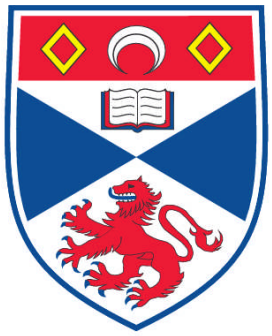
ON THE DYNAMICS AND SELECTIVE TRANSPORT OF FATTY ACIDS AND ORGANOCHLORINES IN LACTATING GREY SEALS (HALICHOERUS GRYPUS)

Aline Arriola Ortiz

A Thesis Submitted for the Degree of PhD
at the
University of St. Andrews



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On the dynamics and selective transport of fatty acids and organochlorines in lactating grey seals (*Halichoerus grypus*)

A thesis submitted to the university of St Andrews for the degree of Doctor of Philosophy

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DECLARATION

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I was admitted as a research student in September 2004 and as a candidate for the degree of Doctor of Philosophy in September 2005; the higher study for which this is a record was carried out in the University of St Andrews between 2004 and 2009.

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ABSTRACT

This thesis examines fatty acid (FA) and polychlorinated biphenyl (PCB) dynamics in a marine top predator, the grey seal (*Halichoerus grypus,*) and their transfer during lactation from mother to offspring. It examines regional and annual variations in FA composition and PCB loads, and also how the physical and chemical characteristics of these molecules (e.g. their polarity and size) can affect the rates of accumulation, mobilization and transfer of specific FAs or PCBs. Two UK grey seal colonies (North Rona (NR) and Isle of May (IOM) were studied during three consecutive years (1996-1998 and 2004-2006). Lactating grey seals and their pups were repeatedly captured during the lactation period and sampled for blubber, serum and milk and analysed for FAs and PCBs.

Overall, the two colonies were clearly distinguished from each other, suggesting that the main prey species had different FA composition, and possibly that the seals from these colonies had different diets . These differences are probably a direct consequence of differences in prey community structure in the two regions where seals from these two colonies are thought to feed. Within each colony, annual differences could be detected between some years but not between others. During 1996-98, IOM seals showed a clear change in their FA profiles while NR seals did not. In contrast, during 2004-2006 NR seals showed a clear change while IOM seals did not. The changes observed in IOM during 1996-1998 are consistent with the large-scale regime shift that occurred in the North Sea during the 1990's.

The relative proportions of each FA that were mobilized from blubber and transferred to the milk during lactation were very similar between colonies, and could be explained to a large degree by their physico-chemical properties. For a given carbon chain length the mobilization increased with increasing number of double bonds; and for a given number of double bonds the mobilization decrease with increasing carbon chain length. However, the mobilization also appeared to be influenced by the specific nutritional requirements of the growing pups. For instance, FAs that are considered essential for pup development or efficient energy storage (e.g. saturated FAs) were more highly mobilised than expected. This selectivity was also reflected in the FA composition of the different body compartments (maternal

blubber and milk, pup blubber) that persisted throughout lactation. These changes were also similar between the colonies.

Colonies could also be clearly distinguished by their blubber PCB profiles. IOM seals had higher total concentrations on average than NR seals (1327.9 vs. 680.2 ng/g lipid in 2005 and 1199.7 vs. 819.0 ng/g lipid in 2006). IOM seals also had higher total amounts in both years (79.2 vs. 38.0 mg in 2005 and 61.7 vs. 53.4 mg in 2006). One of the main differences between colonies was that females from IOM had higher concentrations of highly chlorinated congeners than NR seals.PCB concentrations in blubber increased towards the end of lactation. Serum and milk PCB concentrations also increased rapidly, especially for the highly chlorinated congeners. These results were consistent with other studies showing the increase in concentrations as a result of lipid loss. Serum concentrations stayed constant during the first part of lactation and increased at late lactation. This was also observed in milk PCB concentrations. The changes in the PCB profiles in the three body compartments were very similar between colonies. However IOM seals always had higher total concentrations of PCBs in all of the body compartments. The concentrations of individual congeners relative to PCB-153 showed that blubber contained higher proportions of the highly chlorinated PCBs relative to other tissues. There were no clear changes in these proportions in blubber during lactation, but the relative proportions of highly chlorinated PCB In serum and milk increased throughout lactation while the less chlorinated PCBs stayed constant. The highly chlorinated PCBs were found in lower concentration in the milk compared to the less chlorinated compounds suggesting a selective release from blubber to blood and a selective transfer of PCBs to the milk.

CONTENTS

1 General Introduction

INTRODUCTION

This thesis explores the dynamics of lipids in lactating grey seal females and their pups. It examines the benefits to animals, i.e. the mobilisation of fatty acids to cover the mother's metabolic needs and the transfer to offspring for their metabolic requirements and essential building materials. The thesis also examines the negative corollary of this lipid mobilization and transfer: the mobilization and transfer of organochlorines.

Most marine mammals have a largely lipid based metabolism. They rely to a large extent on accumulated lipid stores during long periods of fasting, for instance during moulting, breeding or migration. Thus, blubber has played an important role in the evolution and adaptation of marine mammals to the aquatic environment (Koopman et al. 2002). Blubber in marine mammals has several functions: it is the main site of energy storage, it provides crucial insulation, and it can be used to adjust buoyancy and optimise streamlining (Ryg et al. 1988, Iverson et al. 2002). Blubber can constitute up to 50% of the total body mass of an animal (Iverson et al. 1995, Kirsch et al. 2000, Iverson et al. 2002).

Persistent organic pollutants (POPs) such as PCBs, dioxins and dieldrins are highly lipophilic man-made compounds that are very resistant to degradation. These characteristics cause them to bio-magnify and accumulate through the food web, particularly in organisms with high lipid contents and lipid-based metabolisms. Consequently they are found in high concentrations in the blubber of marine top predators. PCBs have been shown to have a wide variety of biotoxic effects as the compounds and their metabolites are structurally analagous to important biological molecules. Stored pollutants in the blubber do not necessarily pose a direct threat to the organism (Pomeroy et al. 1996, Antunes et al. 2007). However, when the lipids from the blubber are utilized for instance during periods of negative energy balance in association with breeding and moulting fasts, the toxicants can be release. The stored pollutants are released into the circulatory system, potentially exposing vital organs to dangerous levels of the toxics (Norstrom & Muir 1994) and increasing the negative effects in the organism. During lactation pollutants are also circulated to the mammary glands

and become incorporated into milk, and are thus transferred to the offspring. Since pups are undergoing significant growth and development of many vital organs, they are particularly vulnerable to these compounds, which can seriously affect their normal development and survival.

Because of the high affinity of PCBs for lipids, the mobilisation, dynamics and transfer of these pollutants are closely linked with the mobilisation, dynamics and transfer of lipids. Thus, understanding the dynamics of lipids, especially of fatty acids, can give us a better understanding of PCB dynamics in marine mammals.

GREY SEALS

LONGITUDINAL STUDIES OF TWO UK GREY SEAL COLONIES

In this study we make use of two long-term research programs of the reproductive behaviour and performance of grey seals; one at North Rona (NR) (59°06'N, 05 °50'W) off the North-western tip of the Scottish mainland and the other at the Isle of May (IOM) (56°10'N, 2 °33'W) in the Firth of Forth on the Scottish east coast (Fig. 1). Reproductive parameters of seals from these two colonies have been studied for several decades, and in some cases their diet and pollutant levels have also been examined (Boyd & Laws 1962, Fedak & Anderson 1982, Pomeroy et al. 1994, Pomeroy et al. 1996, Pomeroy et al. 1999, Walton et al. 2000, Walton & Pomeroy 2003). The North Rona and the Isle of May breeding colonies are situated in distinct ocean regions that are influenced by very different ocean circulation patterns, climate, antropogenic disturbance and also by different contamination sources. It is likely that these contrasting physical environmental characteristics affect the type of prey and its abundance and also the pollutant levels within the food webs. In this way it is possible that the detailed structure of the food webs in the two areas are likely to be dissimilar which may impact the diet composition of seals feeding in these regions and in consequence the levels and characteristics of pollution.

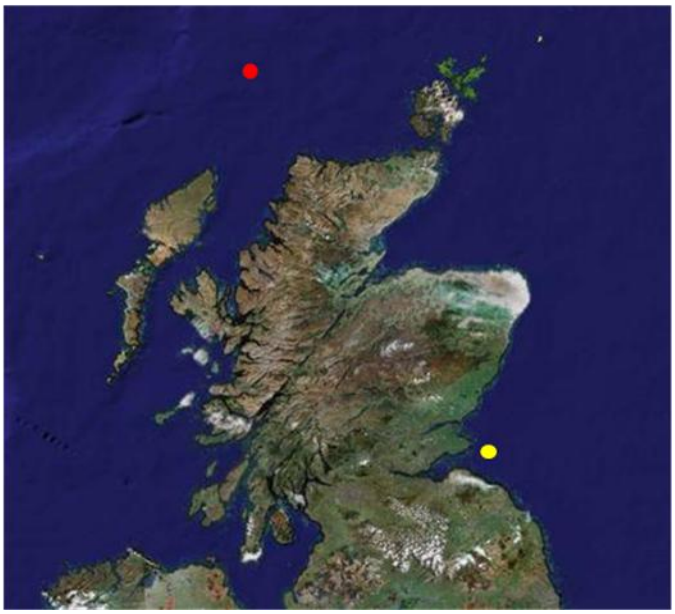


Figure 1. Map of Scotland and Scottish Isles showing the locations of North Rona (red) and Isle of May (yellow).

DISTRIBUTION AND LIFE HISTORY

The grey seal (*Halichoerus grypus*) is a member of the Family Phocidae and the subfamily Phocinae. It occurs in three geographically distinct stocks, one in the western North Atlantic (mainly Canada), one in the eastern North Atlantic and one in the Baltic Sea (Bonner 1981, King 1983). The eastern population can be found from Iceland and the Faeroe Islands to the British and Norwegian coasts. Within the UK, the largest colonies are found in the Outer Hebrides, in the Orkney and Shetland Islands and at the Farne Islands (King 1983, Bonner 1994). While the main colonies are thus found offshore on largely unpopulated Scottish islands, the general population increase over the past decades has been accompanied with an increased occupancy of several mainland sites (Duck & Thompson 2007). The population estimate for grey seals around the British Isles was 113,300 in 2004 (Duck & Thompson 2007).

Female grey seals can reach sexual maturity at 3-5 years of age, and they give birth almost exclusively to a single pup once every year, during the annual breeding season. Lactation usually lasts for 16 to 22 days (Pomeroy et al. 1999), after which females return to sea while weaned pups remain at the breeding

colony for a post weaning fast which usually lasts 10-28 days (Reilly 1991). The breeding season of the three main stocks is different (Bonner 1994). These differences are almost certainly linked to stability of the breeding habitat in the different regions. While grey seals from Canada, and the Baltic breed mainly on sea-ice in late winter, colonies around the UK breed exclusively on land between September and December (Bonner 1981). There is a gradual clockwise cline in the date of peak pupping around the British Isles from southwest to southeast, with populations in Cornwall breeding earliest, the northwest of Scotland breeding between mid-September and mid-November, while peak pupping along the south east coast generally occur between mid-October and mid-December (King 1983).

LAND-BASED REPRODUCTION

In land breeding phocids such as grey seals, feeding and breeding are completely separated in space and time. The evolution of this breeding strategy has been accompanied by several physiological adaptations to allow seals to cope with the high energetic demands of long periods on land without food intake (Trillmich 1996, Crocker & Costa 2002). Grey seals are therefore a typical example of a true capital breeder (Drent & Dann 1980) acquiring all resources necessary for the process of lactation and breeding prior to their arrival on land to give birth. Most importantly, the accumulated body stores will be utilized to cover the metabolic demands of mothers during lactation as well as those of the developing pup. During lactation the metabolism of females is reorganized in order to ensure that appropriate nutrients are transferred to the mammary gland for the production of milk (Iverson et al. 1993, Vernon & Pond 1997).

Lactation has a substantial energy expenditure and the lactation period in land breeding phocids is characterized by high energy transfer rates over a comparatively short time period, which effectively reduces the mother's metabolic overheads (Castellini & Rea 1992). During this energetically demanding period mothers can lose up to 65 kg of their body mass (Fedak & Anderson 1982). Up to 57% of the energy from mobilized lipids will be available to the pup via milk (Iverson et al. 1993, Lydersen et al. 1995, Mellish et al. 1999). In the UK, grey seal

pups ingest around 3 litres of milk daily and achieve growth rates around 2kg/day while mothers lose about 3-4 kg/day (Pomeroy et al. 1996). These high energy transfer rates are possible due to the very high lipid content of the milk, which increases from about 30% at early lactation to as much as 60% at the end of lactation (Iverson 1993, Pomeroy et al 1996). Triacylglycerols (TAG) make up most (~98%) of these lipids (Neville & Picciano 1997) which provides not only the required energy reserves but also essential fatty acids that are critical for growth and development (Iverson et al. 1995, Innis 2005, Innis 2007). As the milk lipid content increases throughout lactation, the water content decreases correspondingly. The protein content of grey seal milk is generally relatively constant at around 11% while carbohydrates make up less than 1% (Oftedal et al. 1987, Lydersen et al. 1995, Mellish et al. 1999). Milk proteins are critically important for growth and development and contain essential immunoglobulin and vitamins (Schweigert & Stobo 1994).

AT-SEA DISTRIBUTION AND MIGRATION PATTERNS

The at-sea movements of grey seals from several haul outs around the UK have been monitored over many years (Thompson et al. 1991, 1996, McConnell et al. 1999, McConnell pers. comm.), and these data have been used to describe the foraging patterns and habitat use of key populations around the UK coastline. Only one at-sea movement study has been conducted at NR, in 2003 when 20 female grey seals were equipped with location-only satellite transmitters at the end of the breeding season (Pomeroy, unpubl.). These seals could be followed only till the tags moulted off, and therefore the period for which data were available represents the post-breeding foraging of mothers until moult and thus might not be comparable with those performed in the east and west coast that have been perfomed mainly outside the breeding season. The animals' movements from NR seals were focussed around a radius of approx 80 miles from NR, with trips to the Hebrides, the Atlantic shelf and the NW mainland. A few animals approached the Orkneys and only one ventured into the northern North Sea after visiting Shetland (Pomeroy, pers comm).

There are no recent tracking studies of postbreeding adult females from the IOM. However, after the moult adult females have been equipped with tracking devices at haul outs in the near vicinity such as the Farnes and Abertay (Thompson et al. 1991, 1996, McConnell et al. 1999, McConnell pers. comm.). It is known that seals from the IOM use these haul outs outside the breeding season (Pomeroy, unpublished). While seals from the IOM colonies frequently switch haul outs (and sometimes venture north to haul outs in the Orkneys and even Shetland), they appear to forage almost exclusively in the North Sea (McConnell, pers comm.). Thus, while individuals are capable of moving over relatively large distances in a matter of days, and do this frequently as they switch between focal haul outs, there appears to be a fairly clear separation between feeding areas of seals along the northwest coast and those from along the North Sea coast, with a delineation running along an axis from the Northeastern tip of Scotland via the Orkney Islands to Shetland (McConnell pers. comm., Pomeroy, pers. comm.). Few seals from the east coast appear to frequent areas in the Northeast Atlantic and similarly, few seals from e.g. the Hebrides venture far into the North Sea. It should be noted however, that these studies are based on a relatively small number of individuals, and there is likely some overlap between the feeding areas of seals originating from haul outs along the east coast and seals from haul outs along the west coast and the Hebrides. Althought the animals tag outside the breeding season in the haul outs from the east and west coast of Scotland might not necesarily represent the animals from NR and IOM colonies, we assume that the seals from the west and east of Scotland utilized different foraging areas.

FEEDING AND DIET

Grey seals are opportunistic predators, they have the capacity of switching prey species depending on the abundance and preference (Pierce et al 1991).Their principal prey consists of a variety of fish species and to a lesser extent molluscs and crustaceans (King 1983). Dietary studies of UK grey seals based on faecal analysis suggest that cod, sandeel (*Ammodytes* spp) haddock, herring, ling and different flat fish species are the most common fish prey (Hammond & Prime

1990, Hammond & Harris 2006, Hammond & Grellier 2006). However, there are large variations between regions, years, seasons and individuals (Hammond & Prime 1990, Hammond et al 1994). The variations lie mainly in the proportions of the major prey species consumed.

The diet composition and prey consumption of grey seals of Western Scotland, Shetland and different haul outs in the North Sea were examined in detail in 1985, 1996-1999 and 2002 (Hall 1999, Hall et al 1999, Hammond & Harris 2006, Hammond & Grellier 2006). In addition, three main haul outs in the North Sea were also sampled quarterly from Nov 1996 to Jan 1999. In the North Sea, the most important prey species in 1995 and 2002 were sandeels, cod and haddock. While the absolute estimated amounts of fish consumed in tonnes for the three main prey species increased from 1985 to 2002, the percentage of the total diet they represent decreased from 1985 to 2002, this occured due to the increased consumption of other prey species such as short spined seascorpion, plaice, whiting and saithe (Hammond & Grellier, 2006). In the same years, grey seals from the Hebrides consumed principally sandeels, herring and cod. In contrast to the North Sea, the consumption of sandeel in absolute amounts were lower in 2002 than in 1985 (Hammond & Harris, 2006) as were their relative representation in the total diet. Herring consumption increased more than 3 times the relative proportion of the diet compared to 1985. Cod represented a similar percent of the total amount of prey consumed in both years (Hammond and Harris, 2006). In general, the changes in diet between 1985 and 2002 were more obvious in the Hebrides, with species such as haddock, lemon sole, rockling, bullrout and dragonet among others increased in the percentage of the total diet (Hammond & Harris, 2006). In 1985 the total consumption of sandeel (tonnes) was higher in the North Sea and represented almost twice the relative proportion of the diet compared to the Hebrides. In 2002, the estimated amounts of sandeel consumed by seals in the North Sea was more than three times that consumed in the Hebrides (Hammond & Harris, 2006). In contrast, herring consumption in the North Sea represented less than one percent of the total estimated diet in both years, while in the Hebrides herring represented 5 and 16 percent of the total diet in 1985 and 2002 respectively. Cod consumption and its relative abundance in the diet were very similar between areas in both years.

In 1996 to 1999 faecal samples were collected from three main haul outs on the UK's east coast: Isle of May, the Tay Estuary and the Farne Islands. Again, cod, sandeels, whiting, plaice and other gadoids were the main prey species. However, there were large variations in the proportions consumed of these main prey species between years and seasons (Hall et al 1999).

It is important to point out that there are no faecal studies performed in North Rona, thus it is imposible to make any dietary comparisons between the studied colonies. However, the dietary studies performed in different haul outs during the moulting season in the east and west coast might give an aproximation and have shown that there are dietary differences between the grey seals from the east and the west coast of Scotland.

NATURAL ENVIRONMENTAL CHARACTERISTICS

The marine environment is highly dynamic and variations across space and time will determine the functioning and distribution of resources within the marine ecosystems.

Physical and chemical properties such as bottom topography, sediment types, ocean circulation patterns, salinity and temperature will affect the supply and distribution of nutrients and thereby the foundation for entire food webs. Thus, marine regions that are geographically close can nevertheless be very different in their ecosystems and food web structure if their physico-chemical characteristics are sufficiently different. The North Sea and the adjoining Northeast Atlantic are good examples.

THE NORTHEAST ATLANTIC

The waters of the western coastline of the UK are influenced largely by the relatively warm and saline water of the Northeast Atlantic (Inall et al. 2008). At a

regional scale, the waters off the northwest coast of the British Isles are better known as the 'Northwest Approaches', and encompass a relatively narrow continental shelf, including the Scottish Shelf, separated from the deeper Atlantic Ocean to the west (Hackett & Røed 1998). The Slope Current is the most important surface flow in this region and flows along the continental slope from the south of Porcupine bank to Faroe Shetland Channel (Fig. 2) (Hackett & Røed 1998). It transports the Northeast Atlantic water into the Northern part of the North Sea. The Slope current has a major impact on the biology of the shelf-break and contributes to the formation and maintenance of the shelf-break front. It also plays a significant role in transporting dissolved and suspended material from the Atlantic northwards into the Norwegian Sea (OSPAR Commission 2000b). One of the two main processes that drive the ocean circulation in the Northeast Atlantic is the balance between the northwards outflow of surface waters across the ridges between Scotland and Iceland into the Norwegian Sea.

There is a strong inter-annual to decadal variability in this process, and these cyclical events may be linked to the biological oscillations observed in primary and secondary productivity and variations in fish stocks (OSPAR Commission 2000b). While it has been difficult to link these biological responses directly to changes in the physical environment, they have been linked to variations in zooplankton production and the distribution of fish (OSPAR Commission 2000b). The combination of the general water masses and the effects of the shelf break strongly influence the structure of the marine food webs along the west coast of the UK (OSPAR Commission 2000b). Changes in the intensity of the Slope Current are thought to be linked to the recent changes observed in the North Sea (Reid et al 2001). While the waters around the Northwest approaches have long been exposed to fishing and more recently to oil and gas exploration, the area is flanked by land with some of the lowest population densities in W. Europe, with correspondingly little in the way of direct industrial effluent.

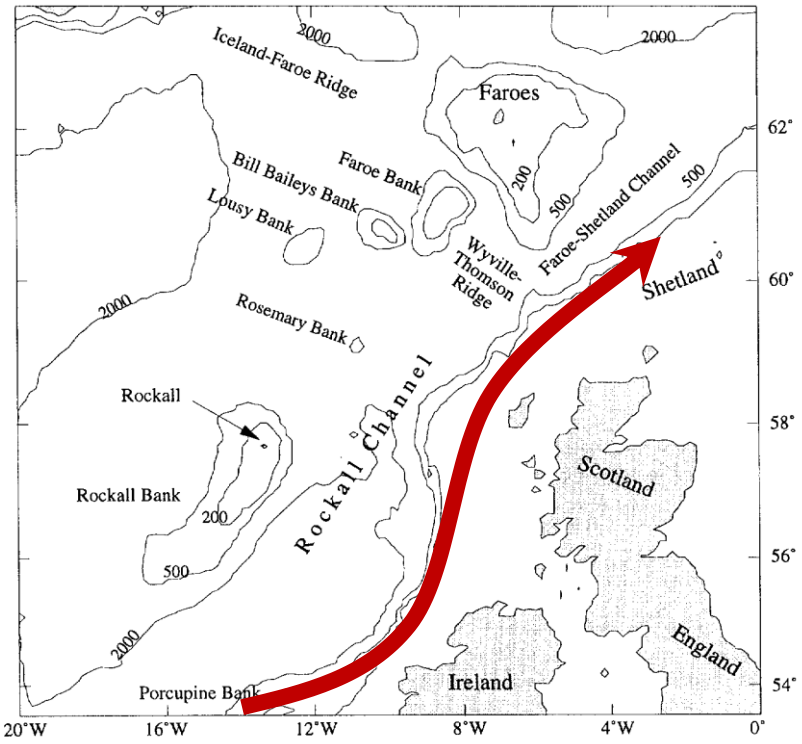


Figure 2. The Northwest approaches to the British Isles. Reproduced from Hacket and Røed (1998). The Slope Current is represented by a red arrow.

The North Sea

The North Sea is situated on the continental shelf of Northwestern Europe. It opens to the Atlantic Ocean to the north and through the English Channel to the southwest, and also connects to the Baltic Sea to the east (OSPAR Commission 2000a). The North Sea is surrounded by many densely populated and heavily industrialised countries. Over 164 million people live around its shores. This region is of economic importance to many of the industrialised surrounding countries. The North Sea has been exploited for many purposes such as fishing, extracting oil and gas from beneath the seabed, and not least as a recipient for the waste products of modern everyday life (Ferm 1996). These activities have resulted in substantial anthropogenic stresses on the marine environment of the North Sea, with many direct negative impacts on the diversity, abundance, growth and health of its marine life.

The North Sea is relatively shallow (less than 200 m in depth) and consists of a mixture of North Atlantic water and the freshwater influx from the many rivers and estuaries along the coasts. The major current comes from the North Atlantic via the Norwegian Sea and the straits between Orkney, Shetland and the Faeroe Islands, and a small portion also enters the North Sea via the English Channel (Fig. 3). The outflow is very limited and occurs mainly via the coast of

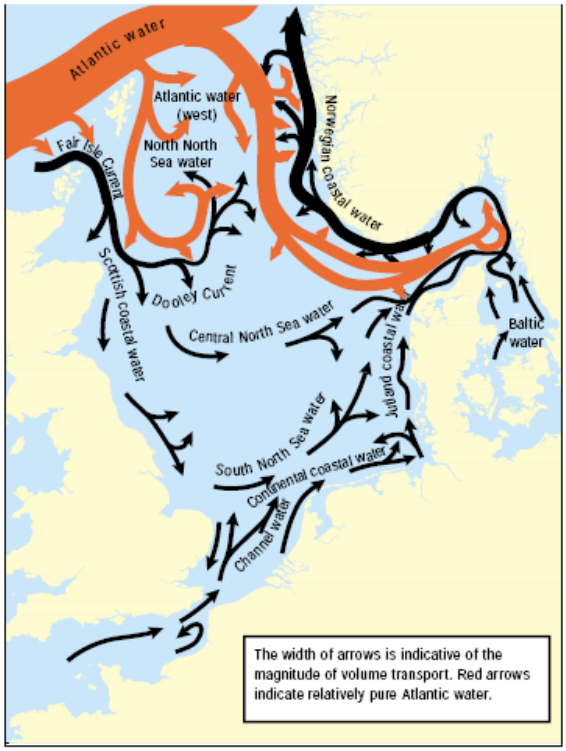


Figure 3. Map showing a schematically diagram of the circulation on the North Sea. Taken from OSPAR 2000

Norway and the deep Norwegian Trench. There is therefore a tendency for water masses to be retained within the North Sea, and to re-circulate around the coasts. This has important consequences in terms of the fate of pollutants that enter into this system and also on the distribution of nutrients (OSPAR Commission 2000a). Due to the limited through flow and shallow depths, the salinity and temperature of different areas within the North Sea are influenced greatly by heat exchange between the ocean and the atmosphere, and by local freshwater supply. Unlike the waters along the west coast, the marine ecosystems in the North Sea are strongly

influenced by the shallow depths and estuarine influences, especially in the southern parts (OSPAR Commission 2000a). The North Sea's physical characteristics are dominated by tides, winds and solar radiation. During the winter, the low radiation and the strong winds and tidal friction leave the water column completely mixed (Scott et al 2006). On the other hand in the spring with increasing sunlight and less wind the vertical mixing decreases. This creates differences in temperature and density and therefore stratification in the water column (Scott et al 2006). The variation in the degree of mixing of the water column drives the timing and the seasonal cycle of primary production and thus affecting the rest of the marine food chain in the North Sea (Scott et al 2006).

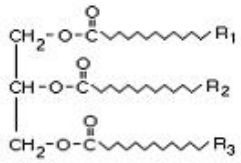
FATTY ACIDS (FAS)

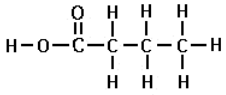
Lipids constitute a class of compounds that contains long chain aliphatic hydrocarbons and derivatives such as fatty acids. They are insoluble in water but soluble in solvents such as ether, chloroform and benzene. Lipids can be classified into several groups. One of them is made up of the triacylglycerols (TAG), which are the most common form of lipids stored in the adipose tissue of animals (Allen 1976). TAGs are the main source of energy for marine mammals, which can store large amounts of TAGs in their blubber. Lipids are the most readily available source of energy, producing approximately twice the amount of energy for the same mass as carbohydrates or proteins. TAGs are formed by three fatty acids attached to a glycerol (Fig. 4A).

Marine fatty acids (FAs) are typically molecules with a carbon chain containing from 14 to 24 carbons (Fig. 4B). They have a methyl terminal at one end and a carboxyl group at the other end (Allen 1976). Fatty acids with shorter chain lengths exist, but they are very uncommon in marine food webs. The only short-chain FA that can sometimes be found above trace amounts is a FA with 12 carbons. There are also FAs with more than 24 carbon atoms, but they are also very uncommon in marine food webs (Budge et al. 2006). Fatty acids also vary in their degree of saturation. Completely saturated fatty acids (SFAs) have no double

bonds linking the carbons along the chain, while monounsaturated fatty acids (MUFAs) have a single double bond and polyunsaturated fatty acids (PUFAs) have two or more bonds.

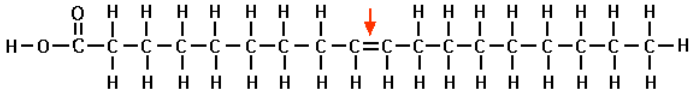
A



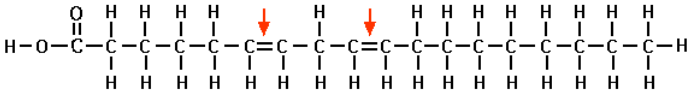


Butyric Acid-Saturated Fatty Acid

B



Oleic Acid- Monounsaturated Fatty Acid



Linoleic Acid- Polyunsaturated Fatty Acid

Figure 4. Chemical structure of A) triglycerides and B) examples of saturated, mono- and polyunsaturated fatty acids. R1, 2 and 3 indicate fatty acids with different chemical structure which can be bound to the same glycerol to form the TAG.

Fatty acids can be referred to in many different ways. Here, I will use their numerical symbols exclusively. These describe the carbon chain length and the position of the first double bond relative to the carboxyl end (Fig. 4B). For instance, the numerical symbol of Eicosapentanoic acid (EPA) is 20:5-n3. This FA has a carbon chain containing 20 carbons. It also has five double bonds, the first of which is located between carbon number 3 and 4 relative to the carboxyl group. A complex hormonal and enzyme biochemical control system ensures that when the body is in negative energy balance, TAGs are broken down into fatty acids and glycerol in order to traverse cell membranes. Lipoprotein lipase catabolizes the hydrolysis of TAGs and also controls the entry of TAGs in to the cell; it will also regulate the uptake of circulating TAGs by most tissues. The free fatty acids (FFAs) released can either be transported directly to other tissues or transported to the liver where the FAs will be incorporated into very low density lipoprotein

(VLDL) before being transported to other tissues via the circulatory system. All tissue can take up the circulating fatty acids as a function of their concentration in blood; however, only the tissues that increase the activity of lipoprotein lipase (LPL) can compete for the fatty acids (Herzberg G.R. 1991, Iverson et al. 1995, Vernon & Pond 1997). During the lactation fast the metabolism of mothers is altered to facilitate the partitioning of nutrients into the mammary gland for milk synthesis and secretion (Hanwell & Peaker 1977, Bauman & Currie 1980, Iverson 1993, Herrera 2002). The FA uptake in the mammary gland is increased by the augmentation in the LPL activity there, and by the reductions in LPL activity in the blubber (Iverson et al. 1995).

FAs are not only important as energy compounds but they are also important as membrane lipids, ligands for receptors and as precursor of eicosanoids among other functions. Many of these FAs are known as essential fatty acids (EFAs). They cannot be synthesized by animals and thus need to be acquired from the diet (Iverson et al. 1995). The recognized EFAs are 18:2n-6 and 18:3n3. These two EFAs can be further desaturated and elongated to form the long chain n-3 and n-6 PUFAs (Iverson et al. 1995) 20:4n-6, 20:5n-3, 22:6n-3. These EFAs are required for normal growth and cell function and are important in brain and retina development (Crawford et al. 1991, Herrera 2002, Innis 2005). Because EFAs can only be obtained from the diet and are indispensable for a normal growth and development, their transfer to the offspring is of extreme importance.

POLYCHLORINATED BIPHENYLS (PCBS)

Large amounts of man-made pollutants have been released to the environment since the start of the industrialized era. In particular, persistent organic pollutants (POPs) have been extensively used and discharged since the 1920s, when the U.S Monsanto Chemical Corporation introduced polychlorinated biphenyls (PCBs) to the world (AMAP 1998). Since then, these compounds were used and manufactured on a worldwide scale. The physicochemical characteristics of PCBs make them very stable and resistant to biological and chemical degradation, which

makes them very useful in a variety of applications (Safe 1984). PCBs have been extensively used as hydraulic and dielectric fluids, flame retardants, in coolant insulation fluids in transformers, in lubricants and plasticizers in paints, surface coating, adhesive, carbonless copy papers among others (Safe 1994, AMAP 1998). Jensen et al. (1969) first detected PCBs as major contaminants in 1966 in seals and herring from the Baltic Sea. Due to their high toxicity and the negative environmental effects their use and production was banned during the late 1970s. However, the wide variety of uses and a lack of substitute compounds for certain applications such as in transformers and capacitors meant that PCBs were still used and manufactured under some limitations until the 1990s (AMAP 1998). There was therefore continued release of several tons of PCBs into the environment annually. Due to their resistance to biodegradation and their affinity for lipids, large amounts of these compounds are still found in the environment.

PCB PHYSICOCHEMICAL STRUCTURE

The chemical structure of PCBs is shown in Fig. 5. Their basic structure is a biphenyl molecule which consists of two benzene rings joined by a carbon-carbon bond, and with one to ten chlorine atoms attached. The number of chlorines will give 10 possible groups of isomers ranging from mono- to deca-chlorobiphenyls. The trivial names of congeners from PCB 1 to PCB 209 are based on the increasing degree of chlorine substitution according to the International Union of Pure Applied Chemistry (IUPAC). The variation in the number of chlorines and their position on the rings will determine the physical and biological activity of the compound (AMAP 1998). Chlorine positions 2, 2', 6, 6' are *ortho* positions; 3, 3', 5, 5' are *meta* positions and 4, 4' are *para* positions (Fig. 5). The number of chlorine substitutions in the ortho positions will determine the planarity of the compounds. Congeners without *ortho* substitutions will adopt a planar configuration, while congeners with the substitution of 2 or more *ortho* positions can adopt a non-planar configuration (Sawhney 1986). The resistance against degradation of these compounds is closely linked to the degree of chlorination and the position of the chlorines. For instance PCBs lacking adjacent un-substituted

positions on the biphenyl rings are very persistent in the environment. Although organisms have a varying capacity to metabolize some of the PCB congeners, the estimated half-lives for the most persistent congeners can be more than ten years (de Boer et al. 1994).

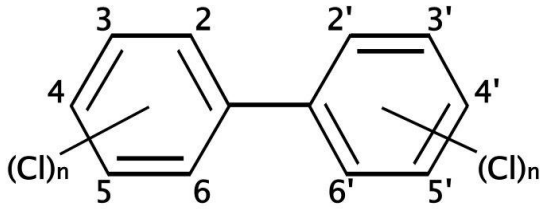


Figure 5. Basic structure of PCBs. One carbon atom is situated at each numbered position around the benzene rings. Double lines between pairs of carbon atoms represent double bonds, but in reality these double bonds flip back and forth between all carbons, forming a very stable structure. 2, 2', 6, 6' are *ortho* positions; 3, 3', 5, 5' are *meta* positions and 4, 4' are *para* positions.

PCB TRANSPORT AND DISTRIBUTION IN THE ENVIRONMENT

Estimates of the total world PCB production since their discovery vary. The most detailed description takes into account the world production until 1990, and estimates a total mass of 2.0 X 10^9 kg of PCBs, only 20-29% of which are estimated to have been released into the environment, and only 1% have reached the oceans (Hutchinson & Simmons 1994). At the time of the report by Hutchinson & Simmons (1994) the possibility of future dispersions of ~30% of PCBs accumulated in dump sites and sediments was recognised, and it was also realised that the 70% of PCBs still in use may ultimately reach the oceans.

PCBs are subject to long-range transport from their initial source, primarily via atmospheric air streams and to a lesser degree via ocean currents. They have been found in all the world's ecosystems, even in remote areas such as the Arctic and Antarctic (Mcclurg 1984, Aguilar 1987, Muir et al. 1988). The distribution of PCBs in the environment is determined firstly by the spatial distribution of point sources and the physicochemical properties of the toxic materials, secondarily by global atmospheric circulation, removal by precipitation and thirdly by biological

pathways (AMAP 1998). Thus pollutant levels found in a given area are closely related to the quantities released from the source area, the transport rate, the detoxification and degradation rate of the chemicals during transport and the residence time in the ecosystem before been absorbed by organisms (Aguilar 1987). The most volatile persistent organic pollutants will be preferentially transported (Bard 1999). Atmospheric transport is the primary way of global distribution of PCBs, with the aquatic environment being the final sink for these contaminants (Antunes et al. 2007). Up to 98% of the PCBs in the ocean are deposited from the atmosphere, and the rest by influx from land runoffs and direct discharge from sea dumping and drainage (Clark 1992). Once in the oceans, PCBs are redistributed to other geographical regions by ocean currents. Marine mammals inhabiting industrialized and therefore highly polluted regions or areas with a limited water circulation such as the Baltic Sea and to some extent the North Sea, will tend to show higher levels of contaminants than those of less polluted areas. It is important to take into account that some hydrological characteristics, such as shallow seas and semi-enclosed areas, can favour the deposition of these contaminants (Hutchinson& Simmons 1994). The high levels of pollution observed in the Baltic and the North Sea are therefore not surprising.

While high levels of PCBs are still recorded in many regions of the world, several studies have reported a decrease in PCB concentrations in organisms since their use and production were banned. This has been seen for instance in harp seals (Beck et al. 1994), sea birds (Elliott et al. 1988) and in Beluga whales from Canadian populations (Muir et al. 1996). However, after initial declines in concentration after the ban, levels now appear to have reached a steady state, for instance those observed in ringed seals in Canada (Addison & Smith 1998).

PCBs IN MARINE MAMMALS

The common characteristics of organochlorine compounds are their low water solubility (high lipophilicity) and their resistance to biodegradation. These characteristics in combination with the low metabolism rate and excretion in organisms cause PCBs to bioaccumulate in the food web. Body burdens (the total

amount of pollutants present in an organism) will also increase with the lifespan of the organism (Ross et al. 2000). PCBs, are initially accumulated by the smaller but abundant organisms such as phytoplankton, or in dead organic matter that readily absorbs PCBs (Bard 1999). Contaminants are then transferred through the food web to zooplankton, crustaceans and fish, all of which use lipid as energy storage and ultimately to top predators such as marine mammals (Mossner & Ballschmiter 1997). This pattern of bioaccumulation and biomagnification means that the longer-lived species that store high amount of lipids and that feed at higher trophic levels accumulate the highest amounts of lipophilic pollutants.

In marine mammals, PCBs are incorporated into blubber lipid stores where they remain until lipids are mobilized for energy and/or production of milk (Addison & Brodie 1977). Blubber can retain as much as 98% of the total body burden (Addison& Brodie 1977, Hutchinson& Simmons 1994). In many species use of stored reserves during breeding, moulting or migration will release the PCBs in to the blood stream where they can exert toxic effects on animals (Hall 2001) due to direct exposure to vital organs.

The most abundant congeners found in marine mammal tissues are PCB-153, -138, -180, 187, 170 and –101, with some variations in their relative concentrations between individuals and tissues (Debier 2001). Individual differences in total and congener-specific concentrations will depend on the concentrations found in the diet (which will be a function of the environmental PCB concentrations and distribution as well as on the structure of the underlying food web), sex, age, nutritional and health status, reproductive history, and on the capacity to metabolize and excrete the pollutant compounds (Bard 1999).

The effects of these persistent organic pollutants in animals have been well documented; they can change the natural function of the reproductive, metabolic, endocrine and immune functions, and this can in turn affect the population dynamics (Helle et al. 1976, Reijnders 1980, Reijnders 1984, Reijnders 1986, Hammond et al. 2005). Indeed, mass mortalities caused by virus epidemics as well as low reproductive success have both been related to high levels of different types of pollutants including PCBs (Reijnders 1986, Hall et al. 1992, Simmonds et al. 1993). Other complications such as occlusion and stenosis of uterine lumens, kidney problems and intestinal ulcers have also been linked to exposure to PCBs

(Bergman & Olsson 1985). While the impact of pollutants on individual organisms has been well documented in laboratory experiments, there is still a long way to go before we can fully describe causes and effects of PCB exposure in marine mammals, especially because chronic exposure can have a delayed effect (O'Hara & O'Shea 2005).

Concentrations of PCBs in different body tissues will be almost entirely dependent on the lipid content of these tissues (Jenssen 1996). In marine mammals the uptake of PCBs occurs mainly from diet via the gastrointestinal tract. It has been suggested that PCBs are transported by passive diffusion across the epithelium, driven by the concentration gradient across the epithelial cells between the gastrointestinal lumen and the blood. Once in the blood, PCBs are believed to be transported in association with chylomicrons, which transport dietary lipids via the blood to the liver where metabolism of the toxics may take place, or to adipose tissue where they can be stored and may remain for long periods. The partition of PCBs between blood and a specific body tissue will be determined by the lipid content of the tissue and by the blood:tissue PCB concentration gradient (Matthews & Dedrick 1984). Due to the high lipophilicity of the compounds they will have a higher affinity for lipid rich tissues and will thus readily be transferred from the blood to these tissues. PCB lipid solubility varies between congeners, and generally increases with increasing numbers of chlorine substitutions. The most common measure of this degree of lipid solubility is the octanol:water partition coefficient, or K_{ow}. Compounds with a high K_{ow} have a higher affinity for lipid and will tend to accumulate more readily in lipid rich tissues. A dynamic equilibrium of PCBs will be established among all body tissues, and the dynamics will be largely driven by changes in requirements for lipids between the tissues according to the nutritional state of the organism.

The excretion of PCBs is minimal before a PCB has been metabolised (Matthews& Dedrick 1984), and one important factor affecting the rate of accumulation is therefore the degree to which animals can metabolize these compounds. This capacity varies between species and organism groups. For instance, marine mammals have a lower capacity for metabolizing PCBs than many terrestrial mammals, but a higher capacity than fish (Matthews& Dedrick 1984). However, the general mechanisms for PCB metabolism appear to be the

same. The kidney and liver are the main organs for PCB metabolism. PCBs are metabolised by the P450 monooxygenase system. The metabolism rate will depend on the number and position of chlorines. The lower chlorinated congeners are more easily metabolised and excreted while the highly chlorinated pollutants appear to be retained and poorly excreted from the body. Sometimes the metabolisation process can also generate metabolites that can be equally or more toxic than the parent compounds (Debier 2001).

PARENT TO OFFSPRING PCB TRANSFER

Elimination of PCBs from the mammalian body mostly occurs passively and in combination with the passage of other substances from the body, especially those that have a high lipid content such as milk and foetuses (Matthews & Dedrick 1984). The amount transferred via milk is much greater than the prenatal transfer. In grey seals it has been seen that up to 30% of the DDT and 15% of PCBs of the maternal burden is transferred to the pups (Hutchinson & Simmons 1994). One reason for the high rates of transfer seen in for instance grey seals is the fact that these animals remain in negative energy balance throughout lactation thus mobilising large amount of lipids and with them the PCBs stored in the blubber. The high rates of PCB transfer to the offspring are of extreme concern due to the vulnerability of developing pups to exposure to high levels of PCBs. While the transfer of PCBs through milk is of concern in terms of the effects on growing offspring, studies show that the concentrations of the more highly lipophilic PCBs in milk are lower than those of less lipophilic congeners (Sørmo et al. 2003, Debier et al. 2003a, Debier et al. 2003b). This may be at least in part explained by a lower rate of mobilization of these PCBs from the adipose tissue into the blood (due to their higher affinity for lipid rich tissues), and thereby a lower rate of transfer to the mammary gland. It is possible that there may be some form of barrier reducing the transfer of these highly chlorinated congeners to the mammary gland.

SUMMARY

This thesis will examine the mobilization and transfer of fatty acid and PCB in grey seals from North Rona and Isle of May, Scotland. The second chapter deals with the geographical and temporal variation in FAs. The second chapter describes a simple model developed to understand the mobilization of FAs from the blubber. The fourth and last FA chapter describes the variations in FAs between compartments and the transfer of FAs to the pups. Two PCB chapters follow these. The first one describes the geographical variation in PCBs and the second examines the differences between compartments and the changes occurring across lactation stages. The aim of this work is to provide greater detail regarding the dynamics of FAs and PCBs. The data of this study will be of fundamental importance for the development of more specific models of PCB dynamics in mammals in general.

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2 Regional, annual and individual differences in blubber fatty acid composition of grey seals (*Halichoerus grypus*) at two UK colonies

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ABSTRACT

Direct observations of feeding in seals are difficult but blubber fatty acid (FA) analysis can be used to obtain information about the diet and also to indicate its variation. In this study we use the blubber FA profiles from breeding grey seal females at early lactation to assess annual, regional and individual variation in the diet over six years in a decade. A total of 127 and 115 blubber samples from Isle of May (IOM) and North Rona (NR) respectevly were analysed. The FA profiles were anlaysed by multivariate analyses: random forest classification analysis. During the six years of study the colonies were clearly distinguished from each other by their FA profiles. There were also annual differences within colonies, which occurred at different time periods in each colony. The FA profile from IOM seals significantly changed during the first three years of study (1996-1998) while NR significantly varied during the next three years (2004-2006). The individuals that were caught in more than two years were similar only in consecutive years. Grey seals are oportunistic feeders, thus feeding in a large variety of species depending on their availibility and preferences. Understanding the geographical and annual variations in diet is important for policy making and mangement of the ecosystem. The posibilities for variation in diet can be a result of a combination of factors, here we disucuss the natural and anthropogenic factors that probably cause such variations in the grey seals FA blubber composition from two Scotish colonies.

INTRODUCTION

Grey seals (*Halichoerus grypus*) are widely distributed in the UK waters with an estimated population size of 113,300 (Duck & Thompson 2007). Approximately 91% are found in Scottish waters and most of the remainder in Wales and England (Duck & Thompson 2007). Grey seals are important generalist marine top predators, and their prey includes gadoids, sandeels (*Ammodytes* spp.) and herring (*Clupea harengus*) (Hammond & Grellier 2006, Hammond & Harris 2006), which are also of commercial importance (Harwood & Croxall 1988). Perceived conflicts between seals and fisheries require realistic models of predator-prey interactions within a larger framework of marine ecosystem management. Formulation of these models must consider geographical, seasonal and annual variations in the diet of grey seal populations, how these may be influenced by the abundance of alternative prey species and also individual preference. Therefore, diet variability must be studied over an extended range and period of time to be useful for management and policy making (Thompson et al. 1996).

Direct observation of foraging behaviour and prey choice of marine mammals is difficult and alternative indirect techniques have been developed to estimate their diet. One of the most widely used methods is the analysis of hard parts collected from scats (Hammond & Prime 1990, Prime & Hammond 1990, Hammond et al. 1994a, b, Thompson et al. 1996, Tollit & Thompson 1996). This method has been used to document seasonal, annual and spatial variations in the diet of a variety of pinnipeds (Pierce et al. 1991, Beck et al. 1993, Tollit & Thompson 1996, Hall et al. 1998, Tollit et al. 1998, Bowen & Harrison 2007), and was used to estimate seasonal and regional variations in grey seal diet and prey consumption in 1985, 1996-1999 and 2002 in the North Sea (Hall et al. 1999, Hammond & Grellier 2006) and only in 1985 and 2002 in the west coast of Scotland and the Shetland Isles (Hammond & Harris 2006). However, scat analysis may provide a biased estimate of seal diet (Cottrell et al. 1996). Typical problems are that otoliths from different prey can be degraded at different rates and some prey species even lack hard parts (Harvey 1989). Furthermore, prey

remains in scats collected on seal haul outs may also be more representative of recently ingested prey from areas close to the haul-outs, rather than providing information on meals ingested over an extended period (Murie & Lavigne 1986). Alternative methodologies for investigating seal diet have emerged in recent years. One such method is based on variations in the fatty acid (FA) composition of blubber, the main storage depot of lipids in marine mammals. Fatty Acid Signature Analysis (FASA) has proved a useful tool in qualitative studies of the feeding ecology of marine organisms (Iverson 1993, Andersen et al. 2004). Fundamentally, many FAs in the marine food web are transferred between organisms with little or no modification, and dietary FAs are deposited in the lipid stores of predators relatively unchanged (Iverson 1993). As blubber FAs are related to the diet this technique uses changes or differences in blubber fatty acids as a qualitative indicator of changes or differences in the diet (Iverson 1993, Kakela et al. 1993, Iverson et al. 1995, Smith et al. 1996, Olsen & Grahl-Nielsen 2003, Thieman et al. 2004). A recent study using captive seals eating a known diet has suggested that FA analysis can be used to assess the diet quantitatively (Iverson et al. 2004) but further controlled feeding experiments are required to assess if this method can be applied reliably to wild populations. Although there is still some debate over the use of blubber FAs to obtain direct information about the diet, the blubber FA profiles can be used successfully to observe relative variations in the diet of marine mammals. FA studies of geographical and temporal variations in diet, for instance between populations or within populations over time, have been widely reported (Kakela et al. 1993, Smith et al. 1996, Iverson et al. 1997, Walton et al. 2000, Moller et al. 2003, Walton & Pomeroy 2003, Beck et al. 2005, Budge et al. 2006, Walton et al. 2007). However, few studies have yet addressed the variability in FA profiles over an extended period of time. In order for FASA to be useful for monitoring dietary variations within and between populations over time, samples must be obtained from specific colonies over a number of years, and preferably also from known individuals to quantify individual variation and assess the stability of individual feeding patterns.

The research presented in this paper is part of a longitudinal study of two UK grey seal breeding colonies where individual adult females have been identified,

studied and sampled over a number of years (Pomeroy et al. 1999). The period covers 3 years during the mid 1990's and 3 years during the mid 2000's. The FA data from 1996-1998 has been previously analysed by Walton et al (2000) and Walton & Pomeroy (2003). The two colonies are situated in markedly different marine regimes: North Rona in the Northeast Atlantic and the Isle of May in the North Sea, separated by approximately 500 km. The two systems have undergone changes in recent decades (Hislop 1996, DeYoung et al. 2004). Changes in the plankton and fish abundance, community composition and phenology in marine ecosystems have been attributed mainly to changes in the ocean climate and physical characteristics such as the temperature and salinity (Edwards et al. 2002, Beaugrand et al. 2003, Beaugrand 2004, Edwards & Richardson 2004). Some changes can occur in response to cyclic patterns of variability. For instance, there is evidence that marine ecosystems undergo decadal fluctutations that are driven by climate (Alheit et al. 2005). The pelagic ecosystem in the North Sea underwent dramatic changes in phytoplankton and fish abundance and distribtion after the mid 1980s (Beaugrand 2004) and early 1990s (Edwards et al 2005). These changes are thought to be a result of changes in regional hydro-climatic parameters such as wind intensity and sea surface temperature which have been related to the North Atlantic Oscillation (Beaugrand 2004, Payne et al. 2009). This triggered a change in the composition and diversity of calanoid copepod species in the North Sea (Beaugrand 2004), and this is likely to have affected the rest of the food web. Increased abundance of warm water species was also observed while species generally associated with colder waters decreased (Beaugrand 2004, Payne et al. 2009). In addition to these environmentally driven changes, marine ecosystems are also influenced by human explotation such as fisheries (Wanless et al. 2007). For instance, it has been suggested that overfishing was the ultimate responsible for the herring colapse in the North Sea. There can be positive and negative feedbacks between human exploitation and environmental conditions. For example, although there were severe measures that lead to the total stop of the herring fishery, the recovery of the stock was slow because the abiotic factors were unfavourable, and the recruitment of herring only increased when the conditions improved (Hislop 1996). Substantial changes in the marine ecosystems as a result of environmental changes and/or antropogenic

factors will significantly alter the composition of prey species available to marine top predators such as seabirds and marine mammals. For instance, the estimated total biomass of principal prey such as cod and sandeel decreased dramatically both in the North Sea and along the Scottish West Coast from the mid 90s to the mid 2000s (ICES 2009).

Grey seals breed synchronously every year in the autumn and tend to be site faithful (Pomeroy et al. 1994). Since grey seals accumulate dietary lipids during the feeding period prior to the lactation fast, blubber collected postpartum offers an integrated but standardised picture of recent dietary history. Although seal diet cannot be estimated directly, we assessed dietary differences and diet variation based on the FA content of blubber in grey seal females at these contrasting colonies. Previous studies have described ecological differentiation between seals from the two colonies and we investigate if the differences persist over a longer time period despite the fluctuations in the regions utilised by the seals. We also investigate if variations in blubber FA profiles are consistent with reported changes in the ecosystems.

METHODS

Lactating grey seals at North Rona (NR) (59°06'N, 05°50'W) and Isle of May (IOM) (56°10'N, 2°33'W) were studied during the breeding seasons in 1996-1998 and 2004-2006. In total 67 and 62 known individual breeding females were captured on IOM and NR respectively, and many females were sampled in more than one year (Table 1). In this study we only consider samples from early lactation, as these represent the blubber energy reserves accumulated during the pre-breeding feeding period. Samples were designated as early lactation if they were collected within the first 4 days post-parturition. This threshold was selceted based on visual inspection of the data. The average sample collection occured in a 2.5 ± 1 days after parturition (range 1-4 days). Lactation duration in this study ranged from 12-23 days (mean ± s.d = 17.5 ± 2.1), and a few samples designated as early lactation could therefore have been collected up to 1/3 into lactation,

however the majority of samples was collected on the first quarter of lactation. The fact that the rate of lipid mobilisation is relatively low at the beginning of lactation suggests that this variation in the timing of sample collection is unlikely to have any major effects on our results. The number blubber samples obtained using this criterion were 127 at IOM and 115 at NR (Table 1).

Table 1. A) Total number of early lactation blubber samples obtained from grey seal (*Halichoerus grypus*) females at North Rona and Isle of May breeding colonies during six years (1996-1998 and 2004-2006). B) Number of females that were sample repeatedly during the six years of study.

	IOM	NR
1996	**33**	**23**
1997	**29**	**25**
1998	**30**	**25**
2004	**10**	**10**
2005	**14**	**14**
2006	**11**	**18**
Total	**127**	**115**

No Captures	IOM	NR
1	33	31
2	18	20
3	8	10
4	6	1
5	2	0
Total	67	62

Females were anaesthetized using an intramuscular dose of 'Zoletil 100' (Virbac, 1.0 ml per 100 kg). A small incision in the skin of the mid pelvic region was made with a scalpel, and a full depth blubber biopsy was extracted using a 6mm diameter biopsy punch (Acu-Punch, Acuderm Inc., USA). Samples were stored in chloroform:methanol (2:1) with 0.05% of BHT as antioxidant until further analysis.

All procedures for handling and sampling animals were performed under UK Home Office project licence and using ASAB (Association for the Study of Animal Behaviour) research guidelines, in consultation with St. Andrews University ethics committee.

LIPID EXTRACTION

Lipid was extracted from the blubber biopsies following the method by Folch et al. (1957). Briefly, the samples were homogenized in 10 ml of dichloromethane:methanol (2:1 vol/vol), water added to form two layers and the aqueous layer discarded. The lipid layer was taken to dryness under nitrogen, weighed and dissolved in toluene at a concentration of 100mg/ml (Walton et al. 2000). Fatty acids were esterified to produce fatty acid methyl-esters (FAME) using 1% (vol/vol) of sulphuric acid in methanol, and incubated for 16 hours at 50^{o}C. The purified FAMEs were dissolved in hexane and analysed by gas chromatography using a Trace GC-2000 (Thermoquest, CE Instruments) equipped with a flame ionisation detector and fitted with a DB23 fused silica capillary column (25 x 0.25mm, J&W Scientific). Hydrogen was employed as the carrier gas. The temperature was programmed to start at 60^{o}C and held for 2 min, then rise to 150 ^{o}C at 20 ^{o}C min^{-1} held for 2 min and then rise to 205 ^{o}C at 1.8 ^{o}C min^{-1}and finally rising to 230 ^{o}C at 5 ^{o}C min^{-1}. Separated components were identified by reference to standards (Walton et al. 2000, Walton & Pomeroy 2003).

STATISTICAL ANALYSIS

All analyses were performed using the R language (R Development Core Team, 2008). All 58 fatty acids detected in the early lactation blubber samples were included in the analysis. Data were in the form of the relative proportion of each of the fatty acids in each sample. Prior to analysis, proportions were arcsine transformed to improve normality. The arcsine transformed FA proportions were used as explanatory variables and Year and Colony were re-coded into a single classifying variable to examine regional and interannual differences.

Random Forest (RF), (Breiman 2001) is a multivariate approach capable of producing unbiased classifications from data sets with a large number of variables. It has proven to be extremely robust and to provide reliable results in a variety of situations (Liaw and Wiener 2002). RF is essentially a tree-based

clustering technique, but rather than creating a single classification tree, RF creates a forest consisting of a user-specified number of trees. The construction of each tree uses a different bootstrap subsample (by default 2/3) of the observations. The remaining 1/3 of the observations are called *out-of bag* (OOB). In a normal tree classification method each node is created using one variable that provides the best split of observations. In RF, each node is created using the best among a randomly chosen subset of variables. By default, the number of random variables used is the square root of the total number of variables. Once a tree has been grown the OOB observations are run down the tree to obtain a predicted classification for each observation. As each new tree is added to the forest, the OOB predictions are used to form an unbiased estimate of the classification error rate. Since random subsamples of the variables are used at each node in each tree, the final forest also provides estimates of the importance of each variable for the classification. Each time an observation is part of the OOB sample, the class into which it is allocated receives a vote. These votes are counted across all the tress built in the RF and an observation is then allocated into the class that received the highest votes for that observation.

In addition to the classification errors and variable importance, RF also provides a measure of the similarity between observations. This is obtained by keeping track of how frequently two specific samples are allocated into the same node when they are run down the tree as part of the OOB sample. This is measured as proximity and stored in a symmetric n x k matrix, where n and k both represent the number of observations. Each time samples n and k end up in the same node when they are both run down the tree as OOB samples, the value in row n and column k is incremented by 1, and the matrix therefore provides an intuitive measure of the similarity between observations. RandomForest is available in the `randomForest` package for R (Liaw & Wiener 2002).

Because of the difficulty in visualising multidimensional proximities we performed multidimensional scaling on the distance matrix (i.e. the inverse of the proximity matrix) to project the multidimensional distances down in to a low dimensional space. A scatterplots of the first two dimensions often provides a useful graphical representation of the clustering into a lower dimensional space while preserving the distances between the observations. The proximity matrix

can also be displayed as a heatmap indicating the proximities between particular observations and classification groups. A heatmap is a representation in a colour gradient of the values obtained by a given variable. Each colour illustrates coded squares that specify a proximity value between samples or groups where a colour image represents similar samples or profiles.

