:4n-6 (AA)	0.62	0.07	0.61	0.15	0.661
20:4n-3	1.14	0.30	1.09	0.30	0.411
20:5n-3 (EPA)	3.30	1.10	2.89	1.13	**0.027**
22:5n-3 (DPA)	2.97	0.85	3.25	0.93	0.298
22:6n-3 (DHA)	10.61	2.61	10.98	2.99	0.714
Sum SFA	24.21	6.27	23.23	5.99	0.43
Sum SC-MUFA	42.93	13.39	42.86	13.69	0.94
Sum LC-MUFA	10.58	3.78	11.40	4.13	0.14
Sum PUFA	22.28	3.13	22.51	3.23	0.68

LL linoleic; AA arachidonic acid; EPA eicosapentaenoic acid; DPA Docosapentaenoic acid; DHA docosahexaenoic acid; SFA saturated fatty acids; MUFA monounsaturated fatty acids; SC short-chain; LC long-chain; PUFA polyunsaturated fatty acid.

3.2. Variation between body sites

The bi-plot of the first two PCs did not reveal any clear separation between the pelvic and thoracic samples, although there was a clustering of blubber samples along PC1 between individuals 505, 506, 516, 605 (negative values on PC1) and the rest of the individuals (Fig. 3). The causes of this particular separation may be due to the variability of blubber FA profiles between males and females (Meynier, 2008; Meynier et al., 2008). This is however beyond the scope of this study, and so will not be further discussed.

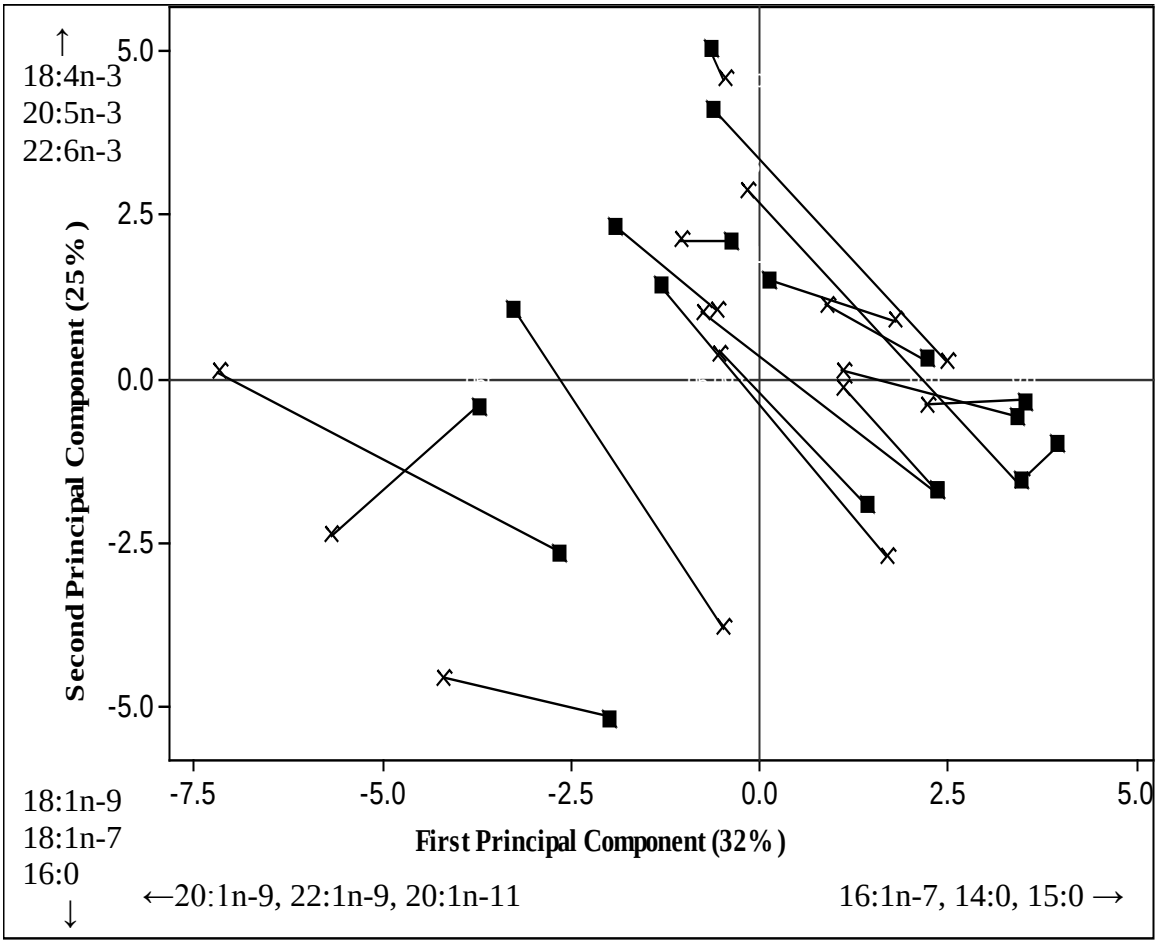

Fig. 3. Bi-plot of the first two principal components (PCs) from the fatty acid (FA) compositions of thoracic and pelvic blubber samples of 18 by-caught New Zealand sea lions (*Phocarctos hookeri*). X indicates pelvic, black squares thoracic. Lines indicate pairing of thoracic and pelvic samples for each sea lion. The three most important FAs driving each PC are displayed on the side of each axis with the direction.

Table 3. Loadings of fatty acids (FAs) for the first three principal components from the FA compositions of the pelvic and thoracic blubber samples of 18 by-caught New Zealand sea lions (*Phocarctos hookeri*). Data are sorted by magnitude to 18:4n-3 on PC1 to 20:4n-6 on PC2 and to 15:1 on PC3. The percentage of variance explained by each PC is in parentheses.

FA	PC1 (32%)	PC2 (25%)	PC3 (15%)
16:1n-7	0.324	0.047	0.163
20:1n-9	-0.317	0.070	-0.082
22:1n-9	-0.316	-0.002	-0.147
20:1n-11	-0.315	0.115	0.051
14:0	0.299	0.009	-0.231
22:5n-3 (DPA)	-0.258	0.210	0.178
15:0	0.249	-0.161	-0.249
16:3n-4	0.246	-0.091	0.257
22:1n-11	-0.243	0.182	-0.249
18:3n-3	0.225	0.267	0.036
18:4n-3	0.211	0.374	0.101
20:5n-3 (EPA)	-0.175	0.342	-0.089
18:1n-9	0.164	-0.321	0.293
18:1n-7	0.151	-0.315	0.260
22:6n-3 (DHA)	0.147	0.313	0.030
18:4n-3	-0.143	0.297	-0.180
16:0	0.117	-0.241	-0.342
18:0	-0.106	-0.223	-0.324
20:4n-6 (AA)	0.100	0.203	0.201
16:2n-4	0.117	-0.009	-0.363
18:2n-6 (LL)	0.164	-0.050	0.237
15:1	-0.051	0.010	0.104

DPA Docosapentaenoic acid; EPA eicosapentaenoic acid; DHA docosahexaenoic acid; AA arachidonic acid; LL linoleic acid.

The first two components derived from PCA accounted for 57% of the variation (PC1 32%, PC2 25%) amongst samples. The most important FAs on the first three PC are presented in Table 3 with the highest loadings. They are 16:1n-7, 20:1n-9 on PC1; 18:4n-3, 20:5n-3 on PC2 and 16:2n-4, 16:0 on PC3.

There were no significant differences found in the proportion of major FA groups between the pelvic and thoracic sample sites (Paired t-tests, Table 2, P-values > 0.05).
Finer scale differences between the two blubber types were only observed between two of 22 FAs (20:5n-3 and 18:2n-6). FA 20:5n-3 was found in significantly lower proportions in the pelvic region compared to thoracic (P-value = 0.027) and 18:2n-6 was found in significantly higher proportions in the pelvic compared to thoracic (P-value = 0.004), (Table 2).

4. Discussion

FASA of blubber is a useful tool for investigating the diet of pinniped species, allowing sampling of both sexes and a large range of age cohorts (Iverson et al., 1997b; Arnould et al., 2005; Beck et al., 2005; Grahl-Nielsen et al., 2005; Budge et al., 2006; Ridoux et al., 2007). It has become apparent, however, that within some marine mammal species, variation in body sample sites may translate into inconsistent FA profiles (Kakela & Hyvarinen, 1996; Koopman, 1998; Samuel & Worthy, 2004; Arnould et al., 2005; Budge et al., 2006; Ruchonnet et al., 2006). Consequently, it is imperative to investigate the species-specific variation of FA profiles between body sample sites to ensure accurate interpretations of diet (Olsen & Grahl-Nielsen, 2003; Budge et al., 2006).

The results of this study have shown a high degree of similarity between the FA profiles of blubber from the thoracic and pelvic body sites currently used for dietary analysis in NZSLs. This is the first investigation into the regional variability of FA profiles in a sea lion species and only the second published for an otariid. Studies on variation of FA profiles between body sites have focused on the investigation of blubber distribution of phocid seals and cetacean species (Best et al., 2003; Samuel & Worthy, 2004; Ruchonnet et al., 2006; Beck & Smith, 1995). In phocids, blubber is shown to have a relatively uniform lipid distribution (Ryg et al., 1988; Beck et al., 1993; Mellish et al., 2007) and to be uniformly mobilised around the body during energy deficits (Slip et al., 1992; Mellish et al., 2007). Such findings have influenced the development of the standardised blubber sample site for FASA within phocids of the posterior flank area/mid pelvic region (most easily accessible) (Iverson et al., 1997b; Grahl-Nielsen et al., 2000; Kirsch et al., 2000; Walton et al., 2000; Best et al., 2003; Bradshaw et al., 2003; Walton & Pomeroy, 2003). Similarly in cetaceans it has been well documented that along the main body trunk there is a relatively homogenous distribution of lipids and blubber FA profiles (Samuel & Worthy, 2004; Rauchonnet et al., 2006). However, the caudal peduncle fin area may display significantly thinner blubber (Koopman, 1998), lower lipid content and different FA profiles to the anterior trunk (Koopman et al., 1996; Koopman, 1998; Hamilton et al., 2004a; Struntz et al., 2004). Therefore, the main body trunk of cetaceans is suggested as a standardised target for remote sampling techniques (Dahl et al., 2000; Hooker et al., 2001; Krahn et al., 2004). Within otariids, the lack of investigation into blubber distribution and

site-specific variation of lipid deposition and mobilisation and FA characteristics, has resulted in a lack of standardised sites for blubber sampling for FASA (Arnould et al., 2005).

4.1. Variation in FA profiles of NZSL

In this study, a variety of statistical analyses found the FA profiles of the pelvic and thoracic regions to be very similar. PCA did not separate the blubber types into separate groupings related to sample site (Fig. 3). Neither the pelvic or thoracic profiles showed any variability in overall FA groupings. Univariate analysis revealed that only two of 22 identified FAs (PUFA 20:5n-3 and 18:2n-6) were found in variable amounts between the sample sites ($P < 0.05$). This variability may be attributed to the differential deposition and mobilisation of individual FAs. Previous research has shown FA molecular characteristics (number of carbon atoms, double bonds and position of the first double bond) to influence mobilisation rates (Raclot & Groscolas, 1993, 1995; Gunstone, 1996; Raclot, 2003; Nieminen et al., 2006). This makes some FAs such as PUFA (especially 22:6n-3 & 20:5n-3), SFA (especially 18:0 & 16:0) and MUFA C20 family metabolised faster than others during energy depletion (Raclot & Groscolas, 1993; Raclot, 2003). Nevertheless, caution must be used when interpreting the univariate results of this study, as all individual FA percentages are dependant on one another. A larger sample size would be required to enable use of more accurate multivariate analysis techniques in order to determine the significance of this variability.

As far as I know, the only other published otariid research available for comparison with this study is that of Arnould et al, on the Cape fur seal. In contrast to our findings, Arnould et al found significant variation in blubber FA profiles between three body sample sites (neck, rump and mammary area). Two of their sampling sites (neck and rump) may not be considered as sites of energy storage in pinnipeds and therefore may be expected to have different FA profiles as a reflection of the differences in blubber function at those sites. Structural blubber has been shown to have a lower lipid content and a higher collagen and protein content (Lockyer et al., 1984; Koopman et al., 1996; Hamilton et al., 2004b; Torneroa et al., 2004), making it more metabolically inert (Koopman et al., 2002) than

blubber functioning primarily as energy storage. In harp seals (*Phoca groenlandica)* (Beck & Smith, 1995), southern elephant seals (*Mirounga leonina*) (Slip et al., 1992), ringed seals (*Phoca hispida*) (Ryg et al., 1988) and steller sea lions (*Eumetopias jubatus*) (Mellish et al., 2007) neck blubber is considered to be predominantly structural in nature. It has a thin blubber layer relative to radius (Slip et al., 1992; Beck & Smith, 1995) and shows little fluctuation in depth during energy deficit (Ryg et al., 1988). This may explain the differences observed by Arnould et al, where the mammary blubber FA profiles might be expected to reflect a more dynamic site of FA deposition and mobilisation than that of the structural blubber of the neck and rump. In this study, the sample sites used are both likely to represent sites of energy storage rather than structural blubber and are not likely to be subject to the function-based differences inherent in the sites used in the Arnould study. More extensive sampling of NZSLs both in terms of body sites sampled and number of animals may help further clarify these differences. When utilising blubber FAs for the interpretation of diet, FA profiles must be obtained from sites of dynamic energy depots in order to be representative of dietary intake (Budge et al., 2006).