While other classification methods require inclusion of a cross validation step to obtain an unbiased estimate of the classification error, RF estimates the overall error and the classification error in each tree and the results are given in the output as a confusion matrix. This is central for the evaluation of the RF classification for the different classes.

TESTING FOR EFFECTS OF REPEATED SAMPLES

Samples collected from the same individual in different years may be more similar than the overall similarity within that colony between the different years. To evaluate if there was any risk of these repeated samples leading to an increase in the error rate between the years for which repeated samples existed, we performed several RF analyses on subsets of the data for each colony. The first subset excluded all individuals for which samples were obtained in more than one year. A new subset was then created by adding samples from a particular individual that was sampled in two different years and randomly deleting one sample each from the same two years to keep the sample size constant. The procedure was then repeated, sequentially substituting two random samples from the same two years as previously with samples from another individual with repeated captures in those two years until all females with repeated samples in the two years were included in the subset. In each RF run the misclassification error rate between the two years was recorded. This procedure was repeated for all possible combinations of years, each time keeping samples from all other years constant. The overall effect of the number of individuals with repeated samples on the error rate was evaluated by a linear model, where the explanatory variable was the number of animals with repeated samples and the response variable the misclassification error rates. Our analysis suggests that the presence of repeated

samples from individuals did not have any effect on the classification error rate ($F_{(1,111)}$= 0.2307, p = 0.632).

Similarity between samples from the same individuals

We also tested if samples from the same individuals in different years were more similar than the overall colony sampled in the same years at the same location. The number of samples collected from the same individuals between years ranged from 0 to 19 (Table 3). The similarity between the same seal in different year was tested by comparing the RF proximity values of samples from the same individuals in different years to the proximity values of unmatched samples in the same years. For each combination of two years within a colony the proximities of matched (animals sampled in both years) and unmatched samples where compared with bootstrapped Wilcoxon tests. Due to the small sample size of matched samples in comparison to the unmatched in any of the pair of years compared, a random subset of the unmatched samples, of the same size as the number of matched samples, was extracted at each of 1000 bootstraps. The Wilcoxon test was then performed between the matched samples and each of the 1000 bootstrapped subsets. The median of the 1000 bootstrap *p* values ($\alpha = 0.05$) was then used as measure of significance.

Results

Blubber fatty acid composition

There were large variations in the FA composition, and these variations appeared to be largely explained by colony differences and annual variability. The FAs that were present in relative amounts greater than 2% in all colony:year groups were 18:1n-9, 22:6n3, 16:1n-7, 16:0, 20:1n-9, 20:5n-3, 18:1n-11, 22:5n-3, 14:0, 22:1n-11, 18:2n-6, 18:1n-7, and 18:4n-3. Altogether these 13 FAs accounted for

approximately 85% of the total fatty acids in both colonies in all years. The remaining 45 FAs were found at much lower percentages (Appendix 1).

The confusion matrix (Table 2) shows the number of samples that were correctly and incorrectly allocated to the different groups, and also shows the classification error for each colony in each year. Samples from NR and IOM were well differentiated in each of the 6 years studied. Only 3 out of 127 IOM and 5 out of 115 NR samples were allocated to the wrong colony.

The degree of misclassification between years within the same colony was higher than the between-colony misclassification rate. For IOM samples, misclassification error rates were much smaller during 96-98 than during 04-06. The misclassifications observed in 96-98 occurred mainly within those three years. Annual differences in the IOM were much less pronounced during 04-06. In contrast, North Rona showed the opposite pattern, with samples from 96-98 being poorly differentiated from each other with high classification errors between the three years, and samples from 04-06 being highly differentiated from each other, with very low classification errors (Table 2).

Plots of the first two dimensions of the multidimensional scaling emphasise the confusion matrix results (Fig. 1). The highest degree of differentiation and of cluster separation from the other samples was observed for samples from IOM 96-98 (Fig. 1a). NR samples from 04-06 appear fairly well differentiated from each other and from the rest of the NR and IOM samples, while IOM samples from 04-06 formed an internally diffuse cluster that was distinct from the rest of the samples from both colonies (Fig. 1b). Samples from NR 96-98 formed a cluster without any obvious differentiation between the 3 years (Fig. 1c), but this entire group (NR 96-98) was well differentiated from all other groups. The apparent overlap between NR 04 and IOM 04-06 seen in Fig. 1 shows the limitation of 2-dimensional scatterplots for visualising multidimensional data. Instead, these differences are more apparent in the heatmap, where a complete picture of the

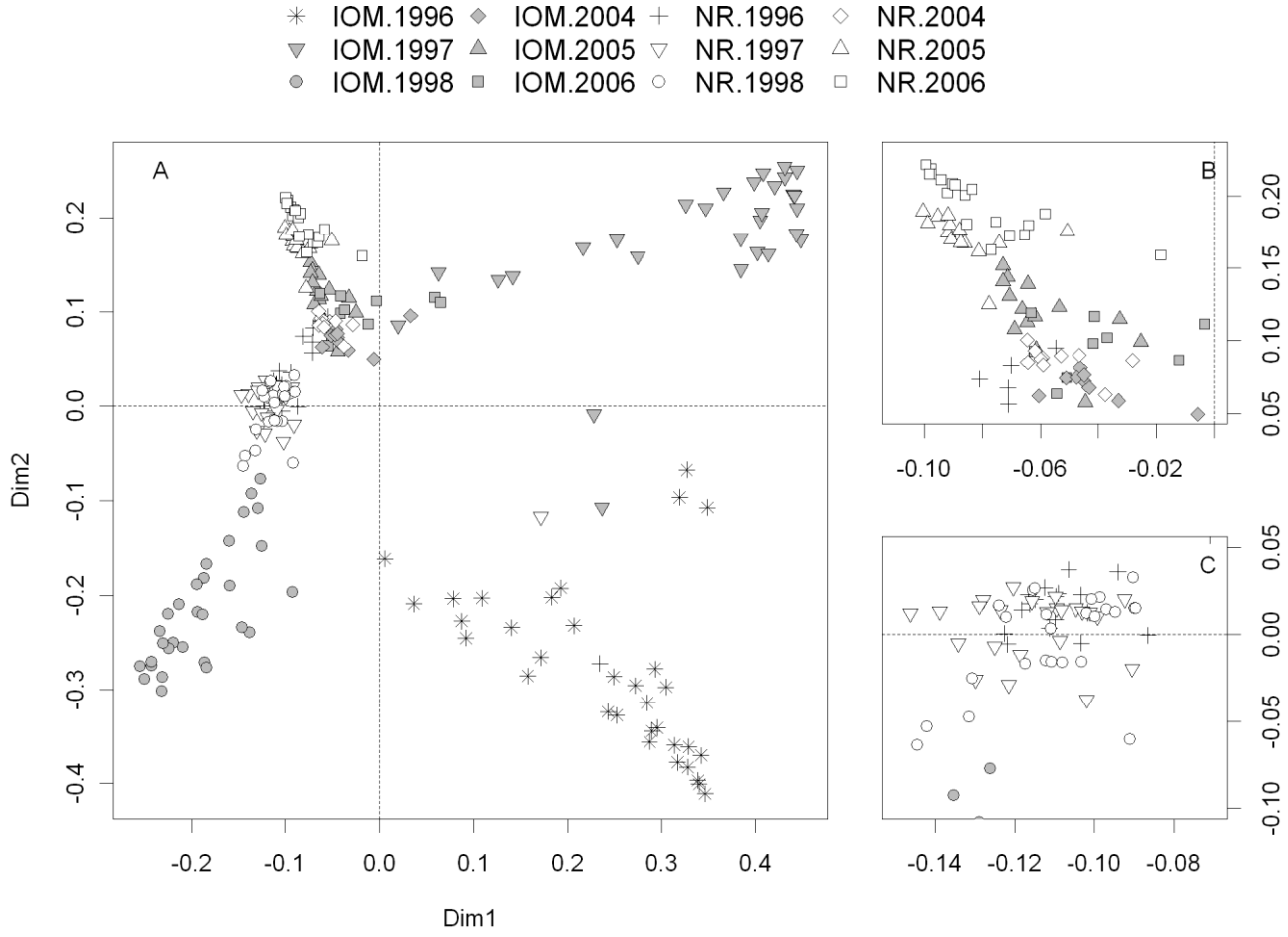

Figure 1. Multidimensional plot of the first two dimensions (Dim1 and Dim2) based in the proximity matrix to visualise clustering and similarities between groups. Plot of early lactation blubber samples from grey seals (*Haliochoerus grypus*) obtained during six years at North Rona and Isle of May. A) Both colonies represented during six years; B) Zoom into the samples from NR and IOM 04-06 and C) Zoom into the samples from NR 96-97.

overall patterns and similarities of samples within and between years and colonies can be observed (Fig. 2). The heatmap shows the high degree of similarity within IOM samples over the last three years and for NR samples over the first three years. The heatmap also highlights the degree of similarity between samples from a specific colony in a specific year, such as for IOM samples from 96-98 and NR 04-06, where the degree of similarity between samples of a given group is higher than that observed in the last three years at IOM and first three years at NR.

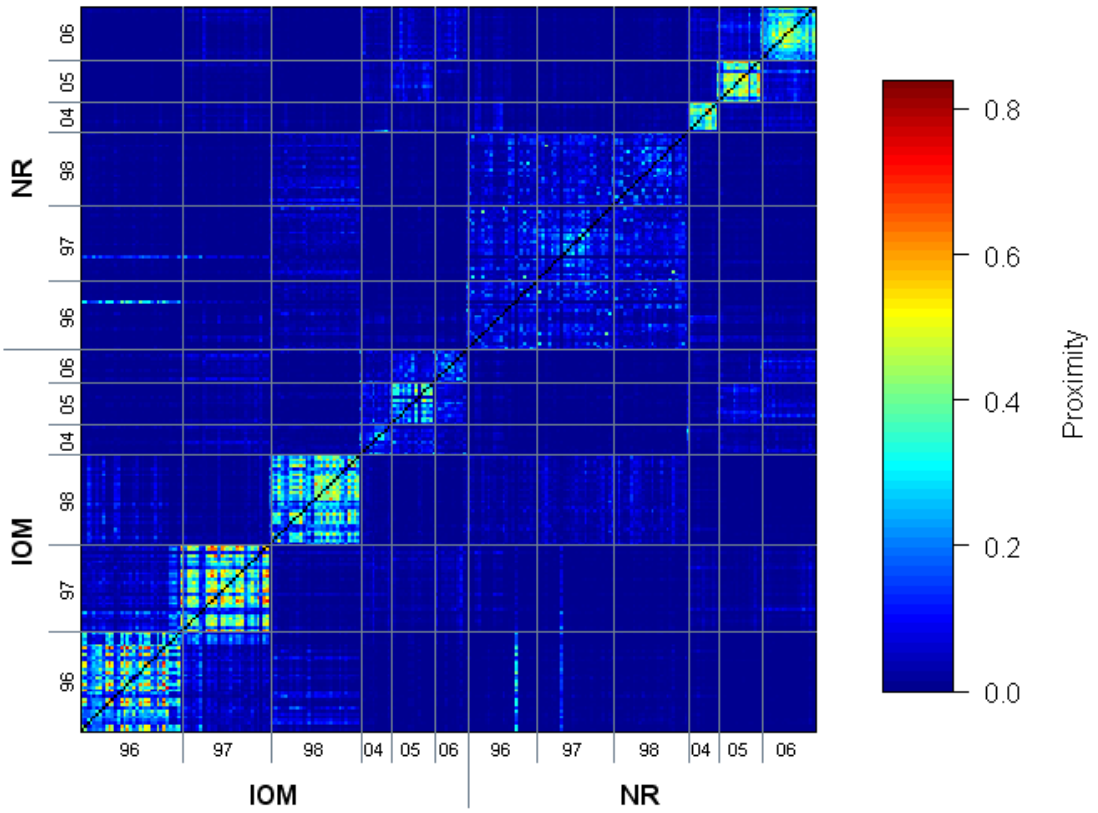

Figure 2. Heatmap of the proximity matrix obtained from the Random Forest analysis of female blubber FA composition at early lactation. Each cell represents one individual in a given year (the same individual can occur in more than one year but can only occur once within a given year). The matrix is symmetric and individuals are organized in the same order on both axes. The cell colour represents the proximity between individuals, ranging from low (blue) to high (red). The diagonal black line represents the proximity between one individual and itself, thus having a value of one. The horizontal and vertical grey lines highlight the delination between squares representing the different colony/year groups. Squares along the diagonal represents the within-group proximities, while squares off the diagonal represents between-group proximities.

Table 2. Confusion matrix showing the samples correctly and incorrectly allocated by random forest classification analysis of fatty acid composition of blubber at early lactation. The overall and group-wise classification had an error rate of 22 %.

Location		Isle of May						North Rona						
		1996	1997	1998	2004	2005	2006	1996	1997	1998	2004	2005	2006	C. error
Isle of May	1996	30	-	3	-	-	-	-	-	-	-	-	-	0.09
	1997	2	26	-	-	-	-	-	-	-	-	-	1	0.10
	1998	-	-	30	-	-	-	-	-	-	-	-	-	0.00
	2004	-	-	-	6	3	1	-	-	-	-	-	-	0.40
	2005	-	-	-	2	9	2	-	-	-	-	-	1	0.36
	2006	-	-	-	1	1	8	-	-	-	-	-	1	0.27
North Rona	1996	1	-	-	-	-	-	11	7	4	-	-	-	0.52
	1997	1	-	-	-	-	-	6	11	7	-	-	-	0.56
	1998	-	-	2	-	-	-	1	4	18	-	-	-	0.28
	2004	-	-	-	1	-	-	-	-	-	9	-	-	0.10
	2005	-	-	-	-	-	-	-	-	-	-	12	2	0.14
	2006	-	-	-	-	-	-	-	-	-	-	-	18	0.00

INDIVIDUAL VARIATION

There was some weak support for the hypothesis that samples from the same individual seals in different years were more similar than unmatched samples from the same colony. Out of 15 annual comparisons, there were only two pairs of years in which matched samples from the same indidivuals in two different years were more similar than unmatched samples from the same pair of years. Interestingly, both these significant results were for consecutive years (96-97 and 05-06, Table 3), while individuals sampled in non-consecutive years did not appear to be more similar. At NR, matched samples were more similar than unmatched samples in 4 out of 15 annual comparisons: 96-97, 96-98, 97-98 and 05-06. Again, these greater similarities for matched samples were mainly observed for consecutive years.

Table 3. Degree of fatty acid profile similarity between samples from the same individuals in different years, compared with the overall similarity of unmatched samples collected in the same year at the same location. The number of animals sampled each year (shown in brackets beside the year) and the number of same individuals compared between years (shown in brackets besides symblos) for each colony and for each year compared. *** indicates year comparisons where similarity of matched samples are significantly higher than for unmatched samples; * indicates matched samples are higher but not significantly; and = indicates that mached and unmatched samples are equally similar. The p value for significance was $\alpha = 0.05$.

Isle of May	**1996** (33)	**1997** (29)	**1998** (30)	**2004** (10)	**2005** (14)
1997 (29)	*** (15)				
1998 (30)	* (15)	= (19)			
2004 (10)	= (5)	= (7)	= (7)		
2005 (14)	= (1)	= (2)	* (2)	= (5)	
2006 (11)	= (3)	* (4)	* (4)	= (4)	*** (5)

North Rona	**1996** (23)	**1997** (25)	**1998** (25)	**2004** (10)	**2005** (14)
1997 (25)	*** (17)				
1998 (25)	*** (12)	*** (9)			
2004 (10)	0	0	= (2)		
2005 (14)	* (1)	= (1)	* (2)	= (2)	
2006 (18)	= (3)	* (3)	* (4)	= (3)	*** (7)

VARIABLE IMPORTANCE FOR CLASSIFYING BETWEEN GROUPS

The most important FAs for distinguishing a particular colony in a given year relative to other years is shown in Fig. 3. The plot is based on the variable importance values generated by randomForest. The most important FAs for classifying the groups are not necessarily the FAs found in high proportions in the blubber. In fact, the six most important FAs selected (18:3n-3, *iso*-18, 18:2n-6, 16:3n-1, 18:3n-1, 22:5n-3) by the RF classification all had relative abundances less than 10% (Fig. 4).

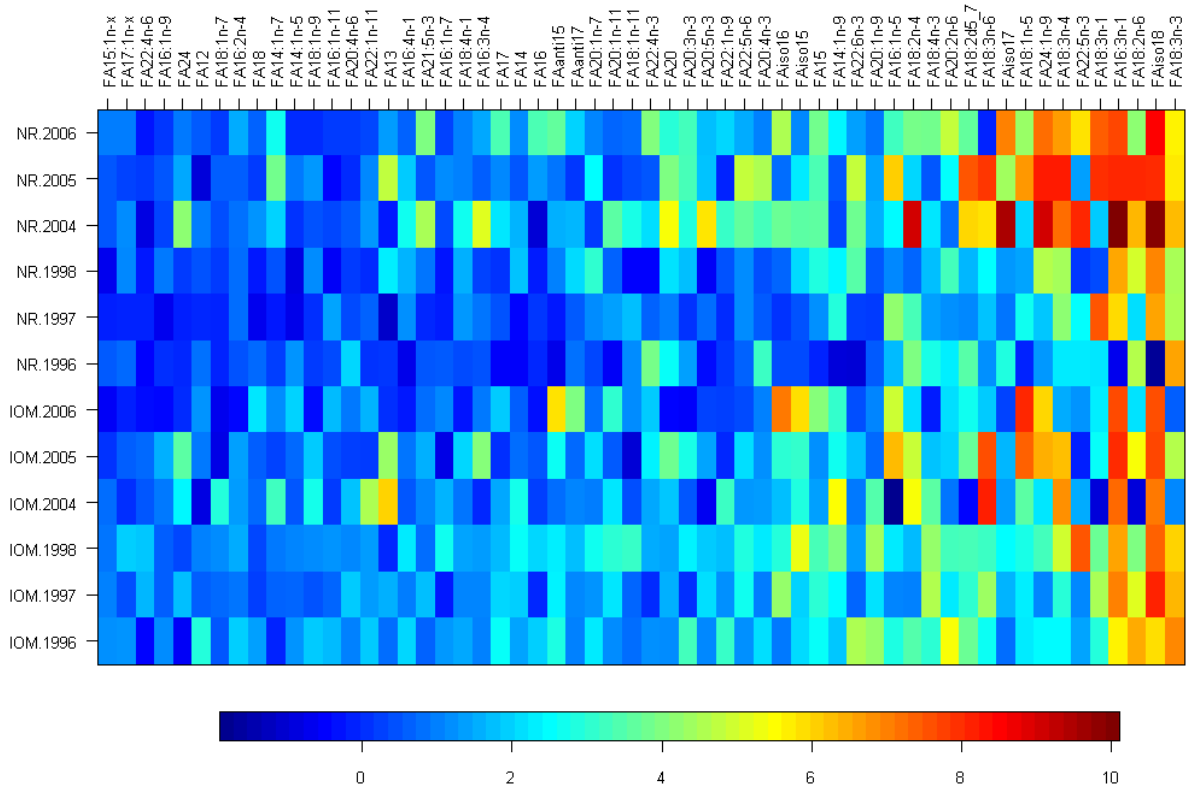

Figure 3. Variable importance plot. Each colour represents a group wise importance for each fatty acid. The continuous colour scale represents the decrease or increase of variable importance obtained from the random forest, where dark red corresponds to the most important and dark blue to the less important variable used to distinguish between groups.

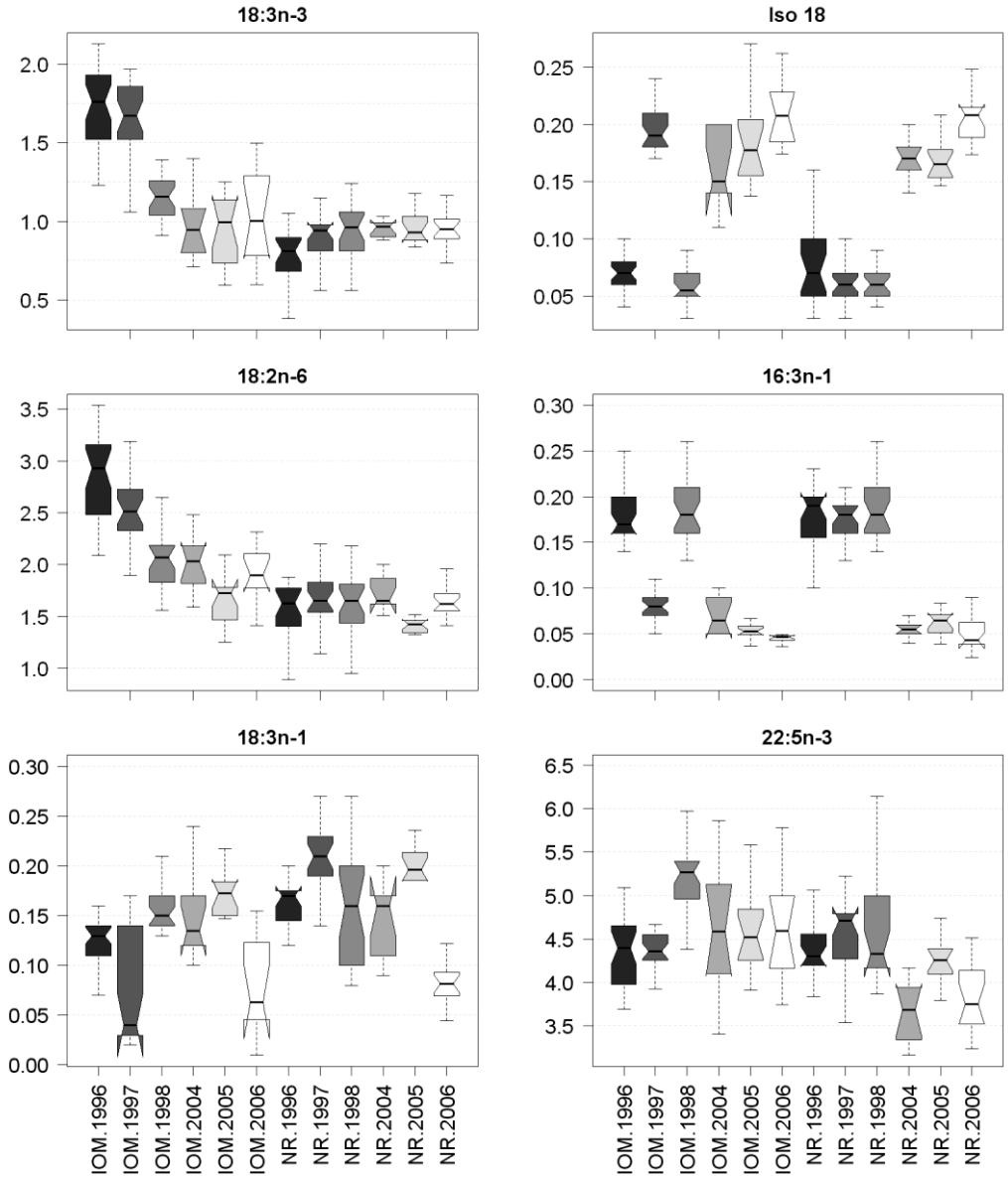

Figure 4. The six most important FA in distinguishing between year and colony groups. Boxes and whiskers represent the range containing 50% and 95% of the data respectively. Notches in the boxes extend to ±1.58 times the interquartile range divided by the square root of the sample size. No overlapping notches in two boxes indicate a ~95% probability that the medians in the two groups are different.

DISCUSSION

The present paper shows that despite substantial year-to-year variability, the differences in FA composition of grey seals from North Rona and the Isle of May that were described by Walton et al (2000) persisted at least throughout a 10-year period from the mid-1990s to the mid-2000s. This suggests that seals utilizing these two breeding grounds are clearly ecologically distinct in terms of their feeding, at least during the time leading up to the breeding season. In addition, this study also indicates that major changes in feeding ecology occurred in both populations, but that these changes did not occur simultaneously. I will later discuss to what extent this may relate to observed ecosystem changes in the North Sea and the Northeast Atlantic. There was also some evidence that the FA profiles of individual seals remained more similar between consecutive years than for the population as a whole, but that these individual signatures disappeared at greater separation between years. The results from this study provide novel and important insights into the regional, annual and individual variations in blubber composition of female grey seals, highlighting the importance of monitoring populations over longer time periods to appreciate the variability and dynamics of their feeding ecology. While several previous studies have documented geographical, annual and seasonal variation in the diet of pinnipeds (Prime & Hammond 1990, Pierce et al. 1991, Bowen et al. 1993, Hammond et al. 1994b, a, Tollit & Thompson 1996, Tollit et al. 1997), these studies have generally approached the issues at a population level, while no longitudinal studies have been carried out expressly to examine variations on the same group of individuals, at an annual and regional level.

There are at least two mechanisms that may explain the general differences in FA profiles between NR and IOM seals. First, if we assume that the FA composition of specific prey species is similar in both regions, the observed differences suggests differences in prey availability between the two regions, leading to (1) the consumption of entirely different prey species by seals from the two colonies or (2) the same prey species being consumed but in different proportions. The diet of grey seals has been described as consisting mainly of

benthic and demersal fish, and while more than 20 potential prey species are represented (Hammond & Prime 1990) only a handful of these account for a major proportion of the diet (Hammond & Prime 1990, Bowen & Harrison 2007) with cod, sandeel, herring, haddock and different flat fish species been the main prey species (Hammond & Prime 1900, Hammond & Harris 2006, Hammond & Greiller 2006). This has been observed in the faecal analyses perfomed in different haul outs in the North Sea including IOM during 1985 (Hammond & Prime 1990), 1996 to 1998 (Hall & Walton 1999) and in 2002 (Hammond & Grellier 2006). While no similar studies have been done specifically for seals at NR, studies in the Hebrides and Orkneys indicate that while many of the same prey species occur in the diet of grey seals from the east and west coasts, they occur in different relative proportions (Hammond & Prime 1990, Hammond & Grellier 2006, Hammond & Harris 2006). For instance, the estimated consumption rate of sandeel in 1985 was higher in the North Sea compared to that in the Hebrides, while in 1985 and 2002 the consumption rate of herring was higher in the Hebrides than in the North Sea. Although our results can not describe directly the diet, the clear differences between colonies in their FA compositions over a decade support the idea that seals from these colonies are ecologically distinct, i.e. that they probably feed in different ecoregions. Indeed, the feeding areas of seals captured at different haul outs around the UK outside of the breeding season have been studied using satellite telemetry (Thompson et al. 1991, 1996, McConnell et al. 1999, SMRU unpublished). The only telemetry study on seals from NR indicates that their main feeding grounds are situated around the northwest coast of Scotland and probably overlap to a certain extent with the feeding grounds of seals from the west coast (Pomeroy, personal communication). The only region that appear to be utilized to any significant degree by seals from both NR seals and seals from the eastern UK haul outs is around the Orkney Islands (Pomeroy, personal communication). In general, these observations support the assumption that there is a general geographic separation between east coast seals, which mainly feed in the North Sea, and seals from the northwest coast, which remain almost exclusively in the northeast Atlantic (McConnell, personal communication).

The ecosystem structures in the Northeast Atlantic and the North Sea are very distinct, and this can partly be explained by the vastly different circulation patterns and general environmental conditions of these two regions (Edwards et al. 2005). The observed colony differences in FA composition of top predators in these systems can occur as a result of differences in the relative proportions of the main prey species available as previously mentioned. However, differences can also occur as a result of differences in the plankton community structures, which ultimately determine the FA composition of the prey of higher trophic levels. Changes in lower trophic leves will also affect directly the diet of the main prey species. Thus, the most likely explanation for the observed differences in FA composition of seals from the two colonies is therefore a combination of 1) differences in the proportion of different prey in the diet and 2) differences in the FA composition of those prey species.

Similar explanations can also be applied to the observed temporal changes in FA composition which were observed at the two colonies but at different times. Drastic changes or shifts in plankton and fish community structure and abundance have been observed in many different marine ecosystems (Bax, 1998). The mechanisms driving these shifts have been linked to climate change, eutrophication and anthropogenic factors such as over-fishing and pollution, or sometimes to a combination of these (Heessen & Daan 1996, Choi et al. 2004, Frederiksen et al. 2006, Litzow et al. 2006, Smith et al. 2006, Alheit 2007). Changes in the oceans induced by any of these factors are likely to affect all trophic levels. Any variations in nutrient composition can have knock-on effects throughout the food web (Alheit 2007) so that changes at lower levels in the food web can have direct and rapid effects on fish community biomass and distribution, ultimately affecting top predator diet (Baker 2005, Frederiksen et al. 2006, Hátún et al. 2009). For instance, dramatic changes in the North Sea have occurred in the last few decades, both in terms of physical characteristics such as increase in temperature, and in biological structure, such as decrease or increase in the abundance of certain species (Heessen & Daan 1996, Hislop 1996, Beaugrand 2004, Edwards et al. 2002, Edwards & Richardson 2004, Alheit 2007). These changes have been linked to antropogenic and environmental factors (Beaugrand 2004, Alheit 2007). The two most distinct regime shifts in the North Sea occured

during the late 1970s and late 1980s, but there was also a clear regime shift in the plankton communituy during the 1990s, from being dominated by cold-water species towards greater abundances of more warmer sub-tropical species (Beaugrand 2004, Edwards et al. 2002, Edwards & Richardson 2004, Edwards et al.2005). After this last regime shift, it appears that the North Sea ecosystem has remained in a warm water state (Edwards et al.2005), although annual variations within this state are also evident (see below). One of the most dramatic and important changes is that of *Calanus* copepods, one of the most important secondary producers in this ecosystem. Data on plankton phenology, abundance and species composition from 1946 to 2003 indicate that the abundance of these copepods declined dramatically during the 1990s (Wanless et al. 2007). Changes in the abundance of some fish species, such as sandeel and gadoids, have been linked to such changes in phytoplankton and zooplankton communities, particularly to these declines in copepods (Fredriksen et al. 2006, Hislop 1996). Similarly, in the northeastern North Atlantic, increases in sea surface temperature have affected the distribution, biomass and seasonality of the plankton and has also produced a shift in the distribution of species, with a northward shift in the occurence of herring, cod and sandeel (Baker 2005). Changes in plankton community structure can not only affect the abundance of various fish species but may also change their lipid composition. For instance, changes in the FA composition of herring larvae occurred when phytoplankton species composition at the base of the food web changed from being dominated by flagellates to higher abundances of diatoms (John & Lund, 1996). A recent study by Hátún et al (2009) have shown that pronounced changes in the fauna have occured in regions from as far south as the English Channel all the way north to the Barents Sea. These changes occured during 1920, late 1960s and again in the early 1990s. They attributed these changes to the water mass dynamics in the North-estern North Atlantic Ocean, showing that a change in the subpolar gyre from a strong state to a weak state impacted the entire ecosystem, from phytoplankton to marine mammals. For instance, blue whiting showed dramatic changes in stock size, shifts in migration patterns and variation in the spawning activity, and these factors were also linked to changes in the occurrence of pilot whales for which blue whiting is a main prey species. Changes in the stock

biomass of two main prey species of grey seals: cod and sandeel, have taken place in both the North Sea and the West of Scotland from the mid 90's to mid 00's. In the North Sea the estimated total stock biomass (TSB) of cod between 1996-1998 was at least 3 times higher in comparison to the estimated biomass for 2004-2005 (ICES, 2009). The TSB for sandeel increased almost the double from 1996 to 1997. However, from 1997 to 1998 it decreased to a similar estimated TSB as in 1996. Since 1998 the estimated biomass has been declining, from alost 2.4 million tonnes it decrease to less than a million in 2004 with further decreases in the following years (ICES, 2009). Although for the west coast there are no estimated total stock biomass

In addition to these bottom-up effects, there is also evidence that top-down mechanisms can affect the FA composition at lower trophic levels. For instance, overfishing of higher trophic level demersal fish species led to a community transition in the North Atlantic (Litzow et al. 2006), and it has been suggested that such 'trophic cascades' can have an impact on the community lipid content at lower trophic levels, ultimately changing the lipid composition of the prey of top predators (Litzow et al. 2006).

Data on the dietary composition of grey seals, whether from faecal analyses of analyses of blubber FA composition, have not been carried out with sufficient regularity and consistency to determine exactly how observed dietary changes are related to the major ecosystem changes discussed above. The latest major regime shift in the North Sea occurred in the early 1990s while our earliest blubber samples are from 1996. However, substantial annual variations have been observed also between these major regime shifts, and these may be linked to natural environmental variability. Natural environmental fluctuations affecting marine ecosystems can occur at various timescales, such as for instance the North Atlantic Osillation which varies on a decadal timescale (Edwards et al. 2002, Baker 2005). The period 1996 -1998 coincides with a period of return from an extreme negative NAO year in 1996. Within the generally warm water state of the North Sea ecosystem since the 1990s, 1996 was notable for a temporary return towards a pre-1990 state, but the period following 1996 again saw a change towards a state more dominated by warm water species (Edwards et al. 2005). The fact that IOM blubber samples from 1996-98 can be relatively clearly

distinguished from each other may reflect a response by upper trophic levels to this change. It is unclear, however, why we do not see evidence for similar annual changes at NR during the same years. Since the response of the marine organisms to physical changes usually occur with some delay, ecological regime shifts cannot always be pinpointed to a specific year. Furthermore, different species can react to environmental change in different ways and at differen rates (Alheit et al 2005). The response of ecosystems may therfore not always be immediate and straightforward, but will be related to physiological, biological and ecological species characteristics, and the way in which they respond to the various environmental signals (Beaugrand 2004), and the environmental signals themselves may be more or less strongly expressed depending on physical structures of different regions. For instance, it is possible that environmental fluctuations, such as for instance those associated with changes in the NAO index, have more dramatic effects on ecosystems in the shallower North Sea than in the northeast Atlantic region, where ecosystems may be more influenced by upwelling of deep water which are less affected by atmospheric anomalies. Ultimately, however, ecosystems in these less directly affected regions may start responding, if the environmental signals become sufficiently strong. There is some evidence that further dramatic changes in ecosystem structure occurred in the northeastern North Atlantic during the mid-2000s (Reid, pers. comm.). Unfortunately the data on plankton community have not yet been sufficiently analysed and synthesized to determine if such a trend is indeed present during this period.

The data of our study can not determine if the variations that we observed in both colonies is due to changes in the prey availability and/or changes in the FAs composition in the prey due to an alteration in the lower food web such as in the community plankton. The fact that NR and IOM are located in two very different environments might explain the differences observed in the variation in diet in different decades. While some of the underlying environmental drivers can be of a global nature, the mechanisms discussed above normally operate on a regional scale and can affect individual species, species groups or sometimes entire ecosystems (Heessen & Daan 1996). All the underlying factors affecting the food web may ultimately impact the prey composition and availability and, if the changes are dramatic enough, may force predator populations to switch diet. The

dramatic decline in gadoid abundance in the North Sea during the 90s (Hislop 1996) and the major shifts in the plankton community fits with the changes in the FA composition observed in the IOM colony during the 96-98 period. In the scat study performed during 1996-1998 at IOM clear changes in the diet were observed, where the proportions of cod were significantly higher during the spring diet between 1997 and 1998 and contained higher proportions of sandeels in the winter diet of 1996-1997 than in the winter diet of 1998-1999 (Hall & Walton 1999).

The large number of known individuals within each colony that were sampled repeatedly during several years allowed us to explore how individual patterns in FA composition develops over time. Previous studies have demonstrated a high degree of individual variation in diet of seals due to factors such as age, sex and body condition (Grahl-Nielsen & Mjaavatten 1995, Beck et al. 2005, Bowen & Harrison 2007). In addition, if individual preferences or specialisation for specific prey species exist, one would assume that the lipid composition of individuals would remain more similar in consecutive years compared to the overall colony. Our analyses found some evidence of such individual similarity between years. Comparisons showed a slightly higher individual similarity compared to the overall year-colony sample, with the degree of individual similarities decreasing as the years between sampling increased. However, the pair of consecutive years that showed a higher similarity within the same individual were few. The lack of similarity at other times could be interpreted as seals changing diet as a response to a changing environment. Individual dietary preferences and strategies will develop or possibly change with age. As the average age in 1996 for the same individuals compared was 22 ± 6 year at NR and 15 ± 5 years at IOM, we suggest that preferences and strategies were well established already, thus age is unlikely to have much bearing on the observed similarities between the same individuals in different years.

While analyses of FA composition is normally done using traditional multivariate techniques such as Principal Components Analysis, Discriminant Function Analysis or Tree analysis, we introduce the use of randomForest in this context. Samples from the first three years had already been analysed using classical multivariate analyses (Walton et al. 2000). The results obtained in this

study by Random Forest agreed well with these previous results, showing that randomForest is a reliable methodology for analysing this type of dataset. However, we believe that Random Forest has some attractive properties that in some ways make it a more appropriate method compared to the classical approaches. Perhaps the most important property is that variable importance measures can be obtained for all variables in the dataset. The randomised nature of Random Forest also means that no splitting of data into training and testing datasets is necessary, and the results from Random Forest has been shown to be very robust in a broad range of applications.

Analysis of blubber fatty acid composition has proved to be useful for investigating dietary differences between regions, between years and also to track individual patterns and how these may change over time. FAs ratios should be studied as trophic biomarkers for an indication of possible dietary sources, since FAs can be attributed to particular phytoplankton, zooplanton and even fish (Budge & Parrish 1998, Stubing & Hagen 2003). Because top predators are likely to be affected by the accumulated changes occurring throughout entire food webs or ecosystems, multiple processes operating at different trophic levels may cause the observed differences. Since these processes may take place over a variety of timescales, long-term studies are essential for monitoring the foraging dynamics of populations and if possible, specific individuals to assess how they respond to major ecosystem shifts. It will be good to obtain complementrary data that monitor variations within and between colonies over extended periods of time to confirm our results.

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3 Selective blubber fatty acid mobilization in lactating grey seals (*Halichoerus grypus)*

ABSTRACT

Blubber is the main energy store in marine mammals. During negative energy balance fatty acids stored as triglycerides are released to cover the metabolic demands of the body. Studies have shown that fatty acids (FA) from adipose tissue are selectively mobilized according to their carbon length and number of double bonds, however the few studies *in vivo* have only focused on fasting and non lactating animals. During lactation, grey seals fast for 18 days mobilizing a large amount of lipids to cover their metabolic demands and the nutritional requirements of a growing pup. We investigated the FA mobilization from blubber in grey seals from two UK breeding colonies, Isle of May (IOM) and North Rona (NR), which are well discriminated by their FA profiles. Both colonies were sampled in 2005 ($n_{IOM} = 9$; $n_{NR} = 12$) and 2006 ($n_{IOM} = 14$; $n_{NR} = 22$). We used linear mixed effects models to examine to what extent the mobilization observed in lactating grey seals in both colonies can be explained as a function of FA carbon chain length and number of double bonds. There was selective mobilization of FAs, such that for a given chain length, mobilization increased with the number of double bonds, and for a given double bond, mobilization decreased as chain length increased. This pattern of selective mobilization was very similar between the colonies. As a result, the degree of mobilization of a specific FA is not simply a function of its occurrence in blubber. Essential fatty acids, which are crucial to pup development but which they cannot synthesize, are more mobilized than predicted based on their chain length and degree of unsaturation, suggesting that selective mobilization of FAs is related not only to the physicochemical characteristics of the FAs but also to the needs of a developing pup.

INTRODUCTION

One major characteristic of mammalian reproduction is the high energetic costs associated with the lactation period. In addition to covering their own metabolic requirements, lactating females must also support those of their growing pups by the production of energy rich milk (Vernon & Pond 1997). Strategies evolved to deal with this high energetic demand, range from pure income breeding to capital breeding (Drent & Dann 1980, Trillmich 1996). At one extreme income breeders obtain the required energy on a daily basis food intake during lactation, while at the other extreme capital breeders rely exclusively on energy stored during a feeding period prior to fasting during reproduction (Trillmich 1996). Land-breeding phocids such as grey seals generally belong to the second category of true capital breeders. Their breeding colonies are often separated in space from their main feeding grounds and therefore they are adapted to fast during this period (Trillmich 1996). Grey seal mothers rely entirely on lipid reserves stored mainly as blubber to cover their own energy requirements as well as that of their growing pups during lactation, which lasts an average of 18 days (Iverson 1993).

Adipose tissue is highly dynamic, and it plays an important role in many physiological functions of mammals (Mustonen et al. 2007). The blubber of marine mammals is composed mostly of fatty acids (FA) stored as triacylglycerols (TAG) (Dalsgaard & St John 2004). The relative amounts of different FAs in blubber are determined by the diet and nutritional state of the individual. During the feeding period FAs from the diet are accumulated with or without modification as TAGs, and during periods of negative energy balance they will be broken down to provide fatty acids for energy production. During the lactation fast, female grey seals typically lose around 40% of their initial body mass and approximately 68% of their initial fat stores (Fedak & Anderson 1982, Pomeroy et al. 1999). The rate of maternal fat mobilization can reach 4.7 kg d^{-1} and as much as 56% of the daily energy expenditure is used for milk production (Mellish et al. 1999). Consequently, grey seal milk is extremely rich in lipid, from 30% fat at early lactation to 60% fat by late lactation (Iverson et al. 1993). Up to 98% of the lipids in the milk are made up of TAGs and most are derived directly from the female

body stores (Neville & Picciano 1997) with minimal *de novo* synthesis of fatty acids in the mammary gland (Iverson 1993).

Lactation is an extremely important period in the development of the offspring in which the newborn is completely dependent on the mother's milk. Phocid milk contains almost no carbohydrate, and the energy required by pups is provided by the high percentage of energy rich milk fats. Milk lipids will also provide essential nutrients such as specific fatty acids that cannot be synthesised in the body. Fatty acids play an important role during growth and development, not only as a source of energy but also as major building material for the growing body. While the body can synthesize some of these, others such as the essential fatty acids (EFAs) have to be acquired through the diet (Herrera 2002, Innis 2005); therefore their transfer during lactation is of extreme importance for the development of the growing pup. For instance, EFAs such as ecosapentanoic acid (20:5n-3; EPA) and docohexanoic acid (22:6n-3; DHA) and arachidonic acid (20:4n-6; AA) are central for the development of cell membranes (Neuringer et al. 1988, Innis 2005), eicosanoid production, hormonal signalling (Pond & Mattacks 1998, Beltowski 2003), and are indispensable for brain and retina function (Neuringer & Connor 1986, Neuringer et al. 1988, Uauy et al. 2001). Therefore, the mobilization of FAs from the blubber is not only essential for the energy requirements of the mother, but is also of extreme importance for the production of milk that will supply essential and non-essential FAs necessary for the maintenance, growth and development of the offspring (Neuringer et al. 1988).

In order for FAs to become available for energy production, milk production or other body functions, they must first be mobilized from the adipose tissue. Early studies of FA mobilisation from adipose tissue were inconclusive regarding the issue of selectivity. Spitzer et al (1966) reported in a study *in vitro* that the release of individual FA from the epididymal fat pads of rats was a simple function of their concentration in the adipose tissue. In contrast, *in vitro* and *in vivo* studies by Hunter et al (1970) and Gavino and Gavino (1992), also on fat pads of rats, showed that the release of FA was selective. Later studies demonstrated that the release of FAs from adipose tissue is not based on the proportions found in the lipid stores (Raclot et al. 1995, Connor et al. 1996) or on the positional distribution of the FAs in the triacylglycerols (Raclot et al. 1995, Raclot 2003). These studies

showed conclusively that the FA release is selective, and Raclot and Groscolas (1993) suggested that this selectivity might be related to the physico-chemical characteristics of individual FAs. Other studies in rats (Raclot & Groscolas 1995), rabbits (Connor et al. 1996), humans (Halliwell et al. 1996, Yli-Jama et al. 2001), American mink (Nieminen et al. 2006) and wild racoon dogs (Mustonen et al. 2007) have also shown that during periods of fasting the differential mobilization of FAs from the lipid stores can be explained by their molecular structure. More specifically, it appears that for a given length of the FA carbon chain, the degree of mobilization increases with increasing number of double bonds (degree of unsaturation), and for a given degree of unsaturation the mobilization decreases with increasing number of carbon atoms (Raclot & Groscolas 1993, Raclot & Groscolas 1995, Connor et al. 1996, Raclot et al. 1997, Raclot 2003). For instance, the highly mobilized FAs are those with 16 to 20 carbons and 4-5 double bonds such as 20:5n-3 or 18:4n-3 ; while the least mobilized ones are those with 20-24 carbon atoms and 0-1 double bond such as 20:1n-9 or 22:1n-11(Raclot 2003).

For young animals with no other nutrient source, milk has to supply all dietary components. As a result, it is possible that the patterns of FA mobilization from maternal adipose tissue in fasting lactating females is not only related to the physicochemical properties of FAs but also to the physiological needs of the developing pup. In particular, this may be the case for EFAs, which can only be obtained from an external source (Samuel & Worthy 2004).

Fatty acids in blubber and in milk have been used as dietary markers for animals at a range of trophic levels, including the higher predators such as pinnipeds. They have also been used to examine temporal, spatial variation in the diet (e.g (Walton et al. 2000, Walton & Pomeroy 2003, Arriola et al. submitted)). FAs have been used mainly as qualitative trophic markers and as a tool to provide information about predator – prey relationships (Dalsgaard & St John 2004). When using fatty acid signature analysis to infer dietary links it is important to understand how FAs are mobilized and deposited. Recent studies have suggested that FAs can be used not only as a qualitative dietary marker but also to estimate quantitatively the relative proportions of different prey species in the diet of predators (Iverson et al. 2004). This technique relies not only on the presence of specific signature FAs but also on the relative proportions of all FAs in a sample.

Clearly, any selective mobilization and transfer of FAs may seriously affect the quantitative estimates, especially if the exact stage of fasting of an individual is unknown when a sample is obtained. A proper understanding of FA dynamics such as differential utilization, mobilization, deposition and FA turnover is essential when applying these quantitative methods.

Selective mobilization of FAs has previously been studied in humans (Halliwell et al. 1996, Yli-Jama et al. 2001), rabbits (Connor et al. 1996) and fat pads of rats *in vivo* and *in vitro* (Raclot& Groscolas 1993, Raclot& Groscolas 1995). Few studies have analyzed FA mobilization in wild animals that fast naturally as part of their life cycle: only three studies to date, on wild raccoon dogs (Mustonen et al. 2007), emperor penguins (Groscolas, 1990) and lactating Weddell seals (Wheatley et al. 2008) can be found in the literature.

The present study examined the mobilization of FAs in naturally fasting lactating grey seals which are good models for lipid research, since they undergo natural periods of fattening at sea and fasting during lactation and moult on land. Our hypothesis is that FAs are not mobilized equally from the blubber. FAs mobilization will be dependent not only on the physico-chemical characteristics of the FAs but also in the physiological needs of the growing pups. If the mobilization of FAs during lactation is dependant simply on their physicochemical structure, the loss of FAs from early lactation blubber should reflect their tendency to be mobilized according to a model based on these properties (chain length and number of double bonds). However, we expect that some FAs will be mobilized more or less than predicted by the physico-chemical model. Thus, indicating a higher or lower preference or requirement during lactation. The grey seals from the two studied colonies are known to be well differentiated by their FA profiles in blubber at early lactation (Walton et al. 2000, Walton& Pomeroy 2003, Arriola et al. submitted). Regardless of their FA profile difference, females from both colonies should mobilize their FAs in a similar pattern if the FA mobilization is a function of both the physico-chemical characteristics and physiological needs of the pups.

METHODS

POPULATION AND SAMPLE COLLECTION

We studied breeding female grey seals at two UK colonies; North Rona (NR) (59°06'N, 05°50'W) and Isle of May (IOM) (56°10'N, 2°33'W) during two consecutive breeding seasons 2005 (n_{IOM}= 9; n_{NR} = 12) and 2006 (n_{IOM} = 14; n_{NR} = 22). Mothers were captured at early (2 - 3 days post partum) and late (15 - 17 days post partum) lactation. They were immobilized using an intramuscular doze of 'Zoletil 100' (Virbac, Cedex; 1.0 ml per 100 kg). Blubber biopsies were obtained from a small incision on the mid pelvic region and a full depth blubber core was obtained using a 6mm diameter punch (Acuderm Inc). The samples were stored until further analysis in chloroform:methanol (2:1) with 0.05% of butylated hydroxytoluene as an antioxidant. At each capture, blood samples were drawn from the extradural vein. At the end of each day blood samples were centrifuged and aliquots of serum and plasma were transferred into microtubes and stored at –20°C until further analysis. Sample sites were treated with topical antibiotic and a mass specific prophylactic intramuscular injection of antibiotic was administered (Terramycin LA, Pfizer S.A.).

Maternal body composition, weight, girth and length were measured at each capture. In order to estimate the absolute amount of each FA in the blubber, information on the fat content of the animals is also required. Body composition was estimated from total body water (TBW), using isotope dilution as described by Reilly and Fedak (1990). A blood sample was collected to determine background tritium levels, before a weighed dose of tritiated water was injected into the extradural vein. Mothers were recaptured for a second blood sample 3-5 hours after the initial injection to allow equilibration of the tritiated water throughout the water pool. Tritiated plasma was analysed using Liquid Scintillation Spectrometry as described in Pomeroy et al (1996). Total body fat (TBF) was estimated from total body water using empirical equations derived for grey seals by Reilly and Fedak (1990), and used to estimate the absolute amounts of each fatty acid in the blubber. While the method of isotope dilution has been shown to

provide reliable estimates of total body lipid (Reilly & Fedak 1990, Bowen et al. 1999), the empirical equations have some uncertainty associated with them. To examine the extent to which these uncertainties may affect the subsequent analyses, we developed a randomised approach whereby the standard deviations given by Reilly & Fedak (1990) for estimating percent body lipid (TBFPC) from TBW were accounted for. This approach is described in more detail below.

All procedures and animal handling were performed under the UK Home Office Licence.

FATTY ACID ANALYSES

Blubber lipid was extracted following the method described by Folch et al (1957). Samples were homogenized with 10 ml of dichloromethane:methanol (2:1 vol/vol). The supertendant was washed by adding chloroform:methanol:water (3:48:47 vol/vol) and treated with anhydrous Na_2SO_4 and dried under nitrogen, weighed and dissolved in toluene at a concentration of 100mg/ml. Fatty acids were esterified to produce fatty acid methyl-esters (FAME) using 1% (vol/vol) of sulphuric acid in methanol. Samples were then incubated overnight at 50^{o}C. The purified FAMES were dissolved in hexane. FAMEs were analysed by gas chromatography using a Trace GC-2000 (Thermoquest, CE Instruments) equipped with a flame ionisation detector and fitted with a DB23 fused silica capillary column (25 x 0.25mm, J&W Scientific). Hydrogen was employed as the carrier gas. The temperature was programmed to start at 60^{o}C for 2 min, then rise to 150 ^{o}C at 20 ^{o}C min^{-1}, then held for 2 min before a further rise to 205 ^{o}C at 1.8 ^{o}C min^{-1}and finally rising to 230 ^{o}C at 5 ^{o}C min^{-1}. Separated components were identified by reference to standards (Chapter 2).

Sixty FAs were quantified in the early and late lactation blubber samples, and the chain length and unsaturation ranged from 12 to 24 carbon atoms and from 0 to 6 double bonds respectively.

DATA ANALYSES

Although in this study we did not measure lipid classes we assumed that blubber is composed mainly of TAG (99.9%) as it was found in Weddell seals (Wheatley et al. 2008). A 95% of the TAG mass in the tissue is represented by the FA mass (Wheatley et al. 2008). Therefore, to obtain the FA mass in the blubber the total mass of fat (kg) obtained from the isotope analysis was multiplied by 0.949 (0.999 x 0.95, (Wheatley et al. 2008)). The resulting value was used to estimate the absolute amount of each FA present in a female's blubber at a given sampling date. We define mobilization as the absolute amount of each FA lost in the blubber from early to late lactation. The proportion of each FA that was mobilised from blubber was calculated from the initial total amount and final total amount of each FA using the following formula:

$$pmFA_i = 1 - \left(\frac{FA_{iL}}{FA_{iE}} \right)$$

where $pmFA_i$ denotes the proportion of FA i lost from blubber, while FA_{iL} and FA_{iE} represents the absolute amount of FA i at late (L) and early (E) lactation. Linear mixed effect models (LME) are a powerful analytical tool for repeated measure data such as those from longitudinal studies (Pinheiro & Bates 2002). LMEs were used to test for differences between populations and lactation stages in their maternal postpartum mass, mass at weaning, total body fat and total body fat percent. An LME was also used to examine to what extent the proportion of each FA lost can be explained as a function of its carbon length and number of double bonds. Models with different combinations of the explanatory variables were compared. Two random effects were also included: 1) *Animal ID*, to account for the repeated measures on the same females and 2) *Year*, to account for possible variations between years within the same colony. With this kind of analysis we can take into account the individual pattern of response, which is likely to depend on different characteristics of each individual including some that are unobserved (Everitt, 2005). We also allow for the fact that the study covered

two years, and without explicitly testing for annual differences this gives us greater confidence in parameter estimates from the fixed part of the model. The general formula for the final model was therefore:

$$pmFA_i \sim f_F(C, DB) + f_R(ID, Year) + \varepsilon$$

where f_F denotes fixed effects, f_R denotes random effects and ε refers to the residual unexplained variance. Candidate models were compared using the Akaike Information Criterion (AIC) and the most parsimonious model was selected based on its relative AIC weight relative to the remaining models. An AIC weight (AICw) for the top model more than twice that of any other model was considered strong support for the top model. The residuals of the model can highlight deviations from the patterns observed, i.e. variation that cannot be explained by carbon chain length, unsaturation or attributed to any of the random effects (*Animal ID* or *Year*). If certain FAs are more (or less) mobilized than predicted (i.e. having high or low residuals) this would indicate preferential mobilization (retention) for these FAs.

As described previously, the estimates of body lipid have uncertainties associated with them. The analyses described above were therefore re-run on several re-sampled datasets, where the originally calculated TBF estimates were replaced by re-calculated estimates with added error. The error here was randomly sampled from a normal distribution with a mean of 0 and a standard deviation taken from Reilly & Fedak (1990). This randomisation step was included in the empirical equation estimating percent body lipid (TBFPC) from percent total body water (TBWPC):

$$TBFPC = 105.1 - (1.47 \times TBWPC)$$

and TBF was then calculated in the usual way using TBFPC and body mass. Using this approach, we created 1000 datasets and re-ran all analyses described above on these simulated datasets.

To examine differences in the rates of mobilisation of specific FAs between populations, we analysed the residuals from the mixed model with a multiple comparisons tests. Multiple comparisons tests provide adjusted p values in an attempt to correct for the increasing probability of committing a Type I error with increasing number of simultaneous tests. In this study we follow the procedure developed by Westfall and Young (1993) for *P* value adjustment for multiple testing using resampling-based techniques. These are available in the `multtest` package for R (Pollard, K. S. Ge Y. Taylor S. Dudoit S. 2008).

RESULTS

Summary statistics of maternal postpartum mass (MPPM), maternal weaning mass (MWM), body composition and the changes throughout lactation are presented in Table 1. North Rona females were significantly heavier than females from IOM in the two years of study when compared by MPPM (t = 5.11, $p <$ 0.0001) and MWM (t = 3.59, $p < 0.0006$). The MPPM differences were biggest in 2006 (t = 2.33, $p = 0.023$). On the other hand there was no significant difference in MWM between the 2 years for either of the two populations. North Rona animals had higher total body fat (TBF) at early lactation ($t_{location} = 2.33$, $p =$ 0.022) compared to IOM animals. At late lactation TBF was significantly lower ($t_{stage} = -11.90$, $p < 0.001$). At the end of lactation there were no significant differences between colonies ($t_{stageLL} = -0.44$, $p = 0.659$) as there were no statistically significant differences between years ($t_{year} = -1.934$, $p = 0.081$). The loss of fat described represents only the fat lost in the interval between the 2 capture dates, and does not represent the total loss over the whole lactation period.

Fatty acids that were more abundant (amounts greater than 1 kg) included 14:0, 16:0, 16:1n-7, 18:1n-9, 18:1n-11, 18:4n-3, 20:1n-9, 20:5n-3, 22:1n-11, 22:5n-3, 22:6n-3. These FAs accounted for around 85% of the total FAs at early and late lactation in both populations.

Monounsaturated FAs (MUFAs) made up the greatest proportion, followed by polyunsaturated FA (PUFAs) and saturated FA (SFAs) being the least common (Table 2). Several FAs differed significantly between the islands at early lactation (Table 2, Fig. 1). All FAs that were significantly different at adjusted p values of ≤0.05 were higher at NR with the exception of FA 18:1n-5 (Table 2).

There were large variations in the way FAs changed from early to late lactation when FAs were expressed in relative proportions, with some FAs increasing while others decreased or remained relatively unchanged. When expressed in absolute amounts, all FAs decreased throughout lactation, but the rates of this decrease varied between FAs. However, both populations followed broadly similar patterns in terms of the proportions of the initial absolute amount for each FA that was lost. Figure 2 show clear increases in mobilization with increasing number of double bonds, especially for FAs with 16, 18 and 20 carbon atoms. For example, FA 20:5n-3 was more readily mobilised in both populations while 20:1n-11 and 18:1n-11 were less mobilized. The mobilization also decreased with increasing chain length, although this was visually less obvious in Figure 2. Among SFAs the proportional mobilization of 24:0 and 20:0 were the lowest while those with 12-18 carbons where more readily mobilized. Among the least mobilised MUFAs, the mobilisation increased in the order 20:1n-11 < 18:1n-11 < 18:1n-9 < 16:1n-9 < 14:1n-7. The most mobilized MUFAs were (in decreasing order of mobilisation) 16:1n-11 > 16:1n-5 > 22:1n-11 > 16:1n-7. In the n-3 PUFA family the proportional mobilization followed the order 20:5n-3 > 18:4n-3 followed by 18:3n-3, 20:4n-3, 22:6n-3, 22:5n-3 being similarly mobilised. The order of decreasing mobilisation among the most mobilised within the n-6 family were 20:4n-6 > 16:2n-6 > 18:3n-6. Among the essential fatty acids 20:5n-3 is preferentially mobilized over 22:5n-3 and 22:6n-3.