An important limitation of this study is the small sample size available (18 animals) and more specifically, the low number of males (n= 4). While the results presented here add important information to the limited amount currently known about blubber profiles in otariids, increasing the sample size in future years will enable better comparisons to be made between age cohorts, sexes, and reproductive classes of NZSLs. Sample collection at a wider range of body sites would also improve our knowledge of blubber function and metabolism in otariids in general.

4.2. Conclusion

This study has demonstrated the homogeneity of blubber FA profiles from the pelvic and thoracic body regions of by-caught NZSLs during the months of February to May 2005 and 2006. This suggests that utilising either the pelvic or thoracic body sample site of by-caught or live sampled NZSLs for blubber FASA during these months will give a comparable interpretation of diet, thus validating current research techniques.

The results of this study present an important foundation for which to develop consistent and accurate blubber sampling techniques in order to investigate the diet of the NZSL through blubber FASA.

Further research is however required in the NZSL and other pinniped species to confirm these results and investigate both general blubber distribution and the deposition and mobilisation of lipids.

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~ CHAPTER 3 ~

The stratification of fatty acids in the blubber of the New Zealand sea lion (*Phocarctos hookeri*) and implications for dietary analysis

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Abstract

Fatty acid signature analysis (FASA) is a powerful tool for investigating the foraging ecology of marine mammals by utilising the fatty acid (FA) profiles from blubber. This technique is based on the principle that FAs from prey species become deposited in a relatively unaltered state into the blubber of monogastric predators. However, studies of cetaceans, phocids and one otariid species have documented the vertical stratification of FAs in blubber. As a result, dietary interpretations may vary depending on the blubber depth sampled. The aim of this study was to investigate the vertical stratification of FAs in the blubber of the New Zealand sea lion (NZSL), *Phocarctos hookeri*. Full blubber cores were collected from the thoracic and pelvic areas of 20 adult NZSLs incidentally caught by the squid fishery around the Auckland Islands (50º42'S, 166º5'E). They were divided into inner and outer halves to determine if stratification occurred. In both the thoracic and pelvic blubber, the inner part displayed much larger concentrations of SFA and LC-MUFA and lower concentrations of SC-MUFA than the outer blubber layer. Both blubber sites showed no significant difference in PUFA levels between the layers. These results indicate that vertical stratification of FAs occurs in the blubber of the NZSL. I suggest that when sampling NZSLs, a full blubber core should be obtained to avoid inconsistent interpretations of diet. This study adds to the limited information currently available about the FA characteristics of otariid blubber.

Keywords: Blubber · fatty acid signature analysis · New Zealand sea lion · stratification

Abbreviations: Fatty acid (FA); Fatty acid signature analysis (FASA); Monounsaturated fatty acid (MUFA); New Zealand sea lion (NZSL); Polyunsaturated fatty acid (PUFA); Saturated fatty acid (SFA).

1. Introduction

Understanding the diet of marine mammal species is a key aspect of marine ecosystem ecology and deciphering food web relationships. Such knowledge enhances species management and conservation practices and improves the accuracy of estimations of anthropogenic impacts such as fishery operations, which may act as competition for marine mammal food sources.

However, an aquatic existence makes investigating the foraging ecology of marine mammals logistically difficult since most prey are consumed below the surface thus inhibiting direct observation. Analysis has therefore relied on indirect methods, entailing the recovery of prey hard parts through stomach content of by-caught or stranded animals (Pauly et al., 1998; Santos et al., 2001; Dehn et al., 2007) and faecal sample analysis of hauled out animals (Reid & Arnould, 1996; Merrick et al., 1997; Childerhouse et al., 2001; Trites, 2001; Sinclair & Zeppelin, 2002). Such techniques have nonetheless shown bias toward the differential gut passage and erosion rates of prey hard parts (Dellinger & Trillmich, 1987; Gales & Cheal, 1992; Bowen, 2000; Staniland, 2002; Arim & Naya, 2003; Tollit et al., 2003) and are restricted to more recent meals.

Recently there has been much interest in developing a biochemical approach to potentially overcome some of the biases of conventional analysis. The fatty acid signature analysis (FASA) of milk (Iverson et al., 1997a; Brown et al., 1999; Iverson, 2002; Lea et al., 2002; Staniland & Pond, 2004) and blubber (Walton et al., 2000; Bradshaw et al., 2003; Olsen & Grahl-Nielsen, 2003; Walton & Pomeroy, 2003; Krahn et al., 2004; Herman et al., 2005; Ridoux et al., 2007) is based on the principle that fatty acids (FAs) pass through the predator gut relatively unaltered and become incorporated into adipose tissues. Adipose tissues may therefore provide a potential record of ingested prey items over a period of time (Iverson, 2002). In pinnipeds, collection of milk is only possible in hauled out lactating females (Brown et al., 1999; Staniland & Pond, 2004), thus severely limiting the age and sex status of the potential sample group. The FASA of blubber may be a better analytical tool as it can be performed on both sexes, all age cohorts (Kirsch et al., 2000;

Falk-Petersen et al., 2004; Beck et al., 2005) and pinnipeds at sea by remote sampling (Hoberecht et al., 2006).

It has become increasingly apparent that FASA of blubber has its own inherent biases. Studies of cetaceans (Koopman et al., 1996; Olsen & Grahl-Nielsen, 2003; Krahn et al., 2004; Smith & Worthy, 2006; Budge et al., 2008), phocid seals (Kakela & Hyvarinen, 1993; Fredhiem et al., 1995; Kakela & Hyvarinen, 1996; Best et al., 2003; Grahl-Nielsen et al., 2005; Wheatley et al., 2007; Strandberg et al., 2008) and a single otariid study (Arnould et al., 2005) have documented the vertical stratification or biochemical layering of FAs from the inner to outer blubber layers. A general pattern of stratification can be seen amongst marine mammals, with variable magnitude between species (Fredhiem et al., 1995; Hooker et al., 2001; Krahn et al., 2004). There is a higher concentration of saturated fatty acids (SFA), long chain monounsaturated fatty acids (LC-MUFA; chain length >18 carbons) and polyunsaturated fatty acids (PUFA) within the inner blubber layer and a higher concentration of short chain monounsaturated fatty acids (SC-MUFA; chain length <18 carbons) within the outer layer (Iverson, 2002; Olsen & Grahl-Nielsen, 2003). Studies suggest that this FA stratification is attributed to the inner blubber (closest to underlying muscle) having a greater metabolic capacity in terms of lipid deposition and mobilisation than the outer (Ackman et al., 1975a; Koopman et al., 1996; Strandberg et al., 2008). If stratification occurs then interpretations of diet may differ depending on the blubber layer sampled (Aguilar & Borrell, 1991b; Koopman et al., 1996; Hooker et al., 2001; Best et al., 2003; Thiemann et al., 2004; Wheatley et al., 2007; Strandberg et al., 2008). This makes it important to investigate species-specific characteristics of FA stratification in order to ensure the best protocol of blubber sampling for FASA is used for the interpretation of diet. This will result in an improved understanding of fishery impacts on prey food sources and translate into appropriate species management solutions (Budge et al., 2006).

There has been little investigation into the presence of FA stratification in otariid seals (Arnould et al., 2005). In the pelvic blubber of some otariids there may also be an additional blubber layer beneath the panniculus muscle, as for example in Steller sea lions (*Eumetopias jubatus*) (Budge et al., 2006). The function of this layer is not yet known,

neither is its presence within most other otariid or phocid species. If the FA content below the muscle is different to more superficial blubber, this may further complicate the interpretation of diet from pelvic blubber samples. For this reason, it is important to address FA variability between adipose tissue stores above and below the panniculus muscle in otariid species to ensure accurate interpretations of diet when pelvic samples are used.

The New Zealand sea lion (NZSL), *Phocarctos hookeri*, is New Zealand's only endemic otariid and classified as a 'threatened' species (Hitchmough et al., 2007), for which the diet is not yet fully understood (Chilvers, 2008). Of particular concern to the species conservation and recovery is the Auckland Island squid fishery operating annually on the sub-Antarctic Auckland Islands shelf (50º42'S, 166º5'E). This is responsible for the indirect capture of sea lions and is a possible competitior for a food source (Wilkinson et al., 2003; Chilvers, 2008). In order to investigate potential interactions between the sea lions and fisheries, research is currently being undertaken into the foraging ecology of the NZSL through FASA of blubber (Meynier et al., 2008). This investigation relies on blubber collected from two body sample sites (dorsal pelvic and ventral thoracic regions) from two sample groups (live-sampling at the Auckland Islands and by-caught sea lions) (see Chapter 2).

The purpose of this study is to determine whether stratification of FAs occurs in either of the two blubber sample sites (pelvic and thoracic) routinely used for current dietary analysis of the NZSL.

2. Materials and Methods

2.1. Sample collection

The sample group was comprised of NZSLs that were incidentally captured by vessels operating on the Auckland Islands shelf squid fishery during the fishing season (February to May) of the years 2005, 2006 and 2007. Carcasses were frozen on board and transported to Massey University, Palmerston North, New Zealand for necropsy. This was under contract with the Conservation Service Providers administered by the New Zealand Department of Conservation and Ministry of Fisheries.

Blubber samples were analysed from the thoracic area of 18 by-caught NZSLs (6 males and 12 females). Due to limited quantities of previously collected blubber tissue (and prior to the knowledge of this study) only eight animals (year 2007) had pelvic blubber samples analysed for stratification (Table 1, see thoracic and pelvic location in Chapter 2). All blubber samples were collected by cutting a 60 x 60 mm blubber piece extending from the outer epidermis to the underlying muscle from both the thoracic (Fig. 1, A & C) and pelvic sample sites (Fig. 1, B & D). In all pelvic samples there was a thin strip of muscle mid-way through the blubber (panniculus) (Fig. 1, B). Samples were then sealed in plastic bags and frozen at -20ºC.

2.2. Lipid Extraction and Esterification

Lipids were extracted from blubber samples employing a modified Folch method (Folch et al., 1957). Thawed blubber samples were extended to their full length and halved, giving two blubber portions, the top (outer) and bottom (inner) (Fig. 1). Any skin or muscle attachment was then removed. Any freeze burned areas indicated by a darkish yellow colour were also discarded. A 0.5 g sub-sample was taken from the center of each blubber section (Budge et al., 2006) and homogenised in a 15 ml, 2:1 chloroform/methanol solution using butylated hydroxytoluene (BHT) as an antioxidant. The extract was filtered and washed with 1% sodium chloride to a final ratio of 8:4:3 chloroform:methanol:saline water.

Fatty acid methyl esters (FAMES) were prepared from 30 g of the lipid extract using 1.5 ml of 10% boron trifluoride in methanol (methylating reagent) and 1.5 mL of toluene. Each

extract was capped under nitrogen, and heated at 50°C overnight. FAMES were then extracted into hexane and stored over anhydrous sodium sulphate at -20°C before chromatography analysis.

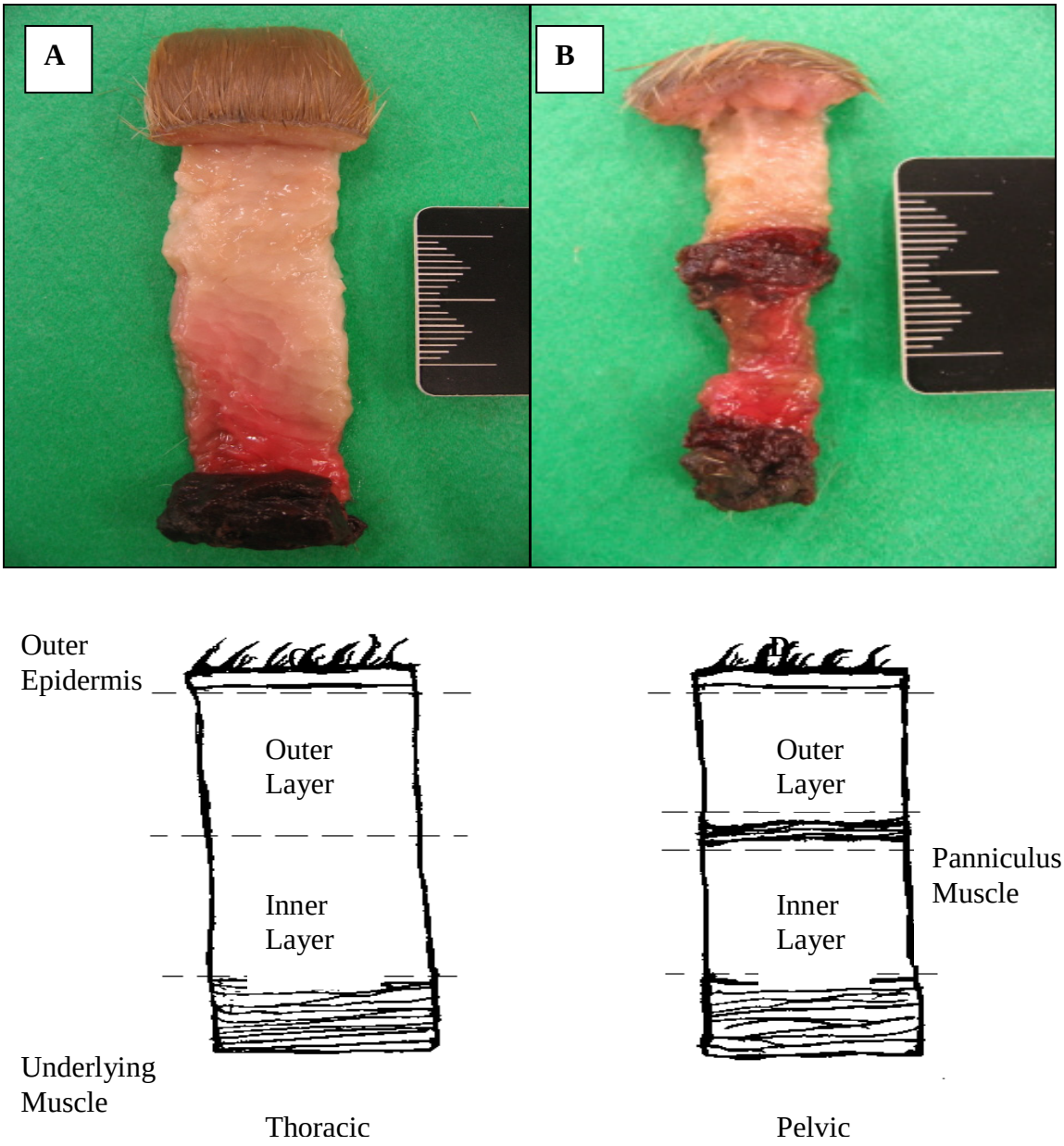

Fig. 1. *Top:* Cross section through the blubber of the (A) thoracic and (B) pelvic areas taken from each New Zealand sea lion (*Phocarctos hookeri*) during necropsy (Scale = 2 cm). *Bottom:* Division of (C) thoracic and (D) pelvic blubber into layers analysed.