Table 1. Mean (± S.D) values for maternal postpartum mass (MPPM), maternal weaning mass (MWM), total body fat (TBF) in kg and as a percentage of the total mass, amount of fat lost and the percentage of fat lost from the initial amount in lactating grey seal females at Isle of May (IOM) and North Rona (NR). Early lactation (EL), late lactation (LL). The number between parenthesis represents the sample size.

		MPPM (kg)	MWM (kg)	TBF (kg)		TBF (%)		Fat lost (kg)	Fat lost (%)
				EL	LL	EL	LL		
IOM	2005 (9)	167.59 ± 21.1	107.72 ± 19.9	59.34 ± 9.62	32.84 ± 10.08	38.34 ± 3.31	29.58 ± 8.24	27.78 ± 11.13	45.26 ± 17.09
	2006 (12)	159.17 ± 22.88	105.6 ± 17.91	52.00 ± 12.82	32.19 ± 12.83	34.61 ± 4.7	28.08 ± 7.54	23.68 ± 11.46	42.29 ± 19.83
NR	2005 (14)	185.09 ± 24.05	119.4 ± 21.33	58.74 ± 10.95	40.06 ± 19.16	34.82 ± 2.96	27.55 ± 7.7	25.65 ± 13.29	39.87 ± 22.41
	2006 (22)	191.79 ± 19.52	120.99 ± 19.43	63.51 ± 17.84	36.46 ± 9.8	35.01 ± 8.12	27.27 ± 4.8	29.33 ± 11.7	33.83 ± 15.73

Table 2. Mean value of the absolute amounts (kg) of fatty acid composition of grey seal females at early and late lactation at Isle of May (IOM) and North Rona (NR). Both years of study have been combined.

	IOM		NR	
	Early (n = 44)	Late (n = 23)	Early (n = 50)	Late (n = 36)
12	0.05 ± 0.01	0.03 ± 0.01	0.06 ± 0.02	0.04 ± 0.02
13	0.01 ± 0	0.01 ± 0	0.02 ± 0.01	0.01 ± 0.01
14	2.54 ± 0.75	1.29 ± 0.56	2.85 ± 1.03	1.72 ± 0.84
15	0.19 ± 0.04	0.09 ± 0.04	0.23 ± 0.06	0.13 ± 0.06
anti15	0.04 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.03 ± 0.01
iso15	0.11 ± 0.02	0.06 ± 0.02	0.12 ± 0.04	0.08 ± 0.03
16	5.22 ± 1.32	2.41 ± 1.23	6.11 ± 1.65	3.17 ± 1.57
iso16	0.05 ± 0.02	0.03 ± 0.02	0.05 ± 0.02	0.03 ± 0.01
17	0.1 ± 0.03	0.05 ± 0.02	0.12 ± 0.03	0.07 ± 0.03
anti17	0.07 ± 0.03	0.05 ± 0.03	0.07 ± 0.02	0.04 ± 0.02
iso17	0.04 ± 0.02	0.03 ± 0.02	0.04 ± 0.02	0.03 ± 0.02
18	0.62 ± 0.14	0.34 ± 0.15	0.73 ± 0.19	0.42 ± 0.17
iso18	0.1 ± 0.03	0.07 ± 0.03	0.11 ± 0.04	0.08 ± 0.03
20	0.05 ± 0.02	0.03 ± 0.01	0.06 ± 0.02	0.04 ± 0.02
24	0.01 ± 0	0.01 ± 0	0.01 ± 0.01	0.01 ± 0
14:1n-9	0.09 ± 0.03	0.06 ± 0.02	0.12 ± 0.05	0.08 ± 0.03
14:1n-7	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
14:1n-5	0.5 ± 0.17	0.37 ± 0.16	0.56 ± 0.19	0.42 ± 0.16
15:1n-x	0.04 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.03 ± 0.01
16:1n-11	0.12 ± 0.03	0.06 ± 0.03	0.15 ± 0.04	0.08 ± 0.04
16:1n-9	0.21 ± 0.05	0.16 ± 0.06	0.24 ± 0.06	0.17 ± 0.05
16:1n-7	6.13 ± 1.89	3.65 ± 1.77	6.59 ± 1.65	3.93 ± 1.47
16:1n-5	0.16 ± 0.04	0.08 ± 0.04	0.13 ± 0.04	0.08 ± 0.03
17:1n-x	0.17 ± 0.06	0.11 ± 0.05	0.22 ± 0.06	0.14 ± 0.05
18:1n-11	2.23 ± 0.75	1.42 ± 0.59	2.56 ± 0.82	1.89 ± 0.66
18:1n-9	7.47 ± 1.56	5.32 ± 1.78	8.91 ± 1.98	6.27 ± 1.86
18:1n-7	1.63 ± 0.59	1.09 ± 0.58	1.77 ± 0.46	1.09 ± 0.37
18:1n-5	0.21 ± 0.05	0.12 ± 0.06	0.16 ± 0.04	0.1 ± 0.04
20:1n-11	1 ± 0.33	0.67 ± 0.27	1.1 ± 0.33	0.83 ± 0.3
20:1n-9	3.73 ± 1.5	2.1 ± 1.19	3.65 ± 1.33	2.59 ± 1.1
20:1n-7	0.13 ± 0.06	0.1 ± 0.05	0.13 ± 0.04	0.09 ± 0.04
22:1n-11	2.1 ± 1.11	0.85 ± 0.68	1.98 ± 1.06	1.17 ± 0.8
22:1n-9	0.19 ± 0.07	0.11 ± 0.06	0.18 ± 0.07	0.12 ± 0.06
24:1n-9	0.1 ± 0.03	0.06 ± 0.03	0.12 ± 0.04	0.08 ± 0.04
16:2n-6	0.04 ± 0.01	0.03 ± 0.01	0.04 ± 0.02	0.03 ± 0.01
16:2n-4	0.24 ± 0.08	0.12 ± 0.06	0.21 ± 0.09	0.12 ± 0.06
16:3n-4	0.16 ± 0.07	0.08 ± 0.05	0.14 ± 0.06	0.08 ± 0.05

Table 2 Continued

	IOM		NR	
	Early	Late	Early	Late
	n = 44	n = 23	n = 50	n = 36
16:3n-1	0.03 ± 0.01	0.01 ± 0.01	0.03 ± 0.02	0.02 ± 0.01
16:4n-1	0.25 ± 0.11	0.11 ± 0.06	0.2 ± 0.11	0.11 ± 0.07
18:2d5_7	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
18:2n-6	0.95 ± 0.22	0.63 ± 0.21	0.9 ± 0.32	0.63 ± 0.23
18:2n-4	0.06 ± 0.03	0.04 ± 0.02	0.05 ± 0.02	0.03 ± 0.01
18:3n-6	0.05 ± 0.02	0.03 ± 0.01	0.05 ± 0.02	0.03 ± 0.02
18:3n-4	0.04 ± 0.01	0.03 ± 0.01	0.03 ± 0.02	0.02 ± 0.01
18:3n-3	0.54 ± 0.18	0.31 ± 0.13	0.54 ± 0.19	0.34 ± 0.15
18:3n-1	0.07 ± 0.04	0.04 ± 0.03	0.08 ± 0.04	0.04 ± 0.03
18:4n-3	1.13 ± 0.43	0.52 ± 0.27	1.1 ± 0.46	0.6 ± 0.34
18:4n-1	0.07 ± 0.03	0.03 ± 0.02	0.06 ± 0.03	0.03 ± 0.02
20:2n-6	0.12 ± 0.04	0.08 ± 0.03	0.13 ± 0.03	0.09 ± 0.03
20:3n-6	0.04 ± 0.01	0.03 ± 0.01	0.05 ± 0.01	0.03 ± 0.01
20:4n-6	0.3 ± 0.13	0.18 ± 0.12	0.37 ± 0.1	0.2 ± 0.08
20:3n-3	0.06 ± 0.02	0.04 ± 0.01	0.07 ± 0.02	0.04 ± 0.02
20:4n-3	0.43 ± 0.11	0.23 ± 0.08	0.52 ± 0.16	0.31 ± 0.14
20:5n-3	2.99 ± 0.83	1.31 ± 0.83	2.75 ± 0.88	1.28 ± 0.73
21:5n-3	0.25 ± 0.06	0.14 ± 0.05	0.23 ± 0.08	0.14 ± 0.06
22:4n-6	0.09 ± 0.05	0.07 ± 0.05	0.12 ± 0.05	0.07 ± 0.03
22:5n-6	0.1 ± 0.04	0.08 ± 0.04	0.16 ± 0.04	0.11 ± 0.04
22:4n-3	0.1 ± 0.03	0.06 ± 0.02	0.1 ± 0.03	0.07 ± 0.03
22:5n-3	2.4 ± 0.46	1.52 ± 0.53	2.36 ± 0.56	1.49 ± 0.53
22:6n-3	6.71 ± 1.32	3.86 ± 1.56	7.86 ± 1.99	4.64 ± 1.92
SFA	9.2 ± 2.2	4.53 ± 2.04	10.6 ± 3.01	5.87 ± 2.77
MUFA	26.24 ± 5.67	16.39 ± 5.38	28.63 ± 6.62	19.17 ± 6.3
PUFA	17.25 ± 3.56	9.58 ± 3.82	18.19 ± 4.71	10.55 ± 4.36
n-3	14.6 ± 3	7.97 ± 3.26	15.53 ± 4.06	8.9 ± 3.77
n-6	1.69 ± 0.38	1.13 ± 0.39	1.82 ± 0.46	1.19 ± 0.41

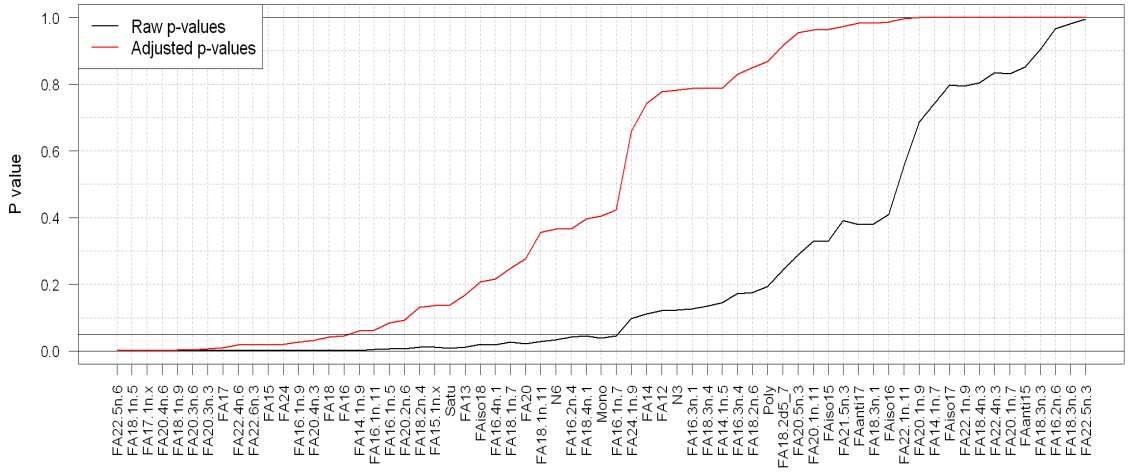


Figure 1. Raw and adjusted p values after applying a multiple comparisons test to compare the absolute values at early lactation between NR and IOM. Multiple comparisons tests were based on the Wilcoxon rank sum statistic. The y axis represents a normal p-value for the Wilcoxon comparison between NR and IOM at early lactation. The black line indicates the p value given to each fatty acid in the standard Wilcoxon tests, while the red line indicates the adjusted p values accounting for multiple comparisons. The traditional threshold value of p=0.05 is indicated by the solid horizontal black line in the bottom of the graph. FAs are arranged in increasing order from left to right according to their adjusted p-values.

Table 3 presents a summary of the top candidate mixed effects models examining the pattern of mobilisation in relation to chain length and degree of unsaturation.

Table 3. Summary of the top candidate of the mixed effect models showing the AIC weights. Mob = Proportion Mobilized, DB = Number of double bonds, C = Carbon chain length, Loc = Location.

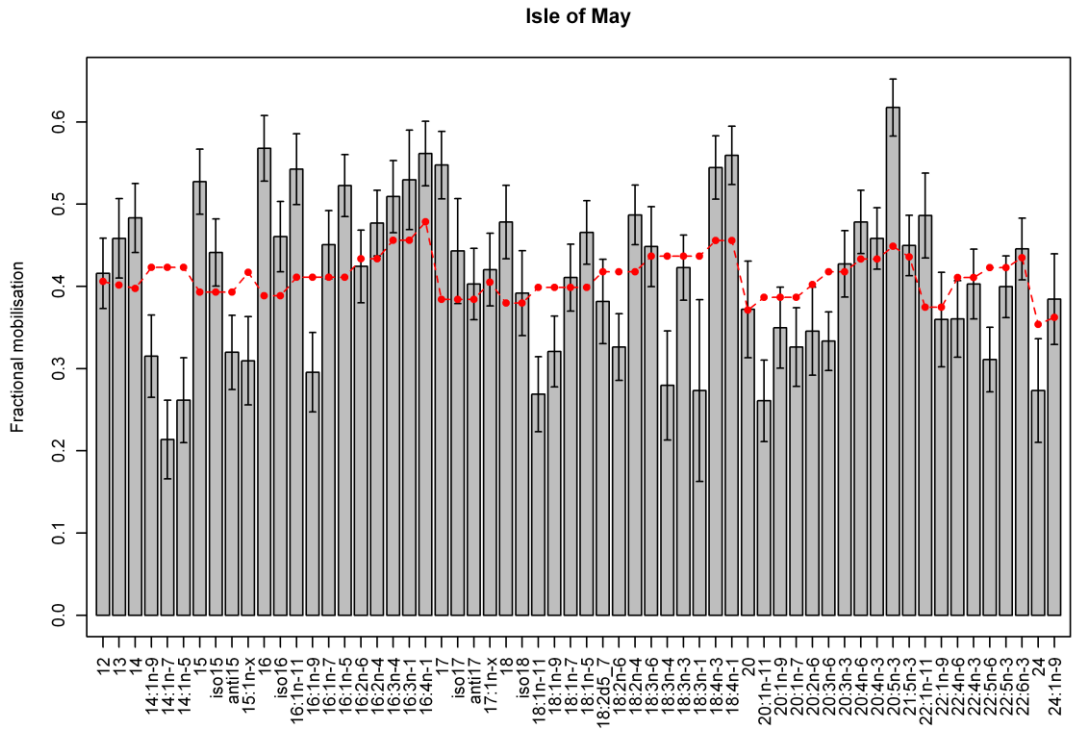
	Response	Fixed	Random	logLik	AIC	dAIC	AICw
Mod 1	**Mob**	**DB,C,Loc,DB*C**	**Year,Animal ID**	**19.88**	**-23.76**	**0**	**0.42**
Mod 2	**Mob**	**DB,C,Loc,C*Loc**	**Year,Animal ID**	**19.48**	**-22.96**	**0.796**	**0.28**
Mod 3	**Mob**	**DB,C,Location**	**Year,Animal ID**	**18.24**	**-22.48**	**1.279**	**0.22**
Mod 4	**Mob**	**DB,C,Loc,DB*C,DB*Loc, C*Loc,C*DB*Loc**	**Year,Animal ID**	**21.29**	**-20.57**	**3.187**	**0.08**

The most parsimonious model included fixed effects for chain length, number of double bonds, location and an interaction term between double bond and chain length. The result from the mixed effects model (Table 4) confirms the general pattern observed in Fig. 2 in both locations. The degree of mobilization was significantly related to both carbon chain length and the number of double bonds. For a given chain length, mobilization increased with the number of double bonds (t_{DB}=2.66, p=0.008), and mobilisation decreased with increasing carbon chain length (t_{CL}= -2.20, p=0.028). Of the remaining variation not explained by the fixed effects, Animal ID accounted for a large part, while year accounted for a much smaller part of the variation (0.33 and 6.57^{-05} respectively, with a remaining overall unexplained residual of 0.231). Results from the randomisation tests to examine the effects of body composition estimate uncertainty are also presented in Table 4. These results are for the same model as the most parsimonious model based on the non-randomised analysis, since for almost every simulated dataset this model was selected as the most parsimonious. The standard errors in parameter estimates caused by uncertainties in body composition were generally small for most of the parameters in the model. Comparisons of the within-model parameter standard errors (SE in Table 4) and the between-model standard errors from the randomisation test (SE-BC in Table 4) provide some indication of the importance of body composition uncertainty. For instance, the between-model standard error on the effect of the number of double bonds represented only ~10% of the within-model standard, and this parameter was considered significant for 99.1% of the 1000 datasets. In the case of the effect of the length of the carbon chain, the between-model standard error represented half of the within-model standard error. This parameter was only considered significant for 38.6% of the 1000 randomised datasets. This can probably be explained by the fact that this effect was overall relatively small (-0.005).

Table 4. Results from the linear mixed effects model of fatty acid mobilisation as a function of double bonds (DB) and carbon chain length (C). SE = Within-model standard error from the non-randomised dataset. SE-BC = between-model standard error for the 1000 randomised datasets including uncertainty in body composition. Sig.Pval represents the proportion of the models run on the 1000 randomised datasets for which a given effect was considered significant.

	Value	SE	SE-BC	t-value	p-value	Sig.Pval
Intercept	0.452	0.080	0.015	5.66	0.000	100.0%
DB	0.051	0.019	0.002	2.66	0.008	99.1%
C	-0.005	0.002	0.001	-2.20	0.028	38.6%
Location NR	-0.074	0.091	0.021	-0.81	0.422	0.0%
DB:C	-0.002	0.001	0.000	-1.81	0.070	4.5%

Figure 2 shows the fractional mobilization of each FA (i.e. the proportion of the initial amount (by weight) that was mobilised. It also shows the predicted fractional mobilizations based on the LME, highlighting the fatty acids that are mobilised more or less than predicted by the model. Fatty acids that were more readily mobilized than predicted included the saturated FAs with 15 to 18 carbon atoms, 16:1n-11 and 22:1n-11 and the EFAs 20:5n-3 and 22:6n-3, while the FAs that were less mobilized than predicted by the model were 14:1n-7, 14:1n-5, 20:1n-11, 18:1n-11 and the EFAs 18:2n-6, 20:3n-6 and 22:4n-6. The multiple comparisons test between the model residuals from NR and IOM showed that there were no significant differences between colonies for adjusted p values at the 0.05 level (Fig. 3).


Isle of May
Fractional mobilisation
0.0
0.1
0.2
0.3
0.4
0.5
0.6
12
13
14
14:1n-9
14:1n-7
14:1n-5
15
iso15
anti15
15:1n-x
16
iso16
16:1n-11
16:1n-9
16:1n-7
16:1n-5
16:2n-6
16:2n-4
16:3n-4
16:3n-1
16:4n-1
17
iso17
anti17
17:1n-x
18
iso18
18:1n-11
18:1n-9
18:1n-7
18:1n-5
18:2d5_7
18:2n-6
18:2n-4
18:3n-6
18:3n-4
18:3n-3
18:3n-1
18:4n-3
18:4n-1
20
20:1n-11
20:1n-9
20:1n-7
20:2n-6
20:3n-6
20:3n-3
20:4n-6
20:4n-3
20:5n-3
21:5n-3
22:1n-11
22:1n-9
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22:4n-3
22:5n-6
22:5n-3
22:6n-3
24
24:1n-9

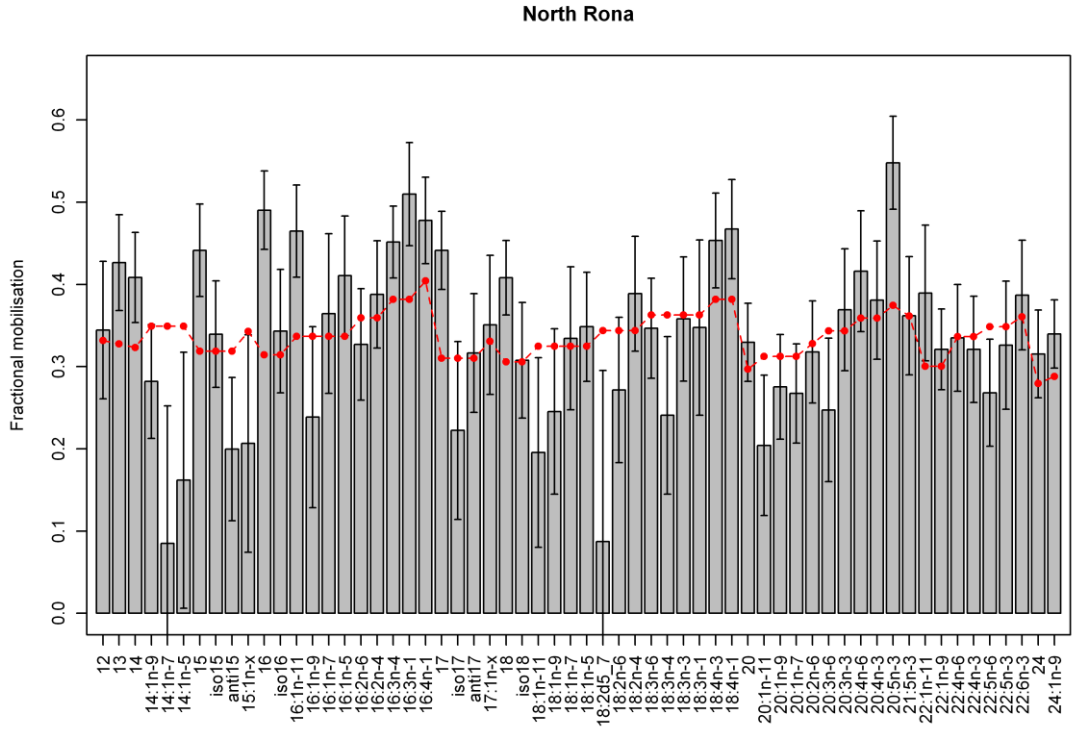
North Rona
Fractional mobilisation
0.0
0.1
0.2
0.3
0.4
0.5
0.6
12
13
14
14:1n-9
14:1n-7
14:1n-5
15
iso15
anti15
15:1n-x
16
iso16
16:1n-11
16:1n-9
16:1n-7
16:1n-5
16:2n-6
16:2n-4
16:3n-4
16:3n-1
16:4n-1
17
iso17
anti17
17:1n-x
18
iso18
18:1n-11
18:1n-9
18:1n-7
18:1n-5
18:2d5_7
18:2n-6
18:2n-4
18:3n-6
18:3n-4
18:3n-3
18:3n-1
18:4n-3
18:4n-1
20
20:1n-11
20:1n-9
20:1n-7
20:2n-6
20:3n-6
20:3n-3
20:4n-6
20:4n-3
20:5n-3
21:5n-3
22:1n-11
22:1n-9
22:4n-6
22:4n-3
22:5n-6
22:5n-3
22:6n-3
24
24:1n-9

Figure 2. (Previous page). Fractional mobilisation (i.e. the proportion of the initial total amount, by weight) of specific fatty acids from maternal blubber throughout lactation. The red lines represent the predicted fractional mobilisation based on the linear mixed effects model of mobilisation as a function of carbon chain length and number of double bonds.

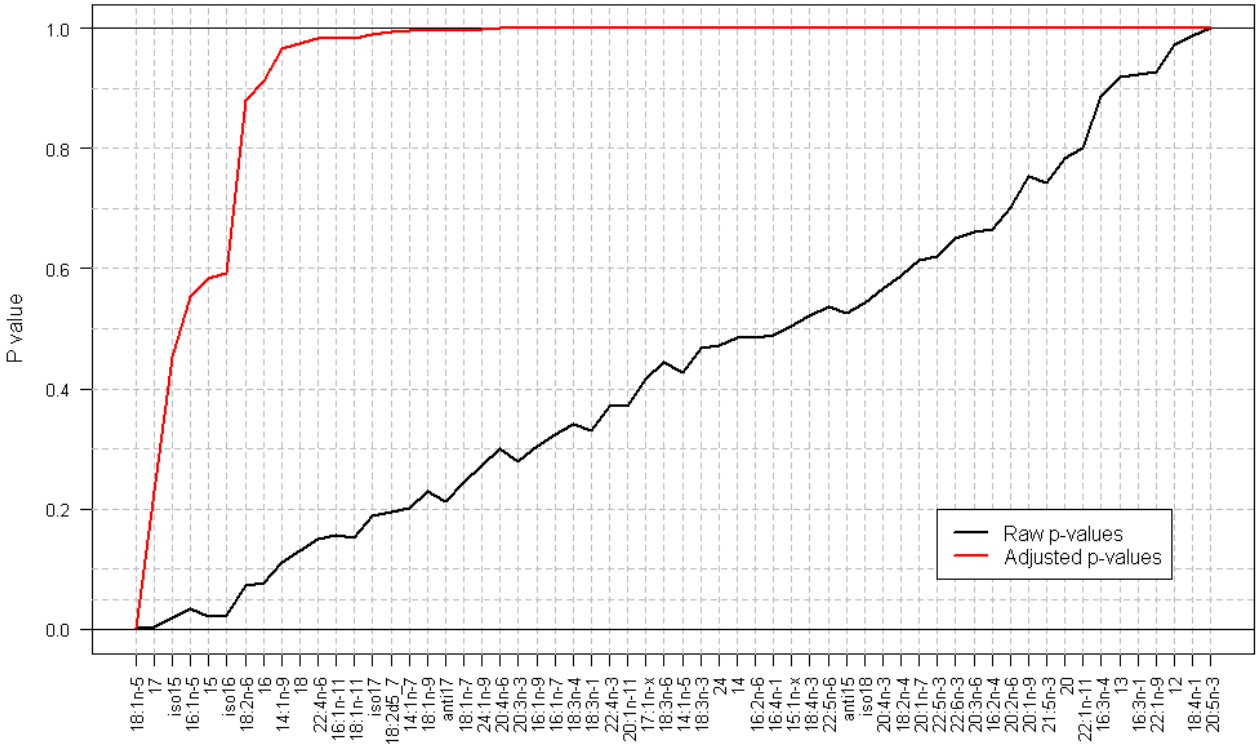

Figure 3. Raw and adjusted p values after applying a multiple comparisons test (Wilcoxon) to compare the proportions of FA mobilized from blubber between North Rona and Isle of May. The black line indicates the p value given to each fatty acid in the standard Wilcoxon tests, while the red line indicates the adjusted p values accounting for multiple comparisons. FAs are arranged in increasing order from left to right according to their adjusted p-values.

DISCUSSION

This study has demonstrated that the release of FAs from the adipose tissue of lactating grey seals is selective, and that the patterns of mobilization are closely related to the FA molecular structure, as has previously been described in both *in-vivo* and *in-vitro* studies in the laboratory. There was a slight decrease in mobilization with increasing carbon chain length and a clear increase in mobilization with increasing number of double bonds for a given chain length. The exact mechanism driving such selectivity is not known, but it has been shown that the mobilization of FAs does not lie in the relative proportions of FA found in the fat cell (Raclot et al. 1995) or in the preferential cleave of FAs in the sn-1 and sn-3 positions in the TAG by the hormone sensitive lipasa. Previous work has shown that the selective FA mobilization from fat cells is not related to the positional distribution of the FAs on the glycerol (Raclot et al. 1995b, Raclot 2003) since weakly mobilized FAs are mainly found in the sn-1 and sn-3 positions (Raclot et al. 1995b, Raclot 2003). The most accepted hypothesis suggests that the differential mobilization of FA may result from differential hydrolysis of TAGs depending on their distribution within a lipid droplet (Raclot& Groscolas 1993). The TAGs are distributed according to their polarity. The TAGs containing more polar FAs will be located at the periphery of the droplet (Raclot 2003, Mustonen et al. 2007) where they will be more accessible to hydrolysis by the hormone sensitive lipase and membrane fatty acid binding protein for transport (Connor et al. 1996, Raclot 2003). Since the polarity of FAs decreases with increasing chain length and increases with the number of double bonds, this provides a mechanism by which longer chain and more saturated FAs can be retained within the lipid droplet (Raclot& Groscolas 1993, Mustonen et al. 2007).

The patterns of FA mobilization were broadly similar between the two colonies; although there appeared to be some more subtle differences between the two (see below). These two colonies are recognized as distinct populations genetically (Allen et al. 1995) and geographically (Allen et al. 1995, Walton et al. 2000). Walton et al (2000) showed that mothers from the two colonies could be differentiated by the fatty acids found in their blubber at early lactation. These

differences appear to have persisted over the past decade (Allen et al. 1995, Walton et al. 2000, Arriola et al. submitted), suggesting that seals from these two breeding locations have different foraging patterns in terms of geographic distribution and/or diet.

While the FA composition at early lactation was different between IOM and NR animals, the changes occurring over lactation were very similar. The overall pattern of FA mobilisation in the two colonies were clearly related to physicochemical characteristics, but our results also suggest that there is another factor influencing the FAs release during the lactation fast. We suggest that in addition the specific dietary requirements of the growing pups influenced the release of specific FAs from the maternal blubber. Because of the lack of enzymes for synthesizing FAs that are critical for the growing pups, preventing growth deficiencies and maintain proper organ functions, these FAs need to be obtained through the diet/milk. In animals from both colonies, Eicosapentaenoic acid (20:5-n3, EPA) was the most highly mobilized of all PUFAs, followed by arachidonic acid (20:4n-6, AA) and linoleic acid (18:2n-6, LA). These very long chain FAs are precursors of eicosanoids (Connor et al. 1996), which are important in the immune system and as messengers in the nervous system among other functions. In particular, the physiological importance of the 20-carbon EFAs (EPA and AA) as precursors of prostaglandins may explain their highly preferential release from the adipose tissue during fasting (Mustonen et al. 2007). This may be particularly important in lactating females providing energy and building materials for their growing pups, since prostaglandins are important signalling molecules acting on a variety of cells but have a very short halflife before being inactivated and excreted. In general, the order of preferential mobilization among n-3 PUFAs in our study is very similar to that found in rats (Raclot & Groscolas 1994) and wild raccoon dogs (Mustonen et al. 2007) where 20:5n-3 is preferentially mobilized over longer-chain n-3 PUFAs such as 22:5n-3 and 22:6n-3. Interestingly, one of the FAs in the n-3 family that has previously been found to be less readily mobilized than other n-3 FAs in rats, rabbits, humans and wild raccoon dogs (Raclot& Groscolas 1995, Connor et al. 1996, Yli-Jama et al. 2001, Mustonen et al. 2007) is Docosahexaenoic acid (22:6n-3, DHA), the most polyunsaturated of the n-3 FAs. In our study this was among the most highly

mobilised of the n-3 FAs after 20:5n-3, along with 18:3n-3, 18:4n-3, 20:4n-3. This is in agreement with Wheatley et al (2008) who found that 22:6n-3 was the most highly mobilized n-3 FA in lactating Weddell seals along with 20:5n-3. While DHA is not important as an energy substrate or as a precursor for eicosanoids, it is known to be important as a component of phospholipids of cell membranes, principally in the brain and in the photoreceptors of the retina (Neuringer& Connor 1986, Neuringer et al. 1988, Farooqui & Horrocks 2004). In rats the period of major brain growth during the postnatal period was associated with rapid increases in concentration of DHA and AA in the brain (Farooqui& Horrocks 2004), and similar results were found for humans and rhesus monkeys during the main period of brain and retinal development during the prenatal period (Neuringer& Connor 1986, Neuringer et al. 1988). While this increased demand for DHA would most likely lead to increased rates of its mobilisation from adipose tissue, previous studies of FA mobilization were not carried out on lactating or gestating subjects, explaining why no such high mobilization rates were found. The high rates of DHA mobilization from the blubber of lactating grey seals (this study) and Weddell seals (Wheatley et al. 2008) are most likely a specific response to the requirements of the pups.

One of the least mobilised FA in both colonies was the MUFA 20:1n11 (Cetoleic acid, CA), and it was less mobilised than predicted by the model based on its physico-chemical structure (Fig 2). This is in accordance with studies performed in vitro (Raclot 2003) and in vivo (Connor et al. 1996). As a general rule, it has been suggested that highly mobilized fatty acids include those with 16-20 carbons and 4-5 double bonds, whereas the weakly mobilized FAs include those with 20 –24 carbons and 0-1 double bonds (Raclot 2003). The remaining FAs, such as the shorter-chain SFA and short chain MUFAs, are considered moderately mobilized (Raclot 2003). Our results agree with this general rule to a certain extent, showing that MUFAs with 14-16 carbons were only weakly or moderately mobilized. In fact, MUFAs with 14 carbons were some of the least mobilised, and were much less mobilised than predicted by the model, while the short-chain saturated FAs were generally more readily mobilised, and also more mobilised than predicted (Fig. 2).

These general patterns of differential mobilization will determine the relative supply of different FAs to various body tissues and organs during periods of negative energy balance (Raclot& Groscolas 1993, Connor et al. 1996). For instance, the selective release of FAs may be an important mechanism by which the EFAs are mobilized in order to be utilized by the body (Connor et al. 1996) and transferred to a developing pup. Moreover, selective release may also regulate the supply of energy-rich FAs depending on the requirements of the animal. While PUFAs are readily oxidized and can be rapidly available as a fuel source saturated FAs are more important for long-term energy storage. High mobilisation of these FAs has been observed in fasting rabbits (Connor et al. 1996) and in wild raccoon dogs (Mustonen et al. 2007). The requirement for these high-energy FAs may be particularly great in fasting lactating females to cover their own energy demands while also supplying their pups with crucial early energy reserves and insulation. This may be particularly important in land-breeding phocids where pups undergo a prolonged fasting period immediately after weaning (Reilly 1991), explaining the high mobilization of saturated FAs with 12-18 carbons observed in this study.

In general, the fact that animals from both populations showed very similar mobilization patterns despite the large differences in FA composition at early lactation is consistent with the idea that differential mobilization is the result of the combined influence of molecular structure and the specific lactation requirements of mothers as well as their pups.

Understanding the dynamics of FA mobilisation and transfer may be important for the use of lipids as dietary and ecological markers. For instance, the FA composition in milk has been used as an indicator of the mother's diet during the previous feeding period (e.g. (Iverson 1993, Iverson et al. 1997)). However, there has been considerable debate about the validity of this method (e.g. Grahl-Nielsen 2000), and the increasing evidence of selective mobilisation and transfer of FAs are relevant for the validation of the methodology. The type of FAs taken up by the mammary gland will depend on the FAs that have been released from the blubber. Selective mobilization of FAs will therefore ultimately affect the type of FAs found in the milk, and the relationship between the dietary FA contribution and the milk FA composition is therefore highly complex. The use of milk as a dietary indicator may give biased information depending when during lactation

the samples were collected, and also on the nutritional status of the mother and pup.

Because of the potential physiological consequences, studies should be carried out to further characterise the mobilization of individual fatty acids in wild animals, especially those who naturally fast and lactate during their life cycle. This study shows that differential mobilization of FAs in grey seals is largely influenced by their physicochemical characteristics, but that the specific requirements of mothers and pups during lactation can play an important role. To determine the extent to which mobilised FAs are utilised by the mother or taken up by the mammary gland and eventually transferred to and deposited by the pup we need to examine the FA composition of milk and pup blubber. If females with different FA profiles, such as those from IOM and NR in this study, have similar patterns of mobilization that are driven by the physiological requirements of their growing pups, we would expect the pups from both colonies to end up with relatively similar FA profiles. However, these similarities may be limited to FAs that are essential for proper growth and tissue development, while FAs that are mainly transferred to provide energy reserves may reflect the dietary differences between females from the two colonies.

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4 Fatty acids in blubber, serum and milk in grey seal mothers and pups during lactation

ABSTRACT

Grey seal mother pup pairs of two UK colonies were sampled for blubber, serum and milk at three different stages of lactation during three breeding seasons (2004-2006) and samples were analysed for fatty acid (FA) profiles. This study provides information on how the dietary differences between populations are reflected in serum, milk and ultimately, in the offspring blubber. We also investigate the differences between compartments within and between lactation stages to give an insight into the changes in FA composition during lactation, but also to determine the milk FA in relation to the blubber FAs and if pups blubber resemble that of their diet. Samples of serum and milk were collected at three lactations stages; blubber from mothers was collected at early and late lactation, while pup blubber was only sampled at late lactation. The results show that blubber, serum, milk and pups blubber could be differentiated from each other during the three lactation stages. The results show that the dietary differences between populations observed in the blubber FAs at early lactation were also seen in other tissues and in pups blubber. Although the dietary differences were transferred to other tissues maternal blubber could still be well distinguished from the FA composition of the milk and from the serum during the different lactation stages. The FAs composition of the milk changed gradually across lactation stages. These changes were similar in both colonies and between years. The FA composition of the pups at late lactation differed from that of their diet and that from their mothers' blubber. Although, the overall FA composition of pup blubber did not differ greatly between colonies, they could still discriminate colonies indicating that proportions of certain FAs that distinguished the mothers are probably transferred via the milk. This study provides important information on the ongoing debate of the possibility of use of milk to infer diet. It also provides a better understanding on the FA dynamics during lactation.

INTRODUCTION

In order to understand the interactions of marine mammals with the marine ecosystems of which they are part, detailed information about the diet of these animals is essential. Determining the diet of marine mammals is challenging, and a variety of approaches such as stomach lavage and faecal analysis have been developed (Pierce & Boyle 1991). Although these have proven to be valuable tools they are also subject to several limitations and potential biases (Jobling & Breiby 1986, Pierce et al. 1993) . For instance, the information obtained from these types of analyses will mainly represent the most recently ingested prey, or may be biased towards prey species that contain hard parts that are less easily digested. Alternative methods such as stable isotope ratios (Gannes et al. 1997) and fatty acid signature analysis (Iverson et al. 1993, Iverson 1993) have demonstrated their usefulness for overcoming some of the problems inherent in faecal or lavage sampling techniques.

The basis for using fatty acids (FAs) as a dietary indicator is that dietary FAs are assumed to be accumulated into the blubber of marine mammals with little or no modification (Iverson 1993, Iverson et al. 1997, Smith et al. 1997) and the analysis of FA composition of blubber has therefore become a popular method for providing information about the accumulated dietary history of the previous fattening period (Iverson 1993, Iverson et al. 1995, Iverson et al. 1997). Several studies at different trophic levels in terrestrial and marine ecosystems have shown that the composition of dietary FAs are reflected strongly in the composition of adipose tissue (Beck et al. 1994, Pond et al. 1995, Castell et al. 1995, Iverson et al. 1997, Smith et al. 1997) and the general approach is now widely accepted despite disagreements over the accuracy of the estimated dietary composition (Beck et al. 1994, Pond et al. 1995, Castell et al. 1995, Iverson et al. 1997, Smith et al. 1997, Grahl-Nielsen 1999). However, there is still considerable debate among researchers as to how the FAs are deposited, transfer and to what extent the FA composition of various body tissues reflects the diet. To determine to what extent blubber FA composition reflects the diet of marine mammals, experiments have been carried out in captivity comparing the FA composition of the blubber of seals

with that of a controlled diet (Tollit et al. 2006, Nordstrom et al. 2008). These studies are generally based on only a few individuals and do not represent the complexity of free-ranging animals. Lactating mothers with their pups have been also used to investigate the influence of the diet in the blubber FAs (Ackman and Jangaard 1965, Iverson et al 1995, Grahl-Nielsen et al 2000, Birkeland et al 2005). In hooded seals (*Cystophora cristata*) it was found that the blubber of the pups was very similar to their milk diet (Iverson et al 1995). However, Grahl-Nielsen et al (2000) found that the blubber FA composition of grey seals (*Halichoerus grypus*) pups was more similar to their mothers blubber than to the milk. Many of these studies describing the deposition and transfer of FAs has been based in a small sample size, some of them are cross-sectional studies and others only take into account samples from an early lactation stage.

Diet inference has also been done using FAs in other lipid-rich body tissues. For instance, Iverson (1993) suggested that the diet of female marine mammals could be inferred from the FA composition of their milk. This is predicted on the assumption that lipids secreted into the milk by a fasting female will be a reflection of the lipids from the blubber and therefore the diet. During fasting the FAs accumulated in the adipose tissue are released into the circulatory system and will be circulated to other organs and tissues, including the mammary gland. Therefore in fasting seals the FA composition of milk will be almost exclusively determined by the FA release from blubber, with very little *de-novo* synthesis occurring in the mammary gland (Iverson 1993). In contrast, the milk FA composition of animals that feed regularly during lactation (such as otariids) will be strongly influenced by FAs from recent dietary intake, since circulating lipids will be directed to the mammary gland (Iverson et al. 1997). If dietary intake is insufficient for milk production, FAs will be mobilized from adipose tissue, and the FA composition of the milk will be a reflection of a combination of both direct dietary and stored blubber lipids (Iverson 1993, Iverson et al. 1995, Iverson et al. 1997). The effect of diet on milk composition has been studied in dairy breeds, humans and domestic animals, but there are few studies that discuss the implications of the use of milk as a dietary indicator for marine mammals (Iverson 1993, Iverson et al. 1995, Iverson et al. 1997, Grahl-Nielsen et al. 2000, Staniland & Pond 2005).

The purpose of this study was to determine the way in which accumulated dietary FA reserves by females are transferred to their offspring via milk and the deposition of this in the offspring blubber. This study also intends to observe to what extent any dietary signature in female blubber is retained in the blood and milk of the female and how the diet of the pups (milk) is reflected in the accumulated FAs of their blubber with the aim to observe if it is possible to use milk as a dietary indicator. Female grey seals give birth to one pup each season, and they remain with their pups throughout the ~18-day lactation period, relying entirely on stored reserves (i.e. blubber lipid). By sampling the blubber, blood and milk of known mothers and their pups at different times throughout lactation we can examine 1) the differences in FA composition of different body compartments at specific time points, 2) the changes within compartments throughout lactation and 3) differences between the FA composition of the pup's blubber and that of the mother's milk and blubber. By studying two geographically separated populations, which have previously been shown to have distinct blubber compositions, presumably reflecting differences in diet (Walton et al. 2000, Walton & Pomeroy 2003, Arriola et al. submitted), we can also examine whether differences in female blubber at early lactation are maintained throughout the different compartments of the female and in the offspring. The FAs that are released during the fast to the bloodstream will have a direct effect on the FA composition of the milk and thus in the FA composition of the pups. We expect to find similarities between the FAs from blubber and milk if the FAs are transferred with no or little modification from one compartment to another. Since the mobilization of FAs from blubber is selective, the variation in FA composition in milk, blood and blubber across lactations stages should be very similar between populations.

METHODS

POPULATION AND SAMPLE COLLECTION

Grey seal mother-pup pairs were studied at North Rona (NR) (59°06'N, 05°50'W) and Isle of May (IOM) (56°10'N, 2°33'W) during three consecutive breeding seasons (2004-2006). The total numbers of females and pup sampled for each year are shown in the FA profiles summary tables, Appendices 2-7. The average lactation duration was 18 days. In 2004, mothers and pups were captured at two lactation stages: early (the first quarter of total duration of lactation) and late (the last quarter of the duration of lactation) with a minimum interval period of 10 to 12 days between them. At both captures females were sampled for blubber, blood and milk. Pups were sampled for blood at both captures and only blubber at late lactation. In 2005 and 2006 females were captured three times at intervals of 6 to 8 days (early, mid and late lactation stages). Females were sampled for blood and milk at every capture and blubber only at early and late lactation. Pup blood was sampled at each of the 3 stages while pup blubber was sampled only at late lactation. Females were immobilized using an intramuscular doze of 'Zoletil 100' (Virbac, Cedex). A small incision on the mid pelvic region was made with a scalpel and a full depth blubber core was obtained using a 6mm diameter punch (Acuderm Inc). Since FA composition can vary across the body depending where the animal is sampled all the blubbers were collected from the mid-pelvic region (Walton et al. 2000). The samples were stored in chloroform:methanol (2:1) with 0.05% of BHT as antioxidant. At each capture blood samples were drawn from the extradural vein using the Vacutainer (Becton Dickinson, UK). At the end of each day, samples were centrifuged and aliquots of serum and plasma were transferred to microtubes and stored at –20°C until further analysis. To stimulate the release of milk 1 mL of oxytocin was intravenously injected and 30 mL of milk were obtained. Samples were collected in a hexane-washed glass jar and stored at –20°C until further analysis. Sample sites were treated with topical antibiotic and a mass specific prophylactic intramuscular injection of antibiotic was administered (Terramycin LA, Pfizer S.A.). Blood samples from pups were either

drawn from the hind flipper or extradural vein. Pup blubber samples were obtained following the same procedure as in females but using a 4 mm diameter punch (Acuderm Inc), following an IV Zoletil dose equivalent to 50% of an adult mass based dose. Body composition was estimated as described previously in Chapter 3. Morphometric measurements: adult mass, girth and length and pup mass were recorded at each capture. All procedures were performed under UK Home Office Licence.

FATTY ACID ANALYSES

Blubber

Blubber lipid was extracted following the method described by (Folch et al. 1957). Samples were homogenized with 10 ml of dichloromethane:methanol (2:1 vol/vol). The top surface was washed by adding chloroform:methanol:H_2O (3:48:47 vol/vol) and treated with anhydrous Na_2SO_4 and dried under nitrogen, weighed and dissolved in toluene at a concentration of 100mg/ml. Fatty acids were esterified to produce fatty acid methyl-esters (FAME) using 1% (vol/vol) of sulphuric acid in methanol. Samples were then incubated overnight at 50^{o}C. The purified FAMEs were dissolved in hexane. FAMEs were analysed by gas chromatography using a Trace GC-2000 (Thermoquest, CE Instruments) equipped with a flame ionisation detector and fitted with a DB23 fused silica capillary column (25 x 0.25mm, J&W Scientific). Hydrogen was employed as the carrier gas. The temperature was programmed to start at 60^{o}C for 2 min, then rise to 150 ^{o}C at 20 ^{o}C min^{-1}, then held for 2 min before a further rise to 205 ^{o}C at 1.8 ^{o}C min^{-1}and finally rising to 230 ^{o}C at 5 ^{o}C min^{-1}. Separated components were identified by reference to standards (Chapter 1).

Milk

Dichloromethane was added to aproximately 0.5 - 1.0 g of milk sample. After a few minutes it was strained with fibre and dichloromethane:methanol 2:1 (vol) was added. Sodium chloride (1%) was added and left for 1 hr. The top surface was removed and the remaining solution was washed by the addition of

dichloroform:methanol:water (2:50:50 vol/vol). Sodium sulphate was added and dried under nitrogen, weighed and dissolved with toluene at a concentration of 100mg/ml. The fatty acids were esterified and analysed by GC as previously described in the analysis of blubber samples.

Serum

To a previously weighed 2 g of serum 10 ml of dichlormethan:methanol 2:1 (Vol) was added followed by 50 µl of C:23. The final mixture was strained with fiberglass wool, and 30 extra ml of dichloromethane/methanol were added. Salt solution (1%) was added and left for 1 hr. After this stage the same procedures as in milk were followed.

STATISTICAL ANALYSIS

Fatty acid concentrations are reported as percentages of total FA. These relative values were arcsine square root transformed prior to statistical analysis. Samples of blubber, serum and milk were divided into three lactation stage categories based on the pup's age when a sample was collected relative to the observed birth and weaning dates. Because lactation duration varied between females, samples collected during the first quarter of the total duration of lactation was designated as early, the last quarter as late and samples collected during the intervening period were designated as mid lactation.

Random forest analysis (see Chapter 2) was used to determine to what extent the compartments (blubber, blood and milk) could be distinguished by their FA profiles and how the FAs vary between lactation stages within a given compartment. This gave a class variable with 8 classes (blubber samples were not collected during the mid-lactation capture). These analyses were performed for all the animals that had a minimum of paired samples for two compartments (blubber-blood, blubber-milk, blood-milk). We also compared the FA composition of mothers with that of pup blood and blubber within the same analysis.

To further examine the degree to which each FA contributed to differences between compartments, lactation stages and colonies observed in the random

forest analysis, multiple pair wise comparisons tests were carried out according to the methods described more fully in Chapter 3. Here we focused our attention to 1) within compartment changes of individual FAs throughout lactation and 2) colony differences of individual FAs in specific body compartments at specific times through lactation.

RESULTS

A total of 630 samples (blubber, blood and milk combined) from mothers and pups were analyzed during the three years of study. Total numbers of females sampled each year are shown in the summary tables, Appendices 2-7, which also present the relative percentages of each of the 60 FAs identified in the three compartments for mothers and pups for both populations for each year. During each of the 3 years the most abundant FAs in all three compartments and in both colonies were the saturated FAs (SFAs) 14:0, 16:0 and 18:0, the monounsaturated (MUFAs) 16:1n-7, 18:1n-7, 18:1n-9, 18:1n-11, 20:1n-9 and 22:1n-11 and the polyunsaturated FAs (PUFAs) 18:2n-6, 18:4n-3, 20:5n-3, 22:5n-3 and 22:6n-3. Overall, MUFAs comprised the highest proportions of the total FAs in all body compartments. In both populations SFAs were more abundant in milk than in blubber, the total MUFAs were higher in blubber while PUFAs comprised were similar proportions in all compartments.

CLASSIFICATION USING RANDOM FOREST

Random forest analysis showed a clear distinction in FA composition between the two colonies, between the three compartments of females, the two compartments in pups and also between mothers and pups (Appendix 8 A and B). NR and IOM were clearly discriminated by the FA composition of all the body compartments. In both colonies, the three body compartments were as well differentiated between each other, only a few samples were misclassified into the wrong body

compartment. The major misclassifications occurred between lactation stages within a compartment, while a small number of samples were misclassified into the same compartment but in the wrong year (11.6%) or the wrong colony (6.8%). Only eight of the 630 samples (i.e. ~1.3%) were incorrectly classified into a different body compartment.

Among IOM samples (Appendix 8A), blubber from 2004 showed major misclassifications between lactation stages with none of the early samples classified correctly. There was also a poor distinction between mothers and offspring. There was greater discrimination among blubber samples in 2005 and 2006, with only a few samples allocated to the wrong year and colony. Milk samples from IOM were well distinguished from the other compartments and between lactation stages. The samples that were misclassified into an incorrect year and/or colony were nevertheless allocated to the correct compartment (i.e. milk) and lactation stage. Blood samples were well distinguished from the other two compartments with not a single sample being misclassified into a different compartment. However, there was relatively poor distinction between lactation stages, and several samples were wrongly allocated to NR blood samples.

The pattern among samples from North Rona was broadly similar to that at the IOM (Appendix 8B). Misclassifications among NR blubber samples in 2004, 2005 and 2006 occurred mainly between lactation stages. Blubber samples from mothers were well discriminated from pup blubber. Milk samples were well discriminated from the other two compartments, with only 1 of 87 samples classified as blubber. Milk samples from 2005 had the poorest classification among milk samples with 27 out of 31 misclassified. In 2006, the misclassifications were relatively low in comparison to the previous years. Blood samples were clearly differentiated from the other two compartments and the misclassifications occurred mainly between the mid and late lactation stages. Mothers and pups were also clearly differentiated. Within pups, blubber samples were relatively well classified between lactation stages.

The FAs within and between compartments varied between lactation stages. At early lactation differences between blubber and milk could be seen in over 20 FAs in both populations for all the years of study to the exception of IOM 2004 where the compartments only differed in 10 FAs. In both colonies, FAs found in

high proportion such as 16:0, 18:0 and 14:1n-5 significantly differed between blubber and milk at early lactation. In addition FAs 16:1n-7 and 20:1n-9 were only different in NR samples. The first three FAs mentioned are part of the most important variables selected by the random forest for splitting between the groups.

CHANGES IN FA COMPOSITION THROUGHOUT LACTATION

Changes in FA composition of the blubber of mothers were described in Chapter 3. Briefly, we found that FAs are selectively mobilized resulting in substantial changes in the blubber FA composition over lactation. Here we focus on maternal blood and milk and on pup blubber.

Overall, the FAs composition in maternal serum remained relatively constant throughout lactation in both colonies (Fig. 1). Only a small number of FAs (0-3 out of 60) changed significantly (Table 1). In contrast, the milk FA composition changed gradually throughout lactation (Fig. 2). While few FAs changed significantly between consecutive stages (2-6 out of 60) the number of FAs that changed significantly from early to late lactation was much greater (24-28 out of 60, Table 1). Interestingly, most of the specific FAs that changed significantly were the same in both populations (Fig. 2), and the changes (increase or decrease in relative proportions) were the same for each of these. The FAs that changed most dramatically were 1) the SFAs 15:0, 16:0, 17:0, 18:0 and 20:0, which all decreased significantly towards the end of lactation, 2) many of the long chain MUFAs, most of which increased, and 3) long chain PUFAs that showed no common direction of change. The serum of pups, as that of the females, remained relatively constant during the lactation period.

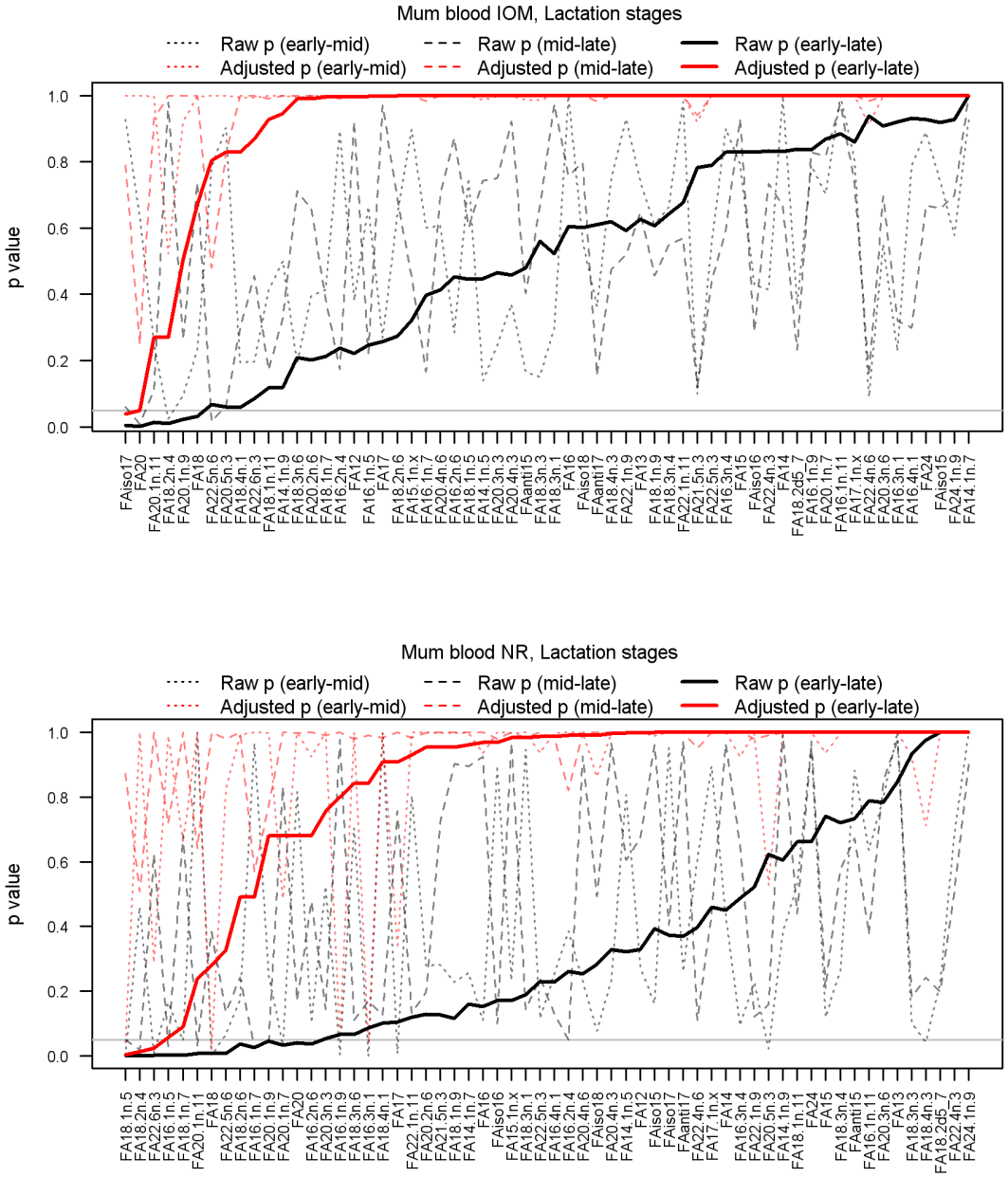


Figure 1. Multiple comparisons tests (Wilcoxon) for differences in FA concentrations in maternal blood between lactation stages at the Isle of May (top) and North Rona (bottom). Black lines represent unadjusted p-values given to each fatty acid in the standard Wilcoxon tests, while the red line indicates the adjusted p values accounting for multiple comparisons, controlling for the family-wise Type I error rate (FWER, see text for further details). Bold solid lines represent the overall difference between early and late lactation. FAs are arranged in increasing order from left to right according to their adjusted p-values.

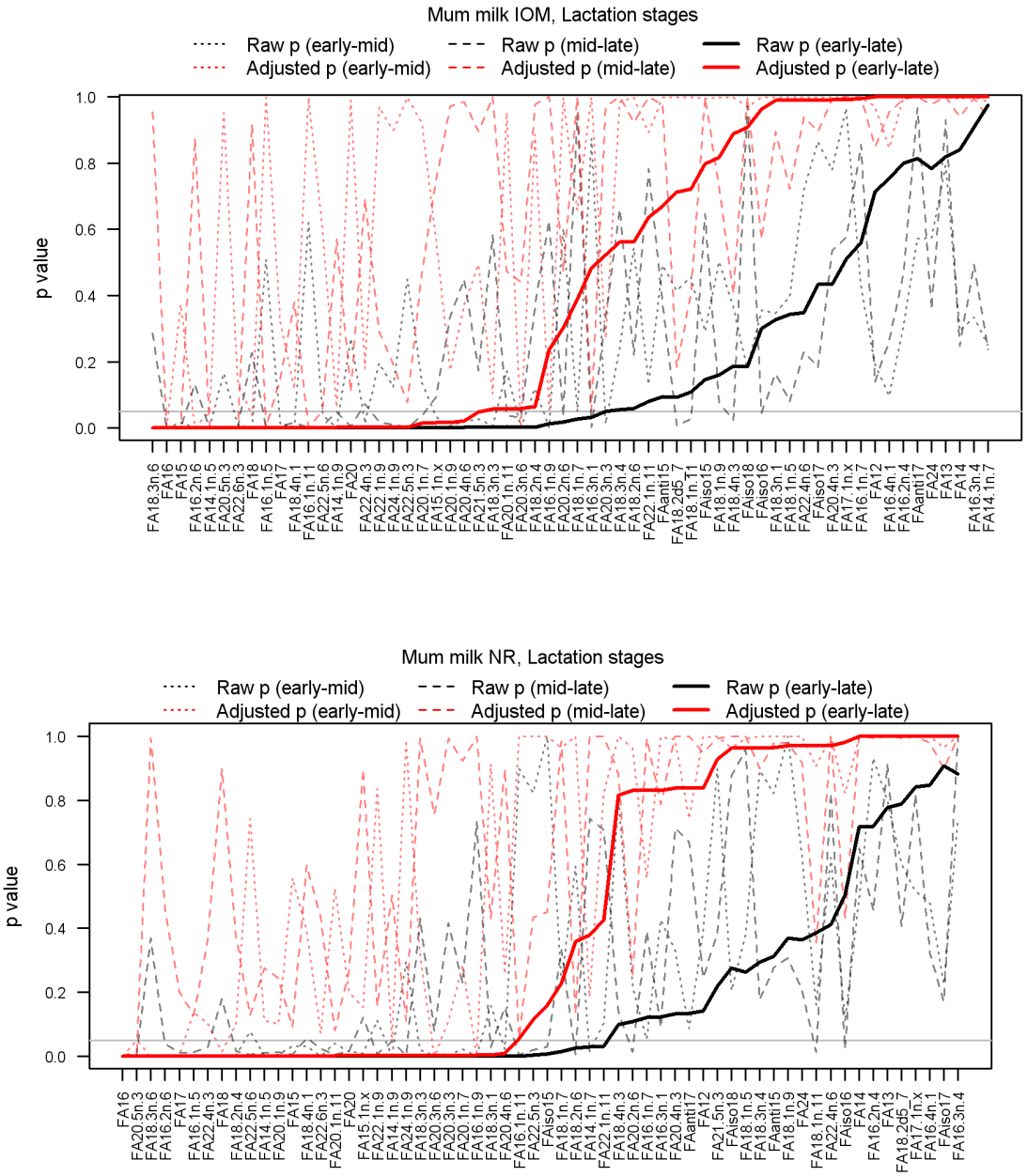


Figure 2. Multiple comparisons tests (Wilcoxon) for differences in FA concentrations in maternal milk between lactation stages at the Isle of May (top) and North Rona (bottom). Black lines represent unadjusted p-values given to each fatty acid in the standard Wilcoxon tests, while the red line indicates the adjusted p values accounting for multiple comparisons, controlling for the family-wise Type I error rate (FWER, see text for further details). Bold solid lines represent the overall difference between early and late lactation. FAs are arranged in increasing order from left to right according to their adjusted p-values.

Table 1. The number of congeners for which raw and adjusted p values were <0.05 in multiple comparisons tests between stages within compartments. Multiple tests were based on the Wilcoxon rank sum test statistic.

Individual	Compartment	Location	Stages	Raw	Adjusted
Mum	Blood	NR	Early - Mid	10	3
			Mid - Late	5	0
			Early - Late	14	3
		IOM	Early - Mid	1	0
			Mid - Late	2	0
			Early - Late	6	2
	Milk	NR	Early - Mid	25	8
			Mid - Late	25	2
			Early - Late	35	28
		IOM	Early - Mid	20	6
			Mid - Late	24	7
			Early - Late	32	24
Pup	Blood	NR	Early - Mid	14	2
			Mid - Late	7	1
			Early - Late	28	11
		IOM	Early - Mid	10	2
			Mid - Late	11	1
			Early - Late	12	4

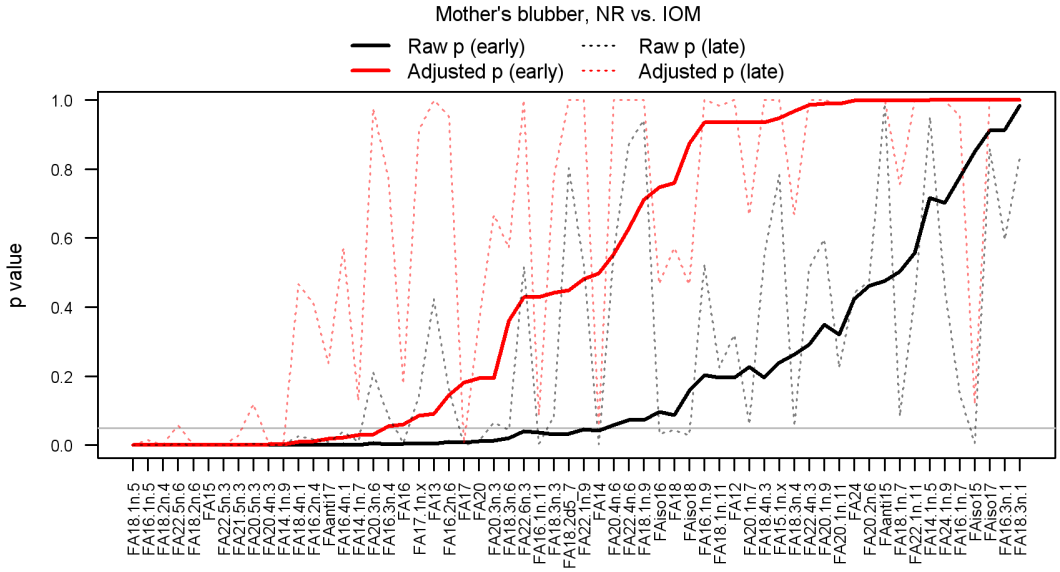
COLONY DIFFERENCES IN FA COMPOSITION

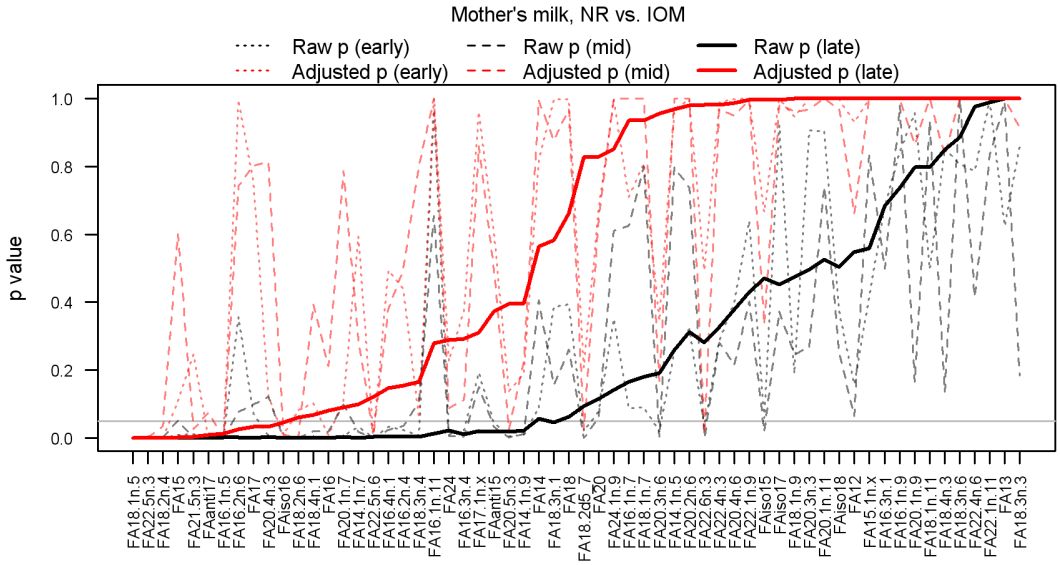
The largest differences between colonies were seen in the blubber of females at early lactation (17 out of 60 FAs) and these gradually decreased towards the end of lactation (10 out of 60 FAs) (Table 2, Fig. 3). The differences between NR and IOM were much smaller in serum (Table 2), only 2 FAs out of 60 were different. Colony differences in milk were larger than in serum but smaller than those in blubber. Once transferred to the pups the differences were again much smaller both in serum and in blubber. The differences observed between colonies in females' blubber and milk occurred mainly in PUFAs (Fig. 3) with only few MUFAS and 3 SFAs present. The few FAs that were different between colonies in blubber pups such as 22:5n-3, 18:2n-6, 18:1n-5 and 18:2n-4 were also different

between colonies in female's blubber and milk (Fig. 3). There were some FAs such as the EFA 20:5n-3 that initially was different between the blubber of females from both colonies, however this FA was not different between the pups blubber from NR and IOM at the end of lactation.

Table 2. The number of congeners for which raw and adjusted p values were <0.05 in multiple comparisons tests between colonies within compartments. Multiple tests were based on the Wilcoxon rank sum test statistic.

Individual	Compartment	Stage	Raw	Adjusted
Mum	Blubber	Early	32	17
		Late	26	10
	Blood	Early	21	2
		Mid	12	2
		Late	11	2
	Milk	Early	26	8
		Mid	23	11
		Late	28	11
Pup	Blood	Early	24	4
		Mid	8	1
		Late	15	3
	Blubber	Late	17	4


Mother's blubber, NR vs. IOM
Raw p (early)
Adjusted p (early)
Raw p (late)
Adjusted p (late)
p value
1.0
0.8
0.6
0.4
0.2
0.0


Mother's milk, NR vs. IOM
Raw p (early)
Adjusted p (early)
Raw p (mid)
Adjusted p (mid)
Raw p (late)
Adjusted p (late)
p value
1.0
0.8
0.6
0.4
0.2
0.0

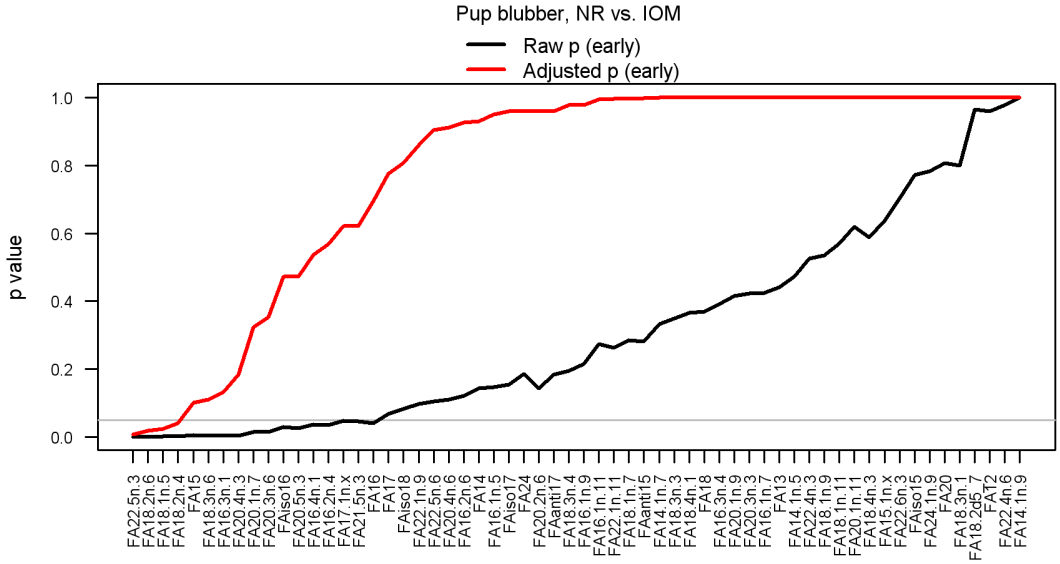

Pup blubber, NR vs. IOM
Raw p (early)
Adjusted p (early)
p value
1.0
0.8
0.6
0.4
0.2
0.0

Figure 3. (Previous page) Multiple comparisons tests (Wilcoxon) for differences in FA proportions between colonies in maternal blubber, milk and pups blubber at different between lactation stages. Black lines represent unadjusted p-values given to each fatty acid in the standard Wilcoxon tests, while the red line indicates the adjusted p values accounting for multiple comparisons, controlling for the family-wise Type I error rate (FWER, see text for further details). Broken dots represent the differences at early lactation, broken lines differences at mid lactation and bold solid lines represent the difference at late lactation. FAs are arranged in increasing order from left to right according to their adjusted p-values.

DISCUSSION

The purpose of this study was to make a parallel comparison of the fatty acid signatures in the blubber, blood and milk of grey seal mother-pup pairs to assess to what extent the dietary differences between populations can be observed in maternal milk and subsequently in the offspring blubber. It also address the questions related to what extent the FAs from female blubber and the blubber deposited in pups can be predicted by the milk.

A general characteristic of the life cycle of pinnipeds is the spatial separation of the feeding and breeding grounds. During the feeding period, fatty acids from the prey are accumulated with minimal modification in the blubber. This will represent a dietary history of the feeding months previous to the breeding season (Budge et al. 2006). Therefore, fatty acids have been used to infer the diet (Iverson 1993) and as a discrimination tool between populations of marine mammals (Iverson et al. 1997, Walton et al. 2000).

During the lactation fast, lipids from the blubber are mobilized and the circulating lipids will be taken up by the mammary gland for the production of milk (Iverson 1993). Thus, according to Iverson (1993), the lipids profiles are conserved through the pathways (from circulating lipids to milk). However, in this study we found that blubber, serum and milk could be well differentiated during the different stages of lactation. These differences between compartments can probably be a result of the selective mobilization of FAs from the blubber and a selective uptake of FA by the mammary gland. A study by Iverson et al (1995) in hooded seals (*Crystophora cristata*) also found differences between blubber and milk at early lactation with the differences also declining towards the end of lactation. Although the study by Grahl-Nielsen et al (2000) in grey seals

(*Halichoerus grypus*) did not analyze late lactation samples, they also found differences between blubber and milk samples obtained one week after birth. The differences between these two compartments, could be due to the fact that the FAs from the blubber are selectively mobilized to the blood stream; some FA will be utilized to cover the mother's metabolic needs, while there may be another selective uptake to produce the milk in order to give the FAs that will be more important for the development, energy and growth for the pup. The differences between blubber and milk or blubber and any other tissue can be also influenced by the outer layer of the blubber that probably has an insulation role (Strandberg et al 2008). The outermost part of the blubber is known to contain mainly saturated fatty acids and long chain monounsaturated fatty acids (Strandberg et al 2008). Based on all these facts, is not surprising that milk FAs did not resemble those from the blubber. The FAs of the serum were as well clearly differentiated of any other compartment; however it is important to acknowledge the fact that the lipoproteins in the blood could have played an important role in this differentiation.

The FA composition of the pups blubber was as well different from the milk they ingested and from their mother blubber. Despite the fact that mother pup pairs have been used to study the deposition of FAs in the blubber, it is important to point out that lactation is a unique period. Milk will provide the FAs that will be essential for the proper development and growth of the pup. Milk will have to also provide the necessary FAs to build up blubber which is necessary for energy storage and insulation. Thus, the blubber FA composition of pups might be influenced by factors related to the physiological and functional properties of the blubber (Birkeland et al. 2005) controlling the FAs that are deposited. On the other hand, many of the FAs from the milk will be utilized and possibly altered in order to cover the physiological needs crucial for survival and development of the offspring. Thus, lactation may not represent accurately the mechanism by which dietary FAs are deposited during the feeding periods.

The differences between lactation stages in serum were not as clear as in other body compartments. In contrast the milk composition varied according to the lactation stages. Both populations showed a gradual change in the FA composition of milk throughout lactation. The changes in milk were similar

between years and colonies, and as lactation progressed, the similarities in milk between colonies became more obvious. Differences in the FA composition of milk throughout lactation have also been shown in Antarctic fur seals (Iverson et al. 1997, Brown et al. 1999). However, in fur seals the changes have been related to the changes in prey in the foraging trips. In contrast, in white whales, which also forage while lactating, females sampled at different lactation stages during the first year of lactation did not show any variation in their FA milk composition (Birkeland et al. 2005). Brown et al (1999) also analyzed milk samples from elephant seals during different stages of lactation and showed a lack of changes in the FA profiles. The similarities between NR and IOM in their milk FAs composition and in the changes of their relative proportion (increase or decrease) as lactation progressed, strongly suggest that there is a common process underlining the changes in the FA composition of the milk independently of the dietary differences in blubber (where the FAs originated in first place) between colonies. The changes in milk composition throughout lactation may reflect the differential mobilization of FAs due to physico-chemical characteristics and/or may also be a response to the specific nutritional demands of the pups. In the latter case, physiological changes of the pups throughout lactation may require adjustments in the proportions of FAs mobilized from blubber and transferred via the milk. For instance, Staniland and Pond (2005) suggested that the observed changes in Antarctic fur seal milk were influenced not only by the changes in prey, but also by changing nutritional requirements of the pups. Milk samples at early lactation had higher amounts of SFA 16:0 and 18:0 than blubber. This was also found in grey seals (Grahl-Nielsen et al. 2000), in hooded seals (Iverson et al. 1995) and in white whales (Birkeland et al. 2005). These FAs are preferentially mobilized from the blubber of grey seals. This may increment the circulating amounts and thus facilitate the uptake of these FA to the mammary gland. However, it is important to mention that these two types of FAs can also be produced by *de novo* synthesis by liver and in some species by the mammary gland, but this is minimum (Iverson 1993). Thus, it is plausible that the high proportions in milk are a combination of both the high levels in the circulating lipids and the production in the mammary gland. Increasing the amounts of SFAs might be a way of transferring energy reserves that are readily catabolised (Grahl-

Nielsen et al. 2000). However, Bryden and Stokes (1969) suggested that the high amounts of 16:0 transferred to the pup are to build up the offspring blubber layer. The fact that MUFAs 22:1n-11 and 20:1n-9 were found in lower proportions in milk than in blubber can be explained by the low rates of mobilization (Chapter 3) from the blubber. Captive harbour seals that were fed on herring showed large increases in the levels of 20:1n-9 and 22:1n-11 in milk as lactation progressed (Iverson 1993). In this study the increase of these two FAs at the end of lactation were also observed even though grey seals were fasting. The increase at late lactation might be a consequence of its low mobilization rate from lipid tissues as it has been described in lactating weddel and grey seals ((Wheatley et al. 2008), Chapter 3). In general, concentrations of PUFAs and EFAs, which are of extreme importance for the development of the pup, were found to be high and constant throughout lactation in milk. Long chain FAs with more than 18 carbons are thought to be the main source of fuel during the fast in both females and pups (Bryden & Stokes 1969).

Colonies were distinguished by their FA profile in blubber, serum and milk during the 3 years of study. However, the changes in the relative proportions of individual FAs throughout lactation in the compartments were very similar for both colonies and between years. Despite these similarities and independently of a similar FAs mobilization (Chapter 3) and milk composition between both colonies, it was surprising that the dietary differences observed in the early blubber FA profile between NR and IOM, persisted on the milk and pups blubber. These differences between colonies observed in pups blubber were perceived only in four main FAs, which were the same FAs that were observed to differ greatly in the blubber and milk between the females of NR and IOM. The high degree of similarities in the FAs profile of pups in parallel to the similarities in milk suggests that the production of milk is strongly influenced by the physiological needs of the developing pups. Thus, even if the females start the lactation with very different FA profiles in the blubber their milk will be very similar. For instance, the differences observed between colonies in their blubber in the EFA 20:5n-3 were not seen in the milk. Milk was found to have higher proportions of this FA compared to the blubber during the 3 stages of lactation suggesting the high amount transferred to the pup. This FA was not different between populations in

the pups' blubber. FAs transferred to the pup not only provide the energy required for body growth, nutrition, thermoregulation and to build up large reserves of blubber (Iverson et al. 1995) but also provide essential fatty acids (EFAs) that are indispensable for visual and brain development and as eicosanoid precursors (Innis 2005, Innis 2007). Thus, many FAs that have been transferred in high amounts will be utilized and will not necessarily be deposited in large amounts in the pups' blubber. The differences between populations in pups blubber was not observed in any of the FAs that are known to be important in the developing of the pups.

In summary, blubber, serum and milk could be clearly differentiated by their FA profiles during the three lactation stages. The changes in the relative amounts of FAs in the compartments was very similar between colonies, this probably due to the patterns of mobilization of FAs from blubber and their selective transfer to milk. Despite these similarities between colonies, females from NR and IOM could still be clearly discriminated by any of the tissues studied probably due to specific ratios of FAs obtained in the diet. This shows that milk might be useful to differentiate populations and lactation stages but not to predict the FA profiles from blubber from females or pups as it has been suggested by other studies (Iverson, 1993). It is clear that diet has an influence on the fatty acid composition of other tissues. Although, the use of milk appears to be useful to infer diet, the knowledge of the physiology and the variation of the fatty acids profile across lactation are necessary in order to interpret properly the results. The different factors influencing the milk production and composition are very complex. Thus, further studies in the understanding of milk FA transfer area are needed before the FAs in milk are used in order to predict the diet in marine mammals. For instance, it needs to be studied in major detail which are the factors that can have an influence on the FA composition of the milk, such as the contribution of *de novo* synthesis of FAs by other tissues and the turnover rate of FAs in the mammary gland (Staniland & Pond 2004). As well studies in deposition of FAs between mother and offspring need to be considered, since pups are in a particular situation where they are utilizing certain FAs for their development, growth and thermoregulation. Especially in marine mammals, the growth of blubber layer is very important due to its insulation properties. This will add a

special requirement by the pups of specific FAs with certain characteristics that will build up the outer layer of the blubber which has primarily a structural and thermoregulation property. In addition, it would be interesting to estimate the absolute amounts of FAs in milk and the amount of milk ingested in order to estimate the total amounts of FAs that have been transferred to the pup. If the total amount of FAs deposited in the pup can be estimated at the end of lactation, it might be possible to estimate the usage of FAs by the pup and mother.

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5 Regional and annual variations in persistent organochlorine levels in UK grey seals

ABSTRACT

Persistent organochlorine (OC) pollutants (PCBs and DDTs) were determined in two UK grey seal colonies: Isle of May (IOM) and North Rona (NR). A total of 10 and 12 adult females were sampled for blubber during 2005 and 2006 respectively at each island. Early lactation blubber samples were analysed for OC concentrations and used to estimate the total body burdens (TBB). We report contamination levels and patterns of PCB congeners and DDT compounds. IOM females had higher total concentration (1613.49 ng/g lipid and 1452.54 ng/g lipid in 2005 and 2006 respectively) than did NR females (850.71 and 1071.77 in 2005 and 2006 respectively). The same was observed for TBB, where IOM females were found to have 96.58 and 74.74 mg of OC in 2005 and 2006 respectively, while females from NR had 47.59 and 66.31 mg of OC in the same years. The PCB profiles in concentrations and amounts were used to investigate the regional variations between these two colonies. IOM and NR could be clearly distinguished by their OC profile, where IOM females presented higher levels of highly chlorinated PCB congeners, while NR had higher concentrations and amounts of lower chlorinated congeners. The geographical differences in OC levels and patterns may be a reflection not only of the fact that IOM seals inhabit a more polluted region but can be as well a consequence of dietary differences between colonies. Analysis of TBBs standardised to a particular age and sex class of animal at a specified time should be a preferred method to monitor spatial and temporal trends in the pollution of marine top predators. This study is the first describing the levels and TBB found in the NR colony.

INTRODUCTION

Persistent organochlorines (OC) such as polychlorinated biphenyls (PCBs) and dichloro-diphenyl-trichloroethanes (DDTs) have been released into the environment since they were first used in agriculture and industry in the early to mid 1900s (Tanabe 2002, Dietz et al. 2004a, Dietz et al. 2004b). These types of contaminants are well known for their high abundance and the potential threat to the environment. Thus, the use of many OCs was banned in the industrialised countries in the late 1970s. However, some organochlorine pesticides are still in use in agriculture and/or to combat malaria in tropical developing countries (Joiris & Overloop 1991, Joiris& Overloop 1991, Aguilar et al. 2002). Unlike DDTs, PCBs did not have any replacements in some of their applications, and they are therefore still in use in some processes (Aguilar et al. 2002) under some limitations and regulations.

The toxic effects of OCs on wildlife have been well documented. These include immunological impairment, reproductive failure, endocrine dysfunction (Safe 1994, Bergman 1999, Tanabe 2002), different types of lesions in organs (Olsson et al. 1994), and alterations in the skeletal growth and ontogenic development as well as the induction of bone lesions (Bergman et al. 1992, Lind et al. 2003). OCs are characterised by their high lipophilicity and resistance to biodegradation (Norstrom & Muir 1994). Therefore, they have long half- lives and bio-accumulate and biomagnify throughout the food webs, resulting in high levels in the top of the trophic chain (Muir et al. 1995). Top predators such as whales, polar bears and seals are among the animals with the highest concentrations (Norstrom& Muir 1994, Bernhoft et al. 1997, Kleivane et al. 2004). Due to the lipophilicity of these compounds, animals accumulate them in their lipid stores. Marine mammals can accumulate up to 98% of the total body burden (TBB) in their blubber (Wolkers et al. 1998).

Pollutants such as organochlorines can be transported long distances either by ocean or air currents and thus have a worldwide distribution. They have been found in all the world's ecosystems, also in remote areas (Aguilar 1987, Wolkers et al. 1998) such as Antarctica (Sladen et al. 1966) and the Arctic (Muir et al.

1992), and are widely distributed in the world's oceans (Joiris& Overloop 1991, Wania & Mackay 1993). However, distribution by air and ocean currents is not uniform. The amount of pollutants found in an ecosystem will vary depending on the rate of transport from the release source, the amount released and the rate of degradation of the pollutant during transport and during its residence in the ecosystem (Aguilar 1987, Stow et al. 1995, Stern et al. 2005).

The North Sea is known to be one of the regions with the highest levels of OCs (Vetter et al. 1996, Vetter et al. 1996, Weijs et al. 2009a, Weijs et al. 2009b) with the major source of contaminant input being the Dutch Wadden Sea (Vetter et al. 1996). In this region concentrations of PCBs were observed to decreased from the 1970s to the 1990s (Aguilar et al. 2002). However, despite these positive reports, it has also been shown that the geographical redistributions of pollutants e.g. entrainment by ocean or air currents can keep levels at a high and steady state in certain areas (Aguilar et al. 2002). OC levels showed a rapid decline in fish, birds, polar bears and seals following restrictions in the use of PCBs and DDT in the 1970s. For instance a report from the West of Scotland showed a decline in the PCB concentration in eggs from Gannets from the late 1970s to late 1990s (Alcock et al. 2002). Another study analysed fish species in the southern part and arctic regions from Sweden and compared samples from as early as 1967 to 1995 showing as well a decline in OC concentrations (Bignert et al. 1998). Decreasing trends have also been seen in cetaceans and seals when data from 1970s and 1990s have been compared in the Baltic Sea, North Sea, Mediterranean Sea and the Atlantic and Pacific coast of USA (Aguilar et al 2002).

A variation of contamination in the oceans have been seen in the tissue of animals, for example Atlantic cod from the North Sea has higher levels of PCBs and DDTs than the cod found in Iceland (Hobbs et al. 2003). Marine mammals inhabiting different regions and feeding on differently contaminated prey might be expected to show different contaminant burdens (Hobbs et al. 2003). Therefore, it is expected that grey seals feeding in the North Sea will have higher levels and different patterns of PCB congeners to those found in the less contaminated waters of the North Atlantic. There is a substantial amount of information on geographical and temporal variations in contaminants in

different marine mammals. However, there are few studies that report the pollution status and geographical variations for grey seals within the UK.

The OC load found in marine mammals are of dietary origin (Kleivane et al. 2000, Borgå et al. 2004), therefore variations in the prey availability, prey composition and/or feeding habits will have an influence on the levels and types of pollutants stored in animals (Kleivane et al. 2000). Variability in contaminant levels can also be attributed in part to individual and/or physiological parameters such as sex, age, nutritional and health status and reproductive stage (Aguilar 1987, Bernhoft et al. 1997, Lie et al. 2003, Dietz et al. 2004b, Stern et al. 2005). Bioaccumulation will also be affected by the degree to which each of the different OC compounds is metabolised, and further complexity is added by the finding that this capacity has been shown to be partly species-specific (Tanabe 1988, Lie et al. 2003). Pollutant loads tend to increase with age because uptake normally exceeds excretion (Aguilar 1987). However, levels can stabilise or even decrease over time in sexually mature females, because they transfer a significant proportion (up to 30% in grey seals) of their burden to their offspring during pregnancy and lactation (Addison & Brodie 1977, Reijnders 1980, Tanabe et al. 1982, Aguilar 1987, Pomeroy et al. 1996). Because of these individual/physiological effects on pollutant levels, it is important to determine the reproductive stage, age and condition of animals when the OC concentrations in blubber are compared between populations and/or individuals (Pomeroy et al. 1996, Hall et al. 2008). Also, when determining the pollutant concentration in seals it is important to obtain information about the stage in the life cycle. Since, many marine mammals are seasonal feeders that rely on their fat stores during the breeding and moulting fasting periods. High rates of lipid turnover and large seasonal variations in the total blubber lipid content during their life cycle will influence the concentrations of OCs in the adipose tissues (Aguilar 1987, Polischuk et al. 1995, Polischuk et al. 2002, Hall et al. 2008). As fasting animals lose fat but not the OCs present in it, the concentration of pollutants in blubber increases (Polischuk et al. 1995, Kleivane et al. 2000, Polischuk et al. 2002, Hall et al. 2003, Debier et al. 2006, Hall et al. 2008) and therefore not providing an accurate estimate of their burden. Thus, it is important to obtain the absolute amounts of pollutants and not only concentrations.

Marine mammals have been used to monitor the environmental contaminants such as PCBs and DDTs in various ecosystems (Aguilar et al. 2002). At the same time pollutants have also been utilized to distinguish between populations, such as harbour seals in different regions of the Baltic Sea (Storr-Hansen & Spliid 1993a, Storr-Hansen & Spliid 1993b), porpoises from Norway and the Baltic Sea (Berggren et al. 1999) and from the North Sea, Baltic Sea and Greenland (Bruhn et al. 1999, Krahn et al. 2004, Krahn et al. 2007). Studies of variations in contaminant levels in organisms across space and time are usually done using pollutant concentrations in lipids. However, this does not necessarily reflect absolute body burdens, since lipid concentrations will depend on the nutritional state of an animal (Kleivane et al. 2004). To avoid this potential problem it is useful to also estimate TBBs. This also needs to be considered when using marine mammals as bio-monitors of the ecosystem in order to get a complete picture of the overall contamination status of the animals (Weijs et al. 2009b).

Regional and temporal variations in OC pollutants have been documented for many marine mammals. Particularly in grey seals regional and temporal variations have been documented in Canadian colonies and in the Baltic Sea. However, few studies have documented regional variation in OC levels in UK grey seals. This study provides information on the regional variations between two UK colonies in their PCBs and DDTs concentrations and TBBs during 2005-2006. In addition, it provides the first measurement of pollutant levels in females from North Rona which we expect to be less polluted that the IOM colony.

METHODS

SAMPLES

Breeding female grey seals at two UK colonies; North Rona (NR) (59°06'N, 05 °50'W) and Isle of May (IOM) (56°10'N, 2 °33'W) were studied during two consecutive breeding seasons 2005 (IOM=10, NR=10) and 2006 (IOM=12,

NR=12). Mothers were captured 2-3 times during the lactation period, early (2 - 4 days post partum), mid (5 – 14 days post partum) and late (15 - 18 days post partum). They were immobilized using an intramuscular doze of 'Zoletil 100' (Virbac, Cedex; 1.0 ml per 100 kg). Blubber biopsies were taken at early and late lactation. A small incision was made on the mid pelvic region using a scalpel, and a full depth blubber core was extracted using a 6mm diameter biopsy punch (Acuderm Inc). Samples were wrapped in aluminium foil and stored at -20^{o}C until further analysis. The procedure for blood sampling, body composition estimation and morphometric measurements were followed as described in Chapter 3.

In most cases, samples were collected from known-age individuals as part of the ongoing demographic studies at IOM and NR e.g. (Pomeroy et al. 1999, Pomeroy et al. 2000). In some cases, new individuals with unknown ages were captured, and thus an incisor tooth was extracted for age determination.

DETERMINATION OF PCBS AND PESTICIDES

Blubber samples were thawed at room temperature. The samples were transferred to a test tube and the lipids were extracted by placing the samples in a microwave oven for 1 minute at 650 W. The samples were purified using acid and Florisil clean-ups. A mixture of sulphuric acid (3 ml, 95%) and hexane (3 ml) were added to the extracted lipid samples and then vortexed for 2 min and centrifuged at 1810 g at 25 ^{O}C. The organic phase was transferred and the acidic phase was vortexed and centrifuged one more time as previously described. The new organic phase was reduced to approximately 1 ml under nitrogen. The next clean up was performed with Florisil solid phase cartridges. Cartridges were conditioned by adding 5 ml of acetone, 5 ml of acetone-hexane (50:50 vol) and 12 ml of hexane. Once the cartridges were ready the samples were added. Tubes containing the samples were rinsed with 3 ml of hexane and then added to the cartridge. Finally 3 ml of hexane were added directly to the column. The sample in the test tube was then completely evaporated under nitrogen.

The final extracts were analysed by gas chromatography using a Thermo Quest Trace 2000 gas chromatograph equipped with a 63 Ni ECD detector (Thermo Quest, Trace 2000, Milan, Italy) and automatic injector. One to five µl of the purified extracts were injected. The PCB congeners were separated on a 30 m x 0.25 mm (0.25 µm film) DB-XLB capillary column (J&W Scientific, USA). The temperature program was as follows: 2 min at 60^{O}C, gradual heating from 60^{O}C to 140^{O}C at the rate of 20^{O}C min, 3 min at 140^{O}C, gradual heating from 140^{O}C to 270^{O}C at the rateof 2.5 ^{O}C/min and 12 min at 270^{O}C. The carrier gas was hydrogen with a flow rate of 4 ml/min and a pressure of 130kPa, and the make-up gas was Ar/CH4 (95:5) at a flow rate of 30 ml/min. The injector was at ambient temperature and the detector was kept at 300 ^{O}C. PCBs were identified according to their retention times. Data were recorded using Chrom-Card 1.19 software. Quantification was performed by comparison with external standards using a calibration curve.

STATISTICAL ANALYSES

All statistical analyses were performed using the R language, version 2.8.0 (R Development Core Team 2008). All results for organochlorines were log transformed before any statistical analysis in order to normalize the data distribution.

In this study we were interested in the total composition of blubber in order to obtain an average representation of total concentrations and the TBB. TBB was calculated from the total concentration estimation for each seal by:

$$TBB_i = TBF \times [OC]_i$$

where *TBF* represents total body fat mass and $[OC]_i$ represent the concentration of the i^{th} organochlorine, assuming that the blubber core was representative of the females distribution of OCs in blubber throughout the body.

Random forest (Breiman 2001) analysis was used in classification mode to examine the annual and regional variability in TBBs and concentrations of OCs in

blubber samples from early lactation. Comparisons at early lactation are ideal, since females have not yet mobilized large amounts of lipids and OCs present provide an integrated representation of the level of contamination up to and including the last feeding period. The analyses were performed using a spectrum of 14 PCB congeners as well as DDT and its metabolites DDE and DDD. Random forest analysis has been described in more detailed in Chapter 2.

A series of mixed effects models were developed to examine to what extent the total OC concentration and TBB, considered separately, could be explained by location, year, total body fat and postpartum mass. Animal ID was included as a random effect to account for the lack of independence between samples obtained from the same individual and to account for the longitudinal nature of the data. Age was also added as a random effect to control for the likely effect of age on overall contaminant levels. The different linear mixed effect models were compared using AIC weights. A model was considered significantly better if its AIC weight was ≥ 2 times higher than the AIC weight for the next model in descending order of AIC weight. All models were tested for normality in their residuals using Shapiro-Wilk's test. All model parameters were considered significant at $p < 0.05$. In order to observe the power of age as an additional explanatory variable, age was included in new models as a fixed factor. However, the models did not improve. A Wilcoxon pair test analyses was performed in order to analyse if the amounts of TBB in the females capture in consecutive years significantly changed from 2005 to 2006. Only 4 females in NR and 6 in IOM were caught in consecutive years.

Multiple comparison tests were performed to examine the degree of significance in comparisons of concentrations and TBB of each individual OC congener between the two populations and the two years. Multiple comparisons tests provide adjusted p values in an attempt to correct for the increasing probability of committing a Type I error with increasing number of simultaneous tests. This type of analyses and the procedure followed are explained in Chapter 3.

RESULTS

Differences in body mass and total body fat at early and late lactation between colonies and years have already been described (see Chapter 3, Table 1). To reiterate the results relevant to the current analyses, animals from NR were on average heavier (kg) and had higher amounts of total body fat (kg) than animals from IOM at early lactation. But they did not differ significantly in their body condition (relative amount of fat).

The average age in NR females was 14.8 and 17.2 years old in 2005 and 2006 respectively and for IOM females 17.4 and 19.3 years old.

Group summary statistics for OC concentrations and TBB for each PCB congener and DDT and its derivatives are shown in Table 1 A-B. The most abundant PCBs in both concentrations and absolute amounts were PCB-153, -180 and -138 and DDE in both populations in both years. During the two years of study, IOM animals had higher concentrations of total PCBs and total DDT. They also showed higher TBB (See below). During 2005 IOM females had almost double the amount of OCs as NR females. However, the difference was much less in 2006 due to an increase at NR and a decrease at IOM.

LINEAR MIXED EFFECT MODELS

Candidate mixed effect models run with total OC concentration and TBB are presented in Table 2 A-B. The OC concentration top model included *Location, Year, Maternal Postpartum Mass* (*MPPM*) *and TBF* as fixed factors, with interaction terms between *Location* and *Year* and between *MPPM* and *TBF*. There was strong support for this model, since its AIC weight (0.53) was > 2.5 times the AIC weight of the next best model (0.21). The significant colony effect ($t_{location}$= -4.61 $p < 0.001$) showed that North Rona animals had significantly lower concentrations of total OCs at early lactation. Furthermore, there were significant effects of *MPPM* (t_{MPPM}=-3.492, p = 0.012) and *TBF* (t_{TBF}=-2.880, p=0.028), as well as a positive interaction between these two effects (0.0004, t=-

3.202, p=0.018). The Shapiro-Wilk's test showed that the residuals were normally distributed (W = 0.9833, p-value = 0.88). Among the random effects, both *Animal ID* and *Age* explained substantial amounts of the variance (0.1817 and 0.1494 respectively, compared to a final residual of 0.0650). To check for any possible effect of age on PCB concentrations, predictions were obtained by fitting this model to data where all variables except the random variable *Age* were kept constant. This analysis showed that despite the substantial contribution to the variance, there was no consistent trend of PCB concentrations either increasing or decreasing with age.

Table 2B lists the top candidate mixed effects models for explaining the variation in TBB. The top model had an AIC weight (0.567) of 2 times higher than the second 'best' model (0.230). The top model contained *Location* and *Year* with interaction as fixed factors, and *Animal ID* and *Age* as random effects. The Shapiro-Wilk's test showed that the residuals were normally distributed. NR animals had significantly lower TBB ($t_{location}$ = 3.760, p < 0.0001) than animals from IOM. Overall the TBB was lower in 2006 compared to 2005 (t_{year} = 2.692, p=0.024). A large part of the variance was explained by the random effects *Animal ID* (0.359) and *Age* (0.114), with a residual variance of 0.070. The Wilcoxon paired test showed that the TBB of females capture in consecutive years did not show a significant decrease or increase from 2005 to 2006 (p = 0.891).

Table 1. Geometric mean range values (in parentheses) corresponding to values between 25th and 75th quantiles of (A) OC concentrations (ng/g of lipid) and total body burden (B) found in blubber at early lactation in grey seals from Isle of May and North Rona during two consecutive years. The number in brackets indicate sample size.

A	Isle of May		North Rona	
	2005 (10)	2006 (10)	2005 (12)	2006 (12)
101	50.45 (43.69 - 58.59)	45.55 (33.62 - 59.15)	48.88 (37.1 - 79.28)	58.54 (44.46 - 79.29)
149	16.31 (12.7 - 25.54)	19.3 (16.84 - 33.12)	7.28 (3.66 - 15.84)	20.04 (11.89 - 31.72)
118	9.99 (7.49 - 20.39)	15.02 (12.84 - 17.48)	11.1 (6.58 - 18.26)	21.13 (15.52 - 28.2)
153	435.06 (375.22 - 528.45)	246.27 (181.07 - 311.73)	252.7 (193.49 - 311.63)	165.78 (110.04 - 237.07)
138	264.07 (225.05 - 313.94)	312.73 (249.83 - 406.77)	146.02 (114.14 - 182.89)	200.93 (128 - 253.43)
187	116.97 (93.46 - 142.92)	110.4 (87.87 - 162.45)	48.18 (34 - 65.41)	78.41 (49.24 - 111.16)
183	62.5 (49.15 - 78.41)	54.92 (38.65 - 77.02)	18.06 (13.89 - 25.72)	32.24 (17.93 - 53.4)
128	12.89 (11.29 - 24.36)	14.5 (8.14 - 18.77)	12.71 (6.81 - 29.27)	8.63 (5.96 - 14.05)
156	8 (4.3 - 23.63)	26.69 (21.55 - 37.98)	3.64 (3.17 - 7.3)	18.11 (10.63 - 28.25)
180	225.8 (200.51 - 269.67)	241.64 (191.58 - 346.92)	95.56 (65.29 - 125.98)	157.78 (97.94 - 235.95)
195	19.59 (9.45 - 35.09)	15.08 (7.94 - 29.55)	1.74 (0.66 - 2.93)	7.28 (3.63 - 12.12)
194	44.1 (28.59 - 72.85)	42.76 (21.18 - 79.03)	9.77 (8.21 - 18.25)	24.25 (12.56 - 48.59)
206	17.94 (9.29 - 38.37)	11.84 (5.22 - 25.35)	3.78 (2.09 - 6.15)	10.25 (4.68 - 18.58)
209	11.24 (3.33 - 28.75)	8.83 (4.03 - 16.23)	0.55 (0 - 0.09)	6.33 (3.1 - 9.97)
DDE	246.64 (200.94 - 322.26)	229.81 (192.48 - 267.88)	152.1 (129.85 - 178.67)	180.28 (138.31 - 215.69)
DDD	8.68 (1.52 - 15.37)	2.09 (1.46 - 6.26)	5.87 (4.39 - 11.96)	6.13 (4.04 - 7.46)
DDT	30.63 (22.55 - 44.57)	5.87 (2.28 - 15.59)	8.02 (6.13 - 10.69)	5.8 (3.73 - 8.27)
Σ PCB	1327.9 (1155.7 - 1608.3)	1199.72 (965.73 - 1612.18)	680.17 (508.37 - 903.87)	819.01 (505.6 - 1114.11)
Σ DDT	288.59 (227.49 - 371.41)	244.94 (204.25 - 286.74)	167.24 (144.16 - 196.04)	195.67 (144.94 - 258.57)
Total	1618.49 (1383.19 - 2011.43)	1452.54 (1169.98 - 1928.57)	850.71 (641.37 - 1099.91)	1017.77 (648.16 - 1340.42)

Table 1. Continued

B	Isle of May		North Rona	
	2005 (10)	2006 (10)	2005 (12)	2006 (12)
101	3.01	2.34	2.73	3.81
	(2.6 - 3.35)	(1.7 - 3.3)	(2.3 - 4.7)	(2.39 - 5.62)
149	0.97	0.99	0.41	1.31
	(0.78 - 1.23)	(0.85 - 1.71)	(0.23 - 0.83)	(0.7 - 2.18)
118	0.58	0.77	0.62	1.38
	(0.41 - 1.08)	(0.64 - 0.89)	(0.37 - 1.13)	(0.95 - 2.07)
153	25.96	12.67	14.14	10.8
	(19.82 - 37.1)	(9.47 - 18.84)	(13.09 - 16.26)	(7.57 - 17.6)
138	15.76	16.09	8.17	13.09
	(12.19 - 21.23)	(13.33 - 20.79)	(7.82 - 9.37)	(9.1 - 21.57)
187	6.98	5.68	2.69	5.11
	(5.71 - 7.92)	(4.76 - 6.68)	(2.25 - 3.58)	(3.47 - 8.77)
183	3.73	2.83	1.01	2.1
	(3.06 - 5.11)	(2.17 - 3.5)	(0.76 - 1.35)	(1.25 - 3.95)
128	0.77	0.74	0.71	0.56
	(0.63 - 1.19)	(0.43 - 0.81)	(0.43 - 1.54)	(0.3 - 1.17)
156	0.48	1.37	0.2	1.18
	(0.25 - 1.18)	(1.1 - 1.81)	(0.17 - 0.45)	(0.73 - 1.89)
180	13.47	12.43	5.35	10.28
	(12.57 - 19.1)	(9.7 - 17.88)	(4.42 - 6.92)	(6.99 - 16.96)
195	1.17	0.78	0.1	0.47
	(0.62 - 1.77)	(0.39 - 1.48)	(0.03 - 0.16)	(0.24 - 0.87)
194	2.63	2.2	0.55	1.58
	(1.9 - 4.11)	(1.43 - 3.86)	(0.47 - 0.96)	(0.89 - 2.95)
206	1.07	0.6	0.21	0.67
	(0.63 - 1.96)	(0.27 - 1.25)	(0.13 - 0.27)	(0.32 - 1.08)
209	0.65	0.45	0.03	0.42
	(0.22 - 1.56)	(0.21 - 0.8)	(0 - 0.01)	(0.22 - 0.59)
DDE	14.72	11.83	8.51	11.75
	(12.19 - 18.49)	(9.74 - 14.18)	(7.18 - 10.7)	(7.11 - 18)
DDD	0.51	0.11	0.33	0.4
	(0.1 - 0.73)	(0.09 - 0.29)	(0.22 - 0.64)	(0.22 - 0.62)
DDT	1.83	0.3	0.46	0.38
	(1.37 - 2.44)	(0.12 - 0.87)	(0.27 - 0.68)	(0.26 - 0.54)
TBB PCB	79.24	61.74	38.05	53.36
	(69.42 - 101.94)	(51.21 - 83.63)	(34.58 - 45.91)	(35.94 - 92.84)
TBB DTT	17.22	12.6	9.36	12.75
	(13.79 - 19.8)	(10.13 - 15.48)	(7.6 - 11.99)	(7.61 - 19.45)
TBB	96.58	74.74	47.59	66.31
	(84.17 - 121.74)	(62.1 - 97.66)	(43.24 - 57.12)	(46.13 - 113.27)

Table 2. Candidate Linear Mixed Effects Models of total PCB concentration as a function of fixed factors and random covariates. Model selection was done using Akaike Weights (AICw). Loc=Location, Y = Year, MPPM= Maternal postpartum mass, TBF = Total body fat

A	Response Variable	Fixed	Random	logLik	AIC	dAIC	AICw
Mod 1	Total Conc	Loc,Y,MPPM,TBF,Loc*Y,MPPM*TBF	ID,Age	1.729716	16.54057	0	0.53
Mod 2	Total Conc	Loc	ID,Age	-4.2049705	18.40994	1.869	0.208
Mod 3	Total Conc	Loc, Y	ID,Age	-3.8208717	19.64174	3.101	0.112

B	Response Variable	Fixed	Random	logLik	AIC	dAIC	AICw
Mod 1	TBB	Loc,Y,Loc*Y	ID,Age	-7.174532	28.34906	0	0.567
Mod 2	TBB	Loc	ID,Age	-10.074229	30.14846	1.799	0.23
Mod 3	TBB	Loc, Y	ID,Age	-9.200589	30.40118	2.052	0.203

MULTIPLE COMPARISON TESTS

The multiple comparison analysis in concentrations for specific congeners showed that IOM 2005 had significantly higher concentrations of the highly chlorinated congeners: 138, 153, 180, 183, 187, 194, 195, 206, 209 and the pesticide DDT with its metabolite DDE than NR 2005 (Fig. 1a and Table 1a). These congeners were significantly different in both raw and adjusted p values. In contrast, there were no significant differences between colonies for any OC in 2006 with neither raw nor adjusted p values (Fig. 1b). Annual differences in OC concentrations within colonies were not the same at IOM and NR. At IOM, the most dramatic annual difference was the significantly lower concentrations of DDT in 2006 (Fig. 2a). In contrast, annual differences at NR rely mostly on the congeners 149, 156, 195 and 209, all of which were significantly higher in 2006 (Fig. 2b). In terms of TBB, significant colony differences were observed for 10 to 11 (raw and adjusted p value respectively) PCBs in 2005 (Fig. 3a) and for 0 to 3 (raw and adjusted p values) congeners in 2006 (Fig. 3b). The differences in TBBs between NR and IOM in 2006 are mainly due to higher quantities of PCB congeners 101 and 118, and DDD at NR. (Fig. 3b, Table 1B). The TBB of PCBs that varied between years within a colony were different for IOM and NR. In IOM, DDT, DDD and PCB-153 were significantly lower in 2006, while in NR only PCB –149, -156 and -206 had significantly higher levels in 2006 (Fig. 4a and 4b).

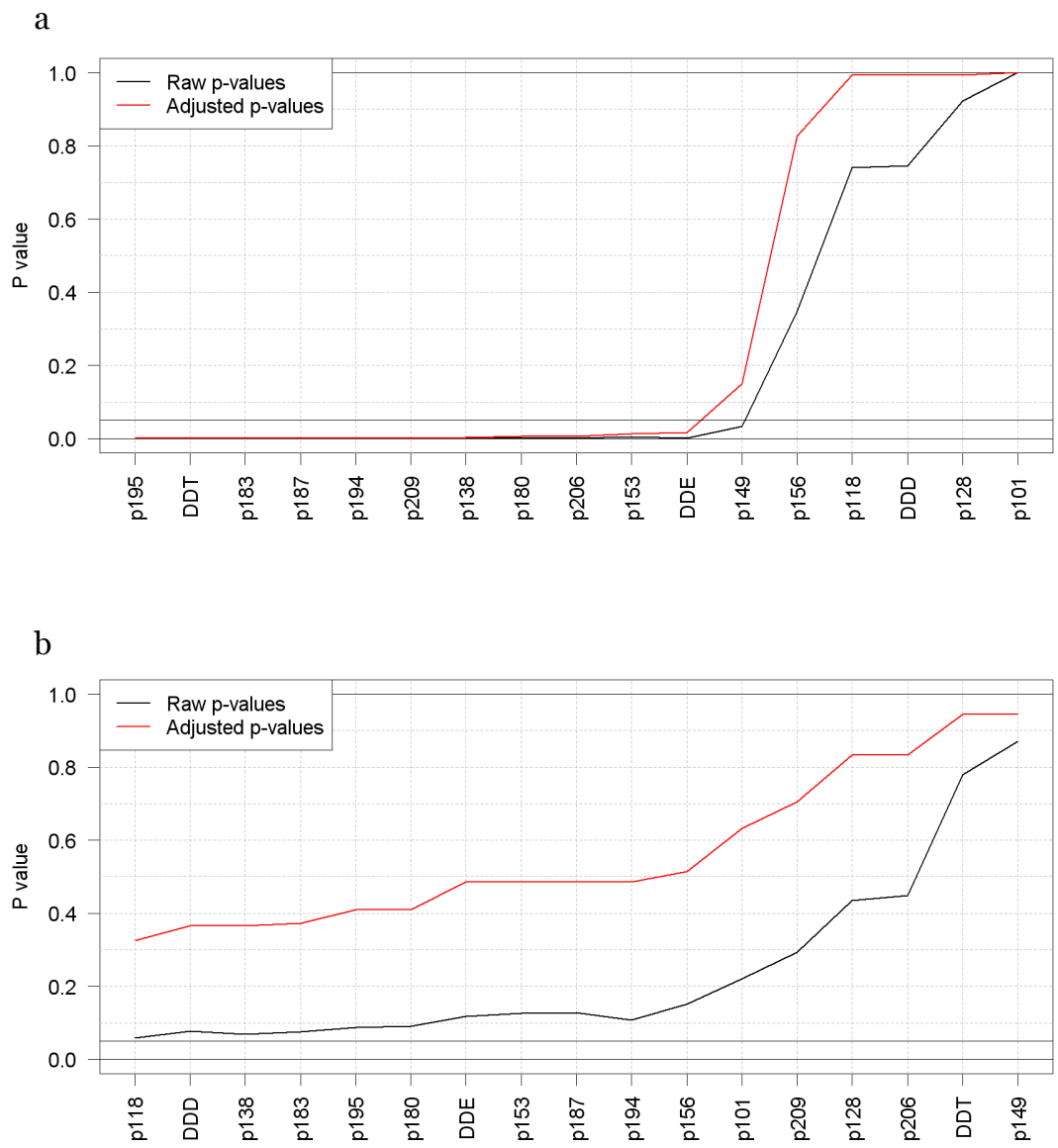

Figure 1. Raw and adjusted p values obtained by multiple comparisons tests comparing OC concentrations between NR and IOM at early lactation in a) 2005 and b) 2006. Multiple tests were based on the Wilcoxon rank sum statistic. PCBs are arranged in increasing order from left to right according to their adjusted p-values.

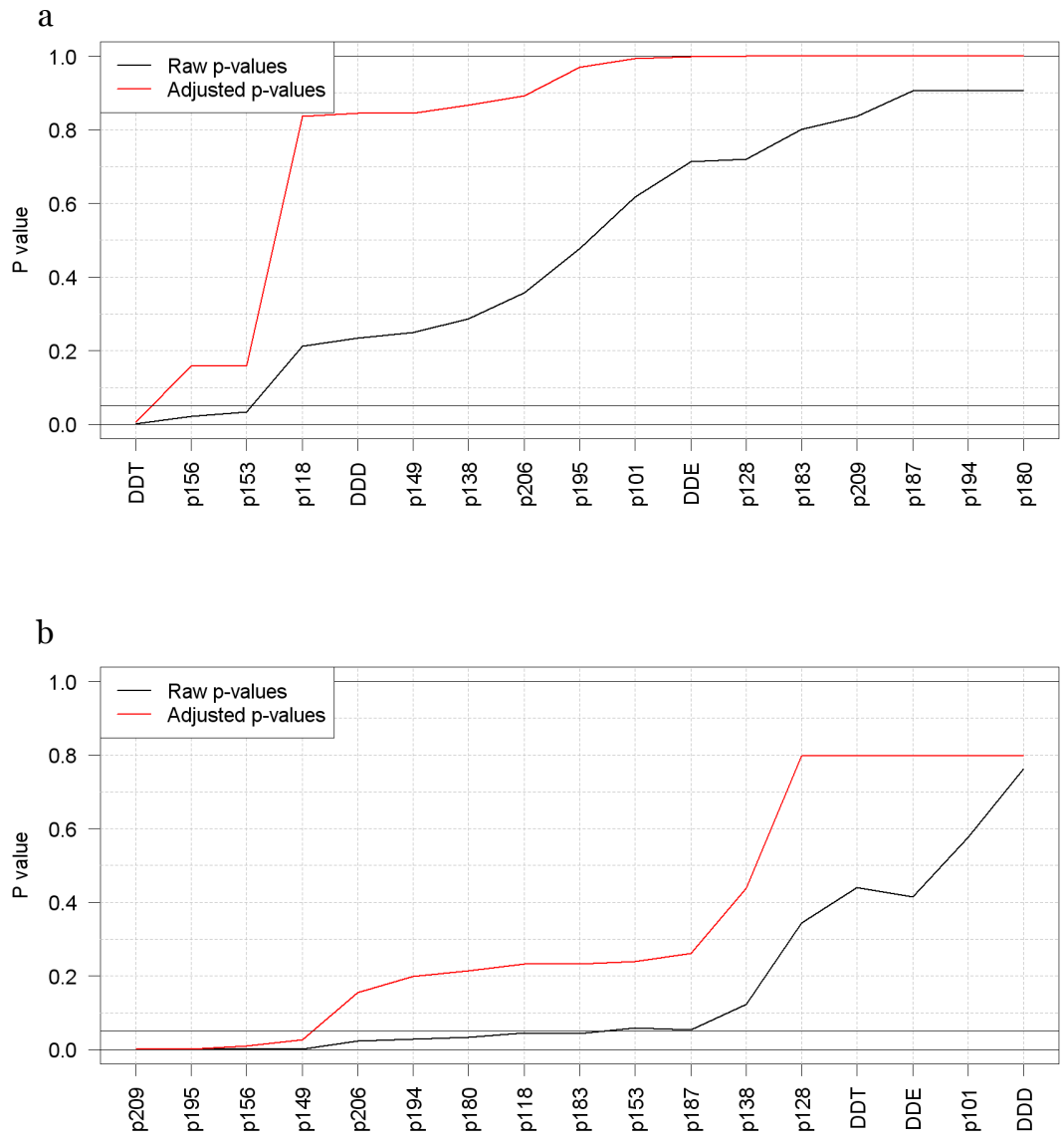

Figure 2. Raw and adjusted p values obtained by multiple comparisons tests comparing OC concentrations between 2005 and 2006 at early lactation at a) Isle of May and b) North Rona. Multiple tests were based on the Wilcoxon rank sum statistic. PCBs are arranged in increasing order from left to right according to their adjusted p-values.