Table 1. By-catch year, identification number (I.D. No.) and sex of New Zealand sea lions (*Phocarctos hookeri*) analysed with the blubber sample sites collected.

* L indicates lactating female, F non-lactating females and M males

(Y) indicates 'collected', (N) indicates 'not collected'

Sea lion			**Sample Type**	
Year	**I.D. No.**	**Sex**	**Thoracic**	**Pelvic**
2005	503	M	Y	N
2005	504	M	Y	N
2005	505	L	Y	N
2005	506	M	Y	N
2005	507	F	Y	N
2005	508	L	Y	N
2005	509	M	Y	N
2005	514	M	Y	N
2005	515	L	Y	N
2005	516	F	Y	N
2006	601	F	Y	N
2006	602	L	Y	N
2007	701	L	Y	Y
2007	702	M	Y	Y
2007	703	L	Y	Y
2007	704	L	Y	Y
2007	705	L	Y	Y
2007	706	L	Y	Y
2007	707	L	N	Y
2007	708	M	N	Y

2.3. 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 and fitted with a 30 m × 0.25 mm i.d. column coated with 50% cyanopropyl polysiloxane (0.25 μm film thickness; J&W DB-23; Folsom, Calif.). Helium was used as the carrier gas. FAMES (1 ul) were injected 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}, hold for 5 min, then finally rise to 236°C at 2°C min^{-1}.

2.4. Identification and quantitation of FAs

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-chek 68D standard was injected regularly to check the quanitification of each FA. Peak areas were measured by an electronic integrator attached to the gas chromatograph (CLASS-VP version 7.3, Shimadzu Scientific Instruments, Inc., Columbia, MD). Each chromatogram was manually checked to ensure the accurate identification of FA peaks and adjusted if incorrect. FAs were expressed as mass percent of total FAs and were designated by the shorthand IUPAC (International Union of Pure and Applied Chemistry) nomenclature of carbon chain length: number of double bonds and location (n-x) of the double bond nearest to the terminal methyl group.

2.5. Data analysis

A combination of multivariate and univariate statistical analysis was carried out to examine the variability of the inner and outer thoracic and pelvic blubber layer FA profiles (Minitab Release 15.0 MINITAB Inc. 2007). The data was normalised by arcsine square-root transformation prior to parametric testing to reduce heterogeneity of data. FAs were grouped into total saturated fatty acids (SFA), short chain monounsaturated fatty acids (SC-

MUFA; chain length <18 carbons), long chain monounsaturated fatty acids (LC-MUFA; chain length >18 carbons) and polyunsaturated fatty acids (PUFA). Their percentages were tested along with individual FAs.

Multivariate analysis.

Due to the multivariate nature of FA analysis, principal component analysis (PCA) was used to view the data in two dimensions and to investigate the possibility of upper and lower groupings of FAs in the thoracic and pelvic blubber portions. A bi-plot of principal component 1 (PC1) and principal component 2 (PC2) was produced describing most of the variation among the samples.

Univariate analysis.

Paired-t tests were carried out to detect any differences between individual FAs and major FA groupings of the inner and outer portions of thoracic and pelvic blubber.

3. Results

Thirty-two FAs were consistently identified in all thoracic and pelvic blubber samples. Individual FAs present at < 0.5% of total FA concentration were excluded from analysis and these were 10:0, 12:0, 15:1, 18:3n-4, 18: 3n-6, 20:2n-6, 20:3n-3, 21:5n-3, 22:5n-6. This reduced the total number of FAs included in the analysis to 23, which accounted for approximately 97- 99% of total FAs observed in the inner and outer thoracic and pelvic blubber. All FAs were present in both layers although some were in more variable concentrations (Table 2).

3.1. Thoracic blubber stratification

The first two components derived from principal component analysis (PCA) accounted for 54% of the variation (PC1 28%, PC2 26%) amongst samples. The PCA revealed a clear separation of the inner and outer blubber layers into two distinct groups (Fig. 2) separated along PC2 driven by high positive values (indicating outer blubber) of 20:4n-6, 18:1n-7, 18:3n-3 and high negative values (indicating inner blubber) of 20:1n-11, 20:0 and 14:0 (Fig. 2).

Three sea lions (602, 701 and 702) showed variable levels of SFA, MUFA and PUFA contents, falling outside the average values and general cluster of points on the PCA graph (Fig. 2). Notable FA differences were higher than average values of 14:0, 16:0 and lower than average values of 18:1n-9, 20:5n-3 and 22:6n-3.

There were significantly variable proportions of grouped FAs between the inner and outer thoracic blubber layers; greater proportions of SFA and LC-MUFA within the inner compared to the outer layers (SFA: t= -5.17; P= 0.000; LC-MUFA: t= -5.35; P= 0.000). The reverse was observed for SC-MUFA (SC-MUFA: t= 10.99; P= 0.000). However, there was no significant variation observed in the proportion of PUFA between the layers (PUFA: t= 0.21; P= 0.839), with average proportions being highly similar (Table 2, Fig. 3).

Table 2. Fatty acid (FA) profiles from the inner and outer layers of thoracic (n=18) and pelvic (n=8) blubber from 20 by-caught New Zealand sea lions (*Phocarctos hookeri*) (% mass of total FAs ± S.D). P-values for paired-t tests area also shown, highlighted values indicate significance (P-value < 0.05).

FA	Thoracic Blubber			Pelvic Blubber		
	Inner layer (Mean %) ± S.D	**Outer layer (Mean %) ± S.D**	**P-value Paired-t**	**Inner layer (Mean %) ± S.D**	**Outer layer (Mean %) ± S.D**	**P-value Paired-t**
SFA						
14:0	7.62 ± 2.61	6.37 ± 2.72	**0.023**	9.41 ± 2.30	8.38 ± 2.46	0.209
15:0	0.51 ± 0.13	0.48 ± 0.11	0.337	0.52 ± 0.11	0.47 ± 0.10	0.246
16:0	15.93 ± 1.70	13.58 ± 1.67	**0.000**	18.66 ± 1.13	15.01 ± 2.70	**0.009**
17:0	0.40 ± 0.13	0.34 ± 0.13	**0.007**	0.45 ± 0.10	0.32 ± 0.14	**0.000**
18:0	3.02 ± 0.68	1.91 ± 0.88	**0.000**	3.93 ± 0.93	1.91 ± 1.62	**0.001**
20:0	0.16 ± 0.03	0.05 ± 0.22	**0.007**	0.13 ± 0.12	0.06 ± 0.11	0.074
SC-MUFA						
14:1n5	0.22 ± 0.23	0.36 ± 0.12	**0.007**	0.20 ± 0.49	0.52 ± 0.12	0.077
16:1n-7	7.10 ± 3.11	9.87 ± 2.67	**0.000**	6.39 ± 3.08	10.26 ± 1.74	**0.001**
17:1	0.71 ± 0.36	0.87 ± 0.17	0.137	0.53 ± 0.26	0.69 ± 0.15	0.064
18:1n-5	0.30 ± 0.07	0.30 ± 0.07	**0.000**	0.30 ± 1.10	0.71 ± 0.09	0.545
18:1n-9	29.19 ± 2.84	33.14 ± 2.56	0.838	23.29 ± 10.62	27.52 ± 3.93	**0.000**
18:1n-7	3.85 ± 0.47	4.20 ± 0.44	**0.000**	3.78 ± 8.01	7.03 ± 0.47	0.182

LC-MUFA						
20:1n-11	2.01 ± 0.30	1.51 ± 0.32	0.080	1.59 ± 0.33	1.56 ± 0.19	0.842
20:1n-9	9.07 ± 2.39	7.92 ± 3.18	0.274	9.79 ± 2.74	8.63 ± 1.68	0.096
22:1n-11	2.20 ± 0.77	1.37 ± 0.81	**0.000**	2.73 ± 0.83	1.62 ± 1.16	**0.000**
PUFA						
18:2n-4	0.17 ± 0.03	0.16 ± 0.04	0.955	1.16 ± 0.35	1.42 ± 0.11	**0.001**
18:2n-6 (LL)	1.49 ± 0.22	1.57 ± 0.19	**0.001**	0.24 ± 0.43	0.28 ± 0.17	**0.018**
18:3n-3	0.37 ± 0.14	0.42 ± 0.15	0.149	0.37 ± 0.12	0.36 ± 0.16	0.402
20:3n-6	0.14 ± 0.03	0.09 ± 0.15	0.421	0.68 ± 0.04	0.45 ± 0.038	0.383
20:4n-6 (AA)	0.47 ± 0.24	0.67 ± 0.25	**0.019**	3.84 ± 0.15	2.79 ± 0.64	**0.026**
20:5n-3 (EPA)	2.54 ± 0.84	2.45 ± 0.99	0.574	2.65 ± 1.32	2.30 ± 1.82	0.275
22:5n-3 (DPA)	2.84 ± 0.64	2.87 ± 0.78	0.734	9.52 ± 0.78	7.72 ± 1.00	**0.030**
22:6n-3 (DHA)	9.65 ± 2.09	9.47 ± 2.41	0.783	1.16 ± 3.15	1.42 ± 3.11	**0.001**
Sum SFA	27.65 ± 3.36	22.73 ± 3.49	**0.000**	33.00 ± 4.54	26.10 ± 2.42	**0.000**
Sum SC-MUFA	40.35 ± 3.47	47.57 ± 3.31	**0.000**	34.50 ± 4.17	46.74 ± 5.03	**0.000**
Sum LC-MUFA	13.80 ± 3.18	11.19 ± 3.36	**0.000**	14.10 ± 2.64	11.80 ± 3.67	**0.023**
Sum PUFA	17.50 ± 2.79	17.64 ± 2.75	0.839	18.45 ± 3.79	15.34 ± 3.70	0.155

LL linoleic acid; AA arachidonic acid; EPA eicosapentaenoic acid; DPA Docosapentaenoic acid; DHA docosahexaenoic acid; SFA saturated fatty acids; MUFA monounsaturated fatty acids; SC short-chain; LC long chain; PUFA polyunsaturated fatty acid.