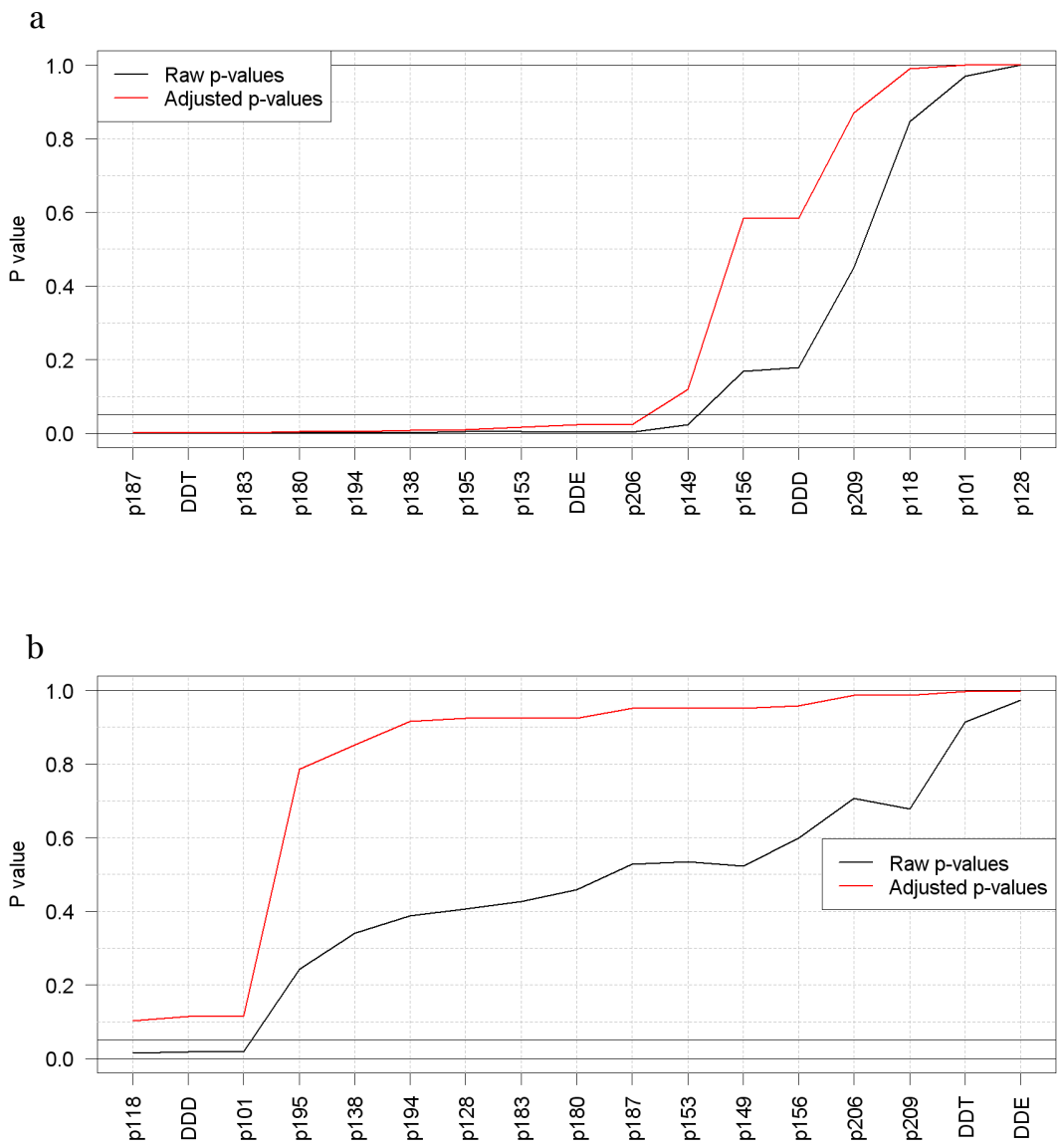

Figure 3. Raw and adjusted p values obtained by multiple comparisons tests comparing OC total body burdens between Isle of May and North Rona at early lactation in a) 2005 and b) 2006. Multiple tests were based on the Wilcoxon rank sum statistic. PCBs are arranged in increasing order from left to right according to their adjusted p-values.

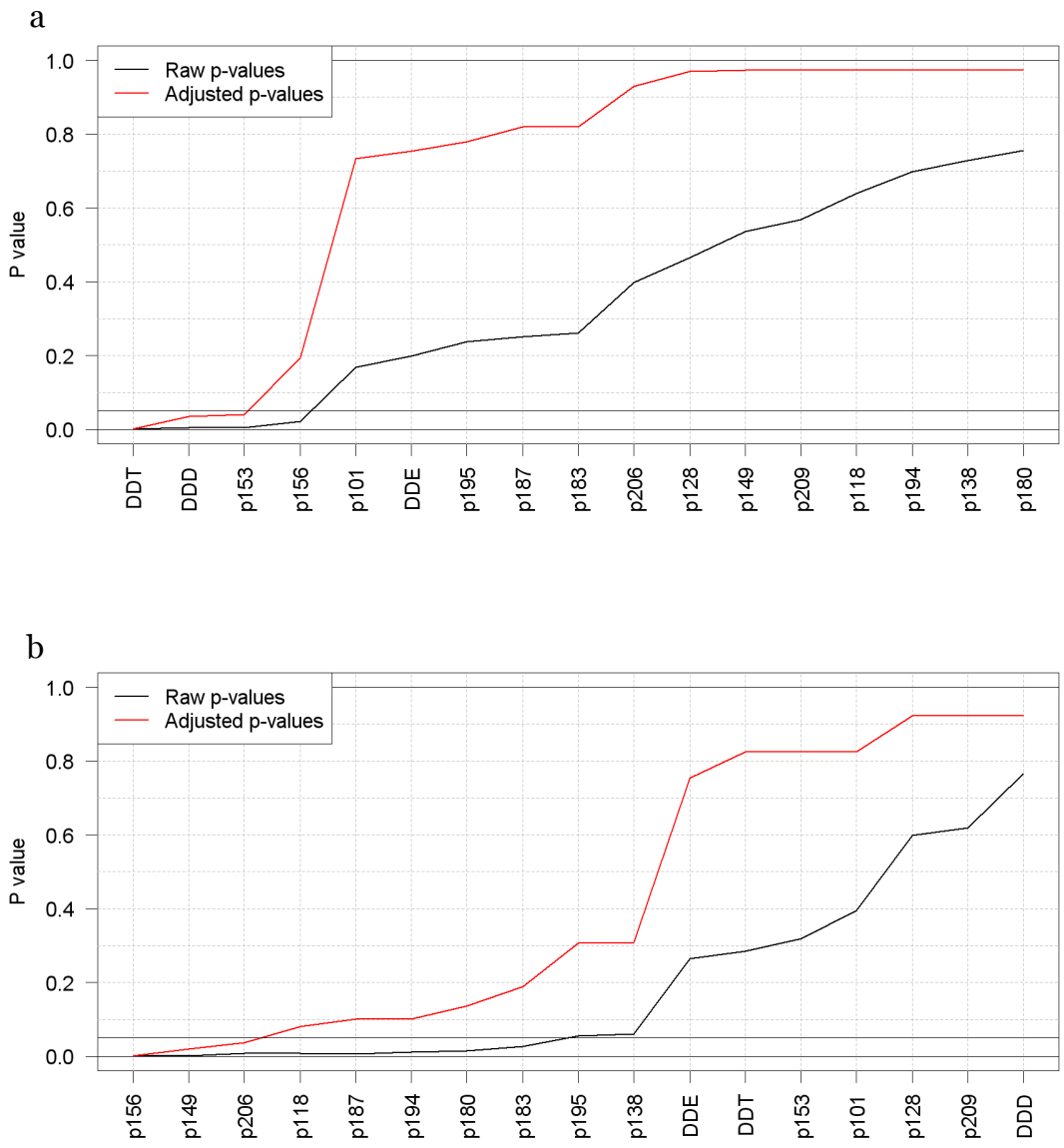

Figure 4. Raw and adjusted p values obtained by multiple comparisons tests comparing OC total body burdens between 2005 and 2006 at early lactation at a) North Rona and b) Isle of May. Multiple tests were based on the Wilcoxon rank sum statistic. PCBs are arranged in increasing order from left to right according to their adjusted p-values.

RANDOM FOREST ANALYSES

Multivariate analyses with Random Forest was performed on congener concentrations and congener body burdens separately. The two colonies and years were relatively well discriminated by both measures (Table 3 a-b).

Table 3. Confusion matrix from Random Forest classification analysis of organochlorine A) congener concentrations and B) congener body burdens. Correct group belongings are given by rows and predicted group belongings are given by columns.

A		Isle of May		North Rona		
		2005	2006	2005	2006	Error
Isle of May	2005	9	1			0.10
	2006	1	6		4	0.45
North Rona	2005			9	1	0.10
	2006	1	4	3	4	0.66

B		Isle of May		North Rona		
		2005	2006	2005	2006	Error
Isle of May	2005	9	1			0.10
	2006	2	7		3	0.42
North Rona	2005			9	1	0.10
	2006	1	1	4	6	0.50

The analysis with the total concentrations showed that during 2005 none of the 20 samples were misclassified between colonies. The only misclassifications in 2005 occurred between years within the same colony. However, in 2006 colonies were poorly distinguished. Misclassifications of 2006 samples within the same colony were higher among NR samples.

Results from the random forest analysis on total amounts displayed very similar patterns as those based on relative amounts (Table 3b). Again, misclassifications between colonies only occurred among samples from 2006.

Misclassifications between years were more common overall (12/44 = 27.3%), and were more common among NR (5 of 22) compared to IOM samples (3 of 22).

DISCUSSION

The present study describes the PCB and DDT concentrations and TBBs in adult female grey seals from the Isle of May, and in addition provides a comparison with females from North Rona, which has been sampled for first time. In general, our result suggests that grey seals breeding along the North Sea coastline have significantly higher contaminant loads compared to those in the northeast Atlantic.

When assessing pollutant levels and/or addressing geographical and temporal variation in OC contamination it is important to ensure that the individuals sampled are comparable, for instance by selecting animals from the same sex, at the same reproductive stage and if possible of similar age. In this study, we analyze samples from females within the first few days of giving birth. The age was known for all females allowing us to control for any systematic age dependent variation by including this as a random effect in the mixed models. The overall variability explained by age in the mixed models has also been documented in other studies (Addison & Brodie, 1977, Bernt et al. 1999). Lactation functions is an important way of eliminating PCB burdens. It has been noticed that concentrations of PCBs in sexually mature females decrease with age. However, it has also been shown that females reach a certain level of PCB concentration that is eliminated via milk in each successful reproductive period but is balanced back during the feeding period (Addison & Brodie, 1977). Indeed, in this study the females captured in consecutive years did not show any decrease or increase from one year to another in their TBB, probably due to the reason explained above. We neither observed a specific trend between age and TBB. This needs to be interpreted carefully due to the small sample size.

While selection of animal based on these criteria (same sex, similar lactation stages and known age) is often straightforward, other individual

characteristics are more difficult to control for. For instance, variations in nutritional status between individuals will always be present and can have important effects on pollution levels in samples (Polischuk et al. 2002). For example, we found that heavier females at early lactation had significantly higher concentrations of PCBs. However, it is more important to include measurements of body condition (i.e relative lipid content) in order to assess the proper levels of pollution. This allows estimating the importance of variations in body composition on the degree of contamination in individual animals. When comparing populations it is important to not only obtain information on the OC concentration in the lipid tissues but also on TBB, especially if we are interested in estimating the total exposure to pollution. For instance, samples from two seals with similar TBBs will have very different concentrations in blubber if their fat content is different. The toxic effects of OCs are largely a function of concentration and the type of congener present in the tissues, both for a female and her suckling pup. Conclusions made in the pollution in the environment, the degree of exposure and uptake by seals based on OC concentrations in their blubber can be misleading (Kleivane et al. 2000, Polischuk et al. 2002, Kleivane et al. 2004, Hall et al. 2008). For instance, IOM animals had significantly higher OC concentrations but they were as well "smaller" animals. Thus, it could have been wrongly assumed that the high concentrations of PCB were mainly due to a lower amount of fat. Thus, the estimation of the TBB could prove that IOM animals are truly more polluted than NR animals in both relative and absolute terms, rather than the higher concentration simply being a consequence of a smaller lipid volume. However, it is important to take into account that some of the variation observed between animals in their TBB is influenced by the error estimates in first place in the total body fat. Thus, further analyses of the data will need to take this variation into consideration.

ENVIRONMENTAL, ECOLOGICAL AND PHYSIOLOGICAL PROCESSES AND OC LEVELS

Variations in contamination levels in top predators will be an accumulated result of several processes acting across a range of scales in space and time. These processes can be physical/environmental, ecological and physiological. I will discuss each of these in turn.

Influences of the physical environment

The high degree of pollution in IOM animals in contrast to those of NR can be an effect of the differences in habitat use. Satellite tracking over many years has shown that seals from the IOM almost exclusively exploit feeding grounds within the North Sea, and while feeding trips to the east coast of Orkney and Shetland are also relatively common, they very seldom migrate further to the west into Atlantic waters (McConnell, pers.comm). In contrast, tracks from NR animals show that post moult-feeding grounds utilized are generally confined to waters off North-western Scotland (Pomeroy, pers. comm). The North Sea is a shallow sea with relatively poor water circulation and exchange with surrounding oceans. The main influx occurs from the Atlantic water along the western slope of the Norwegian Trench with only a small part flowing down south along the coast of Scotland (OSPAR Commission 2000). The North Sea is exposed to direct influx of waste from the many surrounding industrialised countries. The low degree of water turnover will directly affect the retention of the pollutants in the system and may also influence the pollutants' distribution. The current patterns and their magnitude in the North Sea can experience substantial variations between seasons and years (OSPAR Commission 2000), which may also have a direct effect on pollutant distribution. In contrast, waters to the northwest of Scotland are further away from most of the large pollution sources, and are also more exposed to the strong currents associated with the North Atlantic Drift (OSPAR Commission 2000). Although it is considered that atmospheric movements are the main

transport for many OCs on a global scale, the water-associated transport may play a more important role in regional seas (Boon et al. 2002).

Physical environmental variations between areas will influence the pollution of the areas. Consequently, the degree of contamination found in organisms' tissues would ultimately reflect the levels in the waters they inhabit. Geographical variations in OC levels between populations of ringed seals (Muir et al. 2000), harbour seals, polar bears (Muir et al. 2000, Dietz et al. 2004b), killer and minke whales (Hobbs et al. 2003), have been shown to be consistent with pollutant levels in the environment, and decreasing OC levels in some populations are also consistent with decreases in their environment.

Ecosystem and food-web structure

Organochlorines are present in all the organisms along the food web. The variation between species, between populations and between individuals can be explained by the diet (Skaare 1996). Therefore, variations in OC burdens and also the OC profiles between NR and IOM could be a reflection of the different feeding regimes. Dietary studies based on blubber fatty acids from both colonies (Walton et al 2000, Chapter 2) and analyses of scats from several colonies around the Scottish coastline (Hammond & Harris 2006, Hammond & Grellier 2006), have described major dietary differences between the colonies off the northwest coast and those along the east coast, potentially reflecting large differences in the food webs in the Northeast Atlantic and the North Sea (see discussion in Chapter 2). While the differences in blubber fatty acid composition between colonies persisted over several years of study (Walton et al. 2000), Chapter 2), they also indicated that temporal changes had occurred within each of the two colonies (Chapter 2).

The variations in PCB levels and patterns could therefore be the result of regional prey differences (Lie et al. 2003) and the trophic level of the prey (Muir et al. 2000). The OC profiles of the prey will be influenced by the underlying structure of the food web and the different pathways by which OCs can move up the trophic levels (Hobbs et al. 2002, Lie et al. 2003) affecting the levels in prey species of grey seals. Species along different pathways may have different

capacities to metabolize specific congeners. The cytochrome P450 enzymes are responsible for PCB metabolism (Timbrell 1991). The metabolism of different PCBs is dependent on their structure. The presence or absence of vicinal hydrogen's in meta-para and/or ortho-meta positions as well as the number of ortho chlorines will determine if a molecule can be metabolized by P450 isozymes (Bruhn et al. 1995). The degree of metabolisation appears to be a function of the PCB concentrations. The high concentration of PCBs will induce the P-450 enzymes and will likely leave the congeners with higher chlorination content (Wolkers et al. 1998, Debier 2001). IOM females had higher concentrations and total amounts of the highly chlorinated PCBs. Thus, is possible that the variation in the PCB profiles that we observed between IOM and NR might be to some extent by the higher induction of the P-450 enzyme by IOM females. The selective metabolism and accumulation will lead to an increase in pollutant concentrations and a higher proportion of more persistent contaminants in top predators than animals in the lower trophic levels such as fish (Wolkers et al. 2006).

The increase in the trophic level and the compositional pattern of accumulated OCs differs between species in the food chain (Lie et al. 2003). These variations in type of prey consumption and metabolism capacity will gradually modify the original OC profile observed at the source, and this can ultimately give rise to different OC profiles in top predators within the same food-web that acquire their OC burdens via different food-web pathways as we have observed in both colonies.

Individual and physiological factors

Differences in OC burdens and profiles of seals from IOM and NR may in part also be caused by differences in life history. Events throughout an animal's life can influence the accumulation of contamination that we observe. For instance, the amounts and patterns of OCs received as pups, amounts accumulated prior to the first reproductive event, the number of subsequent breeding events, the success in feeding events, prey choice and how much of their TBB they transfer to their offspring in relation to how much fat they transfer during the lactation period will

ultimately affect their amount and pattern of OCs. Although these aspects are more related to individuals life history, if the members of a population share certain characteristics, these individual factors will lead to population difference. For example, individuals from a population can be more vulnerable to reproduction disorders if they are exposed to high levels of OCs therefore accumulating more pollutants. The age differences between females can also be seen as an explanation to the observed variation. Indeed a high percent of the variation observed on the total concentrations and TBB was due to the age of the females although it was not found a clear trend between the age of the animals and a decrease in TBB. Lactation and gestation is one of the major ways of elimination of PCBs. A decrease in the concentrations of PCBs with age has been seen in mature females; however, it seems that females can reach a steady state in their tissues: they eliminate via milk and accumulate again by food (Addison & Brodie 1977). In this study although the age explained a good percent of the variation as the mixed models showed, we did not find a clear correlation between the age of the females and the total concentration. This could probably be due to the small sample size. However, the factors already described previously such as the foraging sites, the reproductive history or the temporal trends in contamination in the environment may play a more important role in the observed variations since the age difference between colonies was not significantly different.

TEMPORAL TRENDS

Temporal changes are impossible to assess with only two years of study. Although we have shown a slight decrease in total OC concentration and TBB at the IOM and a comparatively large increase at NR from 2005 to 2006, these results should be interpreted with caution. The major difference between the years at IOM was in the most abundant and persistent PCB 153 (decreasing by almost 50%) and DDT (almost a 6-fold decrease). Interestingly, the increases at NR were mainly observed for PCBs such as 149 and 156. These increases may indicate that NR seals were either feeding lower on the food web in 2006 or that the trophic

pathways from phytoplankton to seals were longer and/or more complex in 2005. There is evidence that grey seals feed in two well-distinguished regions. Thus, the variation in the PCB profile between colonies can be explained by difference in the foraging site and the temporal trends in the contamination of the marine ecosystem and the prey availability. It could also be explained if a higher amount of PCBs was eliminated via the milk than the amount ingested during the next feeding season. It is also important to mention, that the differences between years for example in IOM, especially the decrease in the recalcitrant PCB – 153, can be a function of the high degree of induction of P-450 triggered by the high concentrations in IOM females. The more polluted the organism the higher the degree of induction and metabolisation of PCBs.

The few females sampled in consecutive years in both islands did not show a significant decrease in their PCB burdens as it has been suggested in the literature for sexually mature females (Aguilar & Borrell 1994, Muir et al 1995). The lack of changes from one year to another in the same female can be a consequence of the small sample size as previously stated.

COMPARISONS WITH PUBLISHED DATA

Comparisons of results from this study with studies of grey seals from other regions such as the Baltic and Canada would be valuable, but they are problematic because of differences in sampling methods and analyses, and also because many of these studies were done on juvenile and adult males or on pups. Furthermore, results from these studies are published as concentrations, and the body composition was usually not known. The stage in the annual cycle also varied between studies. While there are several studies from earlier years of harbour seal colonies in similar regions, intra-specific comparisons are problematic because of likely differences in feeding habits and capacity to metabolize different OC compounds.

Data on OC concentrations and/or TBB of other grey seal colonies around the UK during the same period were not available. To observe temporal trend in pollution levels in the same region within the same species was as well very

difficult. There were few recent studies in grey seals from the same colony (IOM), however none of them reported the levels of pollutants found in female grey seal blubber at early lactation. Due to a variety of reasons, direct comparison between other studies and our results was problematic. For instance, while a recent study by Pomeroy et al (1996) described OC concentrations in seal from the IOM, their results were focused on the PCB transfer from mother to pups and therefore blubber samples from adult females were not described. Kalantzi et al. (2005) described OC levels at IOM colony but this was performed on 0 - 1-year-old grey seals, therefore the comparison with our study was impossible. Another study described OC levels in dead grey seals in 1988 from the Wash and Farne Islands (Green et al 1989). However, samples from dead seals may not be representative of the entire population. The samples that are probably most relevant for comparison come from a study by Debier et al (2003) that described blubber OC concentrations of females from 1998-2000. However, their samples were divided into inner and outer layers and values from the middle layer were not presented. Since we were interested in the total concentration or TBB our study is not comparable to values reported by (Debier et al. 2003).

However, the levels from our study were lower than those reported by Debier et al. (2003) in the outer blubber (the most contaminated) from IOM seals in 2000. While the mean concentration at early lactation was in average 3160 ng/g of lipid in the year 2000, females from our study had in average 1300 ng/g of lipid (range: 1155-1608) in 2005 and range (965-1612 ng / g of lipid)in 2006. North Rona animals had even lower concentrations in both years than those from the east coast of Scotland. Grey seals from England have been reported to range between 5.7-33 μg/g wet weight (Law et al.1989). Our results are as well lower than those found in Baikal seals that reported the equivalent to 11000 ng/g of lipid (Nakata et al. 1995) or in grey seals from the Gulf of St Lawrence with 8500 ng/g of lipid. The blubber concentrations that we report in IOM were in similar levels to those reported in the ringed seals by Severinsen et al (2000) that ranged between (1400-1600 ng/g of lipid) and those in lactating harp seals in Greenland (980 ng/g of lipid) (Espeland et al. 1997). Our results are ten times lower than those reported from grey seals from Sable Island, Canada from 1976 and 1987. Overall, it seems that grey seals from IOM and NR have lower levels than the other regions

of the UK and in comparison to those from Canada. In general, the concentrations reported in lactating grey seals from NR and IOM are below the levels at which severe health and reproductive problems have been observed (Baker 1989, Jepson et al. 1999). As mentioned previously the comparison of PCB concentration with the literature needs to be carefully interpreted due to differences in sex, age, in blubber analyses, body condition among other factors.

This study examined the spatial variation in two UK colonies, and for first time describes the PCB and DDT concentrations and total amounts in NR colony. We have shown that blubber OC concentration profiles can be used to distinguish between grey seal colonies. We have described not only the concentrations of pollutants but have also estimated the TBB in order to assess and monitor changes in the absolute contaminant load resulting from feeding in regions with different degrees of pollution levels. It is important that we keep monitoring these temporal and geographical trends in the levels of persistent organic pollutants. There was a consistent decreasing trend from the 1970s to the 1990s, especially in highly polluted areas such as the North Sea and Baltic Sea (Nyman et al. 2002). In other and less polluted regions such as the Arctic levels initially decreased but appear to have been stable since the 80's to the 90's (Muir et al. 2000). At the moment the concentrations reported for lactating grey seals from the IOM and NR seem to be lower and seem to be declining in comparison to previous studies of similar regions. Although, while PCBs are no longer in industrial use, levels are unlikely to decline more in the near future (Aguilar et al. 2002), and levels in the marine environment are still a threat to both marine organisms and ultimately humans. Comprehensive databases containing OC measurements from various marine organisms across a range of geographical areas over longer periods are needed. Long-term series assessments are important in order to monitor the changes of pollutants exposure in marine mammals from different geographical regions. The assessment of the levels of OCs in the environment and the geographical variation of the pollutants are important factors in the management and conservation of marine mammal populations. Long-term monitoring within the UK of populations of top predators such as seals should be a priority in order for a better assessment of the long-term consequences of human impacts on marine ecosystems. I hope

this study gives the basis for future comparisons when assessing pollutant levels in grey seals.

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6 Changes in PCB levels in blubber, serum and milk over the lactation period of grey seals

ABSTRACT

During the lactation fast grey seal females mobilize a large amount of lipids from the blubber and with them they release PCBs, accumulated during the animal's life history, to the circulatory system. From there they are absorbed and transfered to other body tissues. The understanding of PCB mobilisation from blubber to blood and transfer to milk is limited. The present study investigates PCBs in blubber, serum and milk in lactating grey seal females from North Rona (NR) and Isle of May (IOM). Ten mother – pups pairs were studied in each colony in 2005 and eleven in 2006. Females and their pups were captured up to 3 times during lactation providing samples representing early, mid and late lactation. Total PCB concentrations were found to be higher in blubber, followed by milk and serum. The total concentration in blubber increased at the end of lactation as a result of lipid loss. Serum concentrations stayed constant during the first part of lactation and increased at late lactation. This was also observed in milk PCB concentrations. These increases in milk were probably a result of the increase observed in serum which are at the same time a reflection of the increase in blubber. This suggests that there is a higher release of PCBs from blubber during the second half of lactation. Total concentrations of PCB in blubber and milk were found to be higher in IOM than in NR animals, while PCBs concentrations in serum were similar between colonies. The relative concentrations of individual congeners to PCB-153 showed that blubber contained higher proportions of the highly chlorinated PCBs relative to other tissues and there were no clear changes across lactation. In serum and milk the relative proportions of highly chlorinated PCB increased throughout lactation while the less chlorinated PCBs appeared to stay constant. Milk had relatively higher proportions of the lower chlorinated PCBs. The highly chlorinated PCBs were found in lower concentration in the milk compared to the less chlorinated compounds suggesting a selective release from blubber to blood and a selective transfer of PCBs to the milk. This selective transfer is possibly a consequence of the differential solubility of the compounds and the lipid dynamics during lactation. The changes in the PCB profiles in the three body compartments was

very similar between colonies however IOM seals always presented higher total concentrations of PCBs in any of the body compartments.

INTRODUCTION

Persistent organic pollutants such as PCBs and DDTs have been released into the environment since early in the last century. Long-range transport via air and ocean currents has spread these compounds from industrialized areas into less developed regions across the globe (Pacyna & Oehme 1988, Oehme 1991a, Oehme 1991b). These compounds can therefore be found in high concentrations even in remote areas such as the Arctic (Muir et al. 1988, Oehme 1991a, Norstrom & Muir 1994). Organochlorine (OC) pollutants are highly lipophilic and extremely resistant to biodegradation. These characteristics make them highly persistent in ecosystems, and the high affinity to lipid causes them to concentrate in lipid rich tissues of organisms and to bio-accumulate throughout the food web. Marine mammals are top predators, with high amounts of lipid tissues and have a long life span, thus they accumulate high levels of persistent OCs throughout their lives (Muir et al. 1988, Mossner & Ballschmiter 1997).

Total levels of OCs and relative concentrations of the various congeners can vary between populations and individuals (Mossner& Ballschmiter 1997, Kleivane et al. 2000), Chapter 5). Such variations can be a result of a combination of factors, such as differences in the OC concentrations found in the regions they inhabit, differences in the OC loads of the prey and/or species-specific differences in their metabolism and excretion (Tanabe et al. 1984, Tanabe 1988, Norstrom& Muir 1994, Mossner& Ballschmiter 1997). Within the same species OC concentrations can also vary depending on sex, age, reproductive and health state (Kleivane et al. 1995, Wolkers et al. 1998, Kleivane et al. 2000). Exposure to OCs has been associated with many harmful effects in marine mammals, for instance in the neurological, reproductive, endocrine and immune systems (Safe 1984, Ross et al. 1996, Tanabe 2002, Hammond et al. 2005), and they can also seriously affect skeletal tissues (Bergman et al. 1992, Olsson et al. 1994, Lind et al. 2003). Organochlorine compounds have also been implicated in the seal PDV epizootic events that occurred during the end of the 1980's (Hall et al. 1992).

The storage of large amounts of fat during the feeding period is crucial for the grey seal female's reproductive success. They rely completely on their reserves during the land-based lactation fast in order to produce milk and to cover their own metabolic demands. Due to the high amount of lipids deposited in the blubber and their position in their food web, many marine mammals accumulate a large quantity of pollutants, which will be stored in the blubber. Marine mammals can store up to 98% of their body OC burden in the blubber (Wolkers et al. 2002). When fatty acids are mobilized during lactation a large amount of OCs will be released into the circulatory system and thus other body organs such as the liver may experience increased exposure to their toxic effects (Polischuk et al. 2002, Lydersen et al. 2002). In the case of reproductively active females, a large proportion of the OCs received from their prey and stored in their blubber will end up in the milk during lactation and be passed to offspring (Addison & Brodie 1977, Addison & Brodie 1987, Polischuk et al. 1995, Ramos et al. 1997). Grey seal mothers can transfer up to 30% of their PCB burden to their pups via milk during each reproductive event (Pomeroy et al. 1996). Although some placental transfer of OCs does occur during gestation, the amount transferred via milk is far greater (Addison & Brodie 1987, You et al. 1999). Thus, while lactation allows females to offload some of the accumulated pollutants from their body, pups receive high exposures in a critical developmental period during which even low doses can have serious negative effects on the endocrine, nervous and immune system (Colborn & Smolen 1996, Polischuk et al. 2002, Hammond et al. 2005, Lamb et al. 2006).

The relative amounts of different congeners can vary from tissue to tissue as a result of congener-specific physicochemical properties and as a result of the tissue perfusion and lipid content. Lower chlorinated PCBs appear to be released more readily during fat mobilization in comparison to higher chlorinated PCBs. This has been observed in California sea lions (Hall et al. 2008), lactating grey seals (Sørmo et al. 2003, Debier et al. 2003b) and in northern elephant seal weaned pups (Debier et al. 2006). Debier et al (2003b) found that PCBs were retained in the blubber approximately during the first half of lactation (8 to 10 days), after which large amounts were released to the blood, also showing up in the milk. The transfer of pollutants to the milk has been shown to be selective,

with some form of barrier against the higher chlorinated congeners either in the transfer from blubber to blood or from blood to the mammary gland (Addison& Brodie 1987, Pomeroy et al. 1996, Espeland et al. 1997, Sørmo et al. 2003, Debier et al. 2003a, Debier et al. 2003b, Wolkers et al. 2004). It is possible that the differences in congener composition observed between tissues are related to the selective mobilization of fatty acids from the blubber.

In this study, we describe the variations in total amounts and relative concentrations of different OC congeners in lactating female grey seals from two UK breeding colonies. Specifically, we examined the variations in various body compartments, blubber, blood serum and milk to determine the degree to which the release and transfer of these compounds depend on their physicochemical properties. By comparing these patterns between two colonies characterised by different overall OC levels and, which differ in terms of their physiological states, we can determine to what extent variations in the transfer of PCBs are influenced by the physicochemical characteristics of the compounds and the total amount of PCBs that females start with. Although, IOM females have a higher concentration of contaminants than NR animals, it is expected that both colonies will follow the same changes in their PCB profile through lactation. However, the amounts transfer from blubber to the other tissues would be expected to be greater in IOM females, transferring a higher amount of PCBs to their offspring. Few longitudinal studies describe the dynamics of transfer of PCBs between compartments, and very few have addressed the links to nutritional status and possible selective mechanisms.

METHODS

SAMPLE COLLECTION

The study was carried out on two UK grey seal breeding colonies: NR and IOM during two consecutive years. Ten mother-pup pairs were studied in each colony in 2005, and eleven in 2006. Females and their pups were captured up to 3 times during lactation providing samples representing early, mid and late lactation. Daily observations and recordings of new births and pups observed alone determined the birth and weaning days. Blubber was obtained only at early and late lactation while blood serum and milk samples were collected at each capture. The procedure for collection of blubber, serum and milk samples is described in chapter 5, 3 and 4 respectively. All animals were handled under UK Home Office licence.

CHEMICAL ANALYSES

Blubber sample preparation

PCB analysis was carried out as previously described in chapter 5. Briefly, blubber samples were thawed at room temperature and transferred to a test tube. The fat was extracted by putting the sample in a microwave oven for 1 minute at 650 W.

Serum samples preparation

Serum was de-proteinised by adding 100 µl of triethylamine and 2.5-10 ml of formic acid depending on the volume of sample. The mixture was stabilised for 30 minutes in an ultrasound bath (Julabo USR 05, Seelbach, Germany). PCBs were extracted by solid phase extraction using a C18 micro column (Baker, Deventer, Holland). The column was conditioned with 10 ml of methanol followed by 10 ml of distilled water using a Supelco elution (Visiprep DL,

Bellefonte, PA) and a vacuum pump (ABM, Werheim, Germany). After adding the de-proteinised sample to the column and allowing it to pass through, the column was rinsed with 3 ml of distilled water and dried for 20 minutes using the vacuum pump. The PCBs in the column were then eluted with 5 ml of Hexane (Debier, 2001).

Milk samples preparation

The frozen milk samples were thawed at room temperature and homogenized with an Ultra-Turax (Ika-Werk 18/10 Janke & Kunfel, Staufen, Germany). Two-gram aliquots of the thawed milk samples were lyophilised for 20 hrs and dry matter was determined gravimetically. Milk lipid was extracted using an accelerated solvent extractor. Approximately, 0.600 g of lyophilised milk was weighed and 0.5 g of anhydrous sodium sulphate was added along with a surrogate of PCB 143. The lipid extraction was performed 3 times with a mixture of hexane, dichloromethane and methanol at 80^{O}C under a pressure of 1500 psi. The extracted fat was collected in a pre-weight vial and was evaporated under nitrogen flow (Turbovap LV Zymark, Hopkinton, MA) at 50 ^{O}C. The fat content was determined gravimetically. Finally, the lipids were dissolved in 3 ml of hexane and collected into a test tube and vortexed for 1 minute.

Clean up procedure of the preparations

The clean-up procedure of the blubber, serum and milk samples was performed as described in Chapter 5, but will be described briefly, all the samples were purified by acid and Florisil clean ups. A mixture of sulphuric acid (3 ml, 95%) and hexane (3 ml) were added to the extracted lipid samples and then vortexed for 2 min and centrifuged at 1810 g at 25 ^{O} C. The organic phase was transferred and the acidic phase was vortexed and centrifuged one more time as previously described. The new organic phase was reduced to approximately 1 ml under nitrogen. The next clean up was performed with Florisil solid phase cartridges. Cartridges were conditioned by adding 5 ml of

acetone, 5 ml of acetone-hexane (50:50 vol) and 12 ml of hexane. Once the cartridges were ready the samples were added. Tubes containing the samples were rinsed with 3 ml of hexane and then added to the cartridge. Finally, 3 ml of hexane were added directly to the column. The sample in the test tube was then completely evaporated under nitrogen.

Analyses

The final extracts were analysed by gas chromatography using a Thermo Quest Trace 2000 gas chromatograph equipped with a 63 Ni ECD detector (Thermo Quest, Trace 2000, Milan, Italy) and automatic injector. One to five µl of the purified extracts were injected. The PCB congeners were separated on a 30 m x 0.25 mm (0.25 µm film) DB-XLB capillary column (J&W Scientific, USA). The temperature program was followed as described in Chapter 5.

Blanks and Quality control

Blanks and quality controls were run for all batches of samples analysed to check that no contamination occurred during the clean-up procedure. In the case of milk, blanks were also used to check for contamination during the lyophilisation and extraction procedure. Bovine milk cream and serum with a known concentration of PCBs were used as quality controls for milk and serum samples respectively. The PCB recovery rate was calculated based on the concentration of the surrogate standard PCB-112.

STATISTICAL ANALYSES

Summary tables of concentrations within groups were created based on geometric means and their bootstrapped standard errors. PCB concentrations in organisms are generally characterised by having log-normal distributions, and the geometric mean provides a better estimate of the central tendency for such

data than the arithmetic mean. For the same reason, all statistical analyses were run on log-transformed data.

The first set of analyses focused on the total body burdens (TBB) of PCBs in blubber. We estimated TBB by simply multiplying the total blubber PCB concentration with the amount of body lipid, estimated separately using isotopically labelled water (see Chapter 3). These calculations are based on the assumption that PCBs are distributed equally within the body lipids (Debier et al. 2005) supported by observations in northern elephant seal pups where PCBs do not seem to vary between body area (Debier C, unpublished data). Linear mixed effects (LME) models were used to examine to what extent variations in TBB could be explained by population and lactation stage. LME models allow the use of correlated data and unequal variances and are widely used in longitudinal studies where individuals are sampled more than once. In our case, animal ID and age were included as random effects to quantify and control for consistent individual differences, and to control for the tendency of PCBs to accumulate with age. The linear models were carried out in the TBB, but also on groups of congeners according to their degree of chlorination compared to PCB-153. The first groups included tetra and penta PCBs. The second group had hexa-PCBs and third and last included hepta, octa and nona PCBs (Debier 2001).

We also used LMEs to analyse total concentration of PCBs in different body compartments. A series of LME models were used to examine variations in the total PCB concentration in blubber, milk and blood in relation to lactation stage, population, K_{ow} index of the compound, and random effects such as mother ID, age and year of study. Several models were tested for each body compartment and model selection was done using AIC weight (Breiman & Anderson 2003). The response variable was the total concentration of PCBs for blubber, serum and milk separately, since PCB concentrations are not directly comparable between the three compartments (see below). Year was included as a random rather than fixed effect since meaningful annual trends cannot be examined with only two years of data. Including Year as a random effect nevertheless allowed us to assess the variation accounted by this factor, and thereby isolate this variation from the fixed part of the models.

The next set of analyses examined the PCB profile differences between compartments at a given stage of lactation as well as the differences between populations for each compartment at each stage. These analyses were performed on the ratios of each congener to PCB-153, which is normally the most abundant and persistent congener in biological tissues (Debier 2001) and thus is not easily metabolized. Thus it is accepted that changes in relation to PCB 153 can give a good estimation of changes in other PCB congeners. We refer to these ratios as R-153. We performed a serie of multiple comparisons tests between pairs of groups (populations, compartments or lactation stages), using Wilcoxon ranks-test as described in previous chapters. First, within each colony, congener ratios were compared between blubber -serum, serum - milk and blubber - milk of the mothers to examine the degree of specificity in the release and transfer of different congeners. Second, congener ratios at each lactation stage and each compartment were compared between colonies to examine the degree to which the large variations in PCB loads for IOM and NR seals affected the patterns of congener release and transfer.

RESULTS

The major congeners in the three body compartments were PCB- 153, 138, 187, 183, 180 and 101. Together these congeners accounted for more than 80% of the total PCB concentration in blubber and milk, and more than 85% of those in serum. The geometric mean of the total and for each congener concentrations in maternal and pup body compartments are shown in Appendices 9-12.

TOTAL BODY BURDENS

TBB at early and late lactation are shown in Appendix 9, while candidate LME models are presented in Table 1. The top LME model, which was strongly

supported (AICw=0.66) relative to the second model (AICw=0.32), showed that NR had significantly lower levels of TBB ($t_{locationNR}$ = -4.011, p<0.001). In both colonies TBBs were also significantly lower at late lactation (t_{stage} = -3.120, p = 0.003). There was a significant negative interaction between lactation stage and location ($t_{stageLL\ x\ locationNR}$ = -2.128, p=0.042), showing that there was a significant decrease at NR in the TBB of congeners in chlorination group 1. For PCBs allocated in chlorination group 2, amounts were significantly lower at NR overall ($t_{locationNR}$ = -5.352, p < 0.000), and were also significantly lower at late lactation (t_{stage}= -3.168, p = 0.003). The TBBs of congeners in chlorination group 3 were significantly lower at NR than at IOM ($t_{locationNR}$ = -5.878, p < 0.000).

TOTAL PCB CONCENTRATIONS

General patterns

Colony differences for specific congeners were more apparent in 2005, with IOM having higher concentrations than NR at early and late lactation for most congeners. The most notable exceptions were for congeners PCB-101, -118 and –126, for which concentrations were similar between the two colonies. During 2006 there was no clear difference between colonies in the overall trends and in the concentrations of individual pollutants.

Blubber

Total concentration of OCs in blubber increased as lactation progressed (Fig. 1). The patterns for each congener are shown in Appendix 13 A-B. The candidate mixed effects models of total PCB concentrations are shown in Table 2 A. There was an overwhelming support for the top model (AICw=0.937) compared to the second model (AICw=0.063). There was a significant negative relationship between the K_{ow} index and the concentration of congeners (t_{kow} = -2.69, p = 0.007). Overall NR females had significantly lower total concentrations of PCBs than animals from IOM ($t_{location}$ = -2.69, p = 0.011).

Table 1 Top mixed effect models ran for total body burden (TBB). S = Stage, Loc = Location, Y = Year, ID = Animal ID.

	Response	Fixed	Random	logLik	AIC	dAIC	AICw
Mod 1	TBB	S,Loc	Y, ID, Age	-319.878	653.756	0	0.66
Mod 2	TBB	S, Loc, S * Loc	Y, ID, Age	-319.617	655.2335	1.477	0.316
Mod 3	TBB	Loc	Y, ID, Age	-324.281	660.562	6.806	0.022
Mod 4	TBB	S	Y, ID, Age	-326.699	665.398	11.642	0.002

Table 2 Top mixed effect models. A) Blubber, B) Serum and C) Milk concentrations. Loc = Location, K = Kow, S = Stage of lactation, Y = Year, ID = Animal ID.

A

	Response	Fixed	Random	logLik	AIC	dAIC	AICw
Mod 1	[Blubber]	K,S,Loc ,S*Loc	Y, ID, Age	-6271.711	12563.42	0	0.937
Mod 2	[Blubber]	K,S,Loc,K* S * Loc	Y, ID, Age	-6271.413	12568.83	5.403	0.063
Mod 3	[Blubber]	K	Y, ID, Age	-6314.03	12642.06	88.853	0
Mod 4	[Blubber]	K, S	Y, ID, Age	-6286.643	12599.48	36.062	0
Mod 5	[Blubber]	K,S,K * S	Y, ID, Age	-6285.413	12600.91	37.488	0
Mod 6	[Blubber]	Loc, K, S, K * S	Y, ID, Age	-6275.044	12582.17	18.751	0

Table 2 Continued

B

	Response	Fixed	Random	logLik	AIC	dAIC	AICw
Mod 1	[Blood]	K, S, Loc, S * Loc	Y, ID, Age	-1298.252	2618.504	0	0.991
Mod 2	[Blood]	K,S,Loc,K*S*Loc	Y, ID, Age	-1297.928	2627.856	9.352	0.009
Mod 3	[Blood]	K	Y, ID, Age	-1362.753	2737.506	124.623	0
Mod 4	[Blood]	K,S	Y, ID, Age	-1310.608	2637.217	24.334	0
Mod 5	[Blood]	K,S,K*S	Y, ID, Age	-1309.079	2638.157	25.275	0
Mod 6	[Blood]	S,K,Loc,K*S	Y, ID, Age	-1307.266	2636.533	23.651	0

C

	Response	Fixed	Random	logLik	AIC	dAIC	AICw
Mod 1	[Milk]	K,S,Loc,K*S*Loc	Y, ID, Age	-9360.52	18755.04	0	0.974
Mod 2	[Milk]	S,K,Loc,K*S	Y, ID, Age	-9369.723	18763.45	8.405	0.015
Mod 3	[Milk]	K	Y, ID, Age	-9369.982	18763.96	8.923	0.011
Mod 4	[Milk]	K,S	Y, ID, Age	-9380.71	18779.42	47.911	0
Mod 5	[Milk]	K,S,K*S	Y, ID, Age	-9386.378	18790.76	35.716	0
Mod 6	[Milk]	K, S, Loc, S * Loc	Y, ID, Age	-9377.503	18777.01	21.965	0

For both islands samples from late lactation had significantly higher PCB concentrations (t_{stage} = 8.86, p < 0.001), but the differences between early and late stages in NR were smaller than for IOM ($t_{location\ x\ stage}$ = -4.42, p<0.001). Only very minor random variations were attributable to *Year* ($S.D._{year}$ = 0.021), while relatively large amounts of random variation could be attributed to *Animal ID* and *age* ($S.D._{ID}$ = 27.62 and $S.D._{age}$ = 94.67). The standard deviation of the unexplained residual of the model was 133.16.

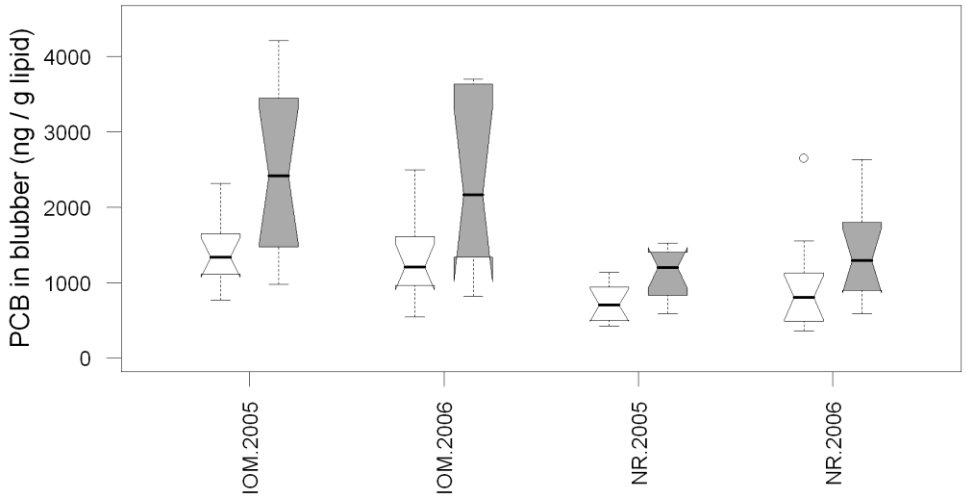


Figure 1 PCB concentration (ng/g of lipid) in blubber during 2 stages of lactation early (white) and late (dark grey) in NR and IOM grey seal females during 2 consecutive breeding seasons.

Serum

Total OC concentrations in maternal serum during early, mid and late lactation are shown in Fig. 2 and the changes in concentrations for each congener in Appendix 14 A-B. There were no obvious colony differences for individual congeners. This was partly supported by the LME models (Table 2 B). There was very strong support for the model (AICw = 0.991) compared to the second model (AICw=0.009). Like in blubber there was a significant negative relationship between K_{ow} index and OC concentration (t_{kow} = -2.78, p = 0.006).

There was no significant effect of colonies. It was also shown that while concentrations did not changed from early to mid lactation, they significantly increased from mid to late lactation (t_{stage} = 9.319, p < 0.001). The differences between early and late were smaller for NR than for IOM (t_{stage} = -4.503, p < 0.001). Some of the variation could be attributed to *Animal ID* ($S.E._{ID}$ = $S.E._{age}$ = 0.250) and *age* ($S.E._{ID}$ = $S.E._{age}$ = 0.241). The standard deviation of the unexplained variance was 0.523.

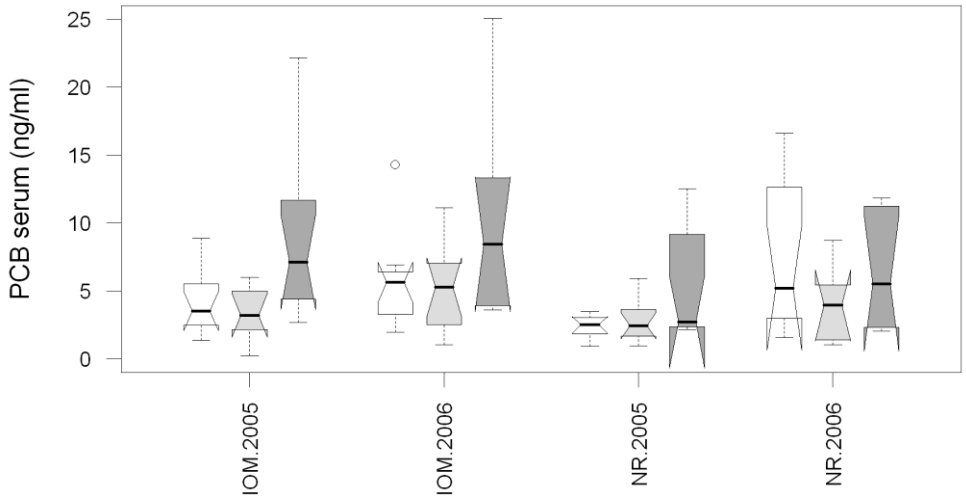


Figure 2. Total PCB concentration (ng/ml) in serum during 3 stages of lactation early (white), mid (light grey) and late (dark grey) in NR and IOM grey seal females during 2 consecutive breeding seasons

Milk

The total and congener specific OC concentrations and changes across lactation are shown in Fig. 3 and Appendix 15 A-B. The best models can be seen in Table 2 C. There was a strong support for the best model (AIC_w = 0.974) in comparison to the second best model (AIC_w = 0.015). As in the previous compartments, Kow had a significant effect on OC concentration (t = -31.78, p < 0.001). There was no significant effect of location. Similar to the results from serum, the concentrations did not change significantly from early to mid-

lactation, while they significantly increase from mid to late (t_{stage} = 3.66, p<0.001). As for blubber and serum the random variation attributed to *Year* was small ($S.E._{year}$ = 0.010). *Animal ID* and *age* explained ($S.E._{ID}$ = 18.00, $S.E._{age}$ = 49.10) respectively, and the standard deviation of the unexplained variance of the model was 31.69.

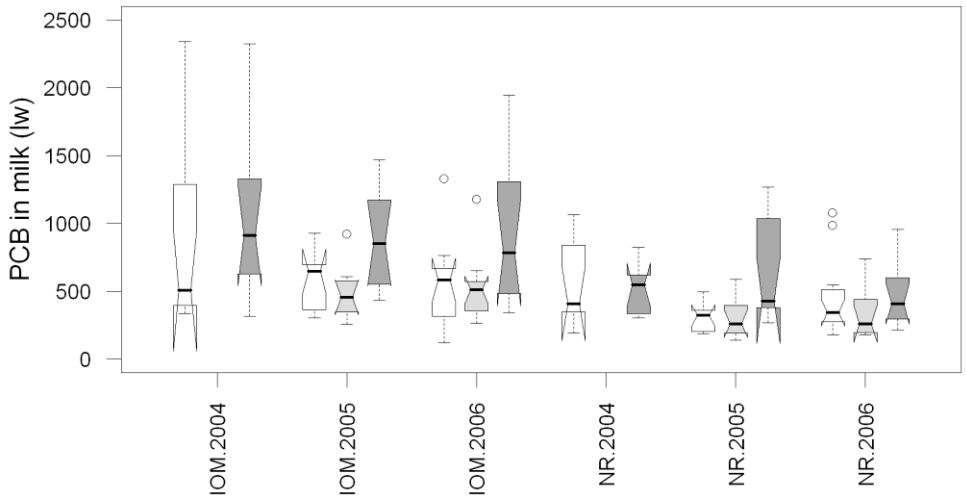


Figure 3. Total PCB concentration in milk during 3 stages of lactation early (white), mid (light grey) and late (dark grey) in NR and IOM grey seal females during 3 consecutive breeding seasons. Lw = lipid weight

Differences in congener PCB ratios between compartments

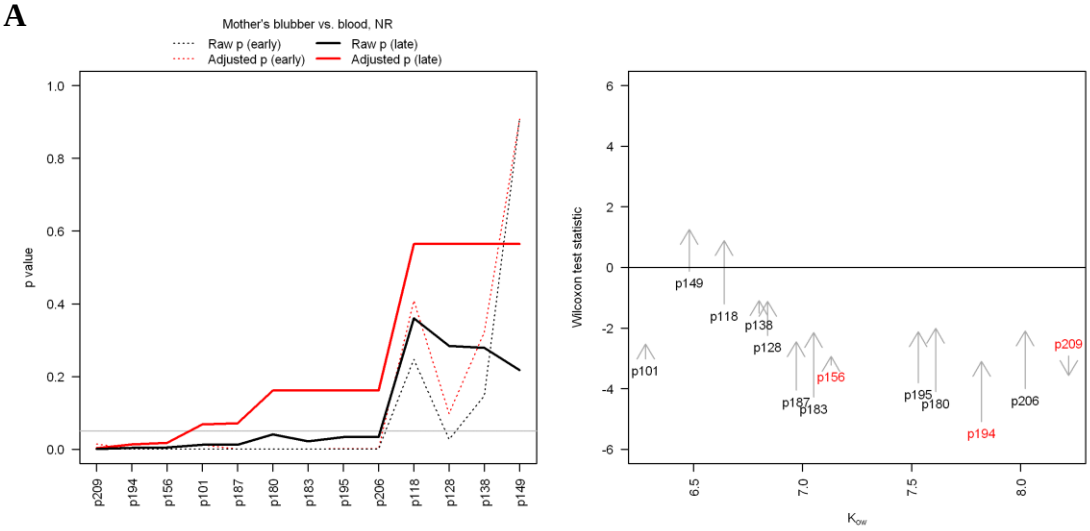
In both populations the values of R-153 for almost all congeners significantly differed between blubber and serum at early lactation, with the exception of the lower chlorinated PCBs: 101, 118, 138 and 149 (Fig. 4a and Fig. 5a). There appeared to be a threshold where congeners with a K_{ow} index greater than ~6.8 had significantly higher ratios in blubber than in serum, while congeners with a lower K_{ow} index had similar ratios in both compartments (Fig. 4a and Fig. 5a). As lactation progressed the differences between these two compartments decreased, especially in NR animals where only 3 congeners differed significantly between compartments at late lactation. While the differences

between compartments also decreased in IOM seals, 7 congeners, all with medium to high K_{ow} indices, were still significantly different at late lactation (Fig. 5a).

The equivalent comparisons between serum and milk at early and late lactation only showed significant differences for a small number of low-chlorination congeners having higher ratios in milk than in serum in both populations (Fig. 4b and Fig. 5b). Towards the end of lactation, differences between serum and milk were again smaller for NR seals, but there was a general pattern of low chlorination congeners being relatively enriched in milk and high chlorination congeners being enriched in the serum. In IOM samples there was a clear increase in the ratios of lower chlorinated PCBs in the milk towards the end of lactation.

Not surprisingly, the comparisons of ratios in blubber and milk revealed much more distinct differences than those found in the comparisons blubber-serum and serum-milk (Fig. 4c and 5c). There was a very clear trend with lower K_{ow} congeners being significantly enriched in milk compared to blubber, while congeners with higher K_{ow} values were significantly reduced in milk relative to blubber.

A


Mother's blubber vs. blood, NR
Raw p (early)
Raw p (late)
Adjusted p (early)
Adjusted p (late)
p value
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Wilcoxon test statistic
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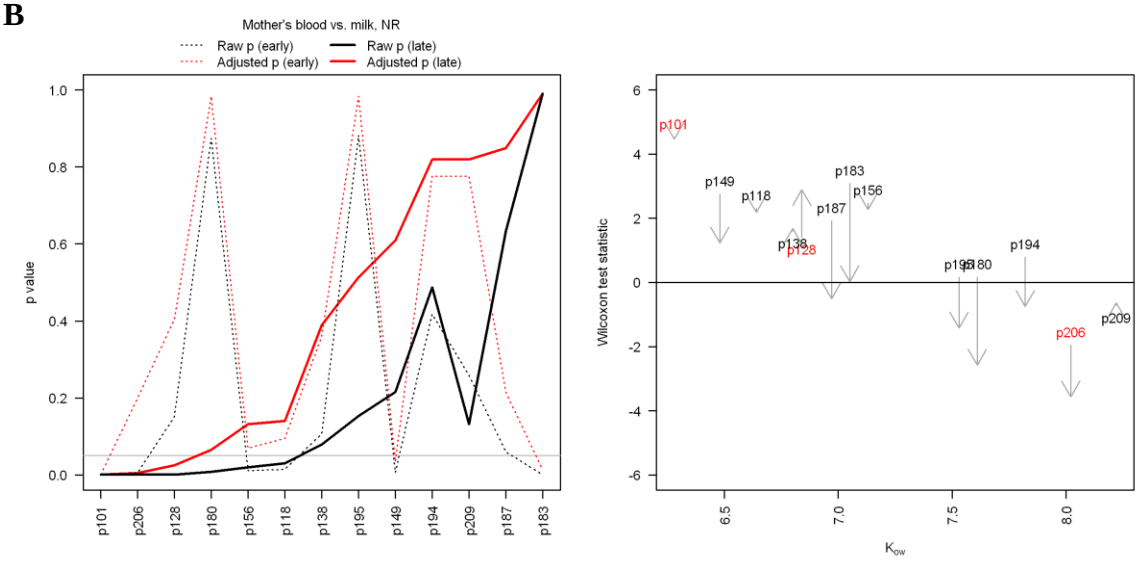
Kow

B


Mother's blood vs. milk, NR
Raw p (early)
Raw p (late)
Adjusted p (early)
Adjusted p (late)
p value
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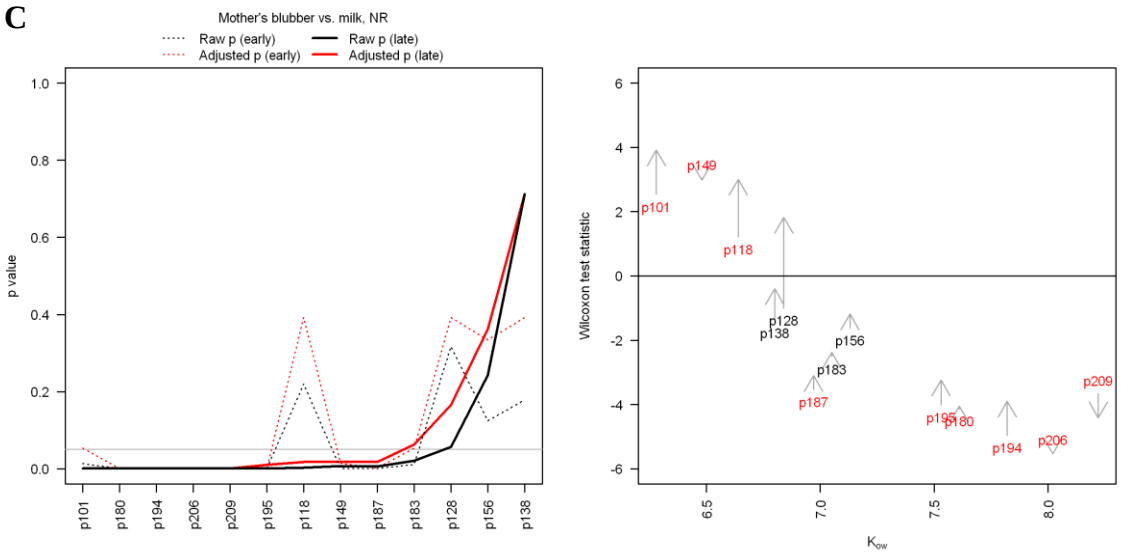
Wilcoxon test statistic
p101 p149 p118 p183 p156 p187 p138 p128 p194 p180 p209 p206
Kow

C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Raw p (late)
Adjusted p (early)
Adjusted p (late)
p value
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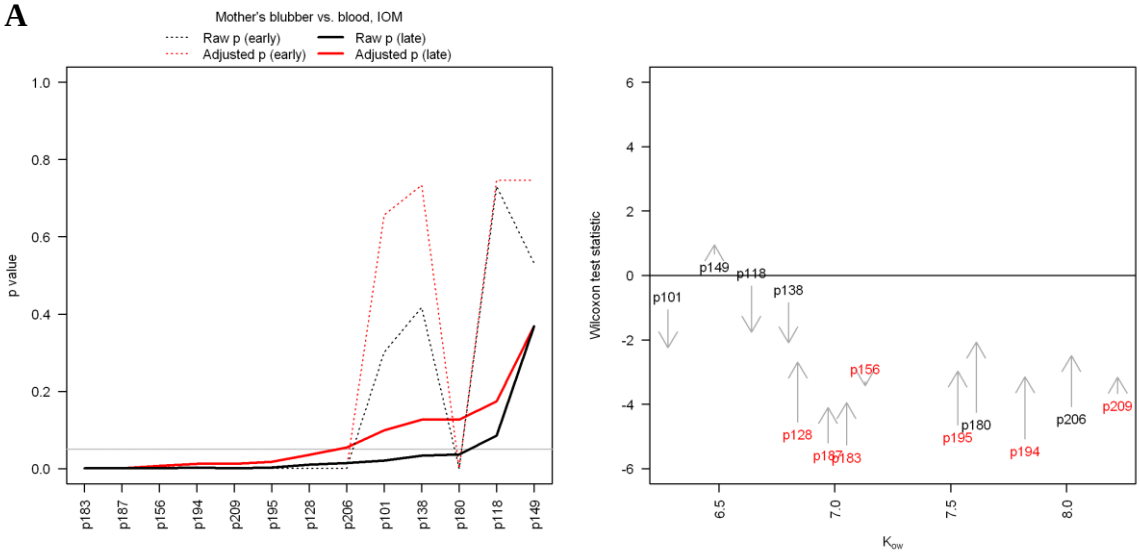
Wilcoxon test statistic
p101 p149 p118 p128 p138 p156 p183 p187 p195 p180 p194 p206 p209
Kow

A


Mother's blubber vs. blood, IOM
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Adjusted p (early)
Raw p (late)
Adjusted p (late)
p value
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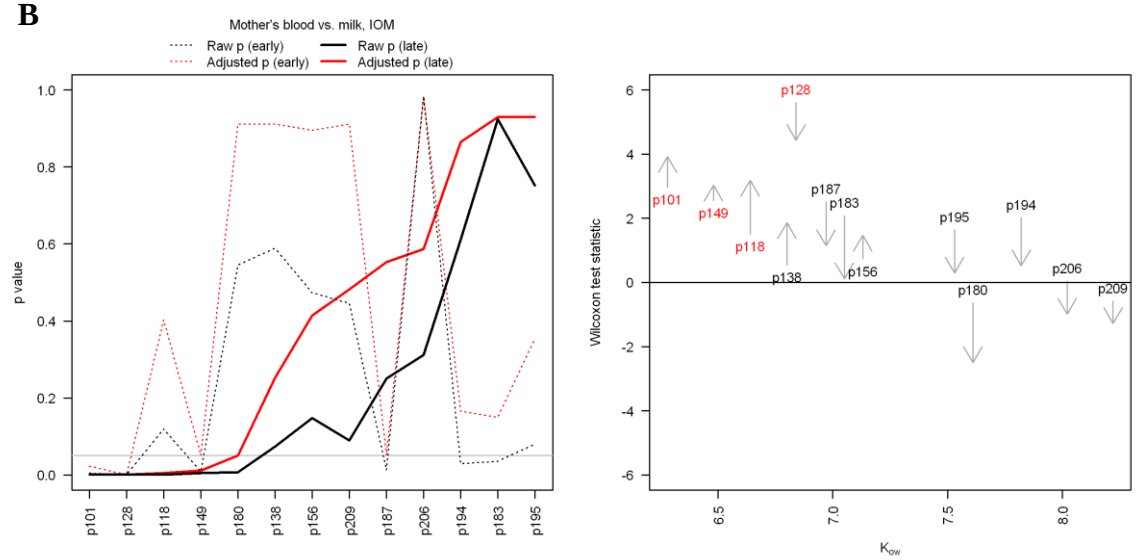
Wilcoxon test statistic
p149 p118 p101 p138 p156 p128 p187 p183 p195 p180 p194 p206 p209
K_ow

B


Mother's blood vs. milk, IOM
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Adjusted p (early)
Raw p (late)
Adjusted p (late)
p value
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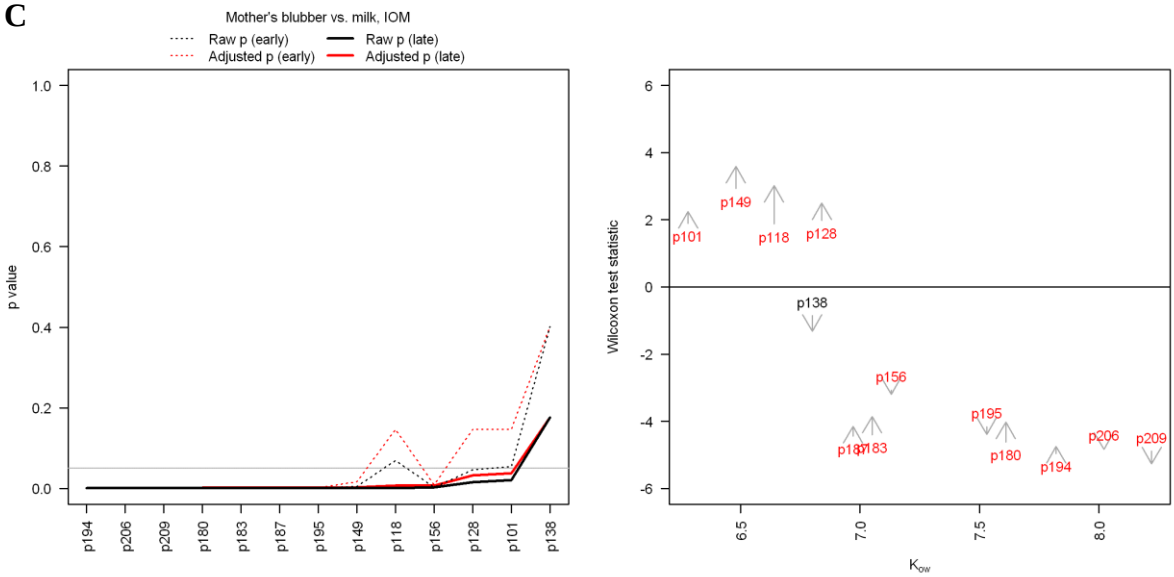
Wilcoxon test statistic
p128 p101 p149 p118 p187 p183 p138 p156 p195 p194 p180 p206 p209
K_ow

C


Mother's blubber vs. milk, IOM
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Adjusted p (early)
Raw p (late)
Adjusted p (late)
p value
p194 p206 p209 p180 p183 p187 p195 p149 p118 p156 p128 p101 p138
Wilcoxon test statistic
p149 p101 p118 p128 p138 p156 p187 p183 p195 p180 p194 p206 p209
K_ow

Figure 4 and 5. (Previous pages). R-153 comparisons between compartments at early and late lactation for each colony. First three graphs represent comparisons in North Rona and the last three in Isle of May. The panel on the left shows the raw and p values from the multiple comparison test. PCBs are arranged in increasing order from left to right according to their adjusted p-values. The right panel shows the values for the Wilcoxon rank sum statistic. The arrows represent the change in the values from early to late lactation; the red numbers highlight the PCBs that were significantly different between compartments at late lactation. In both figures A) comparison between blubber and serum, B) comparison between serum and milk, C) comparison between blubber and milk.

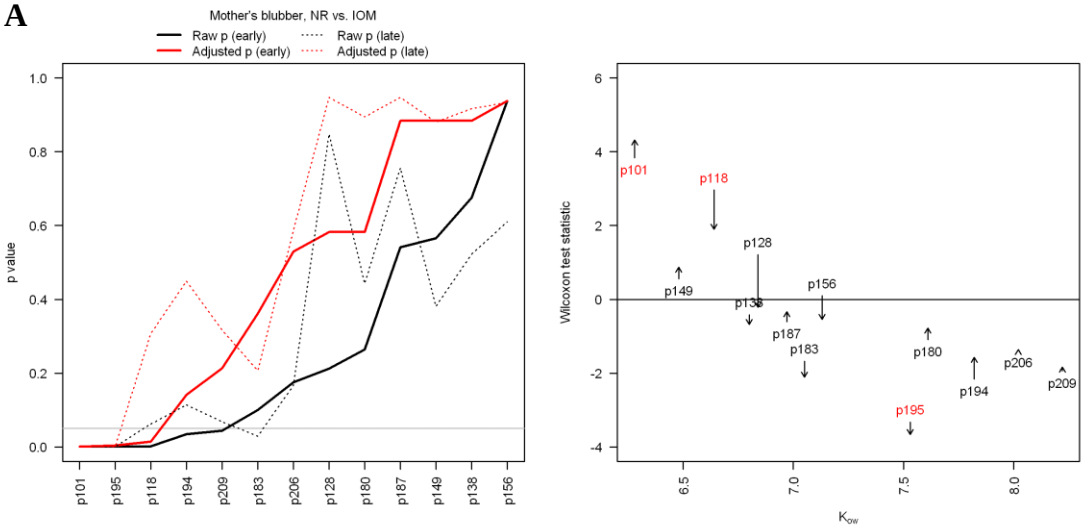
As lactation progressed, the differences between blubber and milk increased. At the end of lactation the pattern of high relative enrichments of low-chlorination congeners in milk and high-chlorination congeners in blubber was very clear, especially in the case of IOM where all congeners except PCB 138 were significantly different between blubber and milk (Fig. 5c)

Differences between colonies

The differences in R-153 between colonies within the same compartment were relatively minor but showed broadly similar patterns in relation to K_{ow} index in the three compartments. Animals from NR always had higher ratios of the lower chlorinated (lower K_{ow} index) PCBs, while IOM seals had higher ratios of the highly chlorinated (high K_{ow} index) congeners. This trend in differences in relation to the K_{ow} index is again clearly seen in the plots of the Wilcoxon test statistic in relation to K_{ow} index (Fig. 6a-e). The ratios of the low-chlorination PCB-101 and PCB-118 were significantly and consistently higher at NR compared to the IOM, and in terms of the blubber at late lactation IOM animals had significantly higher ratios of the high-chlorination PCB- 195.

The differences in PCB ratios in pup serum (Fig. 6d) between the colonies were almost identical to the patterns observed in their mothers. Pups from NR had higher ratios of the low-chlorination congeners, and again these differences were only significant for PCB-101 and PCB-118. Interestingly, in blubber samples obtained from pups at late lactation, there were very small differences in PCB ratios between the colonies (Fig. 6e), and there was no pattern in terms of the relationship between these minor differences and the K_{ow} index.

A


Mother's blubber, NR vs. IOM
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Adjusted p (early)
Raw p (late)
Adjusted p (late)
p value
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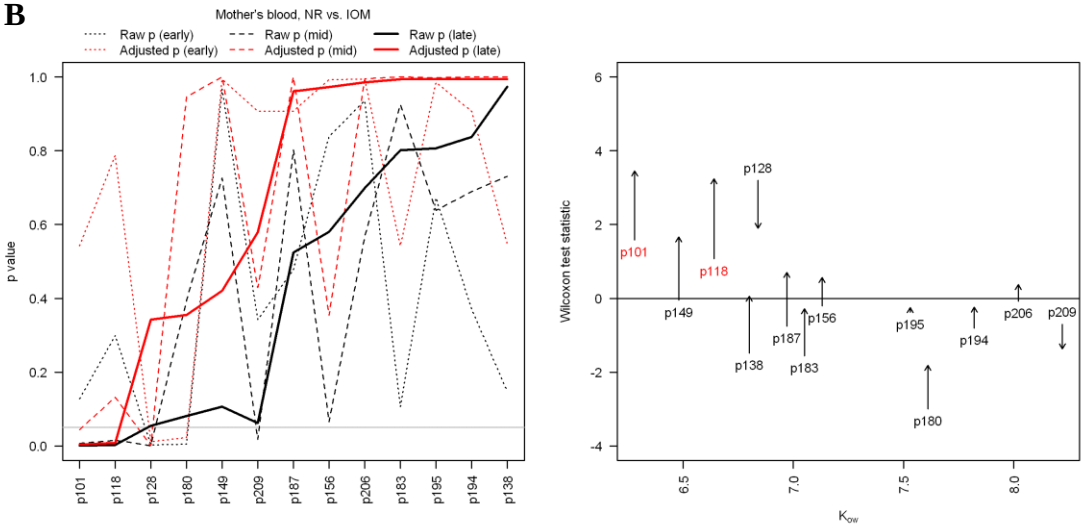
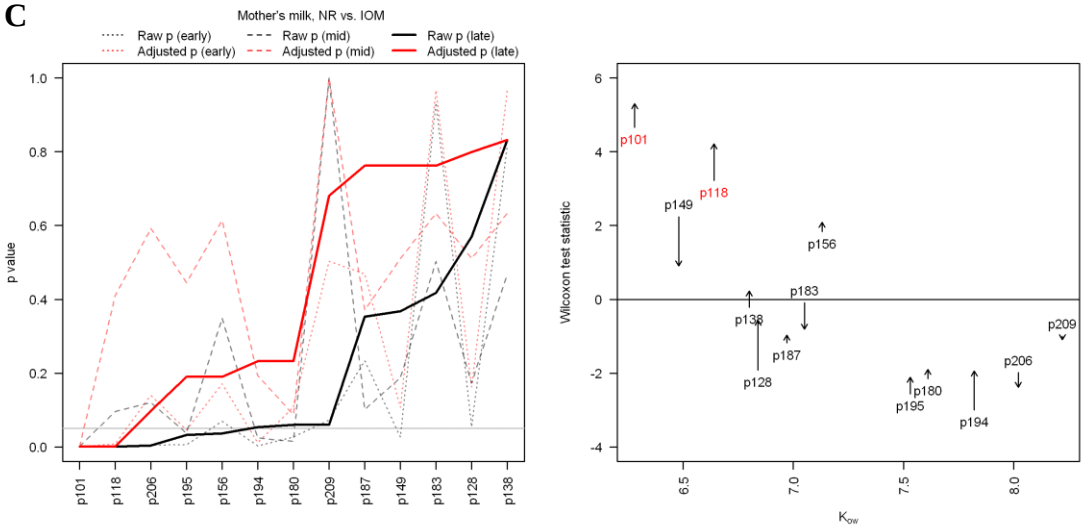
Wilcoxon test statistic
p101 p118 p128 p149 p138 p156 p187 p183 p180 p195 p194 p206 p209
K_{ow}

B


Mother's blood, NR vs. IOM
Raw p (early)
Adjusted p (early)
Raw p (mid)
Adjusted p (mid)
Raw p (late)
Adjusted p (late)
p value
p101 p118 p128 p180 p149 p209 p187 p156 p206 p183 p195 p194 p138
Wilcoxon test statistic
p101 p149 p118 p128 p138 p187 p156 p183 p195 p180 p194 p206 p209
K_{ow}

C


Mother's milk, NR vs. IOM
Raw p (early)
Adjusted p (early)
Raw p (mid)
Adjusted p (mid)
Raw p (late)
Adjusted p (late)
p value
p101 p118 p206 p195 p156 p194 p180 p209 p187 p149 p183 p128 p138
Wilcoxon test statistic
p101 p149 p118 p156 p183 p138 p209 p187 p128 p206 p180 p195 p194
K_{ow}

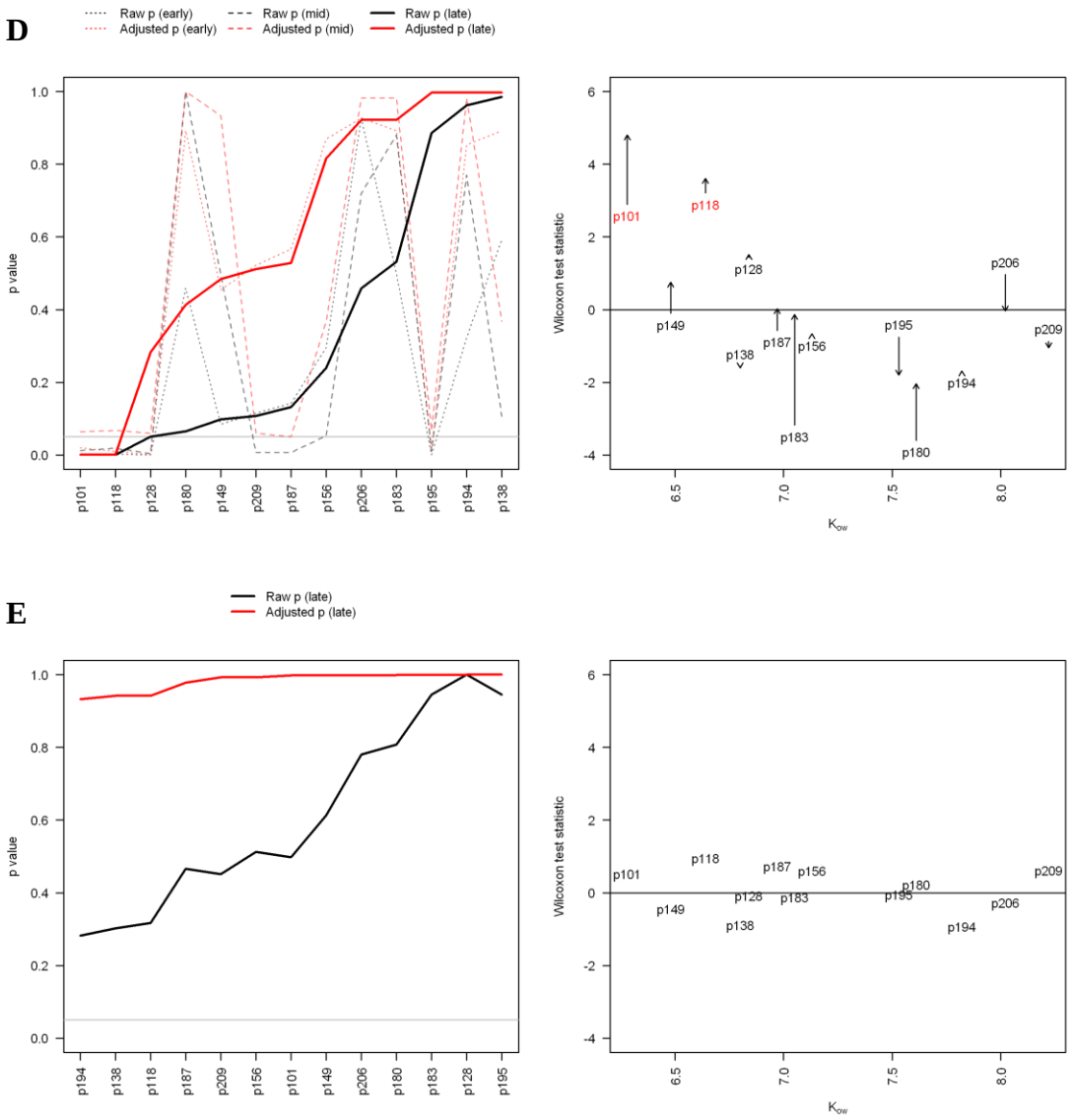


Figure 6. Comparisons between populations by the R-153 for each congener. The panel on the left shows the raw and p values from the multiple comparison test. While the right panel shows the values for the Wilcoxon rank sum statistic. PCBs are arranged in increasing order from left to right according to their adjusted p-values. The arrows represent the change in the values from early to late lactation; the red numbers highlight the PCBs that were significantly different between populations at late lactation. Comparisons between colonies were A) Blubber, B)Serum, C) Milk, D) Pup serum E) Pup blubber.

DISCUSSION

In this study we have attempted to describe the dynamics of PCB release from blubber, and the transfer between body compartments and to pups throughout the lactation period of grey seal females. This study examines the longitudinal changes within individuals of a free-ranging marine mammal. We could compare these patterns between two colonies with different contamination levels. The use of LME models allowed us to examine in greater detail the relative importance of a range of variables, ranging from regional differences to maternal physiological state, to the overall patterns of variation.

TOTAL PCB CONCENTRATIONS AND BURDENS

The total concentration of PCBs in females remained relatively constant throughout the first half of lactation, and thereafter increased dramatically. This increase was particularly remarkable in females from the IOM, where the total concentrations often doubled from early to late lactation. As expected, the total body burdens decreased as females transferred substantial amounts of PCBs to their pups via milk. These patterns are consistent with the expected fate of lipophilic compounds in fasting animals relying almost exclusively on stored lipids. Lipophilic pollutants, such as PCBs, are partitioned into fatty tissue along with lipids originating from the diet during periods of positive energy balance. During periods of negative energy balance lipids are mobilised from the adipose tissue, and the accumulated pollutants such as PCBs can then be released into the circulatory system and be transported to other tissues such as the mammary gland. It was initially suggested that the PCB partition between the circulatory system and various body tissues was determined largely by the relative lipid contents and PCB concentrations of the blood and each specific tissue, and by the solubility of the compounds in each of these compartments. For instance, the transfer from blubber to blood has been explained by a simple equilibration process whereby compounds are partitioned based on their lipid solubility

(Matthews & Dedrick 1984). Adipose tissue is made up of mostly non-polar triacylglycerols (TAG), while the blood consists mainly of polar lipids and lipoproteins (Espeland et al. 1997), and the lipophilic nature of PCBs causes them to be less mobilized and thus retained in the adipose tissue. PCBs are then redistributed in the blubber resulting in gradual increases in concentration in the blubber as the lipids are mobilised throughout the period of negative energy balance (e.g.(Addison & Stobo 1993, You et al. 1999, Lydersen et al. 2002, Debier et al. 2003a). Indeed, the concentration of PCBs in blubber was observed as lactation progressed. At some point however, the PCB concentrations in blubber may reach a retention limit, beyond which the release of PCBs increase dramatically. Such patterns have previously been described by e.g. (Sørmo et al. 2003, Debier et al. 2003a, Debier et al. 2003b), and our results are in agreement with these previous studies (see also below).

DIFFERENTIAL RELEASE AND TRANSFER OF CONGENERS

This study also shows that the release and transfer of different PCB congeners is not uniform, since the PCB patterns differ from one compartment to another. In general, more highly chlorinated congeners (i.e. those with higher K_{ow} index and therefore higher affinity for lipid) are preferentially retained in blubber, and therefore become enriched relative to lower chlorinated congeners. This pattern has been described by several previous studies, but the exact mechanisms are not well understood. The fact that PCBs are so highly lipophilic favours the idea that the patterns of PCB mobilization and transfer are intimately linked with the patterns of FA mobilization and transfer e.g. (Espeland et al. 1997). The mobilization of FAs is dependent on the chain length and degree of unsaturation ((Raclot 2003), Chapter 3). For a given carbon chain length the rate of mobilisation increases with increasing number of double bonds and for a given number of double bonds the mobilisation decreases with increasing chain length ((Raclot & Groscolas 1993, Raclot 2003), Chapter 3). At the lipid/water interface of a fat droplet, TAGs are believed to be positioned according to their polarity. The most polar TAGs which are rich in unsaturated and/or short chain

FAs are believed to be more abundant at the water interface, possibly leading to preferential hydrolysation of these more polar compounds (Connor et al. 1996, Raclot 2003). Consequently, the less-polar lipids may remain for longer periods in the blubber as the fast progresses, and it is possible that highly lipophilic PCBs will tend to remain in the lipid stores due to their stronger affinity for these non-polar lipids. This may explain the observed increase of PCBs in blubber concentration over lactation, which is more dramatic for the more lipophilic and highly chlorinated PCBs. Debier et al. (2003b) have also described this phenomenon in grey seals. The sudden increase in the concentrations of PCBs in blood and milk towards the end of lactation may be caused by the blubber reaching a limit. The blubber capacity for retaining PCBs become reduced after a large amount of lipids has been mobilised (Debier et al. 2003b). Interestingly, this dramatic increase in serum and milk during the second half of the lactation period was mainly seen for the most highly chlorinated and highly lipophilic congeners. The more dramatic increase observed in IOM seals compared to those from NR may therefore suggest that the more polluted animals at the IOM reach this retention limit at an earlier stage of lactation probably due to their lower amount of fat. As the release of highly lipophilic congeners increases, we also expect the differences in PCB composition of blubber and blood to be reduced. Our results do indeed show such a pattern, and while the differences in ratios between blubber and blood decreased over lactation in both populations, IOM seals nevertheless showed slightly larger differences between these compartments at late lactation compared to seals from NR. It is possible that the levels of highly chlorinated congeners in blubber at the IOM are so high that the levels in blood remain much smaller, even despite the dramatic increases towards the end of lactation.

Once in the blood, PCBs can be taken up and metabolized by other tissues (primarily the liver), excreted, or transferred to the offspring via milk. The degree to which different congeners follow these different pathways are again likely to be influenced by their lipophilicity, and the mechanism of any barriers against the transfer of highly chlorinated compounds between compartments are probably linked to variations in polarity (Matthews& Dedrick 1984, Safe 1984).

In addition to giving support for the theory of selective mobilization from blubber during lactation, our study also suggests that the transfer efficiency between blood and milk is lower for the more highly chlorinated PCBs. The differences in congener ratios in milk compared to blubber and serum observed in our study have also been observed in hooded seals (Borga et al. 2005) and grey seals (Addison et al. 1999, Debier et al. 2003a), and suggest that some barrier exists against the highly chlorinated PCBs at the mammary gland. Between serum and milk there was an increase of the lower chlorinated PCBs and a decrease of the highly chlorinated ones. While the idea of a barrier for highly chlorinated PCBs is not new (e.g Addison& Brodie (1977)) the specific mechanism of this process (from a selective release of PCBs in relation to the lipids mobilization or a mechanism that prevent the highly chlorinated PCB to be transferred to the milk) is still unknown. While the preferential transfer of more polar low-chlorination OCs from blubber to circulating lipids can be explained by equilibration partitioning between the non-polar blubber lipids and the more polar circulating lipids and lipoproteins (Matthews& Dedrick 1984), it is unclear how this mechanism can explain the apparent selectivity against any non-polar circulating OCs at the mammary gland.

While the existing barrier against the most chlorinated OCs entering the mammary gland will increase the risk to the female, the consequences to the pup if these OCs were transferred to the milk may be far worse, since pups do not have the same capacity for detoxification (Boon et al. 1992) and are at a more vulnerable stage. Transfer of highly chlorinated PCBs to the offspring can have adverse effects on the immune, endocrine and neurological systems, increasing the susceptibility to infections and therefore having an indirect effect on the mortality (Beckmen et al. 1999). The effects on the endocrine system may lead to postnatal reductions in weight gain and additional adverse effects on development in humans and laboratory animals (Lamb et al. 2006).

In agreement with previous studies in grey (*Halichoerus grypus*) and harbour seals (*Phoca vitulina*) (e.g.(Green et al. 1996, Sørmo et al. 2003, Debier et al. 2003b, Wolkers et al. 2004)) we found that total concentrations of PCBs in serum and milk were significantly lower than those found in blubber. This finding along with the patterns of PCB change across lactation in the three

different compartments gives support to the idea that the transfer of OCs from blubber to blood and from blood to milk is to some degree selective, with potentially important consequences in terms of preventing the most highly chlorinated congeners being transferred to the offspring. Selectivity against release of highly chlorinated congeners from the blubber would reduce the risk of adverse health consequences for pups and mothers (stored PCBs may be functionally inert). Alternatively, if highly chlorinated PCBs are released to the blood but a second barrier reduces their uptake by the mammary gland, the risk of adverse health consequences for pups is also reduced, but the mothers may become more exposed to highly chlorinated OCs in the circulatory system. Our results are in accordance with studies by Wolkers et al (2004 & 2005), who found that PCB congeners with a lower degree of chlorination were selectively transferred to the milk.

The way body lipid reserves are utilized during the fast will directly affect the OC concentrations in the adipose tissue (Polischuk et al. 2002), and the energetic and metabolic costs associated with the lactation fast will also influence the amount of lipids mobilized (Espeland et al. 1997) and therefore the total amounts of PCBs transferred to the pups. It is possible that females with smaller lipid reserves may reach the saturation point in blubber earlier during lactation than fatter females, therefore releasing higher amounts of total PCBs, and particularly the highly chlorinated compounds, during earlier stages of lactation. The increased PCB concentrations in milk towards the end of lactation coupled with the higher milk fat content and increased pup milk consumption during the final stage of lactation (3 kg in contrast to 2.3 kg at early lactation) (Pomeroy et al. 1996) implies that pups will be more exposed to highly chlorinated PCBs if females have a longer lactation duration and transfer higher amounts of lipids.

In previous chapters we have shown that the mobilization of fatty acids from blubber to blood is selective (Chapters 3) and that the transfer from blood to the mammary gland may also be selective. This may be a natural process that ensures the delivery of essential building blocks to the growing pups. Due to their highly lipophilic characteristics, it is possible that OC dynamics are closely linked to those of fatty acids. If this was the case, it is tempting to think of a

'barrier' to the transfer of pollutants as an analogous or even homologous process to that for fatty acids. However, it is important to keep in mind that while fatty acid selectivity may have evolved naturally in response to the selective pressure for pups to obtain essential lipids, any selective advantage to barriers against the transfer of man-made pollutants would be relatively new in evolutionary terms. It is therefore likely that these barriers are coincidental, and again linked in some way to the physicochemical properties of PCBs and how this influences their degree of uptake in the mammary gland. The nature of the lipids transferred to the mammary gland at different stage of lactation can have also have an influence in the efficiency of the transfer of PCBs to the milk.

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7 General Discussion

DISCUSSION

Marine predators obtain both fatty acids (FAs) and pollutants, such as polychlorobiphenyls (PCBs), exclusively from their diet which, in the case of pups, is the mother's milk. The levels of these compounds that are ultimately observed in different tissues and different life stages will be a result of the initial levels ingested, the life history of the individual and the way lipids and PCBs are metabolized, mobilized, excreted and transferred to offspring. Thus, there are multiple factors operating at a range of different scales that will determine the levels and profiles of these chemicals, ranging from environmental variation to the animals' physiology, and the characteristics of the compounds in question. PCBs are highly lipophilic compounds, and their occurrence is tightly linked to that of lipids. Their fate in an organism depends to a very large extent on their degree of lipophilicity, how tightly associated they are with specific FAs, and the dynamics within an organism of these specific FAs. Understanding the large and small scale processes that determine the variations in FAs and PCBs will therefore improve our understanding of the fate of these compounds in marine ecosystems and organisms.

Current physiology-based pharmacokinetic (PBPK) models for marine mammals (e.g. (Hickie et al. 2005, Klanjscek et al. 2007) are restricted to using published average values for lactation parameters such as lipid mobilization rates and milk transfer rates. Details about lipid dynamics, such as selective transfer of specific FAs and the potential effects that this may have on the transfer of different PCB congeners, are not considered because the details about these processes are seldom known. Therefore, it is of fundamental importance to establish an understanding of lipid dynamics to elucidate the dynamics of lipophilic compounds.

The purpose of this thesis was to examine how levels of FAs and PCBs differ and change in time in grey seals from two UK colonies, and the factors that

determine FA and PCB mobilization and transfer to the offspring. Although this thesis did not examine explicitly the link between PCBs and FAs, it provides a good basis to obtain information that can be relevant to models that attempt to explain pollutant behaviour in animals. The examinations cover a range of scales from environmental to physiological and these will be discussed in turn, including some general hypotheses about how PCBs maybe be linked to FAs during their mobilization and transfer.

The main findings of this thesis were 1) large variations in both FAs and PCB blubber profiles between two UK grey seal colonies, 2) the mobilization of these compounds was shown to be selective and partly a consequence of their physicochemical properties, and 3) in addition to the effects of physicochemical properties, specific FAs were also selectively mobilized probably in response tom the needs of the growing pups.

REGIONAL AND TEMPORAL VARIATIONS

The factors that will determine the FA composition and PCB load of an animal are strongly linked to their diet (milk in case of pups), since these clases of compounds are exclusively obtained from the ingested prey. Differences in feeding areas and the food web in which animals feed is likely to strongly affect the degree of exposure to pollutants and the type of lipids deposited in the tissues.

We found that the FAs that dominate the grey seal female blubber composition at early lactation were the ones that have been reported to be most prevalent in many other marine mammals (West et al. 1979, Iverson et al. 1997, Dahl et al. 2000). However, there was variability in the relative abundance of different FAs between seals that bred at the Isle of May (IOM) and at North Rona (NR). These differences are probably mainly attributable to differences in diet. However, age, habitat type, food availability, food web structure of the region etc. will all influence the diet (West et al. 1979, Innis and Kuhnlein 1987, Grahl-Nielsen and Mjaavatten 1995, Käkelä et al. 1993, Iverson et al. 1997, Dahl 2000). The time of sampling (fasting or fattening seasons) will also influence the FA

profiles that are found in blubber. Since the diet is ultimately influenced by all these factors, they need to be considered together since they are not mutually exclusive (Thompson et al 1998).

Previous studies have shown that the FA composition of marine mammal blubber is largely determined by diet (*e.g.* Iverson 1993; Käkelä et al. 1993; Iverson et al. 1995b, 2004; Käkelä and Hyvärinen 1998; Kirsch et al. 2000) and that variability in FA signatures can reflect spatial differences in predator foraging (Iverson et al. 1997). The results of this thesis confirmed that grey seal females from two geographically separated colonies in the UK display persistent differences in their FA composition despite substantial annual variations. We cannot determine conclusively that these differences in FA composition are caused by differences in diet. However, given the substantial influence of diet on blubber FA composition this seems a likely explanation. Scat studies from a range of non-breeding haulouts have showed large differences in the diet of grey seals from the east and west of Scotland (Hammond and Grellier 2006, Hammond and Harris 2006), although these were non-breeding haulouts more or less distant from NR and the IOM respectively. Furthermore, satellite tracking studies performed at haulouts from the east and west of Scotland have shown that seals from the east coast forage almost exclusively in the North Sea, while seals from the west remained to the west of Orkney Islands (Thompson et al. 1991, 1996, McConnell et al. 1999, McConnell pers. comm.). None of these tracking studies have been done on breeding females from either of the two breeding colonies studied here. However, outside the breeding season seals from IOM are known to frequent the haulouts at which tracking studies have been done. Similarly, we cannot be certain that any of the animals tagged along the west coast of Scotland actually breed at NR, or to what extent NR seals utilize these haulouts outside the breeding season. The adult females tracked as part of the post-breeding telemetry study conducted at NR generally foraged within 80 km of the island and did not venture into the North Sea (Pomeroy, pers comm). While they do not provide conclusive evidence, these telemetry studies nevertheless support the assumption that seals from NR and the IOM tend to forage in different ocean regions.

The persistent differences in FA composition that were observed between

regions suggest that regional/colony differences tend to be larger than interannual variations. Seal blubber FA analyses carried out in this study supplemented earlier work by Walton et al (2000) and Walton and Pomeroy (2003). Together these data revealed clear differences in FA composition between colonies during six years over a 10-year period of study that may be the result of differences in prey availability and/or individual prey preference. However, ecosystem dynamics in the two regions may also affect the FA composition of the prey and thus the FA profiles of top predators. Our analyses also revealed that seals breeding at the IOM colony appeared to go through rapid changes in their blubber FA composition during the mid-1990s, although the composition remained relatively stable during the mid-2000s. This temporal pattern was reversed for NR animals, which showed a relatively stable blubber FA composition during the mid-1990s but rapid annual changes during the mid- 2000s. This could have been a result of changes in the availability of prey to seals in the North Sea, where a decline of cod, one of the main prey species for grey seals, occurred during the mid-1990s. A similar change occurred in Atlantic waters to the northwest of the UK about a decade later. However, it is also possible that the change was a consequence of a shift in the lipid composition of the main prey species due to regime shifts occurring at lower levels in the food web.

The physical and biogeochemical characteristics of the North Sea and the North Atlantic fluctuate substantially on seasonal, annual, decadal and longer timescales. Such changes can have large influences on ecosystem function and trophic structure, and may ultimately affect the species and/or lipid composition of the prey of top predators. The main change in community structure of the North Sea has been from a mainly cold-water community in the 1960s to a warmer-water community in the 1990s. This change can be linked to changes in the sea surface temperature across the Northeast Atlantic during this time (Edwards et al 2008). One of most distinct shifts occurred at the end of the 1980s and the beginning 1990s, after which the plankton community has generally remained in a warm-water state (Edwards et al 2008). There also appears to have been a similar regime shift in community structure in the North Atlantic in 2000, mainly characterized by an increased prevalence of warm-

water copepod species (Edwards et al, 2008). Such changes can in turn have knock-on effects further up the foodwebs, affecting the fish community and top predators (Alheit 2007, Hatun et al. 2009).

In addition to these gradual trends, changes in the decadal climate variability, such as the one observed in the North Atlantic Oscillation (NAO) index, may have induced major changes to the ecosystem structure, ranging from phytoplankton to top predators (Alheit 2007). For example, Alheit and Hagen (1997) describe the impact of the NAO on the growth and spatial distribution of herring and cod in the North Atlantic. Changes in ocean temperature and precipitation linked to NAO fluctuations can also affect the production and distribution of phyto- and zooplankton, which can in turn affect larval growth and mortality of certain species of fish, and ultimately the timing of high food availability for higher predators (Irigolen et al 2000, Reid et al 2001, Beaugrand et al 2002, Edwards et al 2002, Alheit 2007).

If we assume that the blubber fatty acid composition of seals at early lactation is largely determined by their diet, to what extent are our results consistent with these environmental and ecosystem changes? It is unfortunate that the time series of sampling does not cover the period during which the most dramatic ecosystem regime shift occurred (i.e. late 80s to early 1990s), and that there is a gap between 1998 and 2004. The change in FA composition of seals at the IOM was observed in 1996-98, i.e. a few years after the observed regime shift in the North Sea, while the change at NR was observed in 2004-06, i.e. a few years after the documented regime shift in the Northeast Atlantic. But why would there be a delay between the ecosystem shifts and the changes in blubber FA composition? As suggested by Beaugrand (2004) one year shift can be observed in physical data, but it might be too short in biological time. His study showed that, according to the species or taxonomic group, the timing of the shift may change. Beaugrand and Reid (2003) pointed out that difference in timing between species can be expected, since they can react differently to hydro-climatic forcing depending on their particular physiological processes or their life history. The different spatial distribution of species is also likely to explain this change in timing. In this way changes in lipid composition of lower trophic

levels in response to changes in phytoplankton community structure may occur relatively rapidly, it is possible that changes in lipid composition of top predators only become apparent after some longer time period, especially if these predators feed on prey with a lifespan of more than one year.

In summary, I suggest that the observed changes in the FA composition of top predators are most likely caused by both changes in the abundance of specific prey species and changes in their lipid content and FA composition, which have been caused by environmentally induced changes at lower trophic levels. This study could not determine if the observed variations in FA composition were a result of the relative occurrence of different prey in their diet, or caused by variations in the lipid composition of the prey species themselves. This could be achieved by building a comprehensive FA library of potential prey species covering the relevant ocean regions and seasons. This is obviously a major undertaking, but would be worth the effort considering the variety of benefits to diet studies and broader ecological investigations.

The amounts of pollution found in an ecosystem will be influenced by several factors, including the distance from sources of pollution, the transport rate of the pollutants, their size and volatility, and the rate at which the pollutant degrades while in transport. Since PCBs can be present in sediments and as suspended particles in the water column, organisms are exposed to these pollutants in a variety of ways. The most important is through ingestion of PCBs associated with organic material, largely derived from organisms from lower down in the food web. Fish can also acquire water-borne pollutants through their gills, but by far the greatest uptake comes through their diet. Exposure to pollutants in marine mammals is almost exclusively through the diet, and differences in feeding areas and the food web structures in these regions can strongly affect the degree of exposure to pollutants. The North Sea is surrounded by a large number of sources of pollution. In coastal waters the waterborne inputs of PBCs are dominant. The main source is electrical equipment. While the use of PCBs has been banned for several decades, they continue to be released into the environment. The estimated inputs to the North Sea by direct and river input ranged from 530-1500 kg/year between

1990 and 1996 (OSPAR 2000). This in contrast to the relatively clean water masses in the North Atlantic. Thus it is not surprising that IOM animals had higher total concentrations and total body burdens in contrast to seals from NR.

The species composition of the diet of top predators and the position of different prey species in the food web will affect not only the FA profiles but also the pollutant congener profiles found in the blubber of seals. The food web characteristics will affect the type of PCBs accumulated due to the variations in the metabolization and/or excretions across that can differ between the species across the food web. The PCB congener profiles differed between colonies, with IOM seals exhibiting higher levels of the highly chlorinated congeners and lower levels of the lower chlorinated ones compared to NR. The differences are probably a result of seals from IOM having a high rate of pollutant uptake through the prey. This in combination with the low rate of metabolization and excretion of highly chlorinated congeners will have caused these compounds to accumulate at a higher rate over time in seals that forage in the North Sea.

Another factor that may have contributed to the differences in PCB congener profiles between IOM and NR is that the lower chlorinated compounds are more volatile and therefore are more readily distributed via the atmosphere to regions further away from the point sources, such as the North Atlantic. The less volatile high chlorination congeners remain in the North Sea, closer to the numerous sources (Muir et al. 2000).

Since this study only covers only two seasons of PCB sample collection, we could not address any annual trends in levels, and the variations observed within the two populations between years should be interpreted with caution.

In summary, regional and temporal variation in FA composition were observed between the two grey seal study sites. Higher body burdens of PCBs were found in IOM grey seal than their NR counterparts. The FA and PCB patterns also differed significantly between the two colonies, possibly reflecting between-colony differences in the rate of metabolism of PCBs, proximity to areas of historical PCB discharge, difference in oceanography patterns, differences in prey availability or preferences, and reproductive history of individual females.

MOBILIZATION AND TRANSFER OF FAS AND PCBS

The levels of PCBs found in an organism are a result of the net effect of the processes of uptake, distribution, metabolism and excretion. In order to understand the toxicokinetics of PCBs it is important to study their mobilization and transfer, and how these are related to the mobilization and transfer of lipids. Few previous studies have described the toxicokinetics of PCBs during periods of negative energy balance, when rates of lipid mobilization and transfer are high. The mobilization of both FAs and PCBs appear to be highly selective, and in part determined by their molecular structure. This suggests that their dynamics are linked through their physico-chemical structures. While the mechanisms behind these links are not well understood they are likely related to the polarity of the various FAs and PCB congeners.

Highly lipophilic PCB congeners are well known to have a greater tendency to remain in more non-polar body compartments, such as blubber, rather than being mobilized into more polar compartments, such as blood (Crawford et al. 1991, Debier et al. 2003, Debier et al. 2006). Similarly, Raclot & Groscolas (1995), Raclot et al. (1995), Connor et al. (1996) and Raclot (2003) have demonstrated that FAs mobilization rates are related to their polarity. In this thesis, we have shown that highly chlorinated PCB congeners are released from blubber to the circulatory system at much lower rates than less chlorinated ones. Also, saturated FAs were generally mobilized at lower rates compared to more saturated ones. The subsequent transfer from the circulatory system to milk followed a similar pattern.

Since all PCB congeners are relatively lipophilic (depending on their degree of chlorination), they all have a tendency to be retained in the blubber. Consequently, PCB concentrations in blubber remained relatively stable during the early stages of lactation, but increased dramatically during the second half of lactation. These results are in accordance with previous studies on lactating female seals and fasting weaned pups (Debier et al. 2003, Kleivane et al. 2004, Debier et al. 2006). They also support previous suggestions (Sørmo et al. 2003, Debier et al. 2003, Debier et al. 2006) that the blubber reaches a PCB saturation

point beyond which the rates of release increase dramatically. Once this saturation point has been reached congeners are readily released from the blubber, increasing also the concentrations of pollutants in blood and milk.

It is clear from the previous discussion that a more detailed understanding of the underlying processes by which FA and PCB dynamics are coupled is essential for monitoring and modeling the fate of PCBs in ecosystems and organisms. One aspect that has received attention in the past is that of blubber stratification. Some researchers have suggested that the generally low and selective mobility of PCBs may be explained by the stratification of the blubber tissue into distinct layers in marine mammals, so that PCBs stored in less metabolically active layers will be less readily mobilized (Aguilar and Borrell 1994, Severinsen et al. 2000, Debier et al. 2003). During lipid build up, PCBs may be diluted by the deposition of lipids in the more metabolically active inner layer, whereas concentrations in the more metabolically static outer layers remain unaffected (Severisen et al. 2000). Similarly, the utilization of lipids from the inner blubber layer during fasting may result in increased concentrations of PCBs in this layer while the outer blubber layer remains relatively unchanged. Debier et al (2003) found that PCB concentrations in the outer blubber layer of grey seals remained relatively unchanged during periods of lipid loss during lactation, suggesting that PCBs were mainly mobilized from the inner layer. IN this study we do not address this issue for several reasons. Firstly, the blubber of seals appears to be much less clearly stratified than, for instance, that of small cetaceans (see e.g. Koopman et al XX). For FAs, Strandberg et al. (2008) found that the relative concentration of different FAs changed gradually throughout the entire blubber layer, and it is likely that the relative concentrations of PCB congeners change in a similarly gradual way rather than being stratified. Any analyses of FA and PCB concentrations in different tissue layers is therefore likely to depend to a large extent on the exact place where a blubber core is divided. This may lead to biases in concentrations, especially towards the end of lactation when the blubber layer has become relatively thin. It may be possible to examine the variations in PCB congener concentrations as a function of its relative position within the blubber layer in large samples from dead animals,

similar to what was done for FAs by Strandberg et al (2008). Such an analysis would make a useful contribution to the understanding of PCB dynamics and its link to the position of specific FAs within the blubber layer, and on blubber lipid metabolism and mobilization in general.

Regardless of the issue of the position within the blubber layer, the mobilization of FAs and, the PCB congeners that m,ay be associated with them, is believed to be linked to their position within the cells of the blubber tissue. Blubber is comprised of a fixed number of adipose cells, called adipocytes or fat droplets, that shrink as lipids are mobilized during fasting and expand again as lipid is laid down during fattening periods (Koopman et al 2002). The selective mobilization of FAs is believed to be related to their arrangement within a fat droplet, and this is believed to be a function of their polarity (Raclot 2003). Fatty acids are stored in the adipocytes as triacylglycerols (TAG) (Glycerol and 3 FAs). Raclot (2003) suggested that the selective mobilization of FAs is related to the heterogeneous arrangement of TAGs at the lipid water interface of a fat droplet, and that this arrangement is driven by the polarity of the FAs contained in a given TAG. According to this model, TAGs containing more unsaturated (less lipophilic) FAs will organize themselves closer to the water phase of the cytosol, where they will be more accessible to the hormone sensitive lipases which hydrolyse TAGs into free FAs (Iverson 1993). It is possible that the lipophilicity of PCBs also dictate their distribution in the fat droplet according to a similar pattern. In addition, if the strength of the covalent bonds between PCB congeners and FAs depends on the polarity of both types of compounds, the association and arrangement of specific FAs and PCB congeners at the lipid water interface may be even more tightly coupled. For instance, lower chlorinated and therefore more polar PCBs may be preferentially bound to polar TAGs, and therefore readily released from the fat droplet when these TAGs are hydrolysed. In contrast, the more lipophilic hepta, octa and nona PCBs may be retained within the fat droplet in close association with the less polar TAGs. This is supported by the fact that low levels of highly chlorinated PCBs are observed in blood and milk during the early stages of lactation. However, as the lipid stores are utilized, there is a subsequent increase, especially of the highly chlorinated compounds, towards the end of

lactation. In other words, it is possible that the less lipophilic PCBs are released during early lactation as a result of the preferential mobilization of the more polar lipids that are in the periphery of the fat droplet. Once the lipid stores have been depleted, and the fat droplets have become sufficiently reduced in size, the mobilization of less polar FAs increases, leading to increased release of the less polar and more lipophilic PCBs.

The existence of some mechanism at the mammary gland for PCB selectivity cannot be explained from an evolutionary perspective, since PCBs have not been present in the environment over an evolutionary time period. But there is nevertheless some process that results in some selectivity in the transfer of PCB congeners via milk to the offspring (Debier et al, 2003, Sørmo et al. 2005). It appears that despite dramatic increases in the concentration of highly chlorinated congeners in blood and milk towards the end of lactation, low-chlorination congeners are preferentially transferred via milk to the pups, while the more highly chlorinated ones are retained in the female body. In this study, this general pattern was observed at both study colonies, although milk and pup blubber from IOM always had higher total concentrations and generally higher levels of highly chlorinated compounds compared to NR. The increase in PCB concentrations in milk towards the end of lactation also appeared to be more dramatic in animals from IOM. It is possible that the higher initial blubber PCB concentrations at the start of lactation causes IOM females to reach the blubber saturation point (described previously) at an earlier stage of lactation, leading to a dramatic increase in the release of higher chlorinated PCBs from the blubber at an earlier stage than seals at NR. If this is the case, it could have a direct impact on the amounts and types of PCBs transferred to the pups via the milk. Less contaminated females will transfer proportionately less of the most lipophilic and chlorinated PCBs. This process will also be strongly influenced by the body condition and of a female, and how she utilizes her lipid stores. For instance, a female with average lipid content who undertakes a longer lactation will transfer higher total amounts of PCBs to her pup, not simply because of the additional number of days but because she will be more likely to reach the blubber

saturation point. Each day spent lactating after this saturation point has been reached will lead to disproportionately higher amounts of PCBs, particularly the highly chlorinated congeners, being transferred to the pups. The consequences of these processes for pups will depend on the total amount of PCBs transferred and deposited. This will be the topic of future analyses of this dataset. We did however examine the ratios of PCB -153 in pup blood and blubber. While these ratios in blubber were relatively similar in the two colonies, there were significant differences in blood, with pups from NR having significantly higher ratios of the less chlorinated PCBs.

This study has also provided some insights in to the ongoing debate of the possibility of using FAs in milk to predict the diet of top predators (Iverson 1993, Iverson et al 1995b). In capital breeders, such as grey seals, the composition of lipids transferred from mothers to pups via milk is ultimately derived from the mother's stored blubber lipid reserves, and it has therefore frequently been suggested that dietary signals observable in the blubber of mothers can be equally well detected in other body compartments, and also in the tissues of the offspring. This suggestion relies on the assumption that FAs are mobilized as a simple function of their relative amounts in the blubber. However, we found that the release of FAs from the blubber is not proportional to their relative or absolute abundance at early lactation, but that it is to a large degree influenced by the physicochemical characteristics of the individual FAs. In addition, our results also suggest that the release of FAs during lactation is influenced by the physiological needs and growth requirements of the nursing pups. For instance, the higher than predicted rates of mobilization of the essential FAs is in accordance with extreme importance of these compounds for growth, development and the immune system (Burdge & Wootton 2002, Burdge et al. 2002, Innis 2005, Innis 2007). Mechanisms similar to those involved in the selective release of FAs from blubber may operate in the uptake of FAs from blood by the mammary gland, ensuring that the milk composition is appropriate to the specific needs of the pups. Such a mechanism may also explain the changes in milk FA composition across lactation, which may be a response to changes in the

requirements of pups at different developmental and growth stages. These processes of selectivity in mobilization and transfer may explain the fact that the milk composition of seals at NR and the IOM were much more similar than would be expected based on their blubber composition, and that the changes in milk composition throughout lactation were also similar in the two colonies. Despite these similarities in mobilization and transfer patterns, samples from the two colonies could nevertheless be clearly distinguished also based on their milk and blood FA composition. In short, colony differences were greatest when comparing blubber samples from early lactation, but despite the similar and selective patterns of mobilization and transfer in the two colonies, there is still sufficient differences remaining in other compartments to allow the distinction between samples from different colonies.

This study is the first to describe concentrations and total body burden from the NR colony. I hope the data presented for both colonies will be useful for future comparisons when assessing geographical and temporal trends in pollutant exposure. This study has showed that concentration of PCBs increases and how PCBs are selectivity release as the seals mobilized their lipids. It is recommended that monitoring programs should report not only the congener concentration but also the total body burden of PCBs, and follow a standardized procedure, trying to obtain whole blubber samples before a large amount of lipids has been mobilized. When analyzing levels of pollutants is important to obtain as much information on parameters that can influence an organism's PCB levels, such as sex, age, reproductive phase, nutritional and health status and environmental characteristics.

FUTURE DIRECTIONS

It is my hope that this study will form the basis for future work examining in greater detail the interactions between FA metabolism and PCB dynamics. Given the evidence provided here for selectivity in both these processes, and the fact that

this selectivity can partly be explained by physicochemical properties related to polarity, this may be a fruitful starting point. Models of PCB dynamics frequently incorporate the K_{ow} index to model the partition of congeners between body tissues. Extensions of these models, taking into account the potential influence of the mobilisation and transfer of FAs with differing degrees of polarity, will be a formidable challenge, but I suspect that it would be a challenge worth meeting. *In-vitro* studies are currently underway to examine the links between specific FA mobilisation and PCB congener release. Results from these studies should be helpful when parameterizing these models. The additional FA selectivity driven by nutritional and developmental needs will add to the complexity, but this results suggest that some initial assumptions may be made to account for these effects. Ultimately, such a model should be challenged by field data from naturally fasting (lactating and non-lactating) animals, and land-breeding phocid seals offer an ideal situation for such validation tests.

Complementary studies could also bring new information to the data presented. Some of the limitations of this study were the lack of dietary information of seals during the years of study. It would be of great value to obtain dietary information through scat analyses as well as obtaining information of FAs from the main candidate prey species from relevant regions and seasons. The lack of telemetry data, especially for NR also needs to be addressed. While a large number of seals have been instrumented on several non-breeding haulouts along the west coast, we cannot assume that these results are indicative of the feeding areas utilized by NR seals. It would also be important to establish, on a larger dataset, to what extent the tracks from post-breeding NR seals obtained previously are representative of this breeding population. Similarly, tracking studies should ideally be carried out on breeding females at the IOM, since this has not previously been done.

Another study that would be worthwile in order to gain further information about the factors that influence the mobilization of FAs and PCBs should be focussed on sampling seals during the moult. Our results could not determine to what extent the mobilization and transfer of specific FAs is a consequence of the specific needs associated with lactation. By

comparing moulting and lactating seals we could gain not only a better understanding of lipid dynamics in marine mammals but also of the degree to which the nutritional and developmental demands of pups influence the patterns of FA mobilisation and transfer. Ideally, this comparison should involve the same individual. While such comparisons cannot easily be done for grey seals, due to their gradual moult and unpredictable moult locations, elephant seals offer an interesting alternative system. These land-breeding seals often return to their preferred breeding beaches during their annual moult, and can therefore be re-located and captured.

The understanding of changes of FAs and PCBs through lactation are normally based on two or three stages during lactation: early, mid and late. Sampling between these stages could give us a better idea of when the changes occur and allow us to examine in greater detail the timing of the dramatic increases in PCB concentrations and its relation to the changes in FA mobilization, milk composition and milk output. These details would be extremely valuable in the formulation of PBPK models which rely to a large extent on equations and parameters for the flux of compounds between compartments.

In this study the different layers of blubber were not considered. This could be important given that blubber serves a variety of functions, including thermoregulation, streamlining the body, and energy storage. Hence, its composition varies with the different functions that dominate as one moves from the metabolically active core outwards to the more structural and less metabolically active skin (Strandberg et al 2008). Therefore, future work could compare the release of FAs and PCBs from the different layers found in the blubber. However, I believe that the analyses of different blubber layers at different stages of lactation can be difficult and must be done with caution. As the lipid is mobilized from the blubber the fat cells collapse, comparisons of layers between different lactation stages can be misleading. One alternative, as stated above, is to conduct these analyses on material from dead animals, as done by Strandberg et al (2008) for FAs. While this would not provide information at specific times of lactation, it would nevertheless provide useful information about the structure and degree of stratification of compounds within the blubber layer.

Continuing the dietary and toxicology research at NR and IOM is of great value due to the large dataset of known individuals, which provide a study system for monitoring trends in variations in FAs and pollutants. Regular sampling of the same females and their pups could give valuable information for models of the transfer of pollutants and lipids to the pups which could be used to predict lipids and pollutants dynamics in other species.

In summary, this thesis contains detailed data on the selective mobilisation and transfer of FAs and PCBs during lactation. I hope that it will be a useful reference point and a basis for further work examining the links between the vitally important dynamics of lipids and the adverse dynamics of organochlorines. The major fraction of all PCBs produced since their discovery have yet to find their way into the environment (Aguilar and Borrell 2005). Also, while some lipophilic pollutants are gradually disappearing from industrial use, others with similar characteristics are introduced. Given their very negative effects, particularly in ecosystems, further research into the fate and effect of organochlorines in organisms is critical.

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8 Appendices

Appendix 1. Fatty acid composition of blubber samples from grey seals (*Halichoerus grypus*) adult females at early lactation at Isle of May and North Rona. Values are the mean weight percent ± standard deviation. Number in parenthesis is the sample size for each year.