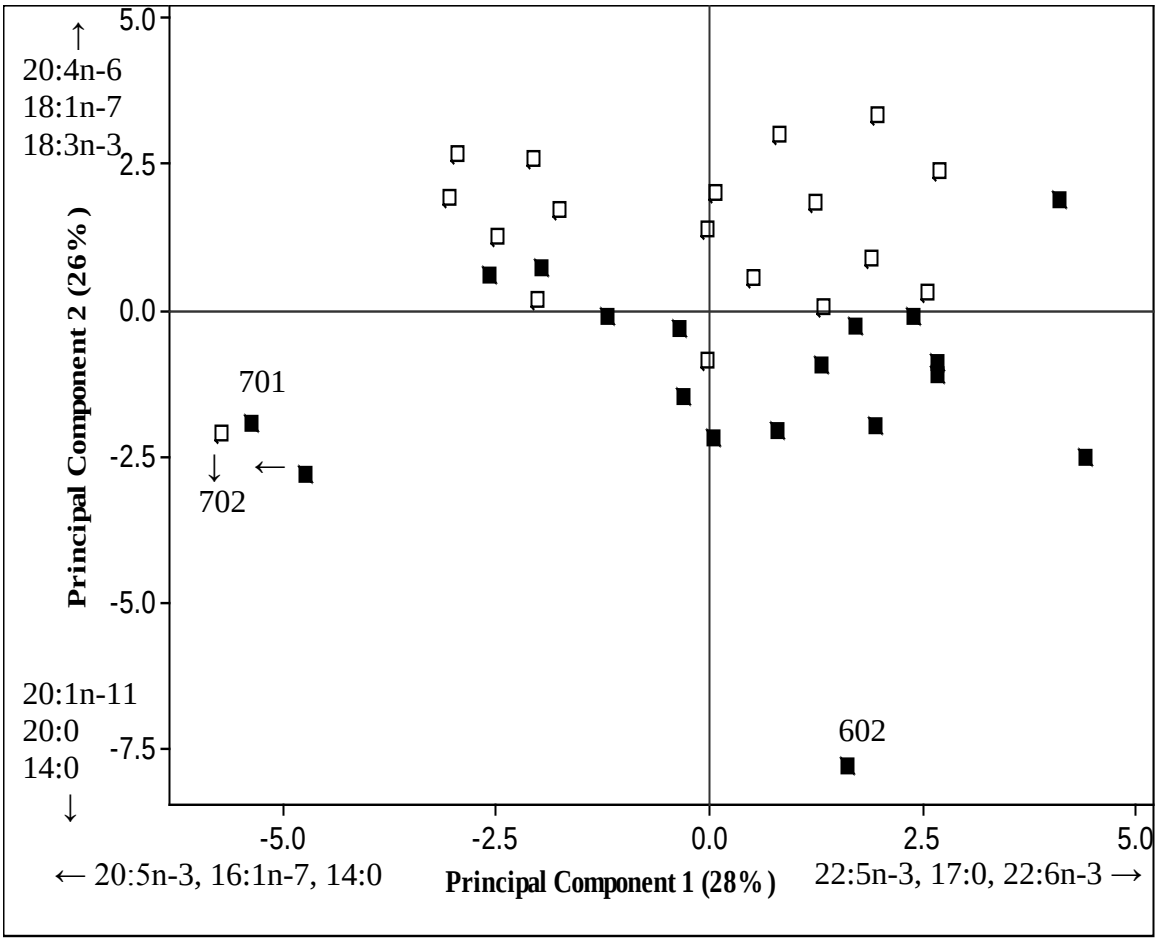

Fig. 2. Bi-plot of the first and second principal components (PCs) derived from the fatty acid (FA) composition of the inner (black squares) and outer (white squares) thoracic blubber layers of 18 by-caught New Zealand sea lions (*Phocarctos hookeri*). The three most important FA driving each PC are displayed on the side of each axis with direction. Outliers are indicated on graph.

Twelve of 23 FA were found in significantly different proportions between the inner and outer layers (Table 2). The inner layer had significantly greater proportions of 14:0, 16:0, 17:0, 18:0, 20:0, 22:1n-11 while the outer layer had significantly greater proportions of 14:1n-5, 16:1n-7, 18:1n-5, 18:1n-7, 18:2n-6, 20:4n-6 (Table 2). The most abundant FAs within the inner blubber layer were 18:1n-9, 16:0, 22:6n-3, 20:1n-9, 14:0, 16:1n-7 and in the outer layer were 18:1n-9, 16:0, 16:1n-7, 22:6n-3, 20:1n-9, 14:0 (Table 2).

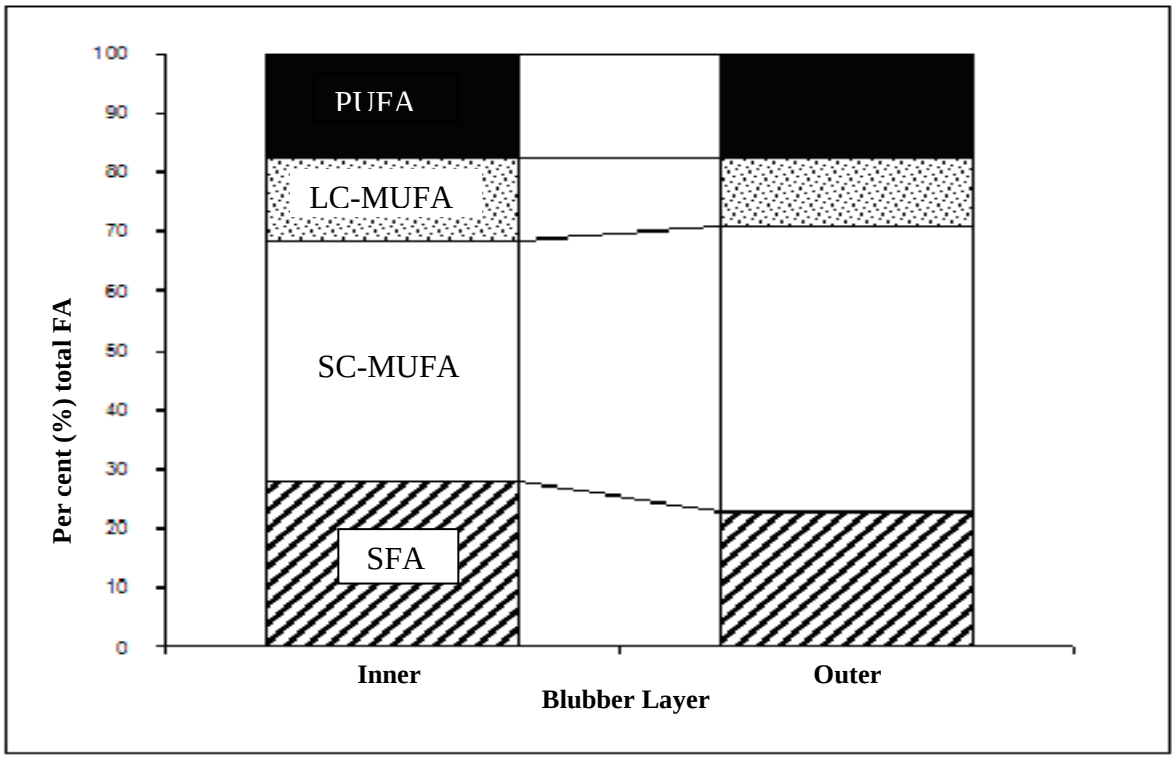

Fig. 3. Mean proportion of polyunsaturated fatty acids (PUFA), saturated fatty acids (SFA) and short chain monounsaturated fatty acids (SC-MUFA) and long chain monounsaturated fatty acids (LC-MUFA) in the inner and outer blubber portions of thoracic blubber from 18 by-caught New Zealand sea lions (*Phocarctos hookeri*).

3.2. Pelvic blubber stratification

With the pelvic data, the number of FAs utilised for PCA was reduced from 23 to 16, due to small number of blubber samples analysed (16) that initially gave more individuals than variables. The original 23 FAs were reduced to 16 by eliminating seven FAs with the lowest overall standard deviations: these seven FAs were 15:0, 17:0, 20:0, 17:1, 18:2n-4, 18:3n-3, 22:5n-6.

The first two components derived from PCA accounted for 59% of the variation (PC1 40%, PC2 19%, PC3 14%), amongst samples. The PC plot revealed a clear separation of the inner and outer blubber layers into two distinct groups similar to thoracic blubber analysis (Fig. 4). The observed separation was also largely driven on PC2 by the high positive

values (indicating outer blubber) 14:0, 20:1n-9 and 18:1n-5, and high negative values (indicating inner blubber) 14:1n-5, 22:6n-3 and 16:1n-7.

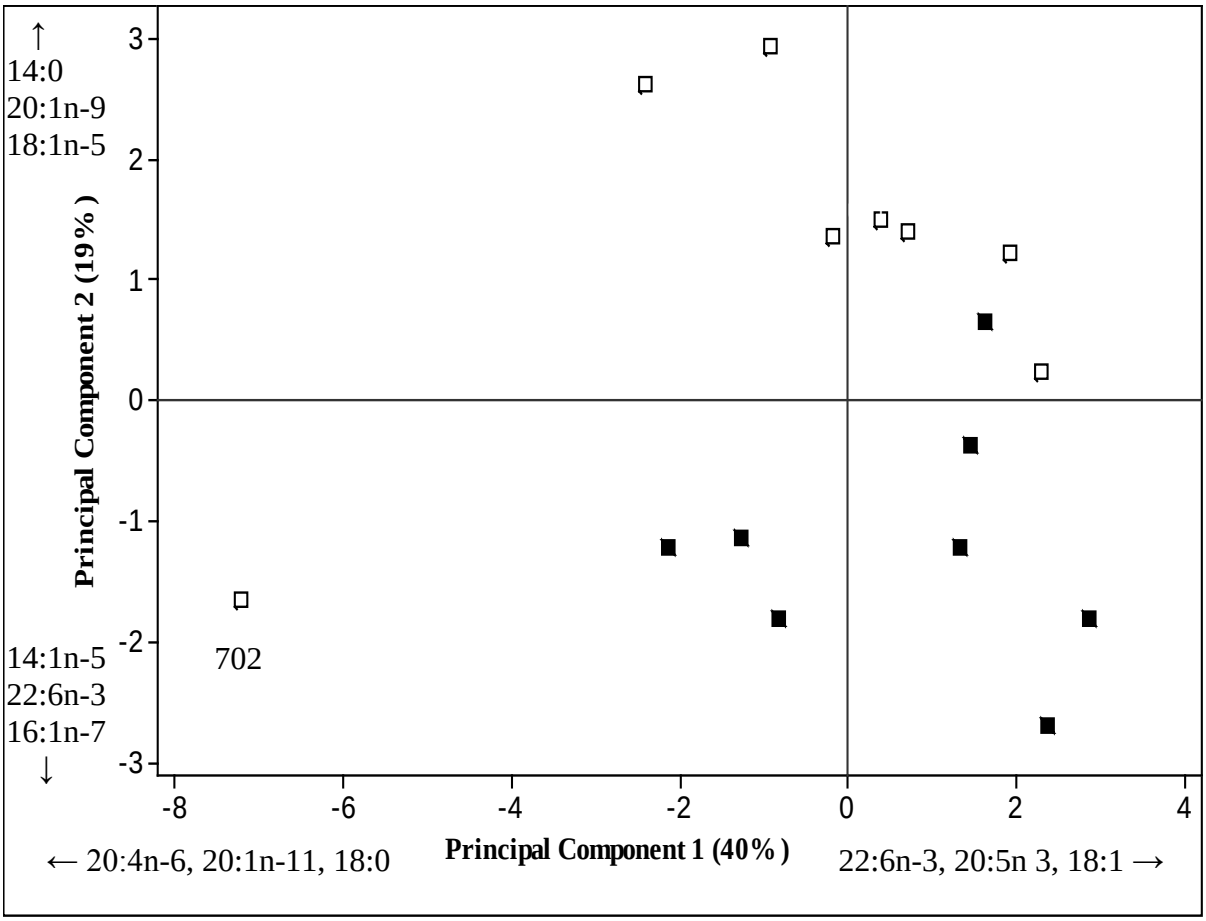

Fig. 4. Bi-plot of the first and second principal components (PCs) derived from the fatty acid (FA) composition of the inner (black squares) and outer (white squares) pelvic blubber layers of eight by-caught New Zealand sea lions (*Phocarctos hookeri*). The three most important FAs driving each PC are displayed on the side of each axis with direction. Outliers are indicated on graph.

Sea lion (702) was displayed as an outlier from the main group on PCA graph (Fig. 4). It had much higher proportions of SFA (particularly 14:0, 16:0), lower proportions of PUFA (particularly 20:5n-3, 22:6n-3) and also lower proportions of 18:1n-9 in the outer pelvic blubber layer compared with the other seven animals.

There were significantly variable proportions of grouped FAs between the inner and outer pelvic blubber: greater proportions of SFA and LC-MUFA in the inner compared to the outer blubber layers (SFA: t= -6.64; P= 0.000, LC-MUFA: t= -2.88; P= 0.023) and greater proportions of SC-MUFA in the outer compared to the inner layers (SC-MUFA: t= 11.29; P= 0.000). Like the thoracic blubber, there was no significant variability between proportions of PUFA in the inner and outer blubber layers (t= -1.60; P= 0.155).

Eleven of 23 FAs showed significant variability between the upper and lower pelvic layers (P-values < 0.05). The inner blubber had significantly greater proportions of 16:0, 17:0, 18:0, 22:1n-11, 20:4n-6, 22:5n-3 and the outer blubber had significantly greater proportions of 16:1n-7, 18:1n-9, 18:2n-6, 18:2n-4, 22:6n-3 (Table 2). The most abundant FAs within the inner blubber layer were 18:1n-9, 16:0, 20:1n-9, 14:0, 16:1n-7 and 18:1n-9, 16:0, 16:1n-7, 20:1n-9, 14:0, 22:6n-3 within the outer (Table 2).

3.3. Specific comparison of pelvic and thoracic stratification (six sea lions)

The six sea lions (701, 702, 703, 704, 705, 706), which had both thoracic and pelvic blubber samples analysed (Table 1) were directly compared to investigate the similarity of FA profiles between the inner and outer layers at each site.

The first two components derived from PCA accounted for 53% of the variation (PC1 35%, PC2 18%) amongst samples. The PCA revealed a clear separation of the inner and outer blubber layers into two distinct groups (Fig. 5), again separated along PC2 driven by high positive values (indicating outer blubber) of 18:1n-9, 16:1n-7, 18:2n-6 and high negative values (indicating inner blubber) of 16:0, 18:0, 17:0 (Fig. 5).

Outliers included sea lions 701 and 702 that had both thoracic and pelvic blubber fall outside clustering of main points due to variable proportions of SFA and PUFA compared to average values of other sea lions in conjunction with previous PC results (Fig. 2 & 4). In general, the proportion of grouped FAs between the inner and outer layers of both sample sites were very similar. However, the outer thoracic blubber of all sea lions had

significantly higher concentrations of PUFA than observed in the outer pelvic blubber (P= 0.009) (Table 5, Fig. 6).

Only three of 23 individual FAs (20:4n-6, 22:5n-3 and 22:6n-3) (PUFA) showed significant variability between the sites and this was within the outer layers (20:4n-6, P= 0.0031; 22:5n-3, P= 0.030, 22:6n-3, P= 0.009). All three FAs were found in consistently greater concentrations within the thoracic outer blubber than the pelvic.

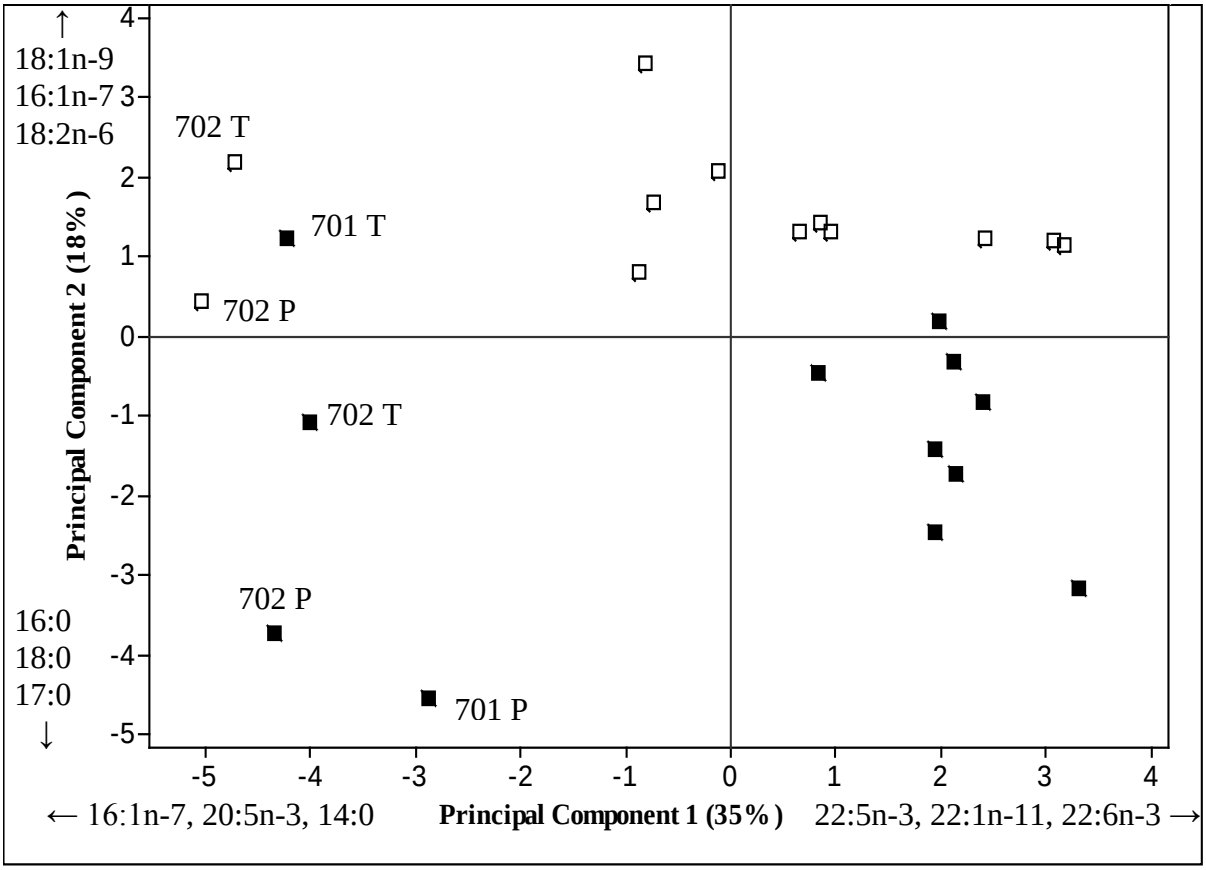

Fig. 5. Bi-plot of the first and second principal components (PCs) derived from the fatty acid (FA) composition of the inner (black squares) and outer (white squares) blubber layers from the thoracic and pelvic sample sites of six by-caught New Zealand sea lions (*Phocarctos hookeri*). The three most important FA driving each PC are displayed on the side of each axis with direction. Outliers are indicated on graph.

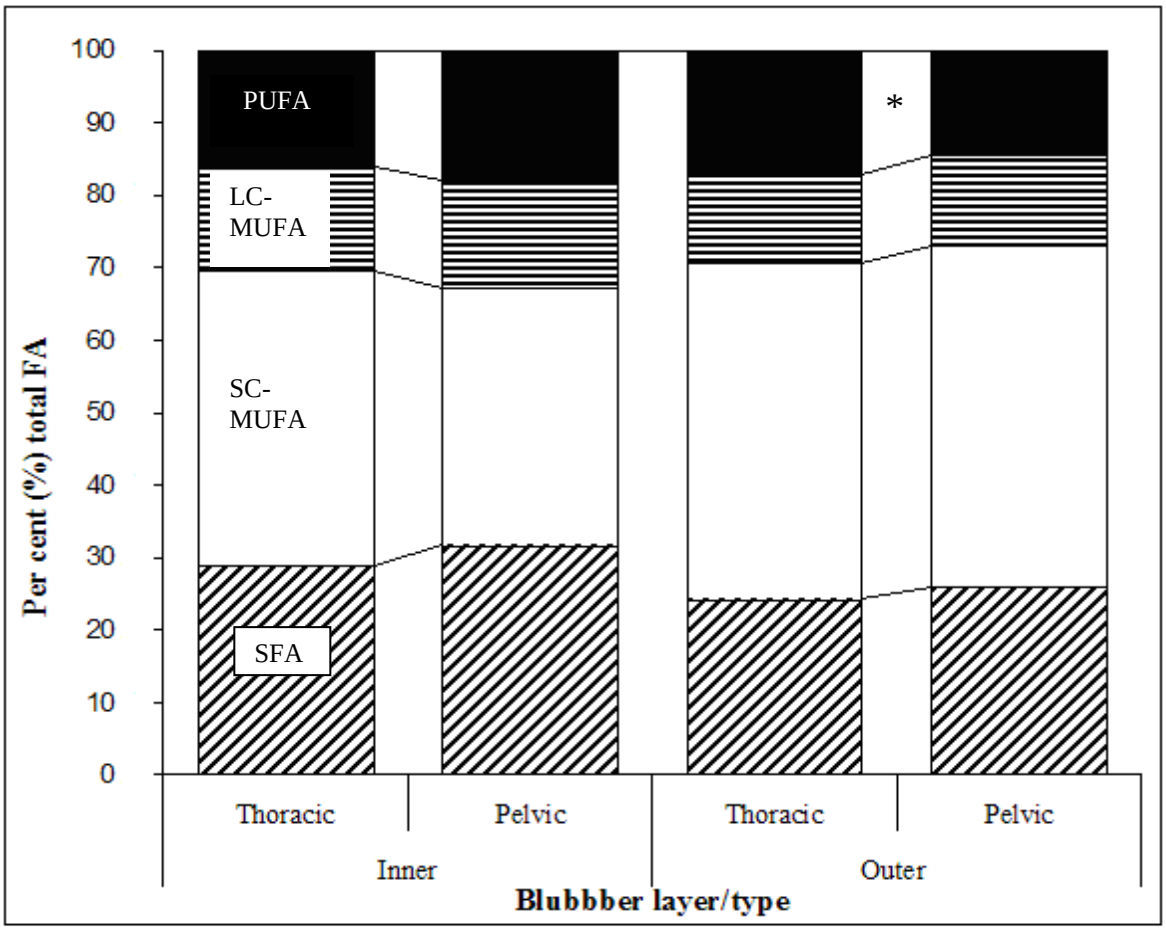

Fig. 6. Mean proportion of saturated fatty acids (SFA), short chain monounsaturated fatty acids (SC-MUFA), long chain monounsaturated fatty acids (LC-MUFA) and polyunsaturated fatty acids (PUFA) in the inner and outer blubber portions of pelvic and thoracic blubber sample sites of six by-caught New Zealand sea lions (*Phocarctos hookeri*). * Indicates the significant variability between the PUFA% of thoracic and pelvic outer layers (P-value < 0.05).

4. Discussion

The results of this study display significant variability between the FA profiles of the inner and outer blubber layers, suggesting that biochemical stratification of FAs occurs in both the pelvic and thoracic blubber of the NZSL. This is the first study to investigate the stratification of blubber in a sea lion species and only the second for an otariid seal. Additionally, this is the first study to address the stratification of the adipose tissue above and below the panniculus muscle in the pelvic blubber of sea lions.

Blubber has at least four primary functions; energy storage, insulation, streamlining and buoyancy (Ryg et al., 1988; Iverson, 2002). The composition of blubber FAs is a combination of direct dietary consumption, modification of ingested FAs and biosynthesis of endogenous FAs. In adipose tissue, FAs are either stored during excess energy intake or metabolised during negative energy input (Young, 1976; Iverson, 2002; Budge et al., 2006). The stratification of FAs has been predominantly attributed to the physiological demands of the inner and outer blubber layers, creating a gradient due to the differential deposition and metabolism of individual FAs (Fredhiem et al., 1995; Koopman et al., 1996; Best et al., 2003; Andersen et al., 2004; Smith & Worthy, 2006; Wheatley et al., 2007; Strandberg et al., 2008). It is generally accepted that the inner blubber closest to the major blood vessels and underlying muscle is likely to be more metabolically active, subjected to higher FA turnover than the outer blubber layer (Ackman et al., 1975a; Ackman et al., 1975b; Lockyer et al., 1984; Koopman et al., 1996); performing as the major site of initial lipid deposition and mobilisation (Koopman et al., 1996; Best et al., 2003; Grahl-Nielsen et al., 2005; Wheatley et al., 2007; Strandberg et al., 2008). During periods of surplus energy intake the inner blubber readily accumulates dietary PUFA (Best et al., 2003) and the FA composition is demonstrated to be closer (although not identical) to that of diet, than the composition in the outer layer (Olsen & Grahl-Nielsen, 2003; Andersen et al., 2004; Grahl-Nielsen et al., 2005; Wheatley et al., 2007). The outer layer is suggested to have a predominantly structural role and be more metabolically inert and contain more endogenously derived FAs (Lockyer et al., 1984; Aguilar & Borrell, 1991b; Kakela & Hyvarinen, 1996; Hooker et al., 2001; Iverson, 2002).

The stratification of FAs may further increase the insulating properties of the tissue (Lockyer et al., 1984; Fredhiem et al., 1995; Iverson, 2002; Olsen & Grahl-Nielsen, 2003). Shorter chain FA and unsaturated FA have much lower melting points compared with longer chain PUFA and SFA (Raclot, 2003). The selective modification and deposition of FAs with lower melting points in the outer blubber will ensure that cell membrane fluidity and function is maintained in the face of cold environments (Pond et al., 1992; Fredhiem et al., 1995; Iverson, 2002; Olsen & Grahl-Nielsen, 2003).

4.2. FA stratification in NZSL blubber

This study generally exhibited similar results to previous pinniped research, showing greater concentrations of SFA and LC-MUFA within the inner blubber layers and greater proportions of SC-MUFA in the outer blubber (Kakela et al., 1993; Best et al., 2003; Andersen et al., 2004; Arnould et al., 2005; Grahl-Nielsen et al., 2005; Wheatley et al., 2007; Strandberg et al., 2008). A recent study of the Cape fur seal (*Arctocephalus pusillus pusillus*) is the only other published otariid research, which also suggests stratification of FAs. However, PUFA concentrations in the inner blubber layers of the Cape fur seal were significantly greater than the outer (Arnould et al., 2005) and were similar to the elephant seal (*Mirounga leonina*) (Best et al., 2003). The NZSL blubber displayed a general uniformity of PUFA concentrations between the blubber layers which is consistent with most phocid species (Fredhiem et al., 1995; Kakela & Hyvarinen, 1996). This species variability in PUFA content may be related to diversity of diets and lipid content of prey items (Arnould et al., 2005; Grahl-Nielsen et al., 2005) or differential lipid deposition and mobilisation between predators (Iverson et al., 2004). The nutritional state of the animal (energy surplus or deficiency) and mobilisation of blubber stores at time of sampling may also have large implications for the degree of stratification and presence of dietary PUFA within the inner layer (Best et al., 2003; Mellish et al., 2007; Wheatley et al., 2007). Studies have frequently documented annual or seasonal fluctuations of blubber depths in pinnipeds but few have related this to FA profiling and lipid content (Kakela & Hyvarinen, 1996; Mellish et al., 2007). It is also important to note that lipid extraction and statistical analysis techniques may vary between studies and result in dissimilar stratification characteristics between species (Budge et al., 2006).