Fatty acids	Isle of May					
	1996 (33)	1997 (29)	1998 (30)	2004 (10)	2005 (14)	2006 (11)
18:1n-9	15.95 ± 3.21	15.29 ± 1.89	16.10 ± 2.45	18.96 ± 5.61	14.67 ± 3.12	15.49 ± 3.08
16:1n-7	10.07 ± 1.94	10.58 ± 1.48	12.72 ± 1.27	12.69 ± 2.12	11.49 ± 1.47	11.76 ± 2.68
20:1n-9	8.39 ± 2.08	6.92 ± 0.95	5.43 ± 1.19	4.93 ± 2.26	6.68 ± 2.15	5.86 ± 2.93
18:1n-11	4.69 ± 0.53	4.29 ± 0.63	4.11 ± 0.81	4.38 ± 1.29	4.15 ± 1.16	3.61 ± 1.40
22:1n-11	3.32 ± 1.52	2.88 ± 0.85	2.36 ± 1.14	1.69 ± 1.96	3.77 ± 1.95	3.01 ± 2.10
18:1n-7	2.76 ± 0.51	2.74 ± 0.42	3.21 ± 0.74	3.78 ± 1.13	3.27 ± 1.14	3.49 ± 1.18
20:1n-11	1.97 ± 0.24	1.81 ± 0.23	1.74 ± 0.29	1.61 ± 0.62	1.96 ± 0.44	1.65 ± 0.66
14:1n-5	0.94 ± 0.25	0.92 ± 0.20	1.15 ± 0.19	1.10 ± 0.31	0.96 ± 0.19	0.88 ± 0.15
22:1n-9	0.59 ± 0.20	0.46 ± 0.13	0.32 ± 0.09	0.29 ± 0.18	0.37 ± 0.14	0.31 ± 0.12
16:1n-9	0.44 ± 0.06	0.43 ± 0.05	0.43 ± 0.08	0.48 ± 0.16	0.38 ± 0.07	0.45 ± 0.10
20:1n-7	0.38 ± 0.17	0.31 ± 0.12	0.33 ± 0.14	0.32 ± 0.19	0.26 ± 0.16	0.32 ± 0.16
18:1n-5	0.33 ± 0.02	0.33 ± 0.02	0.29 ± 0.02	0.36 ± 0.04	0.38 ± 0.06	0.43 ± 0.10
17:1n-x	0.30 ± 0.09	0.28 ± 0.06	0.32 ± 0.10	0.38 ± 0.15	0.34 ± 0.11	0.40 ± 0.15
16:1n-5	0.28 ± 0.05	0.26 ± 0.02	0.23 ± 0.03	0.26 ± 0.05	0.27 ± 0.04	0.31 ± 0.06
14:1n-9	0.21 ± 0.05	0.23 ± 0.04	0.19 ± 0.03	0.11 ± 0.05	0.16 ± 0.05	0.15 ± 0.04
16:1n-11	0.19 ± 0.03	0.22 ± 0.03	0.21 ± 0.04	0.19 ± 0.03	0.22 ± 0.04	0.28 ± 0.05
24:1n-9	0.08 ± 0.05	0.12 ± 0.07	0.04 ± 0.04	0.11 ± 0.08	0.18 ± 0.05	0.17 ± 0.05
15:1n-x	0.06 ± 0.02	0.06 ± 0.01	0.09 ± 0.02	0.08 ± 0.03	0.07 ± 0.01	0.08 ± 0.02
14:1n-7	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.04 ± 0.01	0.05 ± 0.01
22:6n-3	11.94 ± 0.96	13.06 ± 0.87	14.00 ± 1.55	12.84 ± 1.29	13.00 ± 0.69	12.67 ± 1.64
20:5n-3	5.16 ± 0.93	5.71 ± 0.77	6.13 ± 1.14	5.00 ± 0.95	5.58 ± 0.99	5.77 ± 1.24
22:5n-3	4.34 ± 0.38	4.43 ± 0.32	5.21 ± 0.39	4.62 ± 0.73	4.61 ± 0.52	4.67 ± 0.65
18:2n-6	2.83 ± 0.42	2.51 ± 0.33	2.03 ± 0.24	2.04 ± 0.31	1.65 ± 0.24	1.91 ± 0.29
18:4n-3	2.77 ± 0.64	2.99 ± 0.54	1.79 ± 0.29	1.57 ± 0.66	2.03 ± 0.47	1.92 ± 0.69
18:3n-3	1.75 ± 0.25	1.65 ± 0.25	1.16 ± 0.13	1.00 ± 0.17	0.96 ± 0.22	1.04 ± 0.30
20:4n-3	1.02 ± 0.15	0.99 ± 0.15	0.80 ± 0.10	0.81 ± 0.14	0.84 ± 0.10	0.79 ± 0.15
21:5n-3	0.50 ± 0.10	0.50 ± 0.04	0.48 ± 0.05	0.45 ± 0.09	0.47 ± 0.06	0.42 ± 0.03
20:4n-6	0.45 ± 0.10	0.45 ± 0.10	0.60 ± 0.15	0.66 ± 0.24	0.64 ± 0.27	0.64 ± 0.29
16:2n-4	0.36 ± 0.06	0.40 ± 0.06	0.45 ± 0.11	0.39 ± 0.18	0.45 ± 0.11	0.37 ± 0.09
16:4n-1	0.34 ± 0.13	0.44 ± 0.13	0.52 ± 0.21	0.39 ± 0.35	0.47 ± 0.16	0.36 ± 0.13
20:2n-6	0.33 ± 0.04	0.29 ± 0.03	0.24 ± 0.05	0.27 ± 0.09	0.23 ± 0.09	0.27 ± 0.08
16:3n-4	0.22 ± 0.09	0.26 ± 0.07	0.28 ± 0.08	0.24 ± 0.12	0.32 ± 0.09	0.22 ± 0.06
22:4n-3	0.21 ± 0.05	0.21 ± 0.04	0.17 ± 0.03	0.17 ± 0.06	0.20 ± 0.04	0.17 ± 0.06
22:5n-6	0.19 ± 0.04	0.18 ± 0.03	0.22 ± 0.07	0.21 ± 0.10	0.20 ± 0.07	0.21 ± 0.07
16:3n-1	0.17 ± 0.05	0.08 ± 0.03	0.19 ± 0.03	0.06 ± 0.02	0.05 ± 0.01	0.05 ± 0.01
20:3n-3	0.16 ± 0.02	0.14 ± 0.02	0.11 ± 0.02	0.11 ± 0.03	0.11 ± 0.03	0.13 ± 0.03
18:3n-1	0.13 ± 0.03	0.08 ± 0.05	0.16 ± 0.04	0.16 ± 0.05	0.16 ± 0.04	0.09 ± 0.08
22:4n-6	0.13 ± 0.04	0.12 ± 0.04	0.18 ± 0.09	0.16 ± 0.09	0.20 ± 0.11	0.21 ± 0.13
18:4n-1	0.10 ± 0.03	0.12 ± 0.03	0.15 ± 0.05	0.13 ± 0.09	0.14 ± 0.04	0.11 ± 0.02
18:3n-4	0.10 ± 0.02	0.10 ± 0.02	0.13 ± 0.02	0.06 ± 0.02	0.07 ± 0.02	0.09 ± 0.04
18:2n-4	0.07 ± 0.01	0.09 ± 0.02	0.11 ± 0.02	0.13 ± 0.02	0.13 ± 0.05	0.12 ± 0.04
18:3n-6	0.06 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.12 ± 0.03	0.10 ± 0.01	0.08 ± 0.02
18:2d5_7	0.07 ± 0.01	0.06 ± 0.02	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.01
16	8.44 ± 0.95	9.43 ± 1.20	8.35 ± 1.09	9.13 ± 3.04	9.54 ± 1.37	10.13 ± 1.40
16iso	0.05 ± 0.02	0.04 ± 0.01	0.06 ± 0.02	0.07 ± 0.02	0.08 ± 0.03	0.12 ± 0.04
14	4.35 ± 0.41	4.27 ± 0.39	4.08 ± 0.53	3.79 ± 0.98	4.57 ± 0.66	4.54 ± 1.14

Fatty acids	Isle of May (continuation)					
	1996	1997	1998	2004	2005	2006
18	1.01 ± 0.15	1.11 ± 0.14	1.08 ± 0.17	1.20 ± 0.18	1.14 ± 0.24	1.29 ± 0.25
18iso	0.08 ± 0.04	0.19 ± 0.03	0.06 ± 0.02	0.16 ± 0.03	0.18 ± 0.03	0.21 ± 0.03
15	0.28 ± 0.04	0.29 ± 0.03	0.30 ± 0.05	0.31 ± 0.05	0.34 ± 0.03	0.39 ± 0.03
15iso	0.16 ± 0.02	0.18 ± 0.01	0.16 ± 0.02	0.16 ± 0.02	0.20 ± 0.02	0.22 ± 0.02
15anti	0.05 ± 0.01	0.05 ± 0.01	0.06 ± 0.02	0.05 ± 0.02	0.06 ± 0.01	0.08 ± 0.01
17	0.14 ± 0.03	0.15 ± 0.02	0.15 ± 0.04	0.20 ± 0.04	0.19 ± 0.04	0.22 ± 0.04
17anti	0.09 ± 0.02	0.09 ± 0.02	0.10 ± 0.04	0.13 ± 0.03	0.13 ± 0.05	0.18 ± 0.07
17iso	0.07 ± 0.04	0.07 ± 0.03	0.10 ± 0.05	0.07 ± 0.02	0.07 ± 0.02	0.09 ± 0.06
12	0.09 ± 0.01	0.10 ± 0.02	0.10 ± 0.01	0.10 ± 0.02	0.10 ± 0.01	0.10 ± 0.01
20	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.03	0.09 ± 0.01	0.08 ± 0.02
13	0.02 ± 0.00	0.02 ± 0.00	0.03 ± 0.01	0.02 ± 0.00	0.02 ± 0.00	0.03 ± 0.01
24	0.02 ± 0.01	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.01	0.02 ± 0.01

Fatty acids	North Rona					
	1996 (23)	1997 (25)	1998 (25)	2004 (10)	2005 (14)	2006 (18)
18:1n-9	15.94 ± 2.38	15.41 ± 2.78	14.79 ± 2.48	17.53 ± 1.60	14.17 ± 2.06	16.09 ± 2.78
16:1n-7	12.23 ± 1.60	11.62 ± 1.59	11.59 ± 1.15	12.05 ± 1.06	11.19 ± 1.23	11.76 ± 1.82
20:1n-9	6.06 ± 1.22	6.38 ± 1.25	6.45 ± 1.22	6.65 ± 0.93	6.39 ± 1.22	6.32 ± 1.27
18:1n-11	4.77 ± 0.79	4.95 ± 0.95	4.75 ± 0.75	5.39 ± 0.80	4.60 ± 0.82	4.33 ± 0.69
22:1n-11	2.68 ± 0.93	2.83 ± 1.12	3.30 ± 1.27	2.59 ± 0.78	3.61 ± 1.26	3.48 ± 1.43
18:1n-7	3.17 ± 0.66	3.00 ± 0.62	2.95 ± 0.66	3.07 ± 0.42	2.82 ± 0.71	3.12 ± 0.89
20:1n-11	1.97 ± 0.30	2.07 ± 0.38	2.02 ± 0.40	2.25 ± 0.25	1.96 ± 0.31	1.81 ± 0.29
14:1n-5	1.20 ± 0.26	1.15 ± 0.30	1.08 ± 0.22	1.16 ± 0.29	0.94 ± 0.23	1.02 ± 0.19
22:1n-9	0.33 ± 0.10	0.34 ± 0.07	0.32 ± 0.07	0.27 ± 0.06	0.33 ± 0.08	0.30 ± 0.07
16:1n-9	0.43 ± 0.07	0.43 ± 0.07	0.42 ± 0.07	0.47 ± 0.06	0.40 ± 0.06	0.43 ± 0.06
20:1n-7	0.26 ± 0.07	0.26 ± 0.07	0.22 ± 0.02	0.24 ± 0.08	0.19 ± 0.05	0.22 ± 0.06
18:1n-5	0.29 ± 0.02	0.28 ± 0.03	0.28 ± 0.03	0.30 ± 0.03	0.26 ± 0.03	0.28 ± 0.03
17:1n-x	0.36 ± 0.08	0.34 ± 0.08	0.33 ± 0.07	0.37 ± 0.05	0.36 ± 0.09	0.40 ± 0.09
16:1n-5	0.23 ± 0.02	0.22 ± 0.02	0.24 ± 0.03	0.26 ± 0.02	0.22 ± 0.01	0.24 ± 0.02
14:1n-9	0.23 ± 0.04	0.25 ± 0.05	0.25 ± 0.05	0.19 ± 0.08	0.20 ± 0.05	0.23 ± 0.06
16:1n-11	0.24 ± 0.06	0.25 ± 0.05	0.22 ± 0.04	0.23 ± 0.04	0.25 ± 0.06	0.25 ± 0.05
24:1n-9	0.05 ± 0.06	0.03 ± 0.04	0.02 ± 0.02	0.02 ± 0.01	0.23 ± 0.05	0.19 ± 0.04
15:1n-x	0.09 ± 0.02	0.08 ± 0.02	0.08 ± 0.02	0.10 ± 0.02	0.07 ± 0.01	0.08 ± 0.01
14:1n-7	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
22:6n-3	13.51 ± 1.03	13.64 ± 1.04	14.46 ± 1.37	11.94 ± 0.77	14.73 ± 1.48	12.50 ± 1.37
20:5n-3	5.60 ± 1.01	5.62 ± 1.16	5.18 ± 0.86	3.96 ± 0.48	4.92 ± 0.41	4.61 ± 0.57
22:5n-3	4.46 ± 0.41	4.56 ± 0.50	4.52 ± 0.60	3.70 ± 0.37	4.22 ± 0.33	3.80 ± 0.34
18:2n-6	1.63 ± 0.37	1.67 ± 0.38	1.57 ± 0.24	1.75 ± 0.17	1.41 ± 0.14	1.67 ± 0.35
18:4n-3	1.62 ± 0.52	1.77 ± 0.46	1.88 ± 0.40	1.85 ± 0.18	2.05 ± 0.49	1.82 ± 0.42
18:3n-3	0.82 ± 0.26	0.90 ± 0.25	0.92 ± 0.18	0.96 ± 0.06	0.94 ± 0.14	0.93 ± 0.17
20:4n-3	0.71 ± 0.13	0.75 ± 0.14	0.76 ± 0.10	0.91 ± 0.08	0.94 ± 0.10	0.86 ± 0.12
21:5n-3	0.46 ± 0.06	0.47 ± 0.05	0.46 ± 0.07	0.37 ± 0.04	0.42 ± 0.07	0.37 ± 0.07
20:4n-6	0.69 ± 0.18	0.65 ± 0.19	0.61 ± 0.12	0.58 ± 0.12	0.63 ± 0.18	0.60 ± 0.15
16:2n-4	0.46 ± 0.12	0.48 ± 0.13	0.45 ± 0.13	0.37 ± 0.04	0.34 ± 0.09	0.36 ± 0.11

Fatty acids	North Rona (continuation)					
	1996	1997	1998	2004	2005	2006
16:4n-1	0.49 ± 0.18	0.54 ± 0.22	0.46 ± 0.18	0.30 ± 0.07	0.34 ± 0.11	0.34 ± 0.14
20:2n-6	0.23 ± 0.04	0.23 ± 0.04	0.22 ± 0.03	0.25 ± 0.04	0.22 ± 0.05	0.22 ± 0.03
16:3n-4	0.23 ± 0.08	0.27 ± 0.09	0.26 ± 0.09	0.15 ± 0.06	0.27 ± 0.07	0.21 ± 0.06
22:4n-3	0.14 ± 0.03	0.15 ± 0.03	0.16 ± 0.03	0.18 ± 0.03	0.19 ± 0.03	0.16 ± 0.03
22:5n-6	0.21 ± 0.03	0.23 ± 0.05	0.23 ± 0.05	0.26 ± 0.05	0.30 ± 0.07	0.25 ± 0.05
16:3n-1	0.16 ± 0.06	0.18 ± 0.03	0.19 ± 0.03	0.06 ± 0.01	0.06 ± 0.01	0.05 ± 0.02
20:3n-3	0.12 ± 0.03	0.12 ± 0.02	0.12 ± 0.02	0.11 ± 0.01	0.11 ± 0.01	0.13 ± 0.01
18:3n-1	0.16 ± 0.02	0.21 ± 0.03	0.16 ± 0.06	0.15 ± 0.03	0.19 ± 0.05	0.08 ± 0.02
22:4n-6	0.19 ± 0.07	0.17 ± 0.07	0.17 ± 0.05	0.16 ± 0.04	0.20 ± 0.10	0.18 ± 0.07
18:4n-1	0.15 ± 0.04	0.16 ± 0.05	0.14 ± 0.04	0.10 ± 0.03	0.10 ± 0.03	0.11 ± 0.03
18:3n-4	0.12 ± 0.02	0.12 ± 0.02	0.12 ± 0.02	0.13 ± 0.04	0.05 ± 0.02	0.06 ± 0.02
18:2n-4	0.12 ± 0.03	0.12 ± 0.03	0.11 ± 0.03	0.07 ± 0.02	0.09 ± 0.01	0.08 ± 0.01
18:3n-6	0.07 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.07 ± 0.05	0.11 ± 0.01	0.07 ± 0.03
18:2d5_7	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01
16	9.18 ± 1.14	9.01 ± 1.29	9.36 ± 0.93	9.73 ± 1.42	10.42 ± 1.31	10.72 ± 0.98
16iso	0.07 ± 0.02	0.07 ± 0.02	0.07 ± 0.02	0.07 ± 0.01	0.07 ± 0.02	0.08 ± 0.02
14	4.52 ± 0.60	4.68 ± 0.71	4.75 ± 0.77	4.90 ± 0.43	4.81 ± 0.69	5.11 ± 0.91
18	1.19 ± 0.20	1.20 ± 0.19	1.19 ± 0.15	1.12 ± 0.10	1.18 ± 0.15	1.25 ± 0.18
18iso	0.09 ± 0.05	0.06 ± 0.02	0.06 ± 0.01	0.18 ± 0.02	0.17 ± 0.02	0.21 ± 0.02
15	0.34 ± 0.04	0.33 ± 0.03	0.36 ± 0.04	0.38 ± 0.04	0.38 ± 0.04	0.40 ± 0.05
15iso	0.19 ± 0.02	0.18 ± 0.02	0.18 ± 0.03	0.20 ± 0.02	0.20 ± 0.02	0.20 ± 0.03
15anti	0.05 ± 0.02	0.05 ± 0.02	0.05 ± 0.02	0.06 ± 0.02	0.05 ± 0.01	0.07 ± 0.01
17	0.17 ± 0.04	0.16 ± 0.03	0.17 ± 0.03	0.19 ± 0.02	0.20 ± 0.03	0.22 ± 0.03
17anti	0.10 ± 0.02	0.09 ± 0.02	0.08 ± 0.01	0.09 ± 0.02	0.10 ± 0.02	0.11 ± 0.02
17iso	0.11 ± 0.03	0.09 ± 0.03	0.09 ± 0.02	0.19 ± 0.07	0.10 ± 0.02	0.04 ± 0.02
12	0.12 ± 0.02	0.11 ± 0.02	0.11 ± 0.03	0.12 ± 0.02	0.11 ± 0.02	0.10 ± 0.02
20	0.06 ± 0.02	0.06 ± 0.02	0.07 ± 0.02	0.10 ± 0.02	0.10 ± 0.02	0.09 ± 0.02
13	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.02	0.03 ± 0.01
24	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.01

Appendix 2. Fatty acid composition of blubber and milk of grey seal (*Halichoerus grypus*) female and pups at early and late lactation in Isle of May 2004. Values are the mean weight percent and S.D.

	Blubber			Milk	
Fatty acids	Early Mum (n=14)	Late Mum (n=14)	Late Pup (n=16)	Early Mum (n=10)	Late Mum (n=10)
12	0.1 ± 0.01	0.11 ± 0.02	0.11 ± 0.01	0.13 ± 0.01	0.14 ± 0.02
13	0.02 ± 0	0.02 ± 0	0.02 ± 0.01	0.02 ± 0	0.02 ± 0.01
14	3.84 ± 0.83	3.97 ± 0.82	4.19 ± 0.63	4.04 ± 0.94	4.24 ± 0.92
15	0.32 ± 0.05	0.29 ± 0.07	0.33 ± 0.04	0.41 ± 0.03	0.33 ± 0.03
anti15	0.05 ± 0.02	0.06 ± 0.02	0.06 ± 0.02	0.05 ± 0.01	0.06 ± 0.02
iso15	0.16 ± 0.02	0.17 ± 0.03	0.17 ± 0.02	0.17 ± 0.02	0.18 ± 0.02
16	9.46 ± 2.5	8.84 ± 3.11	10.01 ± 2.15	15.85 ± 1.63	11.34 ± 1.93
iso16	0.07 ± 0.02	0.07 ± 0.02	0.07 ± 0.02	0.08 ± 0.02	0.08 ± 0.02
17	0.19 ± 0.05	0.18 ± 0.06	0.19 ± 0.05	0.29 ± 0.06	0.23 ± 0.04
anti17	0.13 ± 0.04	0.12 ± 0.04	0.12 ± 0.03	0.13 ± 0.05	0.13 ± 0.05
iso17	0.06 ± 0.02	0.08 ± 0.06	0.08 ± 0.05	0.22 ± 0.11	0.21 ± 0.08
18	1.23 ± 0.18	1.2 ± 0.31	1.26 ± 0.24	2.24 ± 0.3	1.8 ± 0.25
iso18	0.15 ± 0.04	0.14 ± 0.05	0.15 ± 0.02	0.17 ± 0.04	0.16 ± 0.05
20	0.08 ± 0.03	0.08 ± 0.03	0.09 ± 0.03	0.06 ± 0.01	0.08 ± 0.01
24	0.02 ± 0	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.04 ± 0.03
SFA	15.88 ± 2.94	15.34 ± 4.05	16.86 ± 2.39	23.88 ± 2.07	19.03 ± 2.34
14:1n-5	1.08 ± 0.26	1.19 ± 0.36	1.06 ± 0.27	0.54 ± 0.17	0.76 ± 0.17
14:1n-7	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.05 ± 0.02	0.05 ± 0.01
14:1n-9	0.11 ± 0.06	0.13 ± 0.06	0.13 ± 0.06	0.08 ± 0.04	0.15 ± 0.08
15:1n-x	0.08 ± 0.02	0.09 ± 0.03	0.08 ± 0.02	0.05 ± 0.02	0.06 ± 0.02
16:1n-11	0.21 ± 0.05	0.18 ± 0.06	0.21 ± 0.05	0.23 ± 0.05	0.18 ± 0.03
16:1n-5	0.25 ± 0.04	0.24 ± 0.04	0.26 ± 0.04	0.29 ± 0.03	0.23 ± 0.03
16:1n-7	13.19 ± 2.11	12.77 ± 2.05	13.09 ± 1.5	11.04 ± 1.42	10.69 ± 1.26
16:1n-9	0.47 ± 0.14	0.47 ± 0.11	0.45 ± 0.11	0.48 ± 0.14	0.41 ± 0.12
17:1n-x	0.36 ± 0.16	0.34 ± 0.15	0.34 ± 0.1	0.33 ± 0.15	0.31 ± 0.13
18:1n-11	4.19 ± 1.24	4.76 ± 1.33	4.23 ± 1.32	4.14 ± 1.51	4.84 ± 1.2
18:1n-5	0.34 ± 0.06	0.32 ± 0.05	0.32 ± 0.04	0.34 ± 0.06	0.32 ± 0.06
18:1n-7	3.71 ± 1.2	3.6 ± 0.92	3.58 ± 0.97	4.25 ± 1.17	3.56 ± 0.98
18:1n-9	17.84 ± 5.01	17.44 ± 3.64	16.36 ± 3.18	14.84 ± 2.93	15.73 ± 4.04
20:1n-11	1.58 ± 0.58	1.96 ± 0.57	1.77 ± 0.61	1.35 ± 0.64	1.96 ± 0.67
20:1n-7	0.29 ± 0.17	0.3 ± 0.16	0.26 ± 0.11	0.19 ± 0.07	0.3 ± 0.14
20:1n-9	4.97 ± 2.34	5.87 ± 2.06	5.37 ± 1.87	3.15 ± 1.49	4.96 ± 1.83
22:1n-11	1.93 ± 1.98	2.24 ± 1.69	2.37 ± 1.66	1.44 ± 1.13	2.23 ± 1.75
22:1n-9	0.28 ± 0.16	0.31 ± 0.14	0.27 ± 0.1	0.22 ± 0.08	0.49 ± 0.48
24:1n-9	0.13 ± 0.09	0.13 ± 0.09	0.14 ± 0.09	0.14 ± 0.04	0.19 ± 0.05
MUFAs	51.06 ± 2.98	52.38 ± 3.20	50.30 ± 3.27	43.14 ± 2.96	47.39 ± 3.19

Fatty acids	Blubber			Milk	
	Early Mum (n=14)	Late Mum (n=14)	Late Pup (n=16)	Early Mum (n=10)	Late Mum (n=10)
16:2n-4	0.41 ± 0.17	0.44 ± 0.16	0.45 ± 0.12	0.41 ± 0.17	0.41 ± 0.15
16:2n-6	0.08 ± 0.02	0.08 ± 0.02	0.07 ± 0.02	0.12 ± 0.04	0.07 ± 0.01
16:3n-1	0.07 ± 0.02	0.05 ± 0.02	0.07 ± 0.02	0 ± 0	0 ± 0
16:3n-4	0.27 ± 0.11	0.28 ± 0.1	0.29 ± 0.07	0.29 ± 0.1	0.3 ± 0.12
16:4n-1	0.43 ± 0.33	0.48 ± 0.26	0.47 ± 0.21	0.49 ± 0.31	0.48 ± 0.29
18:2d5_7	0.04 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
18:2n-4	0.13 ± 0.02	0.13 ± 0.04	0.13 ± 0.03	0.16 ± 0.02	0.12 ± 0.02
18:2n-6	1.95 ± 0.28	1.91 ± 0.39	1.92 ± 0.29	1.67 ± 0.27	1.83 ± 0.26
18:3n-1	0.14 ± 0.05	0.12 ± 0.06	0.13 ± 0.04	0.12 ± 0.04	0.12 ± 0.07
18:3n-3	1 ± 0.21	0.92 ± 0.21	1.02 ± 0.26	0.69 ± 0.25	0.88 ± 0.26
18:3n-4	0.06 ± 0.02	0.08 ± 0.03	0.08 ± 0.04	0.09 ± 0.03	0.07 ± 0.02
18:3n-6	0.12 ± 0.03	0.11 ± 0.03	0.12 ± 0.04	0.31 ± 0.12	0.15 ± 0.02
18:4n-1	0.14 ± 0.08	0.15 ± 0.1	0.15 ± 0.08	0.23 ± 0.11	0.13 ± 0.07
18:4n-3	1.64 ± 0.6	1.5 ± 0.36	1.77 ± 0.53	1.77 ± 0.6	1.63 ± 0.6
20:2n-6	0.26 ± 0.09	0.27 ± 0.1	0.28 ± 0.08	0.23 ± 0.07	0.29 ± 0.09
20:3n-3	0.11 ± 0.03	0.1 ± 0.03	0.1 ± 0.02	0.09 ± 0.02	0.1 ± 0.03
20:3n-6	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.02	0.1 ± 0.02	0.15 ± 0.21
20:4n-3	0.78 ± 0.11	0.76 ± 0.1	0.79 ± 0.09	0.73 ± 0.14	0.73 ± 0.11
20:4n-6	0.68 ± 0.26	0.67 ± 0.32	0.65 ± 0.28	1.13 ± 0.51	0.76 ± 0.42
20:5n-3	5.26 ± 0.95	4.64 ± 0.82	5.09 ± 1.13	7.16 ± 1.25	4.24 ± 1.57
21:5n-3	0.46 ± 0.09	0.47 ± 0.09	0.47 ± 0.06	0.43 ± 0.09	0.46 ± 0.09
22:4n-3	0.16 ± 0.06	0.18 ± 0.05	0.17 ± 0.05	0.13 ± 0.04	0.19 ± 0.05
22:4n-6	0.17 ± 0.09	0.18 ± 0.08	0.17 ± 0.08	0.18 ± 0.1	0.25 ± 0.16
22:5n-3	4.62 ± 0.61	4.8 ± 0.76	4.59 ± 0.54	4.06 ± 0.45	4.78 ± 0.66
22:5n-6	0.21 ± 0.09	0.23 ± 0.09	0.21 ± 0.07	0.15 ± 0.05	0.25 ± 0.09
22:6n-3	12.83 ± 1.18	12.66 ± 1.32	12.6 ± 1.39	11.29 ± 1.44	14.26 ± 1.32
PUFAs	32.09 ± 1.79	31.34 ± 2.20	31.90 ± 2.42	32.05 ± 2.61	32.68 ± 2.19

Appendix 3. Fatty acid composition of blubber and milk of grey seal (*Halichoerus grypus*) female and pups at early and late lactation in North Rona 2004. Values are the mean weight percent and S.D.

Fatty acids	Blubber		Milk	
	Early Mum (n= 10)	Late Mum (n=7)	Early Mum (n=10)	Late Mum (n=8)
12	0.12 ± 0.02	0.12 ± 0.01	0.13 ± 0.02	0.12 ± 0.02
13	0.03 ± 0.01	0.02 ± 0	0.02 ± 0	0.02 ± 0
14	5.04 ± 0.5	4.8 ± 0.43	4.95 ± 0.39	4.83 ± 0.55
15	0.39 ± 0.04	0.37 ± 0.06	0.45 ± 0.04	0.4 ± 0.04
anti15	0.07 ± 0.02	0.07 ± 0.01	0.05 ± 0.01	0.05 ± 0.01
iso15	0.21 ± 0.03	0.2 ± 0.02	0.2 ± 0.02	0.2 ± 0.02
16	9.94 ± 1.25	10.06 ± 2.29	16.46 ± 2.12	13.27 ± 1.24
iso16	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01
17	0.2 ± 0.03	0.19 ± 0.04	0.3 ± 0.04	0.25 ± 0.02
anti17	0.09 ± 0.02	0.14 ± 0.11	0.09 ± 0.01	0.1 ± 0.01
iso17	0.21 ± 0.06	0.16 ± 0.09	0.2 ± 0.08	0.2 ± 0.03
18	1.16 ± 0.14	1.81 ± 1.51	2.06 ± 0.34	1.77 ± 0.19
iso18	0.17 ± 0.02	0.16 ± 0.07	0.19 ± 0.03	0.16 ± 0.02
20	0.1 ± 0.02	0.1 ± 0.03	0.06 ± 0.01	0.07 ± 0.01
24	0.01 ± 0	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
SFA	17.8 ± 1.70	18.28 ± 2.60	25.26 ± 2.36	21.53 ± 1.51
14:1n-9	0.2 ± 0.07	0.2 ± 0.08	0.12 ± 0.06	0.16 ± 0.05
14:1n-7	0.05 ± 0.01	0.06 ± 0.01	0.04 ± 0.01	0.03 ± 0.01
14:1n-5	1.12 ± 0.25	1.23 ± 0.29	0.48 ± 0.17	0.63 ± 0.18
15:1n-x	0.09 ± 0.02	0.1 ± 0.02	0.04 ± 0.01	0.05 ± 0.01
16:1n-11	0.24 ± 0.04	0.23 ± 0.06	0.23 ± 0.03	0.21 ± 0.04
16:1n-9	0.46 ± 0.06	0.46 ± 0.06	0.46 ± 0.07	0.4 ± 0.03
16:1n-7	11.69 ± 1.06	12.67 ± 2.12	9.08 ± 0.91	9.67 ± 1.25
16:1n-5	0.26 ± 0.01	0.26 ± 0.04	0.28 ± 0.03	0.24 ± 0.02
17:1n-x	0.37 ± 0.05	0.34 ± 0.14	0.36 ± 0.1	0.4 ± 0.05
18:1n-11	5.31 ± 0.7	7.14 ± 5.18	5.24 ± 0.51	5.39 ± 0.64
18:1n-9	17.09 ± 1.96	14.72 ± 5.48	14.92 ± 1.98	15.54 ± 1.21
18:1n-7	2.98 ± 0.44	2.8 ± 1.35	3.13 ± 0.52	2.96 ± 0.55
18:1n-5	0.3 ± 0.02	0.51 ± 0.57	0.3 ± 0.02	0.29 ± 0.01
20:1n-11	2.23 ± 0.22	2.21 ± 0.64	1.78 ± 0.38	2.17 ± 0.33
20:1n-9	6.82 ± 0.91	6.44 ± 1.81	4.59 ± 1.22	5.82 ± 0.88
20:1n-7	0.24 ± 0.07	0.22 ± 0.05	0.15 ± 0.04	0.17 ± 0.03
22:1n-11	2.8 ± 0.86	2.43 ± 0.91	2.19 ± 0.71	2.38 ± 0.6
22:1n-9	0.29 ± 0.07	0.26 ± 0.09	0.3 ± 0.07	0.34 ± 0.07
24:1n-9	0.02 ± 0.01	0.02 ± 0.01	0.15 ± 0.05	0.18 ± 0.04
MUFAs	52.55 ± 2.11	52.27 ± 3.19	43.82 ± 3.06	47.03 ± 2.21

Appendix 3. Continued

Fatty acids	Blubber		Milk	
	Early Mum (n= 10)	Late Mum (n=7)	Early Mum (n=10)	Late Mum (n=8)
16:2n-6	0.03 ± 0.02	0.05 ± 0.03	0.08 ± 0.02	0.06 ± 0.01
16:2n-4	0.37 ± 0.04	0.35 ± 0.1	0.34 ± 0.07	0.31 ± 0.05
16:3n-4	0.15 ± 0.05	0.24 ± 0.14	0.24 ± 0.05	0.24 ± 0.03
16:3n-1	0.06 ± 0.02	0.08 ± 0.06	0 ± 0	0 ± 0
16:4n-1	0.3 ± 0.06	0.38 ± 0.25	0.31 ± 0.09	0.29 ± 0.07
18:2d5_7	0.03 ± 0.01	0.06 ± 0.09	0.03 ± 0.01	0.03 ± 0.01
18:2n-6	1.71 ± 0.15	1.38 ± 0.6	1.49 ± 0.11	1.53 ± 0.15
18:2n-4	0.07 ± 0.02	0.06 ± 0.03	0.11 ± 0.03	0.08 ± 0.01
18:3n-6	0.06 ± 0.04	0.08 ± 0.06	0.25 ± 0.07	0.15 ± 0.01
18:3n-4	0.13 ± 0.03	0.23 ± 0.33	0.06 ± 0.03	0.05 ± 0.01
18:3n-3	0.97 ± 0.08	0.78 ± 0.31	0.82 ± 0.17	0.89 ± 0.13
18:3n-1	0.15 ± 0.04	0.38 ± 0.59	0.09 ± 0.03	0.16 ± 0.03
18:4n-3	1.91 ± 0.31	1.48 ± 0.72	2.07 ± 0.42	1.82 ± 0.37
18:4n-1	0.1 ± 0.02	0.08 ± 0.02	0.14 ± 0.07	0.09 ± 0.02
20:2n-6	0.26 ± 0.04	0.25 ± 0.03	0.24 ± 0.02	0.24 ± 0.03
20:3n-6	0.07 ± 0.01	0.07 ± 0.01	0.1 ± 0.02	0.08 ± 0.01
20:4n-6	0.58 ± 0.12	0.61 ± 0.18	0.91 ± 0.23	0.81 ± 0.17
20:3n-3	0.11 ± 0.01	0.1 ± 0.01	0.1 ± 0.02	0.1 ± 0.01
20:4n-3	0.93 ± 0.08	0.77 ± 0.13	0.87 ± 0.1	0.85 ± 0.12
20:5n-3	3.93 ± 0.42	4.03 ± 1.26	5.61 ± 1.15	4.27 ± 0.87
21:5n-3	0.38 ± 0.04	0.37 ± 0.05	0.38 ± 0.09	0.37 ± 0.05
22:4n-6	0.16 ± 0.05	0.16 ± 0.06	0.15 ± 0.05	0.17 ± 0.06
22:5n-6	0.27 ± 0.05	0.27 ± 0.06	0.2 ± 0.05	0.27 ± 0.05
22:4n-3	0.18 ± 0.03	0.16 ± 0.03	0.15 ± 0.06	0.19 ± 0.04
22:5n-3	3.67 ± 0.34	3.64 ± 0.22	3.36 ± 0.43	3.58 ± 0.26
22:6n-3	12.14 ± 0.83	12.36 ± 0.98	11.8 ± 1.82	13.87 ± 0.67
PUFAs	28.71 ± 1.11	28.45 ± 1.31	29.87 ± 2.84	30.49 ± 1.27

Appendix 4. Fatty acid composition of blubber and milk of grey seal (*Halichoerus grypus*) female and pups at early, mid and late lactation in Isle of May 2005. Values are the mean weight percent and S.D.

	Blubber			Milk		
Fatty acids	Early	Late	Late	Early	Mid	Late
	Mum (n=24)	Mum (n=11)	Pup (n=11)	Mum (n=11)	Mum (n=10)	Mum (n=11)
12	0.1 ± 0.01	0.09 ± 0.02	0.1 ± 0.02	0.12 ± 0.02	0.12 ± 0.02	0.12 ± 0.03
13	0.03 ± 0	0.02 ± 0.01	0.02 ± 0	0.02 ± 0	0.02 ± 0	0.02 ± 0.01
14	4.65 ± 0.78	3.99 ± 0.7	4.29 ± 0.7	4.79 ± 0.89	4.96 ± 0.82	4.69 ± 0.84
15	0.34 ± 0.04	0.28 ± 0.03	0.38 ± 0.03	0.46 ± 0.05	0.41 ± 0.05	0.37 ± 0.04
anti15	0.06 ± 0.01	0.08 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01
iso15	0.2 ± 0.02	0.19 ± 0.02	0.18 ± 0.02	0.2 ± 0.02	0.2 ± 0.01	0.21 ± 0.02
16	9.48 ± 1.33	7.18 ± 1.21	12.18 ± 1.3	17.14 ± 2.47	14.16 ± 1.23	12.15 ± 1.6
iso16	0.08 ± 0.02	0.09 ± 0.02	0.1 ± 0.03	0.12 ± 0.03	0.12 ± 0.04	0.12 ± 0.02
17	0.19 ± 0.04	0.15 ± 0.03	0.23 ± 0.03	0.31 ± 0.06	0.26 ± 0.06	0.24 ± 0.04
anti17	0.13 ± 0.04	0.16 ± 0.06	0.15 ± 0.07	0.15 ± 0.06	0.15 ± 0.07	0.16 ± 0.06
iso17	0.07 ± 0.02	0.1 ± 0.05	0.06 ± 0.02	0.08 ± 0.05	0.09 ± 0.03	0.09 ± 0.06
18	1.13 ± 0.22	1.1 ± 0.24	1.44 ± 0.2	2.35 ± 0.45	1.9 ± 0.35	1.8 ± 0.24
iso18	0.17 ± 0.03	0.19 ± 0.07	0.2 ± 0.06	0.25 ± 0.07	0.23 ± 0.1	0.23 ± 0.07
20	0.09 ± 0.01	0.1 ± 0.03	0.05 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.07 ± 0.02
24	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0	0.03 ± 0.02	0.02 ± 0	0.02 ± 0.01
SFA	16.73 ± 1.75	13.76 ± 1.72	19.46 ± 1.45	26.15 ± 3.01	22.78 ± 1.24	20.34 ± 1.73
14:1n-5	0.96 ± 0.17	1.16 ± 0.35	0.73 ± 0.13	0.43 ± 0.12	0.56 ± 0.11	0.68 ± 0.16
14:1n-7	0.04 ± 0.01	0.06 ± 0.02	0.06 ± 0.01	0.06 ± 0.02	0.05 ± 0.02	0.06 ± 0.02
14:1n-9	0.16 ± 0.05	0.19 ± 0.06	0.09 ± 0.03	0.13 ± 0.04	0.16 ± 0.07	0.19 ± 0.06
15:1n-x	0.07 ± 0.01	0.09 ± 0.03	0.07 ± 0.01	0.04 ± 0.02	0.05 ± 0.01	0.06 ± 0.02
16:1n-11	0.21 ± 0.04	0.17 ± 0.04	0.2 ± 0.05	0.34 ± 0.23	0.24 ± 0.04	0.21 ± 0.05
16:1n-5	0.27 ± 0.04	0.24 ± 0.04	0.32 ± 0.04	0.33 ± 0.04	0.31 ± 0.05	0.28 ± 0.04
16:1n-7	11.45 ± 1.45	11.17 ± 2.45	13.01 ± 1.96	10 ± 1.96	10.72 ± 1.87	10.63 ± 1.89
16:1n-9	0.38 ± 0.08	0.46 ± 0.08	0.46 ± 0.07	0.55 ± 0.16	0.4 ± 0.06	0.44 ± 0.1
17:1n-x	0.32 ± 0.1	0.34 ± 0.1	0.4 ± 0.12	0.35 ± 0.13	0.34 ± 0.14	0.34 ± 0.12
18:1n-11	4.25 ± 1.02	4.72 ± 1.24	4.41 ± 1.8	4.18 ± 1.57	3.99 ± 1.78	4.4 ± 1.57
18:1n-5	0.38 ± 0.05	0.37 ± 0.05	0.42 ± 0.08	0.43 ± 0.08	0.42 ± 0.09	0.42 ± 0.08
18:1n-7	3.08 ± 0.99	3.37 ± 1.07	3.93 ± 1.43	4.22 ± 1.52	3.86 ± 1.87	3.62 ± 1.22
18:1n-9	14.37 ± 3.44	16.84 ± 2.59	16.28 ± 1.55	14.59 ± 1.7	13.93 ± 1.42	15.57 ± 2.26
20:1n-11	1.91 ± 0.43	2.32 ± 0.78	1.74 ± 0.73	1.58 ± 0.65	1.84 ± 0.52	2.02 ± 0.7
20:1n-7	0.26 ± 0.18	0.31 ± 0.14	0.19 ± 0.06	0.21 ± 0.08	0.22 ± 0.07	0.28 ± 0.1
20:1n-9	6.99 ± 2.17	7.43 ± 3.39	5.14 ± 2.28	4.38 ± 2.07	5.73 ± 2.71	6.26 ± 2.53
22:1n-11	3.96 ± 1.93	3.33 ± 2.19	1.24 ± 0.77	1.97 ± 1.25	2.42 ± 1.39	2.43 ± 1.34
22:1n-9	0.38 ± 0.13	0.37 ± 0.14	0.17 ± 0.07	0.3 ± 0.1	0.3 ± 0.12	0.36 ± 0.1
24:1n-9	0.19 ± 0.05	0.21 ± 0.07	0.05 ± 0.01	0.13 ± 0.03	0.14 ± 0.05	0.17 ± 0.04
MUFAs	49.64 ± 2.26	53.15 ± 3.83	48.91 ± 2.12	44.21 ± 2.59	45.68 ± 2.04	48.39 ± 3.58

Appendix 4. Continued

Fatty acids	Blubber			Milk		
	Early Mum (n=24)	Late Mum (n=11)	Late Pup (n=11)	Early Mum (n=11)	Mid Mum (n=10)	Late Mum (n=11)
16:2n-4	0.47 ± 0.11	0.42 ± 0.1	0.44 ± 0.11	0.43 ± 0.12	0.49 ± 0.11	0.45 ± 0.11
16:2n-6	0.07 ± 0.01	0.08 ± 0.02	0.09 ± 0.02	0.12 ± 0.05	0.09 ± 0.02	0.08 ± 0.02
16:3n-1	0.05 ± 0.01	0.06 ± 0.04	0.08 ± 0.01	0.06 ± 0.02	0.06 ± 0.01	0.05 ± 0.01
16:3n-4	0.32 ± 0.08	0.29 ± 0.07	0.31 ± 0.06	0.3 ± 0.09	0.38 ± 0.1	0.33 ± 0.09
16:4n-1	0.48 ± 0.16	0.38 ± 0.12	0.4 ± 0.11	0.38 ± 0.15	0.51 ± 0.11	0.43 ± 0.14
18:2d5_7	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
18:2n-4	0.12 ± 0.04	0.12 ± 0.04	0.15 ± 0.06	0.17 ± 0.06	0.16 ± 0.08	0.13 ± 0.05
18:2n-6	1.71 ± 0.24	2 ± 0.24	1.69 ± 0.26	1.53 ± 0.23	1.56 ± 0.21	1.7 ± 0.24
18:3n-1	0.17 ± 0.05	0.12 ± 0.06	0.09 ± 0.02	0.04 ± 0.03	0.03 ± 0.03	0.06 ± 0.04
18:3n-3	0.97 ± 0.19	0.97 ± 0.18	0.82 ± 0.24	0.63 ± 0.22	0.82 ± 0.25	0.81 ± 0.2
18:3n-4	0.07 ± 0.02	0.1 ± 0.03	0.12 ± 0.04	0.14 ± 0.07	0.11 ± 0.06	0.1 ± 0.03
18:3n-6	0.1 ± 0.01	0.11 ± 0.03	0.17 ± 0.02	0.28 ± 0.11	0.15 ± 0.02	0.12 ± 0.02
18:4n-1	0.14 ± 0.04	0.11 ± 0.03	0.17 ± 0.04	0.2 ± 0.05	0.18 ± 0.05	0.14 ± 0.04
18:4n-3	2.06 ± 0.5	1.65 ± 0.45	1.9 ± 0.48	1.97 ± 0.68	2.3 ± 0.33	1.87 ± 0.51
20:2n-6	0.22 ± 0.09	0.26 ± 0.06	0.22 ± 0.05	0.19 ± 0.05	0.19 ± 0.05	0.21 ± 0.05
20:3n-3	0.1 ± 0.03	0.11 ± 0.02	0.1 ± 0.02	0.08 ± 0.01	0.1 ± 0.02	0.1 ± 0.02
20:3n-6	0.07 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.09 ± 0.02	0.07 ± 0.01	0.08 ± 0.01
20:4n-3	0.83 ± 0.09	0.79 ± 0.08	0.81 ± 0.14	0.76 ± 0.15	0.77 ± 0.11	0.72 ± 0.1
20:4n-6	0.58 ± 0.23	0.59 ± 0.22	0.88 ± 0.43	1.04 ± 0.5	0.96 ± 0.6	0.8 ± 0.35
20:5n-3	5.55 ± 0.97	4.09 ± 1.24	5.25 ± 1.06	6.68 ± 1.24	6.27 ± 1.26	4.92 ± 1.41
21:5n-3	0.47 ± 0.06	0.48 ± 0.06	0.44 ± 0.06	0.38 ± 0.07	0.43 ± 0.06	0.44 ± 0.07
22:4n-3	0.2 ± 0.04	0.21 ± 0.05	0.17 ± 0.04	0.13 ± 0.03	0.17 ± 0.04	0.18 ± 0.04
22:4n-6	0.17 ± 0.09	0.23 ± 0.13	0.19 ± 0.12	0.17 ± 0.11	0.17 ± 0.12	0.19 ± 0.12
22:5n-3	4.55 ± 0.6	5.24 ± 0.71	4.34 ± 0.51	3.41 ± 0.45	3.68 ± 0.45	4.1 ± 0.75
22:5n-6	0.2 ± 0.07	0.26 ± 0.1	0.16 ± 0.07	0.12 ± 0.04	0.15 ± 0.06	0.19 ± 0.09
22:6n-3	13.03 ± 1.38	13.29 ± 1.76	11.4 ± 1.27	9.06 ± 0.91	10.56 ± 0.87	11.85 ± 1.65
PUFAs	32.73 ± 2.19	32.10 ± 2.95	30.51 ± 1.79	28.40 ± 2.44	30.42 ± 1.84	30.10 ± 3.11

Appendix 5. Fatty acid composition of blubber and milk of grey seal (*Halichoerus grypus*) female and pups at early, mid and late lactation in North Rona 2005. Values are the mean weight percent and S.D.

Fatty acids	Blubber			Milk		
	Early Mum (n=21)	Late Mum (n=21)	Late Pup (n=11)	Early Mum (n=8)	Mid Mum (n=12)	Late Mum (n=11)
12	0.11 ± 0.02	0.1 ± 0.02	0.11 ± 0.02	0.14 ± 0.02	0.14 ± 0.01	0.11 ± 0.04
13	0.04 ± 0.02	0.02 ± 0.01	0.02 ± 0	0.03 ± 0.01	0.16 ± 0.45	0.03 ± 0.01
14	4.68 ± 0.71	4.73 ± 0.65	4.78 ± 0.69	5.11 ± 0.89	4.83 ± 1.05	4.85 ± 1.04
15	0.38 ± 0.04	0.34 ± 0.04	0.43 ± 0.04	0.48 ± 0.05	0.44 ± 0.05	0.41 ± 0.05
anti15	0.05 ± 0.01	0.08 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.02	0.06 ± 0.02
iso15	0.2 ± 0.02	0.22 ± 0.02	0.18 ± 0.02	0.18 ± 0.02	0.18 ± 0.03	0.19 ± 0.02
16	10.38 ± 1.12	8.65 ± 1.28	12.57 ± 0.84	16.84 ± 1.86	14.88 ± 1.54	12.96 ± 1.44
iso16	0.07 ± 0.03	0.07 ± 0.01	0.07 ± 0.01	0.09 ± 0.02	0.09 ± 0.01	0.08 ± 0.01
17	0.2 ± 0.04	0.2 ± 0.04	0.24 ± 0.03	0.31 ± 0.04	0.27 ± 0.03	0.25 ± 0.02
anti17	0.11 ± 0.03	0.12 ± 0.02	0.11 ± 0.02	0.1 ± 0.02	0.11 ± 0.02	0.11 ± 0.03
iso17	0.09 ± 0.02	0.11 ± 0.05	0.08 ± 0.04	0.07 ± 0.03	0.06 ± 0.03	0.08 ± 0.05
18	1.23 ± 0.21	1.23 ± 0.22	1.29 ± 0.2	2.21 ± 0.34	1.97 ± 0.25	1.87 ± 0.19
iso18	0.17 ± 0.02	0.2 ± 0.04	0.2 ± 0.03	0.23 ± 0.03	0.21 ± 0.04	0.21 ± 0.07
20	0.1 ± 0.02	0.12 ± 0.03	0.05 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.08 ± 0.02
24	0.02 ± 0	0.02 ± 0	0.02 ± 0.01	0.01 ± 0	0.01 ± 0	0.02 ± 0
SFA	17.82 ± 1.50	16.21 ± 1.99	20.22 ± 1.15	25.90 ± 2.34	23.47 ± 2.05	21.29 ± 2.20
14:1n-5	0.94 ± 0.21	1.05 ± 0.3	0.81 ± 0.19	0.48 ± 0.12	0.6 ± 0.14	0.7 ± 0.15
14:1n-7	0.04 ± 0.01	0.05 ± 0.02	0.06 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.01
14:1n-9	0.19 ± 0.06	0.24 ± 0.03	0.1 ± 0.02	0.15 ± 0.06	0.16 ± 0.03	0.21 ± 0.05
15:1n-x	0.07 ± 0.01	0.08 ± 0.02	0.07 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.06 ± 0.01
16:1n-11	0.25 ± 0.06	0.2 ± 0.04	0.23 ± 0.05	0.26 ± 0.04	0.26 ± 0.04	0.22 ± 0.03
16:1n-5	0.22 ± 0.01	0.21 ± 0.03	0.27 ± 0.04	0.27 ± 0.02	0.25 ± 0.03	0.22 ± 0.03
16:1n-7	11.28 ± 1.08	10.03 ± 1.53	13.45 ± 1.8	9.45 ± 1.44	10.53 ± 1.56	10.04 ± 1.2
16:1n-9	0.41 ± 0.07	0.44 ± 0.07	0.52 ± 0.08	0.47 ± 0.06	0.43 ± 0.04	0.44 ± 0.08
17:1n-x	0.37 ± 0.09	0.34 ± 0.06	0.45 ± 0.1	0.37 ± 0.09	0.41 ± 0.11	0.39 ± 0.09
18:1n-11	4.49 ± 0.87	5.4 ± 0.73	5.16 ± 0.71	4.63 ± 1.06	4.33 ± 0.86	4.89 ± 0.84
18:1n-5	0.27 ± 0.03	0.29 ± 0.04	0.3 ± 0.05	0.32 ± 0.04	0.31 ± 0.04	0.3 ± 0.03
18:1n-7	2.94 ± 0.79	2.74 ± 0.39	3.4 ± 0.93	3.34 ± 0.83	3.61 ± 1.09	3.32 ± 1.1
18:1n-9	14.62 ± 2.42	16.4 ± 2.48	17.39 ± 2.78	15.27 ± 1.94	16.51 ± 3.02	17.54 ± 4.23
20:1n-11	1.92 ± 0.34	2.52 ± 0.44	1.84 ± 0.28	1.72 ± 0.39	1.84 ± 0.32	2.18 ± 0.34
20:1n-7	0.21 ± 0.07	0.25 ± 0.07	0.16 ± 0.03	0.16 ± 0.03	0.2 ± 0.06	0.23 ± 0.08
20:1n-9	6.27 ± 1.46	7.87 ± 1.22	5.07 ± 1.17	5.03 ± 1.43	5.32 ± 1.44	6.38 ± 1.58
22:1n-11	3.35 ± 1.31	3.54 ± 1.2	1.2 ± 0.53	2.34 ± 0.88	2.34 ± 1.04	2.47 ± 1.18
22:1n-9	0.32 ± 0.08	0.41 ± 0.11	0.18 ± 0.05	0.3 ± 0.07	0.31 ± 0.07	0.38 ± 0.1
24:1n-9	0.22 ± 0.05	0.25 ± 0.04	0.05 ± 0.01	0.15 ± 0.04	0.16 ± 0.03	0.2 ± 0.05
MUFAs	48.36 ± 2.09	52.30 ± 2.45	50.70 ± 2.57	44.80 ± 1.48	47.66 ± 2.79	50.20 ± 2.43

Appendix 5. Continued

Fatty acids	Blubber			Milk		
	Early Mum (n=21)	Late Mum (n=21)	Late Pup (n=11)	Early Mum (n=8)	Mid Mum (n=12)	Late Mum (n=11)
16:2n-4	0.34 ± 0.09	0.35 ± 0.1	0.32 ± 0.1	0.34 ± 0.1	0.3 ± 0.1	0.31 ± 0.12
16:2n-6	0.06 ± 0.02	0.07 ± 0.02	0.08 ± 0.02	0.1 ± 0.02	0.07 ± 0.02	0.07 ± 0.01
16:3n-1	0.06 ± 0.01	0.05 ± 0.01	0.09 ± 0.02	0.07 ± 0.01	0.05 ± 0.01	0.07 ± 0.05
16:3n-4	0.27 ± 0.07	0.24 ± 0.08	0.24 ± 0.07	0.25 ± 0.09	0.21 ± 0.08	0.22 ± 0.11
16:4n-1	0.34 ± 0.12	0.3 ± 0.13	0.27 ± 0.12	0.29 ± 0.11	0.27 ± 0.13	0.25 ± 0.13
18:2d5_7	0.03 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
18:2n-4	0.09 ± 0.02	0.09 ± 0.02	0.09 ± 0.02	0.1 ± 0.02	0.08 ± 0.02	0.08 ± 0.02
18:2n-6	1.41 ± 0.16	1.72 ± 0.2	1.57 ± 0.18	1.41 ± 0.21	1.42 ± 0.24	1.55 ± 0.14
18:3n-1	0.19 ± 0.04	0.15 ± 0.07	0.09 ± 0.05	0.06 ± 0.04	0.09 ± 0.04	0.09 ± 0.04
18:3n-3	0.91 ± 0.15	0.97 ± 0.11	0.88 ± 0.16	0.76 ± 0.23	0.8 ± 0.2	0.85 ± 0.22
18:3n-4	0.05 ± 0.02	0.08 ± 0.02	0.07 ± 0.03	0.08 ± 0.04	0.07 ± 0.03	0.08 ± 0.02
18:3n-6	0.11 ± 0.01	0.11 ± 0.02	0.18 ± 0.02	0.23 ± 0.09	0.13 ± 0.04	0.11 ± 0.02
18:4n-1	0.1 ± 0.03	0.09 ± 0.04	0.13 ± 0.04	0.14 ± 0.05	0.11 ± 0.05	0.09 ± 0.04
18:4n-3	1.98 ± 0.49	1.77 ± 0.41	1.89 ± 0.46	2.03 ± 0.48	1.78 ± 0.55	1.75 ± 0.75
20:2n-6	0.23 ± 0.05	0.25 ± 0.04	0.22 ± 0.03	0.2 ± 0.03	0.2 ± 0.03	0.22 ± 0.04
20:3n-3	0.11 ± 0.02	0.11 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	0.11 ± 0.02	0.11 ± 0.02
20:3n-6	0.07 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.09 ± 0.03	0.08 ± 0.01	0.08 ± 0.01
20:4n-3	0.93 ± 0.1	0.92 ± 0.07	0.89 ± 0.11	0.85 ± 0.11	0.79 ± 0.11	0.78 ± 0.13
20:4n-6	0.66 ± 0.19	0.53 ± 0.09	0.78 ± 0.16	0.87 ± 0.24	0.86 ± 0.2	0.75 ± 0.2
20:5n-3	4.92 ± 0.46	3.3 ± 0.91	4.06 ± 0.68	5.6 ± 0.62	4.73 ± 0.82	3.69 ± 1.29
21:5n-3	0.42 ± 0.06	0.43 ± 0.06	0.37 ± 0.08	0.34 ± 0.08	0.32 ± 0.06	0.34 ± 0.07
22:4n-3	0.19 ± 0.03	0.21 ± 0.03	0.15 ± 0.04	0.13 ± 0.02	0.14 ± 0.04	0.16 ± 0.03
22:4n-6	0.21 ± 0.1	0.2 ± 0.1	0.16 ± 0.08	0.14 ± 0.05	0.18 ± 0.08	0.19 ± 0.12
22:5n-3	4.32 ± 0.47	4.29 ± 0.43	3.47 ± 0.49	3.06 ± 0.42	3.04 ± 0.39	3.21 ± 0.33
22:5n-6	0.3 ± 0.06	0.35 ± 0.14	0.21 ± 0.06	0.18 ± 0.03	0.22 ± 0.07	0.28 ± 0.12
22:6n-3	14.64 ± 1.3	13.83 ± 1.5	11.52 ± 0.76	10.7 ± 1.78	11.65 ± 1.4	12.02 ± 1.01
PUFAs	32.96 ± 1.53	30.54 ± 1.92	27.98 ± 2.11	28.14 ± 2.17	27.73 ± 1.96	27.39 ± 1.96

Appendix 6. Fatty acid composition of blubber, serum and milk of grey seal (*Halichoerus grypus*) female and pup at early, mid and late lactation in Isle of May 2006. Values are the mean weight percent and S.D.