In this study the general proportion of FAs in the NZSL blubber was similar to previous pinniped studies with the most dominant SFAs being 16:0 & 14:0, MUFAs 18:1n-9, 20:1n-9, 16:1n-7 and PUFAs 22:6n-3, 22:5n-3, 20:5n-3 (West et al., 1979b; Fredhiem et al., 1995; Best et al., 2003; Andersen et al., 2004; Grahl-Nielsen et al., 2005; Strandberg et al., 2008). Corresponding variable FAs between the sample sites were 16:0, 17:0, 18:0 (dietary and biosynthesised) and 22:1n-11 (dietary), all with higher proportions in the inner blubber and 16:1n-7 (dietary and biosynthesis) 18:2n-6 and 20:4n-6 (dietary) (Iverson et al., 2004) with higher proportions in the outer layers, results which are also similar to previous pinniped studies (Best et al., 2003; Budge et al., 2004; Arnould et al., 2005; Grahl-Nielsen et al., 2005; Wheatley et al., 2007). This comparable pattern of individual FA proportions and placements within the blubber layers of pinnipeds may demonstrate that the FA composition is specifically constructed to meet the physiological and functional needs of the tissue (Kakela & Hyvarinen, 1996; Grahl-Nielsen et al., 2005).

Previous studies in cetaceans and pinnipeds have shown that the degree of stratification may be influenced by the sex, age and reproductive state (particularly lactation) of the animal (Aguilar & Borrell, 1991b; Andersen et al., 2004; Samuel & Worthy, 2004; Arnould et al., 2005; Strandberg et al., 2008). Assuming that lipid stores will be mobilised foremost from the inner blubber layer, lactation may alter FA composition of blubber by depletion of recently deposited PUFA (Best et al., 2003). This may reduce the concentration of PUFA within the inner blubber layer. Furthermore, specific FAs may also be selectively mobilised for milk production (Iverson et al., 1995) and therefore underestimated in blubber FA profiles (Wheatley et al., 2007). In the current study there was no clear effect of lactation or sex on blubber FA stratification. Both lactating females and males shared amongst the highest and lowest concentrations of PUFA within inner blubber layers. However, when directly comparing the outer blubber layers of thoracic and pelvic blubber of six sea lions, there was consistent variability between PUFA concentrations. In all six animals the thoracic blubber had larger concentrations of PUFA than the pelvic in outer blubber layers, with three particular lactating females (704, 703, 706) showing the largest differences. With such a small number of sea lions to compare, these three females may have skewed the results and thus indicated a strong variation of PUFA concentrations between the outer

blubber layers of the sites. Additionally, this was the only variability recorded between the pelvic and thoracic blubber, with other FA proportions shown to be very similar at each site within the layers. This result may not be reliable and further characterisation of these differences would require a larger sample size.

4.3. Conclusion

This study has demonstrated the presence of FA stratification in blubber of by-caught NZSLs at two body sample sites (ventral thoracic and dorsal pelvic regions) during the months of February to May. As suggested by previous authors (Best et al., 2003; Arnould et al., 2005; Wheatley et al., 2007), I recommend that full depth blubber samples from each body sample site be utilised for FASA in order to minimise variable interpretations of diet.

Limits to this study include the small set of samples (n= 20) resulting in a limited number of males (n= 7) inhibiting the comparison of sexes and producing a small variation in age cohorts. The lack of available blubber also resulted in a limited number of animals (n= 6) available for investigation of stratification above and below the panniculus muscle and the direct comparison of FA stratification between body sites. This study was also confined to specific months and therefore represented only a snap shot over time. It did not address the possibilities of annual variation and effects of blubber thickness, lipid content on stratification.

Future studies should be directed at increasing sample size and extending sampling to include representatives of all age cohorts and reproductive groups. Futher investigation into the variability of FA composition above and below the panniculus muscle in the pelvic region is required to gain further insight to the function of blubber layers and representations of diet. Sampling of females at both body sites pre- and post-lactation may also enable a better understanding of the effects of lactation on FA stratification.

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~ CHAPTER 4 ~

Polychlorinated biphenyls and organochlorines in the blubber of by-caught New Zealand sea lions (*Phocarctos hookeri*)

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Abstract

Persistent organochlorines (OCs) and polychlorinated biphenyls (PCBs) were determined in blubber collected from healthy by-caught New Zealand sea lions, *Phocarctos hookeri*, incidentally caught by the squid fishery around the Auckland Islands (50º42'S, 166º5'E) during 2007. PCBs (45 congeners) and a range of OC pesticides used historically in New Zealand were determined in the thoracic blubber of seven adult sea lions (five females, two males). Data was compared to previously recorded levels of OCs and PCBs in NZSLs that died of infectious disease during a 1997/98 epidemic. The relative concentration of detected contaminants were Σdichlorodiphenyltrichloroethane (DDTs) > ΣPCBs > Dieldrin > ΣChlordane > Σhexachlorocyclohexane (HCH). Aldrin and Heptachlor were unable to be detected in any samples. ΣDDT concentrations (*p,p′*-DDE + *p,p′*-DDD + *o,p′*-DDT + *p,p′*-DDT) ranged from 67.4-512.0 ng/g, with *p,p′*-DDE present in the highest concentration. The average ΣPCBs was 74.86 ng/g. The PCB present in the highest concentration was CB138. Six PCBs (1, 3, 4, 77, 104, 169) were not detected in any samples. The level of OCs and PCBs were slightly higher than previously reported for diseased NZSLs. The ΣPCBs in NZSLs was compared to the proposed threshold for adverse affects (including immunosuppression) in marine mammals of 17 mg/kg; the range for NZSLs was 0.034-0.192 mg/kg lipid. This suggests PCBs are unlikely to play a role in disease outbreaks of the NZSL. Levels of OCs and PCBs in the NZSL blubber were lower than those previously reported in New Zealand cetacean species. This study contributes to the relatively small body of literature addressing lipophilic contaminants in New Zealand marine mammal species.

Keywords: Blubber · by-caught · New Zealand sea lion · organochlorine pesticides · polychlorinated biphenyls

Abbreviations: Dichlorodiphenyltrichloroethane (DDT); Hexachlorobenzene (HCB); Hexachlorocyclohexane (HCH); Long range atmospheric transport (LRAT); New Zealand common dolphin (NZCD); New Zealand sea lion (NZSL); Organochlorine contaminants (OCs); Persistent organic pollutants (POPs); Polychlorinated biphenyls (PCBs).

1. Introduction

Persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs) and organochlorine contaminants (OCs) have been recorded in marine environments since the 1970's (Woodwell et al., 1971). These contaminants are subject to bioaccumulation particularly in aquatic food chains due to their highly lipophilic nature and strong resistance to degradation (Tanabe et al., 1994; Connell et al., 1999).

Marine mammals may be subjected to the effects of POPs due to a number of biological traits; they are usually high trophic level feeders, are generally long-lived and store large amounts of lipid within subcutaneous blubber stores. This facilitates the uptake and retention of contaminants. They also exhibit a reduced capacity to metabolise contaminants compared to terrestrial mammals (Hutchinson & Simmonds, 1994; Tanabe et al., 1994; Fossi et al., 1997; Tanabe, 2002). Studies have linked POPs to numerous adverse affects within marine mammal species, particularly immunosuppression preceding infectious disease outbreaks (Aguilar & Borrell, 1994b; Olsson et al., 1994; Ross et al., 1995; De Swart et al., 1996; Van Loveren et al., 2000; Ross, 2002; Tanabe, 2002; Kajiwara et al., 2008b). POPs may also alter hormonal balance causing reproductive impairments such as reduced implantation rate (Reijnders, 1986), premature pupping and increased neonatal mortality (DeLong et al., 1973; Ylitalo et al., 2005). While direct cause and effect relationships have not been demonstrated, many authors believe that POPs may act synergistically with environmental and anthropogenic stressors to cause adverse effects on marine mammals (Fair & Becker, 2000; Ross, 2002).

Most investigations of POPs and marine mammals have focused on northern hemisphere species, particularly cetaceans and phocid seals inhabiting the mid latitudes of northern Europe and America (e.g. Helle et al., 1983; Lee et al., 1996; Vetter et al., 1996; Mossner & Ballschmiter, 1997; Krahn et al., 2001; Kucklick et al., 2002; Muir et al., 2003; Kannan et al., 2004; Krahn et al., 2004; Kajiwara et al., 2008b). There has been little research on southern hemisphere species (Hutchinson & Simmonds, 1994; Kemper et al., 1994; Aguilar et al., 2002), partly due to the general lower levels of industrialisation and pollutant levels (Aguilar et al., 2002). New Zealand has an agricultural based economy involving the

historical use of pesticides (Buckland et al., 1998a; Scobie et al., 1999) but there have been few investigations into the presence of OCs and PCBs in New Zealand's marine mammal species. The limited studies undertaken have focused on odontocete cetaceans (Buckland et al., 1990; Jones, 1998; Stockin et al., 2007) and there have been few investigations in pinniped species (Baker, 1999).

The New Zealand sea lion (NZSL), *Phocarctos hookeri*, is New Zealand's only endemic pinniped and classified as 'threatened' under the New Zealand Marine Mammals Protection Act (Hitchmough et al., 2007) due to its highly localised distribution within the sub-Antarctic Auckland Islands (50º30'S, 166ºE). The population size is estimated at approximately 11,000-15,000 individuals and is in possible decline (Chilvers, 2008). Infectious disease outbreaks may be a contributing factor toward the population decline (Wilkinson et al., 2003; Wilkinson et al., 2006; Chilvers, 2008). Within the last decade, the NZSL has been subjected to three infectious bacterial disease epidemics during the breeding seasons of 1997/98, 2001/02 and 2002/03, reducing yearly pup production levels and affecting future breeding season recruitment (Duignan et al., 2003; Castinel et al., 2007; Chilvers, 2008). Following the 1997/98 epidemic, a small study measured the levels of OCs and PCBs in the blubber of three sea lions affected by disease, in order to investigate the possible contribution of POPs toward disease outbreaks (Baker, 1999). However, there has been no investigation into the levels of POPs within healthy NZSLs for comparison to the affected NZSLs and to establish any possible relationship between POP exposure and incidence of disease (Jepson et al., 2005). Additionally, as a top-predatory species, the knowledge of contaminant levels in the healthly NZSL population is important data for assessing the health and environmental management of the sub-Antarctic ecosystem.

The purpose of this study was to establish the concentration of PCB and OC pesticides in the blubber of healthy by-caught NZSLs and compare data to previous recorded levels in diseased NZSLs following the 1997/98 epidemic.

2. Materials and Methods

2.1. Sample collection

The NZSL analysed were incidentally captured by vessels operating on the Auckland Islands squid fishery during the fishing season (February to May) of 2007. Carcasses were frozen on board and transported to Massey University, Palmerston North, New Zealand, for necropsy. This was under contract with the Conservation Service Providers administered by the New Zealand Department of Conservation and Ministry of Fisheries. At necropsy all animals were estimated to be in 'good' condition. This was defined by blubber depth measured at the thoracic and pelvic areas and animal weight, in relation to sex and maturity. During analysis, animal condition was also confirmed by percentage of lipid content in blubber.

Blubber samples were collected from the thoracic area of seven by-caught NZSLs (two males and five females). All blubber samples were collected by cutting a 60 x 60 mm blubber piece extending from the outer epidermis to the underlying muscle (see materials and methods Chapter 2). Samples were then sealed in plastic bags and frozen at -20ºC.

Table 1. Identification number (I.D.) and sex of New Zealand sea lions (*Phocarctos hookeri*) analysed (n= 7) for organochlorines (OCs) and polycholorinated biphenyls (PCBs). * L indicates lactating female, F non-lactating female and M male

Sea lion I.D.	Sex
701	L
702	M
703	L
704	L
705	L
706	L
708	M

Asure Quality Ltd, New Zealand, performed the laboratory testing of OCs and PCBs in the blubber of all seven NZSLs, under the laboratory's IANZ Accreditation (No. 131), utilising methods previously published by Stockin et al, 2007. All samples were analysed using the same procedure.

Gas chromatography-high resolution mass spectrometry (HRGC-HRMS) was used to detect and quantify a range of OCs and PCBs; hexachlorocyclohexanes; alpha-HCH, beta-HCH, gamma HCH (lindane), dieldrin, heptachlor, heptachlor epoxide, alpha-chlordane, gamma-chlordane and DDT (plus metabolites *p,p′*-DDE, *p,p′*-DDD (also known as *p,p′*-TDE), *o,p′*-DDT, *p,p′*-DDT and 45 chlorobiphenyl congeners (CB1, CB3, CB4, CB15, CB19,CB28. CB37, CB44, CB49, CB52, CB54, CB70, CB74, CB77, CB81, CB99, CB101, CB104, CB105, CB110, CB114, CB118, CB123, CB126, CB138, CB153, CB155, CB156, CB157, CB167, CB169, CB170, CB180, CB183, CB187, CB188, CB189, CB194, CB196, CB199, CB202, CB205, CB206, CB208 and CB209).

2.2. Instrumental

The HRGC-HRMS analyses were performed on an Agilent 6890 gas chromatograph equipped with a Phenomenex Zebron ZB5 60 m×0.25 mm id×0.25 μmphase thickness column using splitless injection, coupled to a Micromass Ultra high resolution mass spectrometer.

2.3. Blubber preparation

When investigating the presence of POPs in the blubber of pinnipeds, full blubber cores obtained from the main body trunk, should be utilised and homogenised before analysis due to the biochemical layering of blubber lipid components (see Chapter 3). Blubber samples were thawed and a longitudinal sub-sample (measuring from the underlying muscle to the outer epidermis), (see methods, Chapter 2) of approximately 10 g was chopped into small pieces and then homogenised with powdered sodium sulphate. The powder was then extracted from each sample with a Soxhlet apparatus with dichloromethane: hexane (1:1 v/v) for at least 16 h and then evaporated to constant weight by rotary evaporator, and lipid content measured by difference.

2.4. Organochlorine (OC) pesticides

Lipids were removed by column chromatography on florisil, with pesticides being eluted with hexane: diethyl ether (82:18 v/v). Remaining lipids were removed by gel permeation chromatography (GPC) on a Phenomenex Envirosep ABC 300 × 7.8 mm GPC column using ethylacetate: cyclohexane (1:1 v/v). The solvent was removed by nitrogen blow down and the solution reconstituted in 100μL of toluene containing the recovery standard (13C 12–CB) and confirmed by gas-chromatography mass spectromentry (HRGC-HRMS) using multiple ion monitoring as described by Buckland et al (1998).

2.5. Polychlorinated biphenyls (PCBs)

Analysis of PCBs followed the methods outlined in USEPA Method 1668A.
Lipids were removed by chromatography on a reactive multi-column containing sodium silicate and sulfuric acid impregnated silica gel by elution with hexane. The hexane was removed with the residue reconstituted in 100 μL of nonane containing the recovery standards and then analysed by HRGC-HRMS.

The sum of the concentrations of PCB congeners determined was then converted into a lipid basis (mg/kg lipid) using proportion of extractable lipid in each sample (Kannan et al., 2000) for comparison to proposed threshold for adverse health affects in marine mammals of 17 mg/kg lipid.

3. Results

As part of the analysis procedure, the percentage lipid content of each blubber sample was calculated and gave the following values: 701: 83%; 702: 81%; 703: 85%; 704: 85%; 705: 76%; 706: 84%; 708: 83% (Table 3).

3.1. Organochlorine pesticides (OCs)

Organochlorines were detected in all blubber samples (Table 2). DDT was detected at the highest concentration. The ΣDDT concentrations (*p,p′*-DDE + *p,p′*-DDD+ *o,p′*-DDT+ *p,p′*-DDT) ranged from 67.4- 512.0 ng/g. Among the DDT analytes *p,p′*- DDE was present in highest concentration (up to 470 ng/g) followed by, *p,p′*- DDT (up to 50 ng/g), *p,p′*- TDE (up to 20ng/g). The sea lion with highest concentration of DDT was 703, and lowest 704, both lactating females (Table 1).

Dieldrin was present in the second highest concentration, followed by ΣChlordane and ΣHCH, which were very similar. Of hexachlorocyclohexanes (HCHs), β-HCH was present in highest concentration and of chlordanes, α-chlordane was present in highest concentration, γ-chlordane was not detected in sea lion 708. HCB was present in the lowest concentration. Not all OCs were detected in blubber samples. Both Aldrin and Heptachlor had residues below the detection limit. Heptacholor-epoxide was unable to be identified within the chosen matrix, this was reported as Not Quantitated (NQ) (Table 2).

3.2. Polychlorinated biphenyls (PCBs)

The average ΣPCB was 74.86 ng/g (Table 3). Only 34 of 45 PCBs could be detected in all samples. Six PCBs (1, 3, 4, 77, 104, 169) were not detected in any samples and five PCBs (81, 126, 188, 189, 202) were not consistently detected in all samples. CB37 was unable to be quantified (Table 3). Among PCBs the most abundant congeners were CB138 (highest concentration) followed by CB180, CB118, CB101. The sea lion with the highest sum of PCBs was 703 and the lowest was 704. Both were lactating females (Table 1). For each sample, the ΣPCBs was also converted to mg/kg lipid and gave the following values: 701: 0.061, 702: 0.060, 703: 0.192, 704: 0.034, 705: 0.155, 706: 0.048, 708: 0.089 (Table 3).

Table 2. Organochlorine pesticide levels determined in the blubber of seven by-caught New Zealand sea lions (*Phocarctos hookeri)* (ng/g blubber). ND= Not detected, NQ= Not quantified.

Sea lion	α-HCH	β-HCH	γ- HCH	Σ HCH	HCB	Aldrin	Dieldrin	Heptachlor	Heptachlor - epoxide
701	0.12	4.1	0.28	4.5	1.5	ND	6.8	ND	NQ
702	0.14	5.3	0.49	5.93	1.5	ND	5.4	ND	NQ
703	0.15	4.1	0.79	5.01	2.0	ND	8.7	ND	NQ
704	0.14	0.41	0.84	1.39	2.6	ND	4.3	ND	NQ
705	0.20	2.7	0.52	3.42	1.6	ND	8.0	ND	NQ
706	0.15	0.53	0.31	0.99	1.0	ND	5.5	ND	NQ
708	0.17	6.3	0.37	6.84	1.0	ND	2.8	ND	NQ
Mean	0.15	3.35	0.51	4.01	1.6	ND	5.93	ND	NQ

Sea lion	α-chlordane	γ- chlordane	Σchlordane	*p,p′*-DDE	*p,p′*-TDE	*o,p′*-DDT	*p,p′*-DDT	ΣDDT
701	4.0	0.15	4.15	110	9.0	1.1	8.1	128.2
702	4.3	0.32	4.62	93	1.2	0.39	8.2	102.79
703	9.5	0.38	9.88	470	3.4	1.6	37	512.0
704	3.0	0.47	3.47	45	4.7	4.7	13	67.4
705	9.9	0.60	10.5	250	20	9.9	50	329.9
706	2.9	0.44	3.34	55	5.8	3.3	15	79.1
708	5.8	ND	5.8	160	11	1.0	21	193.0
Mean	5.63	0.39	5.97	169	7.87	3.14	21.76	201.77

ΣHCH= sum of α-HCH, β-HCH, γ- HCH; Σchlordane= sum of α- chlordane, γ- chlordane; ΣDDT= sum of *p,p′*-DDE, *p,p′*-TDE, *o,p′*-DDT, *p,p′*-DDT

Table 3. Chlorinated biphenyl (PCB) levels determined in the blubber of seven by-caught New Zealand sea lions (*Phocarctos hookeri*) (pg/g blubber), including conversion of ΣPCBs to mg/kg lipid.

Sea lion	CB1	CB3	CB4	CB15	CB19	CB28	CB37	CB44	CB49	CB52	CB54	CB70	CB74
701	ND	ND	ND	22.7	NQ	305	NQ	202	205	996	ND	72.8	630
702	ND	ND	ND	15.9	NQ	94.7	NQ	64.9	38.7	634	ND	58.7	469
703	ND	ND	ND	18.3	NQ	127	NQ	107	86.4	880	ND	70.2	1040
704	ND	ND	ND	ND	NQ	115	NQ	185	191	510	ND	139	232
705	ND	ND	ND	18.9	NQ	411	NQ	415	528	2070	ND	80.2	1070
706	ND	ND	ND	39.3	NQ	209	NQ	292	232	730	ND	150	313
708	ND	ND	ND	ND	NQ	283	NQ	94.7	95.7	840	ND	34.9	1070
Mean	ND	ND	ND	29.1	NQ	301	NQ	267.23	285.23	1213.33	ND	88.36	817.67

Sea lion	CB77	CB81	CB99	CB101	CB104	CB105	CB110	CB114	CB118	CB123	CB126	CB138
701	ND	ND	2350	3520	ND	1490	1100	118	5640	126	43.9	90000
702	ND	ND	1890	2330	ND	1880	321	130	6630	126	42.6	8120
703	ND	ND	5280	5230	ND	5560	712	405	18700	252	76.9	35700
704	ND	ND	913	2020	ND	615	735	39.2	1960	59.1	ND	4450
705	ND	65.3	4470	7840	ND	2900	2550	221	9650	266	49.4	19600
706	ND	18.2	1180	2420	ND	773	924	53.1	2690	66.8	23.3	5110
708	ND	21.1	3780	4080	ND	2360	1030	159	8830	139	29.4	13000
Mean	ND	34.86	4780	2011	ND	2011	1501.33	144.36	7056.67	157.26	34.03	12570

Sea lion	CB153	CB155	CB156	CB157	CB167	CB169	CB170	CB180	CB183	CB187	CB188	CB189
701	12000	59.0	632	206	641	ND	1570	4460	959	2210	ND	40.9
702	13900	43.3	750	216	491	ND	2860	4440	908	627	ND	34.6
703	48600	161	1630	665	757	ND	7690	18100	3770	2490	ND	ND
704	5730	40.0	371	72.0	232	ND	1190	3950	791	2060	ND	ND
705	24800	160	1120	305	968	ND	4600	15000	3050	7310	45.7	47.2
706	6710	47.3	358	100	304	ND	1690	8270	1140	2650	ND	ND
708	20800	63.7	971	282	683	ND	3480	6430	1310	2400	ND	ND
Mean	18934.29	82.04	833.14	263.71	582.29	ND	3297.14	8664.29	1704	2821	45.7	47.2

Sea lion	CB194	CB196	CB199	CB202	CB205	CB206	CB208	CB209	ΣPCBs Pg/g	ΣPCBs Ng/g	% Lipid content	ΣPCBs Mg/kg
701	433	531	ND	211	24.7	127	90.1	181	50200	50.2	83	0.0061
702	397	430	ND	152	20.1	79.1	50.6	88.1	48300	48.3	81	0.060
703	1540	1940	38.1	652	77.0	365	216	359	163000	163	85	0.192
704	766	892	26.9	NQ	45.1	364	256	816	29800	29.8	85	0.034
705	2100	2260	71.1	898	90.5	909	571	1660	118000	118	76	0.155
706	814	698	20.7	402	48.0	478	343	951	40200	40.2	84	0.048
708	780	730	14.0	233	30.8	192	104	172	74500	74.5	83	0.089
Mean	1231.33	1229.33	35.26	511	56.433	526.33	339.33	927.67	74857.14	74.86	82.428	0.834

NQ= Not quantified, ND= Not detected; ΣPCBs= sum of all 45 listed PCBs, CBs 1- 209.

4. Discussion

The lipid content of the blubber from NZSLs in this study was high, ranging from 76-85%, consistent with values of other healthy pinnipeds (Beck et al., 1993; Arnould et al., 1996; Severinsen et al., 2000) and markedly higher than the values previously recorded for diseased NZSLs in poor condition measuring approximately 12% (Baker, 1999).

The relative concentrations of detected contaminants in the NZSL blubber were ΣDDT > ΣPCB > Dieldrin > ΣChlordane > ΣHCH. Aldrin and Heptachlor were unable to be detected in any samples. ΣDDT levels were approximately three times higher than that of ΣPCB levels. This follows a comparable trend in marine mammal species studied in the southern hemisphere where ΣDDT is generally greater than ΣPCB (Fossi et al., 1997; Evans et al., 2004; Miranda-Filho et al., 2007). Pinnipeds from the northern hemisphere, typically show similar or significantly higher levels of PCBs than DDT (e.g. Mossner & Ballschmiter, 1997; Kucklick et al., 2002; Muir et al., 2003; Fillmann et al., 2007; Wang et al., 2007), although there are some exceptions such as the Baikal seal (*Phoca sibrica*) (Tsydenova et al., 2004), Caspian seal (*Phoca caspica*) (Hall et al., 1999; Watanabe et al., 1999) and California sea lion (*Californianus californianus*) (Kajiwara et al., 2001; Le Boeuf et al., 2002; Kannan et al., 2004) where ΣDDT is greater than ΣPCBs. This is attributed to the close proximity of these species to point sources of DDT pollution (Le Boeuf et al., 2002).

The large percentage of DDE (approximately 67-91%) of the ΣDDTs in NZSL blubber suggests that there have been no recent inputs of DDT to the environment, as DDE is the primary metabolite of DDT and an indicator of biotransformation within the body (Aguilar 1984). The levels of this contaminant have probably arisen as a residue from the historical use of this particular pesticide in New Zealand, which generally ceased usage in the 1970s (Buckland et al., 1998b). This pattern corresponds with the levels previously measured in diseased NZSLs (Baker, 1999) and a recent study of the New Zealand common dolphins (NZCD) (*Delphinus* sp.) (Stockin et al., 2007). However, the DDE levels of NZSLs in this study had a much greater range and were consistently higher (45- 470 ng/g) than diseased NZSLs (21.7-30.6 ng/g) (Baker, 1999). The ΣDDTs in the NZSL blubber of this study were

however lower (45- 470 ng/g) than reported for the NZCD (59- 4430 μg/kg), which also showed the presence of *p'p'*-DDD (Stockin et al., 2007), while the NZSL did not. This may be due to the varying abilities of pinnipeds and cetaceans to metabolise contaminants (Vetter et al., 1996). Additionally, this may also be attributed to to the larger proportion of males to females in the NZCD study (63%) compared to this study (25%), which may have skewed results. Previous studies of pinnipeds and cetaceans have observed varibility of contaminant levels in relation to the sex and reproductive status of animals. Mature males and immature females usually harbor the greatest contaminant burdens as reproductive females off-load POPs to young via mobilisation of blubber stores for milk production (Bacon et al., 1992; Ylitalo et al., 2001; Debier et al., 2003; Metcalfe et al., 2004; Borrell & Aguilar, 2005; Stockin et al., 2007; Kajiwara et al., 2008a). In the present study, no clear distinction could be made between the levels of any contaminants in males and females. Lactating NZSLs showed both the highest and lowest levels of contaminant burdens and no specific differences were seen between OC and PCB concentrations of males and females. However, due to the small number of individuals in this study, the affects of the sex, maturity and lactation on blubber contaminant concentrations cannot be clearly investigated.

The lower concentration of ΣDDTs in NZSL blubber compared to the NZCD may also be related to the further distance of the NZSL population from mainland New Zealand. Generally, coastal marine mammal species are exposed to higher concentrations of contaminants due to close proximity to river mouths and land run-off than more open sea or pelagic species (Jones, 1998; Aguilar et al., 2002). Although considered a pelagic species, the NZCD toxin concentrations are similar to the levels in more coastal New Zealand cetcean species such as the Hectors dolphin, *Cephalorhyncus hectori* (Jones, 1998; Stockin et al., 2007), which may suggest more frequent usage of coastal waters by NZCD than previously thought (Stockin et al., 2007).

The concentration of ΣPCBs in by-caught NZSL blubber was also consistently higher and had a larger range (29-118 ng/g) than levels previously reported for diseased NZSLs (11-23.3 ng/g) (Baker, 1999). This inverse relationship is contrary to previous studies

investigating the possible affects of OCs and PCBs in diseased compared to healthy marine mammals (Jepson et al., 2005). Diseased or emaciated animals generally have lower blubber lipid content and therefore larger concentrations of blubber toxins due to lipid dilution factors (Aguilar et al., 2002; Lydersen et al., 2002). However, it is difficult to establish a proper relationship between the levels of contaminants in the healthy NZSLs in this study and diseased NZSLs due to the small number of individuals utilised for both studies and variation in methods of analysis (Kleivane et al., 2004). The proposed threshold of blubber ΣPCBs for adverse affects on marine mammals in general is 17 mg/kg lipid (Kannan et al., 2000). The NZSLs in this study were all well below this threshold, with the highest value being 0.192 mg/kg from sea lion 703. This may suggest that PCBs in the NZSL are unlikely to be causing adverse health affects such as immunosuppression, although there has been no specific study investigating threshold levels in the NZSL to confirm this result.

The ΣPCBs in NZSLs were lower than previously reported for New Zealand cetacean species (Jones, 1998; Jones et al., 1999; Stockin et al., 2007). The generally low levels of PCBs in New Zealand marine mammals may be due to the remoteness of New Zealand from major sources of global PCB contamination and low levels may reflect the effects of long-range atmospheric transport (LRAT) from the northern hemisphere (Buckland et al., 1998a; Jones et al., 1999). Volatile POPs may evaporate in warmer regions of the globe and be transported by air mass to cooler polar regions where they condense into the environment (Gouin et al., 2004). LRAT is demonstrated by the detection of a wide range of POPs in biota from the remote pristine parts of the globe such as the Antarctica region (Van den Brink, 1997; Connell et al., 1999; Weber & Goerke, 2003; Miranda-Filho et al., 2007). The increase in levels of OCs and PCBs in the NZSLs in the current study compared to diseased in 1998 (Baker, 1999) may suggest that the sub-Antarctic is an increasing sink for LRAT. Due to the very small number of NZSLs in this and the previous study however, no clear conclusion can be made. Assessing and quantifying the LRAT input in the New Zealand environment would be hard to decipher, especially at higher trophic levels where POPs naturally accumulate. Further studies would be required to compile time series data

and assess patterns and progression of POP levels in the NZSL and other marine mammal species.

The general pattern of blubber PCB concentrations was typical of other pinnipeds (Oehme et al., 1996; Vetter et al., 1996; Addison & Smith, 1998; Hall et al., 1999; Kucklick et al., 2002; Fillmann et al., 2007; Miranda-Filho et al., 2007; Wang et al., 2007). PCB138 was present as the highest concentration followed by PCB153, 180, 170 and 187. Certain PCBs such as CB153 are more resistant to metabolism due to their molecular structure and therefore readily accumulate within the body (Oehme et al., 1996; Mossner & Ballschmiter, 1997; Wolkers et al., 1998; Connell et al., 1999).

The absence of Aldrin and Heptachlor in the NZSL blubber is expected as aquatic sediment concentrations from estuarine waters around New Zealand have shown little or no accumulations (Scobie et al., 1999). Similarly, there were also minimal Heptachlor levels reported in the blubber of NZCD (Stockin et al., 2007), although Aldrin was not addressed in that study. Aldrin and especially Heptachlor have had little use in New Zealand compared to other OCs (Buckland et al., 1998b; Scobie et al., 1999). The low levels of Hexachlorobenzene (HCB) seen in the NZSL blubber may not necessarily be due to a low residue levels in the environment since it has been suggested in previous research that pinnipeds may have a good capacity to metabolise this compound (Goerke et al., 2004). Further investigation of HCB at lower trophic levels would be required to confirm its presence within the sub-Antarctic environment. In general, the levels of HCB, chlordane and HCH detected in southern hemisphere marine mammals are minimal compared to northern hemisphere studies (Connell et al., 1999; Aguilar et al., 2002; Miranda-Filho et al., 2007).

It is difficult to compare the burdens of OCs and PCBs from various marine mammal studies due to differences in the analytical methods employed, time of year sampled, method of blubber sampling and sex of animals sampled (Kleivane et al., 2004). The results of the present study suggest the contaminant levels in NZSL blubber are currently lower than those recorded from northern hemisphere pinnipeds (Hutchinson & Simmonds, 1994;

Kajiwara et al., 2001; Le Boeuf et al., 2002). Some of the highest contaminants levels have been documented in California sea lions, up to 1400 μg/g for DDT and up to 410 μg/g for PCBs (Hutchinson & Simmonds, 1994; Kannan et al., 2004). The levels in the NZSL are similar to pinnipeds from the least polluted areas of the globe such as Antarctica (Connell et al., 1999; Vetter et al., 2003; Miranda-Filho et al., 2007) and the Arctic region (Krahn et al., 1997; Addison & Smith, 1998; Wang et al., 2007).

4.1. Conclusion

This study presents the levels of OCs and PCBs in the blubber of healthy by-caught NZSLs during the months of February to May in 2007. The levels of OCs and PCBs were slightly higher than in three NZSLs affected by the disease epidemic of 97/98. With only a small number of NZSLs investigated in both studies, it is difficult to determine any possible temporal increase of contaminant levels in the species. The levels of PCBs in the NZSL are well below the suggested physiological threshold for marine mammals of 17mg/kg lipid. This may suggest that OCs and PCBs are an unlikely contributing factor to disease epidemics, although there is no specific knowledge of the NZSL threshold levels. Management implications suggest there is a need to look to other possible causes of stressors that may be implicating species decline. As a bio-indicator for the sub-Antarctic environment, the low levels of contaminant uptake in the blubber of the NZSL suggest a relatively non-polluted ecosystem.

Limits to this study include financial restraints, which resulted in a small sample set (n= 7) and a lack of variation between sexes and age cohorts. Due to the influence of sex and reproductive status on POP content of blubber, sampling of mature males may give a clearer indication of contaminant levels and possible long-term effects. The study was also confined to specific months and therefore represented only a snap shot over time. It did not address the possibilities of annual variation and effects of blubber thickness and lipid content on concentrations of OCs and PCBs. Due to the profound affect of blubber lipid content on toxin concentrations, more seasonal sampling would give a much clearer conclusion as to the general concentration of toxins seen in the bubber of the NZSL population.

Future directions should also include periodic monitoring (i.e. every 10 years) of OC and PCB levels in the NZSL to establish any possible changes in contaminant burdens and possible future impacts on the population's growth and recovery.

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~ CHAPTER 5 ~

Discussion

1. General discussion

This thesis has described the fatty acid (FA) distribution of two blubber sample sites currently used for fatty acid signature analysis (FASA) in the New Zealand sea lion (NZSL) and the levels of organochlorine pesticides (OCs) and polychlorinated biphenyls (PCBs) in the blubber of healthy by-caught NZSLs. This was the first study to investigate the blubber FA distribution of a sea lion species and only the second for an otariid (Arnould et al., 2005). Additionally, this was also the first investigatation into the OC and PCB levels in the blubber of healthy NZSLs.

The investigation has shown that the FA profiles of the two body sample sites (dorsal pelvic and ventral thoracic) have very similar FA compositions. The FA profiles of full blubber cores obtained from the sites showed relative homogeneity with only two FAs (20:5n-3 and 18:2n-6) present in variable amounts. When the blubber was divided into inner and outer halves for determination of stratification, both sites displayed the same pattern of vertical stratification or biochemical layering of FAs between the two divisions. This comparable FA composition of the thoracic and pelvic blubber suggests that these two body sites have related patterns of lipid deposition and mobilisation and a similar function as energy depots; similar to results obtained from a variety of body sites in phocid seals (Slip et al., 1992; Mellish et al., 2007). Only one otariid study on the Cape fur seal (*Arctocephalus pusillus pusillus*) that utilised different body sample sites is available for comparison (Arnould et al., 2005), making it difficult to draw further conclusions about lipid deposition, mobilisation and the body site variability of FA profiles of otariids in general. However, the stratification of FAs established in both the NZSL and Cape fur seal blubber (Arnould et al., 2005) confirms the complex functioning of otariid blubber, with greater metabolic capacity of the inner blubber, similar to what is documented in phocids (Best et al., 2003; Wheatley et al., 2007; Strandberg et al., 2008).

Previous phocid research has recommended that biochemically layered blubber, as observed in NZSL, should be analysed for OC and PCB levels by obtaining full blubber cores from the main body trunk, which are to be homogenised prior to analysis (Kleivane et al., 2004). For investigating OC and PCB levels in this study, full blubber cores were

therefore obtained from the thoracic sample site and also homogenised. Due to the similarity of FA profiles from the thoracic and pelvic body sites as demonstrated in Chapter 2, it may be expected that either site would also give a similar interpretation of contaminant levels due to the affinity of OCs and PCBs for specific lipids (Kleivane et al., 2004). However, this is only speculation and requires futher investigation to compare contaminant profiles from the two body sites in the NZSL to confirm this hypothesis.

The levels of OCs and PCBs in by-caught NZSLs were slightly higher than those previously detected in three diseased NZSLs (Baker, 1999). Due to the small number of animals analysed and differing analytical methods of both studies (Baker, 1999), it is difficult to determine if OC and PCB levels have increased in NZSLs over the last decade and whether there is a link to the disease epidemics. As the levels of PCBs observed in the NZSL were below the proposed threshold level of 17mg/kg lipid for adverse effects in marine mammals, this might suggest PCBs currently play a minor role in facilitating immune suppression and heightening population susceptibility to disease. However, with no specific knowledge of NZSL contaminant level thresholds, this should only be interpreted as a general guideline value.

2. General conclusion

From the results of this study we can support the current blubber biopsy sampling techniques for FASA of the NZSL, which is obtaining full blubber cores (from the epidermis to the underlying muscle) from either the ventral thoracic (during necropsy) or dorsal pelvic (during field sampling) area. A full blubber core from either sample site will give a comparable interpretation of diet.

Full blubber cores are also required for the investigation of OC and PCB levels in NZSL blubber. No further conclusion however can be drawn as to whether the low levels of OCs and PCBs detected in NZSLs may be acting as a stressor and contributing to population decline via susceptibility to disease.

3. Conservation Implications and future directions

The results of this study contribute toward understanding the characteristics and functioning of NZSL blubber. This knowledge is essential for developing appropriate and consistent blubber sampling protocols that will reduce inter-animal variation of results and improve interpretation of data. Ecological information obtained through blubber sampling will assist in the conservation of the NZSL, by allowing biologists to better assess the impacts of current direct and indirect anthropogenic activities that may be contributing to the species decline and implement appropriate management solutions.

The principal limit to this study was the small number of by-caught NZSLs available for analysis. This was particularly evident in the small number of males and few age cohorts analysed and resulted in an inability to compare sexes and different age cohorts for all aspects of the research. The results of this thesis are principally representative of the mature female NZSL population and future studies should be directed toward analysing mature male blubber for comparison to this research, to further understand the influence of sex and reproductive status on blubber composition. In addition, this investigation was undertaken on NZSLs captured during the months of February to May and therefore reflects the blubber FA and contaminant characteristics during a limited time frame. It did not address any possible affects of variability in blubber thickness and lipid content that may occur during a pinniped's life-cycle on an annual or seasonal basis (Ryg et al., 1990).

Future studies should therefore be directed toward increasing the sample size to include a wider range of age cohorts and greater variation of sex and reproductive status of NZSLs. For FASA, investigating a wider range of body sample sites and more detailed layering of FA stratification would also provide more information on lipid deposition and mobilisation rates of the NZSL and otariids in general. For OC and PCB analysis, longer-term directions should include a bio-monitoring scheme, comparing future analyses with the current study in order to determine any increases or declines in contaminant levels in the NZSL and possible impacts on the population. Investigating OC and PCB levels in mature male NZSLs may also give a more accurate interpretation of contaminant levels in the species due to lower rates of blubber turnover in the male otariid life-cycle.

3. References

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Chapter 5