	Blubber			Milk			Blood					
Fatty acids	Early	Late	Late	Early	Mid	Late	Early	Early	Mid	Mid	Late	Late
	Mum (n=18)	Mum (n=18)	Pup (n=15)	Mum (n=14)	Mum (n=13)	Mum (n=12)	Mum (n=12)	Pup (n=11)	Mum (n=12)	Pup (n=11)	Mum (n=11)	Pup (n=11)
12	0.1 ± 0.01	0.1 ± 0.02	0.1 ± 0.02	0.13 ± 0.02	0.12 ± 0.03	0.12 ± 0.04	0.16 ± 0.11	0.1 ± 0.07	0.11 ± 0.08	0.1 ± 0.06	0.1 ± 0.07	0.1 ± 0.04
13	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0	0.03 ± 0	0.03 ± 0.01	0.11 ± 0.12	0.04 ± 0.03	0.18 ± 0.22	0.04 ± 0.04	0.17 ± 0.18	0.04 ± 0.04
14	4.72 ± 0.98	4.13 ± 1.01	4.62 ± 1	4.59 ± 0.99	4.71 ± 0.94	4.54 ± 0.86	1.8 ± 0.73	2.46 ± 0.59	1.69 ± 0.5	2.5 ± 0.85	1.86 ± 0.95	2.26 ± 0.67
15	0.37 ± 0.03	0.29 ± 0.05	0.41 ± 0.04	0.44 ± 0.03	0.41 ± 0.04	0.35 ± 0.03	0.47 ± 0.16	0.39 ± 0.05	0.43 ± 0.1	0.38 ± 0.06	0.46 ± 0.14	0.36 ± 0.08
anti15	0.08 ± 0.01	0.09 ± 0.03	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.71 ± 0.63	0.26 ± 0.29	0.41 ± 0.6	0.38 ± 0.58	0.46 ± 0.38	0.25 ± 0.31
iso15	0.22 ± 0.02	0.21 ± 0.02	0.19 ± 0.02	0.19 ± 0.02	0.2 ± 0.03	0.2 ± 0.02	0.11 ± 0.04	0.14 ± 0.03	0.1 ± 0.03	0.14 ± 0.02	0.12 ± 0.06	0.13 ± 0.02
16	10.03 ± 1.12	7.06 ± 1.88	12.45 ± 1.38	15.71 ± 1.31	13.67 ± 1.59	10.97 ± 2.76	18.79 ± 3.85	17.4 ± 1.02	18.38 ± 2.41	15.26 ± 2.82	18.23 ± 4.18	13.85 ± 2.75
iso16	0.11 ± 0.03	0.1 ± 0.02	0.11 ± 0.04	0.12 ± 0.04	0.13 ± 0.05	0.11 ± 0.03	0.22 ± 0.14	0.13 ± 0.07	0.17 ± 0.09	0.16 ± 0.12	0.24 ± 0.15	0.14 ± 0.08
17	0.21 ± 0.04	0.15 ± 0.03	0.24 ± 0.05	0.29 ± 0.05	0.26 ± 0.06	0.22 ± 0.05	0.63 ± 0.19	0.39 ± 0.04	0.56 ± 0.13	0.4 ± 0.08	0.56 ± 0.1	0.46 ± 0.1
anti17	0.16 ± 0.06	0.15 ± 0.05	0.17 ± 0.09	0.15 ± 0.06	0.15 ± 0.07	0.15 ± 0.05	0.12 ± 0.06	0.12 ± 0.04	0.1 ± 0.06	0.15 ± 0.07	0.15 ± 0.1	0.16 ± 0.07
iso17	0.09 ± 0.06	0.09 ± 0.05	0.07 ± 0.04	0.07 ± 0.03	0.07 ± 0.03	0.07 ± 0.03	0.06 ± 0.03	0.19 ± 0.1	0.14 ± 0.23	0.13 ± 0.06	0.15 ± 0.09	0.29 ± 0.15
18	1.26 ± 0.27	1.05 ± 0.2	1.72 ± 1.1	2.17 ± 0.24	1.87 ± 0.34	1.72 ± 0.32	11.25 ± 1.99	6.05 ± 0.63	9.93 ± 1.93	7.48 ± 1.59	9.65 ± 2.17	8.93 ± 1.99
iso18	0.21 ± 0.03	0.21 ± 0.03	0.21 ± 0.04	0.22 ± 0.02	0.21 ± 0.03	0.22 ± 0.03	0.07 ± 0.07	0.05 ± 0.06	0.05 ± 0.03	0.14 ± 0.1	0.08 ± 0.09	0.05 ± 0.05
20	0.08 ± 0.02	0.09 ± 0.03	0.22 ± 0.69	0.05 ± 0.01	0.06 ± 0.02	0.07 ± 0.02	0.19 ± 0.03	0.09 ± 0.02	0.2 ± 0.04	0.12 ± 0.03	0.25 ± 0.05	0.16 ± 0.04
24	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0	0.02 ± 0.01	0.02 ± 0	0.24 ± 0.23	0.22 ± 0.56	0.28 ± 0.41	0.09 ± 0.08	0.27 ± 0.25	0.23 ± 0.44
SFA	17.69 ± 1.45	13.76 ± 2.71	20.62 ± 2.19	24.25 ± 1.67	21.96 ± 1.46	18.85 ± 3.06	34.93 ± 6.50	28.03 ± 1.93	32.73 ± 4.42	27.46 ± 3.81	32.73 ± 6.77	27.41 ± 4.18
14:1n-5	0.92 ± 0.22	1.25 ± 0.28	0.8 ± 0.12	0.43 ± 0.08	0.55 ± 0.11	0.7 ± 0.17	0.1 ± 0.07	0.15 ± 0.04	0.14 ± 0.08	0.18 ± 0.07	0.13 ± 0.08	0.19 ± 0.07
14:1n-7	0.05 ± 0.01	0.07 ± 0.03	0.06 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.03 ± 0.03	0.03 ± 0.01	0.03 ± 0.02	0.03 ± 0.01	0.03 ± 0.03	0.03 ± 0.01
14:1n-9	0.16 ± 0.04	0.2 ± 0.05	0.09 ± 0.02	0.1 ± 0.02	0.13 ± 0.03	0.16 ± 0.04	0.08 ± 0.03	0.2 ± 0.06	0.09 ± 0.03	0.23 ± 0.07	0.11 ± 0.04	0.23 ± 0.08
15:1n-x	0.08 ± 0.02	0.09 ± 0.03	0.07 ± 0.02	0.04 ± 0.01	0.05 ± 0.01	0.06 ± 0.02	0.05 ± 0.07	0.11 ± 0.04	0.06 ± 0.08	0.05 ± 0.01	0.07 ± 0.06	0.04 ± 0.01
16:1n-11	0.26 ± 0.05	0.19 ± 0.07	0.24 ± 0.1	0.27 ± 0.06	0.25 ± 0.07	0.19 ± 0.05	0.22 ± 0.1	0.25 ± 0.06	0.19 ± 0.05	0.26 ± 0.07	0.22 ± 0.12	0.2 ± 0.06
16:1n-5	0.31 ± 0.05	0.25 ± 0.04	0.31 ± 0.08	0.36 ± 0.05	0.34 ± 0.05	0.28 ± 0.06	0.23 ± 0.06	0.28 ± 0.04	0.23 ± 0.04	0.24 ± 0.03	0.2 ± 0.06	0.21 ± 0.04
16:1n-7	11.5 ± 2.38	11.42 ± 2.42	12.26 ± 4.04	9.23 ± 1.75	9.96 ± 1.78	9.32 ± 1.75	3.73 ± 1.33	5.68 ± 0.8	4.17 ± 1.51	5.79 ± 0.92	3.42 ± 1.3	5.11 ± 1
16:1n-9	0.44 ± 0.08	0.56 ± 0.15	1.34 ± 3.15	0.49 ± 0.08	0.43 ± 0.09	0.46 ± 0.12	0.36 ± 0.24	0.41 ± 0.12	0.28 ± 0.12	0.31 ± 0.07	0.36 ± 0.29	0.31 ± 0.1
17:1n-x	0.36 ± 0.13	0.36 ± 0.13	0.42 ± 0.2	0.37 ± 0.13	0.36 ± 0.13	0.33 ± 0.11	0.22 ± 0.12	0.25 ± 0.06	0.22 ± 0.1	0.25 ± 0.09	0.23 ± 0.13	0.22 ± 0.06
18:1n-11	3.9 ± 1.22	5.35 ± 1.55	5.66 ± 3.53	4.32 ± 1.68	4.22 ± 1.5	5.12 ± 1.81	1.36 ± 0.65	2.74 ± 1.41	1.5 ± 0.55	2.71 ± 1.42	1.93 ± 0.84	3.05 ± 1.26
18:1n-5	0.42 ± 0.1	0.38 ± 0.08	0.56 ± 0.56	0.46 ± 0.11	0.45 ± 0.12	0.43 ± 0.11	0.33 ± 0.11	0.44 ± 0.09	0.31 ± 0.08	0.37 ± 0.07	0.29 ± 0.1	0.42 ± 0.1
18:1n-7	3.28 ± 1.03	3.36 ± 1	3.68 ± 1.68	3.76 ± 1.04	3.41 ± 1.12	3.18 ± 0.95	4.66 ± 1.37	4.43 ± 0.9	4.29 ± 1.18	3.74 ± 0.94	3.9 ± 1.11	4.06 ± 0.84
18:1n-9	14.85 ± 2.62	18.61 ± 4.43	16.8 ± 4.78	15.21 ± 2.07	14.9 ± 2.78	17.09 ± 4.41	11.11 ± 2.11	13.98 ± 1.12	11.39 ± 2.21	13.95 ± 1.47	11.51 ± 2.07	15.61 ± 1.85
20:1n-11	1.73 ± 0.56	2.41 ± 0.85	2.14 ± 1.72	1.66 ± 0.71	1.79 ± 0.73	2.28 ± 0.9	0.65 ± 0.25	0.99 ± 0.46	0.98 ± 0.88	1.11 ± 0.57	1.27 ± 0.68	1.26 ± 0.6
20:1n-7	0.29 ± 0.13	0.33 ± 0.13	0.21 ± 0.1	0.21 ± 0.09	0.25 ± 0.15	0.31 ± 0.18	0.17 ± 0.07	0.14 ± 0.04	0.16 ± 0.06	0.15 ± 0.07	0.16 ± 0.07	0.19 ± 0.08
20:1n-9	6.4 ± 2.55	7.58 ± 3.17	4.77 ± 2.68	4.63 ± 1.99	5.84 ± 2.57	7.02 ± 2.74	1.86 ± 0.82	2.26 ± 1.03	2.52 ± 1.05	2.74 ± 1.6	3.1 ± 1.41	3.53 ± 1.78

Appendix 6. Continued

Fatty acids	Blubber			Milk			Blood					
	Early Mum (n=18)	Late Mum (n=18)	Late Pup (n=15)	Early Mum (n=14)	Mid Mum (n=13)	Late Mum (n=12)	Early Mum (n=12)	Early Pup (n=11)	Mid Mum (n=12)	Mid Pup (n=11)	Late Mum (n=11)	Late Pup (n=11)
22:1n-11	3.43 ± 1.88	2.88 ± 1.95	1.19 ± 0.7	2.15 ± 1.2	2.45 ± 1.41	2.51 ± 1.34	0.94 ± 0.55	0.91 ± 0.53	0.97 ± 0.73	1.02 ± 0.77	1.14 ± 0.78	1.07 ± 0.64
22:1n-9	0.33 ± 0.11	0.39 ± 0.19	0.17 ± 0.08	0.28 ± 0.11	0.32 ± 0.11	0.41 ± 0.12	2.58 ± 2.01	0.75 ± 0.71	2.63 ± 2.59	1.17 ± 1.25	2.73 ± 1.66	1.43 ± 1.46
24:1n-9	0.18 ± 0.04	0.19 ± 0.07	0.04 ± 0.01	0.12 ± 0.02	0.92 ± 2.82	0.16 ± 0.03	2.11 ± 1.16	1.73 ± 0.71	2.47 ± 1.4	1.88 ± 1.21	2.21 ± 1.51	1.56 ± 0.51
MUFAs	48.88 ± 2.56	55.87 ± 4.27	50.82 ± 2.62	44.15 ± 2.30	46.67 ± 2.73	50.04 ± 5.32	30.80 ± 4.68	35.72 ± 3.16	32.64 ± 5.29	36.18 ± 3.02	33.02 ± 4.72	38.73 ± 5.08
16:2n-4	0.4 ± 0.08	0.37 ± 0.09	0.34 ± 0.09	0.35 ± 0.07	0.38 ± 0.07	0.35 ± 0.08	0.14 ± 0.11	0.19 ± 0.05	0.13 ± 0.05	0.19 ± 0.05	0.12 ± 0.08	0.16 ± 0.04
16:2n-6	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.1 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.06 ± 0.09	0.06 ± 0.01	0.07 ± 0.07	0.06 ± 0.01	0.06 ± 0.06	0.04 ± 0.01
16:3n-1	0.05 ± 0.01	0.03 ± 0.01	0.08 ± 0.03	0.07 ± 0.02	0.06 ± 0.02	0.05 ± 0.01	0.3 ± 0.06	0.27 ± 0.08	0.27 ± 0.08	0.16 ± 0.12	0.3 ± 0.09	0.35 ± 0.14
16:3n-4	0.24 ± 0.06	0.2 ± 0.07	0.22 ± 0.06	0.23 ± 0.05	0.24 ± 0.06	0.21 ± 0.07	0.15 ± 0.27	0.07 ± 0.04	0.06 ± 0.03	0.09 ± 0.04	0.09 ± 0.08	0.06 ± 0.02
16:4n-1	0.4 ± 0.13	0.29 ± 0.11	0.38 ± 0.37	0.33 ± 0.1	0.38 ± 0.12	0.32 ± 0.12	0.08 ± 0.05	0.12 ± 0.04	0.09 ± 0.05	0.14 ± 0.06	0.09 ± 0.06	0.09 ± 0.02
18:2d5_7	0.05 ± 0.01	0.05 ± 0.01	0.07 ± 0.08	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.07 ± 0.05	0.05 ± 0.02	0.05 ± 0.02	0.05 ± 0.02	0.07 ± 0.02	0.06 ± 0.02
18:2n-4	0.11 ± 0.03	0.1 ± 0.03	0.11 ± 0.04	0.12 ± 0.04	0.11 ± 0.04	0.09 ± 0.03	0.09 ± 0.03	0.11 ± 0.03	0.06 ± 0.03	0.1 ± 0.03	0.06 ± 0.02	0.09 ± 0.02
18:2n-6	1.94 ± 0.24	2.27 ± 0.32	2.03 ± 0.61	1.97 ± 0.31	1.98 ± 0.26	2.21 ± 0.36	4.09 ± 1.85	2.55 ± 0.62	4.6 ± 1.89	2.42 ± 0.43	5.17 ± 2.34	2.89 ± 0.58
18:3n-1	0.09 ± 0.07	0.11 ± 0.07	0.08 ± 0.05	0.07 ± 0.04	0.08 ± 0.05	0.09 ± 0.06	0.09 ± 0.12	0.08 ± 0.1	0.08 ± 0.06	0.1 ± 0.08	0.12 ± 0.16	0.14 ± 0.15
18:3n-3	1.08 ± 0.28	1.03 ± 0.21	1 ± 0.26	0.83 ± 0.18	1.03 ± 0.25	1.01 ± 0.21	0.31 ± 0.13	0.46 ± 0.11	0.39 ± 0.13	0.54 ± 0.2	0.43 ± 0.34	0.42 ± 0.2
18:3n-4	0.08 ± 0.03	0.08 ± 0.03	0.09 ± 0.04	0.1 ± 0.02	0.08 ± 0.02	0.09 ± 0.02	0.35 ± 0.4	0.09 ± 0.08	0.37 ± 0.67	0.13 ± 0.09	0.34 ± 0.52	0.15 ± 0.19
18:3n-6	0.08 ± 0.02	0.08 ± 0.02	0.16 ± 0.02	0.29 ± 0.07	0.13 ± 0.02	0.13 ± 0.03	0.13 ± 0.07	0.27 ± 0.09	0.1 ± 0.07	0.12 ± 0.04	0.09 ± 0.04	0.11 ± 0.03
18:4n-1	0.12 ± 0.03	0.09 ± 0.03	0.12 ± 0.04	0.17 ± 0.03	0.13 ± 0.03	0.1 ± 0.03	0.12 ± 0.03	0.11 ± 0.04	0.1 ± 0.03	0.1 ± 0.04	0.09 ± 0.05	0.08 ± 0.04
18:4n-3	2.02 ± 0.64	1.53 ± 0.54	1.77 ± 0.56	2.03 ± 0.52	2.03 ± 0.62	1.8 ± 0.53	0.3 ± 0.13	0.8 ± 0.3	0.36 ± 0.24	0.83 ± 0.41	0.34 ± 0.3	0.59 ± 0.29
20:2n-6	0.26 ± 0.07	0.28 ± 0.07	0.24 ± 0.08	0.22 ± 0.04	0.23 ± 0.05	0.26 ± 0.06	0.4 ± 0.23	0.25 ± 0.09	0.31 ± 0.17	0.18 ± 0.05	0.27 ± 0.09	0.21 ± 0.06
20:3n-3	0.12 ± 0.02	0.11 ± 0.02	0.15 ± 0.16	0.11 ± 0.02	0.12 ± 0.03	0.12 ± 0.02	1.57 ± 4.97	0.05 ± 0.05	0.06 ± 0.06	0.07 ± 0.04	0.11 ± 0.13	0.07 ± 0.04
20:3n-6	0.07 ± 0.01	0.09 ± 0.01	0.11 ± 0.1	0.09 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.8 ± 0.38	0.28 ± 0.12	0.75 ± 0.27	0.2 ± 0.07	0.81 ± 0.32	0.28 ± 0.07
20:4n-3	0.78 ± 0.13	0.72 ± 0.13	0.89 ± 0.64	0.77 ± 0.13	0.75 ± 0.13	0.74 ± 0.11	0.55 ± 0.28	0.38 ± 0.11	0.46 ± 0.21	0.36 ± 0.14	0.48 ± 0.19	0.35 ± 0.11
20:4n-6	0.59 ± 0.24	0.5 ± 0.16	0.72 ± 0.35	0.93 ± 0.34	0.76 ± 0.26	0.66 ± 0.18	10.57 ± 5.3	10.04 ± 2.4	11.83 ± 3.51	9.6 ± 2.83	12.26 ± 5.35	9.95 ± 3.09
20:5n-3	5.7 ± 1.14	3.58 ± 1.15	4.21 ± 1.45	6.99 ± 1.5	6.36 ± 1.55	4.61 ± 1.96	8.4 ± 2.81	9.82 ± 2.84	8.53 ± 2.72	9.75 ± 1.7	6.4 ± 2.03	7.36 ± 2.26
21:5n-3	0.44 ± 0.04	0.42 ± 0.04	0.37 ± 0.03	0.36 ± 0.03	0.39 ± 0.03	0.4 ± 0.04	0.22 ± 0.1	0.16 ± 0.04	0.3 ± 0.58	0.19 ± 0.07	0.34 ± 0.33	0.16 ± 0.09
22:4n-3	0.17 ± 0.05	0.17 ± 0.06	0.14 ± 0.04	0.12 ± 0.04	0.15 ± 0.05	0.18 ± 0.06	0.07 ± 0.1	0.04 ± 0.02	0.03 ± 0.02	0.06 ± 0.04	0.06 ± 0.06	0.05 ± 0.04
22:4n-6	0.19 ± 0.11	0.18 ± 0.08	0.16 ± 0.11	0.15 ± 0.08	0.14 ± 0.08	0.15 ± 0.07	0.18 ± 0.11	0.11 ± 0.05	0.11 ± 0.04	0.11 ± 0.06	0.17 ± 0.09	0.11 ± 0.05
22:5n-3	4.61 ± 0.73	4.76 ± 0.53	3.87 ± 0.46	3.8 ± 0.56	3.91 ± 0.47	4.25 ± 0.34	1.21 ± 0.45	1.82 ± 0.79	1.1 ± 0.3	1.96 ± 0.41	1.16 ± 0.3	2.11 ± 1.2
22:5n-6	0.2 ± 0.05	0.24 ± 0.07	0.15 ± 0.04	0.12 ± 0.03	0.15 ± 0.04	0.2 ± 0.06	0.15 ± 0.04	0.12 ± 0.04	0.14 ± 0.06	0.13 ± 0.05	0.25 ± 0.12	0.16 ± 0.07
22:6n-3	12.43 ± 1.43	11.91 ± 1.74	9.83 ± 1.27	10.01 ± 1.3	10.32 ± 1.89	11.67 ± 1.07	2.91 ± 0.95	6.37 ± 0.9	3.39 ± 1.09	6.97 ± 2.1	3.56 ± 1.4	6.18 ± 2.04
PUFAs	32.3 ± 2.50	32.82 ± 1.91	27.36 ± 2.13	30.39 ± 2.49	30.13 ± 1.85	29.91 ± 2.43	33.29 ± 8.58	34.69 ± 3.42	33.73 ± 7.87	34.62 ± 3.37	33.20 ± 7.71	32.23 ± 3.99

Appendix 7. Fatty acid composition of blubber, serum and milk of grey seal (*Halichoerus* grypus) female and pup at early, mid and late lactation in North Rona 2006. Values are the mean weight percent and S.D.

	Blubber			Milk			Blood					
Fatty acids	Early	Late	Late	Early	Mid	Late	Early	Early	Mid	Mid	Late	Late
	Mum (n=25)	Mum (n=18)	Pup (n=10)	Mum (n=15)	Mum (n=11)	Mum (n=12)	Mum (n=13)	Pup (n=12)	Mum (n=9)	Pup (n=9)	Mum (n=10)	Pup (n=12)
12	0.1 ± 0.02	0.1 ± 0.02	0.07 ± 0.01	0.13 ± 0.02	0.13 ± 0.01	0.13 ± 0.02	0.04 ± 0.06	0.06 ± 0.03	0.06 ± 0.05	0.08 ± 0.03	0.06 ± 0.08	0.08 ± 0.04
13	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0	0.02 ± 0.01	0.03 ± 0	0.02 ± 0.01	0.02 ± 0.03	0.02 ± 0.02	0.01 ± 0	0.02 ± 0.01	0.01 ± 0
14	5.06 ± 0.94	4.48 ± 0.98	4.61 ± 0.71	4.81 ± 0.77	4.94 ± 1.09	5.04 ± 0.9	1.52 ± 0.42	2.95 ± 0.54	1.36 ± 0.44	2.96 ± 0.9	1.45 ± 0.71	2.55 ± 0.91
15	0.4 ± 0.05	0.34 ± 0.04	0.43 ± 0.05	0.46 ± 0.03	0.43 ± 0.05	0.39 ± 0.03	0.4 ± 0.08	0.49 ± 0.13	0.34 ± 0.05	0.4 ± 0.06	0.42 ± 0.17	0.33 ± 0.04
anti15	0.07 ± 0.01	0.08 ± 0.02	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.02	0.07 ± 0.02	0.52 ± 0.74	0.11 ± 0.1	0.29 ± 0.25	0.09 ± 0.14	0.74 ± 1.78	0.04 ± 0.05
iso15	0.2 ± 0.03	0.2 ± 0.02	0.18 ± 0.03	0.17 ± 0.02	0.17 ± 0.02	0.19 ± 0.02	0.08 ± 0.02	0.15 ± 0.03	0.07 ± 0.02	0.12 ± 0.03	0.08 ± 0.03	0.12 ± 0.03
16	10.72 ± 0.95	8.09 ± 1.32	12.5 ± 0.5	17.31 ± 0.61	14.99 ± 1.4	12.71 ± 1.25	20.94 ± 4.14	21.38 ± 2.94	18 ± 2.5	16.98 ± 2.34	19.51 ± 5.46	13.21 ± 1.57
iso16	0.09 ± 0.02	0.09 ± 0.03	0.09 ± 0.01	0.09 ± 0.02	0.09 ± 0.01	0.09 ± 0.02	0.12 ± 0.04	0.13 ± 0.1	0.11 ± 0.03	0.11 ± 0.03	0.15 ± 0.07	0.09 ± 0.03
17	0.22 ± 0.04	0.18 ± 0.03	0.25 ± 0.04	0.31 ± 0.03	0.27 ± 0.04	0.25 ± 0.02	0.64 ± 0.13	0.45 ± 0.08	0.52 ± 0.08	0.42 ± 0.05	0.58 ± 0.19	0.39 ± 0.08
anti17	0.12 ± 0.03	0.12 ± 0.04	0.13 ± 0.08	0.1 ± 0.02	0.11 ± 0.02	0.11 ± 0.03	0.07 ± 0.03	0.1 ± 0.04	0.05 ± 0.04	0.1 ± 0.03	0.06 ± 0.05	0.1 ± 0.03
iso17	0.04 ± 0.02	0.06 ± 0.04	0.06 ± 0.04	0.07 ± 0.03	0.08 ± 0.04	0.06 ± 0.03	0.07 ± 0.03	0.1 ± 0.05	0.08 ± 0.06	0.12 ± 0.06	0.08 ± 0.04	0.11 ± 0.05
18	1.28 ± 0.18	1.14 ± 0.23	1.77 ± 1.3	2.37 ± 0.17	2.01 ± 0.25	1.94 ± 0.21	14.4 ± 3.05	6.69 ± 1.14	10.67 ± 1.47	7.68 ± 1.19	10.63 ± 3.06	8.28 ± 3.48
iso18	0.21 ± 0.03	0.21 ± 0.03	0.26 ± 0.11	0.24 ± 0.03	0.21 ± 0.02	0.23 ± 0.04	0.04 ± 0.02	0.26 ± 0.16	0.12 ± 0.1	0.19 ± 0.12	0.08 ± 0.09	0.26 ± 0.15
20	0.09 ± 0.02	0.1 ± 0.02	0.05 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.09 ± 0.02	0.17 ± 0.07	0.12 ± 0.03	0.19 ± 0.09	0.12 ± 0.03	0.24 ± 0.08	0.16 ± 0.05
24	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0	0.02 ± 0	0.02 ± 0	0.02 ± 0	0.07 ± 0.11	0.04 ± 0.04	0.18 ± 0.32	0.01 ± 0.01	0.11 ± 0.13	0.02 ± 0.14
SFA	18.65 ± 1.63	15.24 ± 2.15	20.49 ± 2.06	26.22 ± 1.05	23.60 ± 2.28	21.35 ± 1.71	39.09 ± 7.67	33.03 ± 3.73	32.05 ± 4.31	29.40 ± 3.44	34.20 ± 8.86	29.10 ± 3.93
14:1n-5	0.99 ± 0.19	1.33 ± 0.31	0.89 ± 0.13	0.4 ± 0.11	0.48 ± 0.14	0.63 ± 0.14	0.06 ± 0.03	0.19 ± 0.07	0.06 ± 0.03	0.22 ± 0.14	0.05 ± 0.03	0.25 ± 0.11
14:1n-7	0.04 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.02 ± 0.01	0.03 ± 0.03	0.01 ± 0.01	0.03 ± 0.01	0.01 ± 0.02	0.02 ± 0.01
14:1n-9	0.22 ± 0.06	0.23 ± 0.06	0.12 ± 0.03	0.14 ± 0.03	0.18 ± 0.03	0.22 ± 0.06	0.12 ± 0.04	0.31 ± 0.09	0.13 ± 0.04	0.35 ± 0.14	0.14 ± 0.05	0.34 ± 0.18
15:1n-x	0.08 ± 0.01	0.1 ± 0.02	0.08 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.05 ± 0.02	0.02 ± 0.01	0.16 ± 0.09	0.02 ± 0.01	0.04 ± 0.02	0.03 ± 0.04	0.03 ± 0.01
16:1n-11	0.26 ± 0.06	0.21 ± 0.05	0.24 ± 0.08	0.25 ± 0.05	0.25 ± 0.04	0.21 ± 0.04	0.22 ± 0.06	0.25 ± 0.07	0.2 ± 0.05	0.28 ± 0.07	0.24 ± 0.1	0.2 ± 0.04
16:1n-5	0.24 ± 0.02	0.21 ± 0.02	0.23 ± 0.07	0.26 ± 0.01	0.23 ± 0.03	0.21 ± 0.01	0.19 ± 0.03	0.27 ± 0.08	0.18 ± 0.02	0.2 ± 0.03	0.14 ± 0.03	0.16 ± 0.02
16:1n-7	11.65 ± 1.76	11.64 ± 1.7	13.08 ± 4.69	8.98 ± 1.32	9.51 ± 1.5	9.19 ± 1.51	3.72 ± 0.8	7.2 ± 1.44	3.72 ± 0.93	6.65 ± 2.68	2.9 ± 0.51	5.69 ± 1.29
16:1n-9	0.43 ± 0.06	0.51 ± 0.09	1.85 ± 4.15	0.51 ± 0.08	0.39 ± 0.05	0.41 ± 0.07	0.37 ± 0.11	0.66 ± 0.31	0.24 ± 0.07	0.31 ± 0.08	0.28 ± 0.14	0.27 ± 0.05
17:1n-x	0.41 ± 0.1	0.42 ± 0.1	0.46 ± 0.15	0.37 ± 0.09	0.38 ± 0.1	0.36 ± 0.12	0.25 ± 0.12	0.35 ± 0.19	0.26 ± 0.09	0.32 ± 0.13	0.21 ± 0.13	0.28 ± 0.08
18:1n-11	4.21 ± 0.74	5.49 ± 1.18	5.94 ± 3.45	4.53 ± 1.04	4.32 ± 0.88	4.81 ± 0.89	1.61 ± 0.4	2.64 ± 0.54	1.52 ± 0.41	2.82 ± 0.94	1.71 ± 0.57	3.09 ± 1.13
18:1n-5	0.28 ± 0.03	0.28 ± 0.02	0.26 ± 0.07	0.28 ± 0.03	0.27 ± 0.02	0.27 ± 0.03	0.25 ± 0.03	0.35 ± 0.09	0.19 ± 0.04	0.26 ± 0.05	0.16 ± 0.03	0.25 ± 0.06
18:1n-7	3.22 ± 0.93	3.39 ± 1	3.4 ± 1.47	3.62 ± 0.87	3.39 ± 1.08	3.07 ± 0.88	4.85 ± 1.24	5.47 ± 1.47	3.74 ± 1.12	3.79 ± 1.3	3.37 ± 0.8	3.66 ± 1.03
18:1n-9	16.06 ± 2.85	19.38 ± 3.8	17.84 ± 5.96	15.93 ± 2.54	16 ± 3.81	16.7 ± 3.43	13.99 ± 2.77	16.03 ± 2.06	12.63 ± 2.68	15.39 ± 3.32	12.25 ± 1.29	15.86 ± 2.1
20:1n-11	1.79 ± 0.27	2.35 ± 0.44	1.69 ± 0.31	1.57 ± 0.38	1.82 ± 0.37	2.12 ± 0.39	0.56 ± 0.14	0.9 ± 0.26	0.57 ± 0.15	1.09 ± 0.34	0.75 ± 0.17	1.27 ± 0.54
20:1n-7	0.23 ± 0.08	0.26 ± 0.09	0.16 ± 0.03	0.15 ± 0.03	0.19 ± 0.06	0.22 ± 0.06	0.11 ± 0.02	0.13 ± 0.03	0.13 ± 0.02	0.14 ± 0.04	0.13 ± 0.02	0.16 ± 0.05
20:1n-9	6.22 ± 1.34	6.88 ± 1.74	4.67 ± 1.01	4.03 ± 0.79	5.35 ± 1.09	6.62 ± 1.46	1.84 ± 0.54	2.08 ± 0.62	1.99 ± 0.33	2.97 ± 0.62	2.45 ± 0.69	3.4 ± 1.53

Appendix 7. Continued

	Blubber			Milk			Blood					
Fatty acids	Early Mum (n=25)	Late Mum (n=18)	Late Pup (n=10)	Early Mum (n=15)	Mid Mum (n=11)	Late Mum (n=12)	Early Mum (n=13)	Early Pup (n=12)	Mid Mum (n=9)	Mid Pup (n=9)	Late Mum (n=10)	Late Pup (n=12)
22:1n-11	3.39 ± 1.4	2.7 ± 1.43	0.91 ± 0.36	1.99 ± 0.76	2.32 ± 0.98	2.69 ± 1.15	0.72 ± 0.42	0.84 ± 0.45	0.75 ± 0.32	1.15 ± 0.46	1.06 ± 0.49	1.16 ± 0.62
22:1n-9	0.3 ± 0.07	0.29 ± 0.09	0.15 ± 0.04	0.27 ± 0.04	0.29 ± 0.05	0.38 ± 0.09	1.76 ± 0.94	1.3 ± 1.89	1.2 ± 0.58	0.48 ± 0.24	2.55 ± 2.2	0.41 ± 0.29
24:1n-9	0.19 ± 0.04	0.19 ± 0.06	0.05 ± 0.01	0.13 ± 0.03	0.14 ± 0.04	0.18 ± 0.05	2.62 ± 1.84	1.12 ± 0.54	2.83 ± 2.03	1.28 ± 0.8	2.55 ± 1.55	1.64 ± 1.15
MUFAs	50.19 ± 2.43	55.90 ± 3.31	52.09 ± 3.86	43.50 ± 2.49	45.60 ± 4.20	48.39 ± 2.98	33.27 ± 5.91	40.27 ± 5.15	30.37 ± 5.53	37.76 ± 7.80	31 ± 3.04	34.30 ± 5.60
16:2n-4	0.36 ± 0.11	0.34 ± 0.1	0.29 ± 0.08	0.34 ± 0.1	0.38 ± 0.14	0.34 ± 0.1	0.1 ± 0.04	0.17 ± 0.06	0.12 ± 0.04	0.21 ± 0.09	0.08 ± 0.05	0.18 ± 0.07
16:2n-6	0.08 ± 0.02	0.08 ± 0.02	0.08 ± 0.01	0.12 ± 0.03	0.08 ± 0.02	0.07 ± 0.01	0.03 ± 0.03	0.06 ± 0.02	0.04 ± 0.04	0.05 ± 0.02	0.05 ± 0.03	0.04 ± 0.02
16:3n-1	0.05 ± 0.02	0.03 ± 0.02	0.08 ± 0.03	0.08 ± 0.04	0.05 ± 0.02	0.05 ± 0.02	0.45 ± 0.16	0.13 ± 0.14	0.22 ± 0.15	0.12 ± 0.13	0.32 ± 0.15	0.11 ± 0.15
16:3n-4	0.22 ± 0.08	0.19 ± 0.08	0.19 ± 0.06	0.21 ± 0.07	0.23 ± 0.08	0.2 ± 0.07	0.08 ± 0.03	0.1 ± 0.03	0.06 ± 0.02	0.11 ± 0.05	0.08 ± 0.05	0.09 ± 0.04
16:4n-1	0.35 ± 0.17	0.27 ± 0.15	0.34 ± 0.41	0.3 ± 0.15	0.4 ± 0.23	0.31 ± 0.14	0.06 ± 0.01	0.09 ± 0.04	0.07 ± 0.03	0.16 ± 0.08	0.05 ± 0.03	0.12 ± 0.06
18:2d5_7	0.04 ± 0.01	0.05 ± 0.01	0.07 ± 0.08	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.02	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.02	0.04 ± 0.01
18:2n-4	0.09 ± 0.03	0.08 ± 0.02	0.09 ± 0.01	0.11 ± 0.02	0.09 ± 0.02	0.08 ± 0.01	0.07 ± 0.02	0.11 ± 0.02	0.06 ± 0.02	0.08 ± 0.03	0.04 ± 0.01	0.08 ± 0.01
18:2n-6	1.65 ± 0.32	1.78 ± 0.22	1.76 ± 0.3	1.71 ± 0.59	1.51 ± 0.17	1.94 ± 0.56	3.53 ± 1.23	1.88 ± 0.45	3.8 ± 0.53	2.44 ± 1.98	4.5 ± 0.97	2.29 ± 1.19
18:3n-1	0.08 ± 0.02	0.08 ± 0.03	0.04 ± 0.02	0.05 ± 0.03	0.06 ± 0.04	0.09 ± 0.04	0.03 ± 0.03	0.09 ± 0.08	0.03 ± 0.02	0.08 ± 0.07	0.08 ± 0.14	0.12 ± 0.07
18:3n-3	0.93 ± 0.18	0.88 ± 0.18	0.78 ± 0.16	0.63 ± 0.14	0.79 ± 0.2	0.88 ± 0.14	0.17 ± 0.07	0.27 ± 0.14	0.25 ± 0.12	0.41 ± 0.14	0.18 ± 0.1	0.45 ± 0.16
18:3n-4	0.06 ± 0.02	0.06 ± 0.02	0.05 ± 0.02	0.08 ± 0.02	0.08 ± 0.02	0.07 ± 0.03	0.16 ± 0.04	0.14 ± 0.05	0.13 ± 0.04	0.09 ± 0.03	0.16 ± 0.07	0.09 ± 0.03
18:3n-6	0.07 ± 0.03	0.06 ± 0.02	0.16 ± 0.02	0.37 ± 0.14	0.15 ± 0.04	0.15 ± 0.05	0.28 ± 0.22	0.26 ± 0.08	0.22 ± 0.12	0.21 ± 0.21	0.14 ± 0.06	0.12 ± 0.03
18:4n-1	0.11 ± 0.04	0.09 ± 0.04	0.11 ± 0.05	0.17 ± 0.06	0.14 ± 0.06	0.09 ± 0.03	0.08 ± 0.04	0.09 ± 0.03	0.09 ± 0.05	0.09 ± 0.04	0.05 ± 0.03	0.07 ± 0.02
18:4n-3	1.83 ± 0.47	1.42 ± 0.44	1.54 ± 0.38	1.88 ± 0.49	1.8 ± 0.62	1.71 ± 0.38	0.14 ± 0.07	0.48 ± 0.24	0.25 ± 0.15	0.73 ± 0.37	0.18 ± 0.14	0.68 ± 0.33
20:2n-6	0.23 ± 0.03	0.24 ± 0.03	0.22 ± 0.02	0.22 ± 0.04	0.22 ± 0.03	0.24 ± 0.03	0.77 ± 0.62	0.35 ± 0.2	0.54 ± 0.34	0.31 ± 0.4	0.4 ± 0.34	0.19 ± 0.04
20:3n-3	0.13 ± 0.01	0.12 ± 0.02	0.1 ± 0.01	0.1 ± 0.02	0.11 ± 0.02	0.11 ± 0.01	0.03 ± 0.02	0.05 ± 0.04	0.04 ± 0.03	0.05 ± 0.01	0.07 ± 0.06	0.05 ± 0.02
20:3n-6	0.08 ± 0.01	0.1 ± 0.01	0.09 ± 0.01	0.12 ± 0.03	0.08 ± 0.01	0.09 ± 0.01	1.02 ± 0.59	0.26 ± 0.09	0.88 ± 0.37	0.41 ± 0.66	0.91 ± 0.42	0.26 ± 0.36
20:4n-3	0.86 ± 0.12	0.81 ± 0.14	0.78 ± 0.13	0.85 ± 0.16	0.8 ± 0.17	0.8 ± 0.1	0.29 ± 0.21	0.26 ± 0.14	0.36 ± 0.16	0.39 ± 0.14	0.36 ± 0.18	0.44 ± 0.12
20:4n-6	0.64 ± 0.17	0.58 ± 0.15	0.77 ± 0.17	1.04 ± 0.21	0.85 ± 0.19	0.74 ± 0.17	11.02 ± 6.91	8.81 ± 3.18	15.03 ± 4.53	9.36 ± 5.79	14.68 ± 7.83	11.22 ± 6.22
20:5n-3	4.68 ± 0.72	3.25 ± 0.82	3.76 ± 0.74	6.34 ± 1.06	5.36 ± 1.48	3.84 ± 0.56	5.37 ± 3.38	5.5 ± 2.65	9.08 ± 4.26	7.81 ± 3.61	6.22 ± 3.3	6.65 ± 2.54
21:5n-3	0.38 ± 0.07	0.38 ± 0.08	0.33 ± 0.07	0.34 ± 0.08	0.37 ± 0.09	0.38 ± 0.09	0.05 ± 0.03	0.07 ± 0.05	0.06 ± 0.03	0.12 ± 0.05	0.07 ± 0.05	0.15 ± 0.08
22:4n-3	0.16 ± 0.03	0.17 ± 0.03	0.13 ± 0.02	0.11 ± 0.03	0.15 ± 0.03	0.17 ± 0.03	0.04 ± 0.07	0.03 ± 0.02	0.05 ± 0.07	0.05 ± 0.02	0.03 ± 0.01	0.06 ± 0.04
22:4n-6	0.2 ± 0.09	0.21 ± 0.1	0.17 ± 0.08	0.15 ± 0.07	0.18 ± 0.11	0.17 ± 0.09	0.14 ± 0.08	0.14 ± 0.08	0.12 ± 0.05	0.13 ± 0.06	0.17 ± 0.09	0.15 ± 0.08
22:5n-3	3.89 ± 0.45	4.19 ± 0.34	3.4 ± 0.31	3.24 ± 0.31	3.49 ± 0.32	3.74 ± 0.36	0.95 ± 0.81	1.31 ± 0.48	1.41 ± 0.87	1.56 ± 0.48	0.99 ± 0.29	1.97 ± 0.73
22:5n-6	0.26 ± 0.06	0.3 ± 0.07	0.2 ± 0.05	0.17 ± 0.04	0.21 ± 0.08	0.26 ± 0.06	0.1 ± 0.08	0.11 ± 0.04	0.16 ± 0.05	0.14 ± 0.05	0.27 ± 0.17	0.24 ± 0.08
22:6n-3	12.6 ± 1.41	11.98 ± 1.52	10.67 ± 1.12	10.36 ± 1.32	12.1 ± 1.11	12.61 ± 0.98	1.74 ± 1.2	4.35 ± 2.33	3.61 ± 1.85	6.04 ± 2.22	3.83 ± 1.01	8.67 ± 2.81
PUFAs	30.03 ± 2.19	27.72 ± 2.21	29.23 ± 1.99	29.13 ± 2.69	29.73 ± 2.44	29.20 ± 1.92	26.74 ± 12.64	25.17 ± 7.70	36.71 ± 8.66	31.16 ± 10.23	33.95 ± 10.91	35.41 ± 4.28

Appendix 8. Confusion matrix showing the number of misclassifications obtained from the Random forest analysis in the fatty acid composition of blubber, serum and milk in grey seal females and pups. The misclassifications between lactation stages for a given compartment, between compartments, between populations and between years. Blue color represents blubber, yellow serum and red blood. A) Isle of May, B) North Rona. E=early, M=mid, L=late lactation.

A

					Isle of May																														North Rona																														
					2004							2005								2006															2004						2005								2006															
					Blubber				Milk			Blubber					Milk			Blubber					Serum							Milk			Blubber			Milk			Blubber					Milk			Blubber				Serum							Milk				
					Mum			Pup	Mum			Mum			Pup		Mum			Mum			Pup		Mum			Pup				Mum			Mum			Mum			Mum			Pup		Mum			Mum			Pup	Mum			Pup				Mum				
					E	M	L	L	E	M	L	E	M	L	L	W	E	M	L	E	M	L	L	W	E	M	L	E	M	L	W	E	M	L	E	M	L	E	M	L	E	M	L	L	W	E	M	L	E	M	L	W	E	M	L	E	M	L	W	E	M	L	Error	*n*
Isle of May	2004	Blubber	Mum	E			1	8				1										1	1																																						1.00	12
				M	1		1																																																								1.00	2
				L	3		3	4							1							1	1												1																												0.79	14
			Pup	L	1		4	3				4				2							1												1																												0.81	16
		Milk	Mum	E					5		1																					2						1																									0.44	9
				M					1																																																						1.00	1
				L							9								1																																												0.10	10
	2005	Blubber	Mum	E	1							19	1							2													1																														0.21	24
				M								3																																																			1.00	3
				L								2	1									4																					2		2																				1.00	11
			Pup	L											1	6		1					2																					1																					0.91	11
				W											7	5																																															0.58	12
		Milk	Mum	E													5	2														3														1																	0.55	11
				M													1	6	1														2																														0.40	10
				L														3	1	1													3	2														1															0.91	11
	2006	Blubber	Mum	E								3								14																													1														0.22	18
				M								2																																																			1.00	2
				L																2		14																													2												0.22	18
			Pup	L											1	1							5	6																				1								1											0.67	15
				W																			6	2																																							0.75	8
		Blood	Mum	E																					5	4	3																																				0.58	12
				M																					2	4	2			1	1																						1	1									0.67	12
				L																					2	4	4																												1								0.64	11
			Pup	E																								8	3																																		0.27	11
				M																									5	4	1																									1		1					0.58	12
				L																									4	5																							1					1					0.55	11
				W																						2																																	4				1.00	6
		Milk	Mum	E																												11	2																											1			0.21	14
				M																			1									1	8	2													1																	0.38	13
				L																		1											4	7																														0.42	12

Appendix 8. Continued

B

					North Rona																												Isle of May																																
					2004						2005								2006														2004							2005								2006																
					Blubber			Milk			Blubber					Milk			Blubber				Serum							Milk			Blubber				Milk			Blubber					Milk			Blubber					Serum							Milk				
					Mum			Mum			Mum			Pup		Mum			Mum			Pup	Mum			Pup				Mum			Mum			Pup	Mum			Mum			Pup		Mum			Mum			Pup		Mum			Pup				Mum				
					E	M	L	E	M	L	E	M	L	L	W	E	M	L	E	M	L	W	E	M	L	E	M	L	W	E	M	L	E	M	L	L	E	M	L	E	M	L	L	W	E	M	L	E	M	L	L	W	E	M	L	E	M	L	W	E	M	L	Error	*n*
North Rona	2004	Blubber	Mum	E	4		3																																								1																					0.50	8
				M	2																																																											1.00	2
				L	5																										1																				1													1.00	7
		Milk	Mum	E				6		1																									1																										0.25	8
				M						2																																																				1.00	2
				L						7																													1																							0.13	8
	2005	Blubber	Mum	E							19								2																																										0.10	21
				M							5								1	1																																											1.00	7
				L							2		8							3																							1																					0.43	14
			Pup	L										4	4							2																						1																				0.64	11
				W										5							2																						2																					0.83	12
		Milk	Mum	E													3	1														4																																		1.00	8
				M												1	2	3														1	4																																1			0.83	12
				L												1	4	2															1	3																																	0.82	11
	2006	Blubber	Mum	E													1		22		2																																											0.12	25
				M																3		2																																											1.00	5
				L								1								5		12																																											0.33	18
			Pup	W										2								7																														1														0.30	10
		Blood	Mum	E																			12																																		1												0.08	13
				M																			2	4	2																																					1						0.56	9
				L																			1	2	6																																		1									0.40	10
			Pup	E																						9	2																																			1							0.25	12
				M																			1			1	6	1																																								0.33	9
				L																								10																																								0.00	10
				W																			1						9																																						0.10	10
		Milk	Mum	E																										14	1																																	0.07	15
				M													3	1												1	5	1																															0.55	11
				L														1			1										1		9																															0.25	12

Appendix 9. Total body burden of organochlorines in grey seal (*Halichoerus grypus*) females at early and late lactation in Isle of May and North Rona during 2005 and 2006.

	Isle of May 2005		Isle of May 2006		North Rona 2005		North Rona 2006	
	Early	Late	Early	Late	Early	Late	Early	Late
101	3.08 ± 0.7	2.53 ± 0.59	2.52 ± 0.99	2.57 ± 1.31	3.34 ± 1.86	2.7 ± 1.29	4.2 ± 1.79	3.01 ± 1.12
149	1.07 ± 0.5	0.89 ± 0.45	1.27 ± 0.74	1.54 ± 1.04	0.55 ± 0.44	0.53 ± 0.32	1.56 ± 0.86	1.23 ± 0.59
118	0.72 ± 0.58	0.71 ± 0.44	0.8 ± 0.2	0.78 ± 0.29	0.76 ± 0.43	0.4 ± 0.24	1.62 ± 0.94	1.1 ± 0.53
153	27.63 ± 9.98	23.59 ± 6.37	14.2 ± 6.54	13.52 ± 7.16	14.74 ± 4.31	12.28 ± 4.8	12.81 ± 7.89	9.56 ± 4.16
138	16.64 ± 5.51	14.31 ± 3.69	17.45 ± 6.69	16.95 ± 7.97	8.49 ± 2.24	7.15 ± 3.14	15.57 ± 10.27	11.45 ± 5.95
187	7.27 ± 2.3	6.88 ± 1.92	6.4 ± 3.65	7.2 ± 4.85	2.86 ± 0.94	2.66 ± 1.05	6.12 ± 4.13	4.78 ± 2.14
183	4.07 ± 1.71	4.06 ± 1.21	3.3 ± 2.2	3.77 ± 3.04	1.1 ± 0.46	1.1 ± 0.62	2.68 ± 1.91	1.95 ± 1.08
128	1.11 ± 0.94	1.22 ± 0.65	1.02 ± 1.51	0.55 ± 0.4	1.01 ± 0.69	0.8 ± 0.5	0.79 ± 0.68	0.54 ± 0.7
156	0.86 ± 0.97	1.57 ± 0.84	1.56 ± 0.8	1.63 ± 1.01	0.32 ± 0.25	0.38 ± 0.32	1.49 ± 1.1	1.08 ± 0.44
180	14.39 ± 4.91	12.71 ± 3.76	14.03 ± 6.81	14.47 ± 8.18	5.71 ± 2.02	5.08 ± 1.92	12.14 ± 7.45	9.93 ± 4.64
195	1.55 ± 1.25	1.77 ± 0.9	1.04 ± 0.85	1.11 ± 0.87	0.1 ± 0.09	0.16 ± 0.14	0.61 ± 0.41	0.53 ± 0.27
194	3.07 ± 1.68	3.4 ± 1.33	2.71 ± 1.71	3.44 ± 3.02	0.72 ± 0.4	0.72 ± 0.5	2.11 ± 1.62	1.93 ± 0.94
206	1.49 ± 1.21	1.89 ± 1.09	0.98 ± 0.97	1.13 ± 1.27	0.26 ± 0.27	0.28 ± 0.2	0.8 ± 0.66	0.83 ± 0.46
209	0.94 ± 0.94	1.35 ± 0.92	0.73 ± 0.9	0.83 ± 1.07	0.02 ± 0.05	0.06 ± 0.1	0.48 ± 0.4	0.51 ± 0.33
DDE	15.54 ± 5.37	12.31 ± 3.45	12.38 ± 3.89	10.92 ± 4.64	8.94 ± 2.86	7.45 ± 3.09	13.43 ± 7.33	9.85 ± 4.89
DDD	0.65 ± 0.76	0.58 ± 0.65	0.19 ± 0.16	0.21 ± 0.18	0.45 ± 0.34	0.29 ± 0.22	0.54 ± 0.55	0.25 ± 0.14
DDT	2.05 ± 1.14	1.79 ± 0.8	0.58 ± 0.51	0.61 ± 0.38	0.48 ± 0.3	0.37 ± 0.23	0.5 ± 0.43	0.35 ± 0.25
Σ PCB	83.89 ± 28.74	57.65 ± 38.08	67.99 ± 29.52	62.54 ± 42.05	39.97 ± 12.14	31.19 ± 16.06	63 ± 39.09	48.43 ± 21.94
Σ DDT	18.24 ± 6.8	11.01 ± 7.62	13.16 ± 4.01	10.56 ± 5.98	9.88 ± 3.34	7.37 ± 4.01	14.48 ± 7.59	10.45 ± 5.01
Total	102.14 ± 35.16	68.65 ± 45.35	81.15 ± 32.75	73.1 ± 47.59	49.85 ± 14.8	38.56 ± 19.91	77.48 ± 46.43	58.88 ± 26.82

Appendix 10. Geometric mean and quantile ranges of OC concentrations in blubber from grey seal (*Halichoerus grypus*) females at early and late lactation.

	Isle of May				North Rona			
	2005		2006		2005		2006	
	Early	Late	Early	Late	Early	Late	Early	Late
101	50.45	80.07	45.55	69.49	48.88	77.8	58.54	79.03
	(43.69 - 58.59)	(57.61 - 115.35)	(33.62 - 59.15)	(47.1 - 80.76)	(37.1 - 79.28)	(67.38 - 106.93)	(44.46 - 79.29)	(65.39 - 93.22)
149	16.31	23.78	19.3	32.79	7.28	12.69	20.04	30.21
	(12.7 - 25.54)	(16.76 - 30.28)	(16.84 - 33.12)	(19.85 - 52.82)	(3.66 - 15.84)	(9.72 - 19.77)	(11.89 - 31.72)	(26.68 - 40.27)
118	9.99	18.39	15.02	23.95	11.1	12.91	21.13	27.69
	(7.49 - 20.39)	(9.81 - 33.36)	(12.84 - 17.48)	(14.91 - 39.71)	(6.58 - 18.26)	(8.05 - 18.07)	(15.52 - 28.2)	(20.25 - 34.69)
153	435.06	746.39	246.27	423.53	252.7	378.68	165.78	246.87
	(375.22 - 528.45)	(495.88 - 963.75)	(181.07 - 311.73)	(283.52 - 659.05)	(193.49 - 311.63)	(302.81 - 494.75)	(110.04 - 237.07)	(195.13 - 331.51)
138	264.07	464.61	312.73	543.25	146.02	211.89	200.93	289.23
	(225.05 - 313.94)	(336.34 - 600.4)	(249.83 - 406.77)	(372.02 - 734.94)	(114.14 - 182.89)	(187.08 - 290.09)	(128 - 253.43)	(195.54 - 414.71)
187	116.97	213.02	110.4	209.8	48.18	82.32	78.41	123.19
	(93.46 - 142.92)	(145.38 - 333.84)	(87.87 - 162.45)	(127.84 - 358.64)	(34 - 65.41)	(65.8 - 102.43)	(49.24 - 111.16)	(92.86 - 170.76)
183	62.5	117.16	54.92	106.4	18.06	33.3	32.24	46.53
	(49.15 - 78.41)	(78.98 - 196.2)	(38.65 - 77.02)	(60.49 - 205.59)	(13.89 - 25.72)	(21.26 - 50.43)	(17.93 - 53.4)	(32.25 - 77.05)
128	12.89	34.92	14.5	30.87	12.71	22.36	8.63	11.78
	(11.29 - 24.36)	(20.46 - 51.35)	(8.14 - 18.77)	(11.56 - 32.5)	(6.81 - 29.27)	(15.34 - 41.05)	(5.96 - 14.05)	(1.63 - 22.53)
156	8	37.45	26.69	49.33	3.64	10	18.11	27.84
	(4.3 - 23.63)	(27.01 - 64.58)	(21.55 - 37.98)	(25.48 - 77.89)	(3.17 - 7.3)	(7.14 - 17.33)	(10.63 - 28.25)	(19.49 - 40.05)
180	225.8	387.17	241.64	445.2	95.56	156.27	157.78	253.18
	(200.51 - 269.67)	(225 - 528.53)	(191.58 - 346.92)	(320.45 - 639.12)	(65.29 - 125.98)	(107.08 - 211.4)	(97.94 - 235.95)	(174.94 - 356.26)
195	19.59	43.73	15.08	33.7	1.74	3.8	7.28	12.87
	(9.45 - 35.09)	(20.41 - 94.87)	(7.94 - 29.55)	(16.32 - 58.44)	(0.66 - 2.93)	(1.35 - 6.5)	(3.63 - 12.12)	(8.4 - 21.79)
194	44.1	90.38	42.76	94.92	9.77	16.82	24.25	48.09
	(28.59 - 72.85)	(51.97 - 157.83)	(21.18 - 79.03)	(48.74 - 192.14)	(8.21 - 18.25)	(10.99 - 34.45)	(12.56 - 48.59)	(32.66 - 82.15)
206	17.94	41.56	11.84	14.89	3.78	6.37	10.25	20.17
	(9.29 - 38.37)	(14.88 - 112.68)	(5.22 - 25.35)	(9.35 - 72.59)	(2.09 - 6.15)	(4.45 - 11.28)	(4.68 - 18.58)	(12.54 - 35.84)
209	11.24	25.82	8.83	19.87	0.55	2.05	6.33	12.24
	(3.33 - 28.75)	(6.78 - 84.01)	(4.03 - 16.23)	(7.23 - 49.81)	(0 - 0.09)	(0 - 1.54)	(3.1 - 9.97)	(7.76 - 19.39)
DDE	246.64	391.01	229.81	358.22	152.1	213.63	180.28	248.95
	(200.94 - 322.26)	(274.96 - 481.22)	(192.48 - 267.88)	(215.72 - 500.02)	(129.85 - 178.67)	(163.25 - 252.22)	(138.31 - 215.69)	(185 - 298.34)
DDD	8.68	8.88	2.09	4.53	5.87	6.28	6.13	6.1
	(1.52 - 15.37)	(1.69 - 27.46)	(1.46 - 6.26)	(2.01 - 7.8)	(4.39 - 11.96)	(2.71 - 11.64)	(4.04 - 7.46)	(4.24 - 8.58)
DDT	30.63	53.84	5.87	14.6	8.02	7.76	5.8	6.76
	(22.55 - 44.57)	(35.84 - 64.73)	(2.28 - 15.59)	(5.77 - 23)	(6.13 - 10.69)	(4.41 - 13.88)	(3.73 - 8.27)	(3.4 - 14.73)
PCB Total	1327.9	2396.44	1199.72	2176.72	680.17	1056.54	819.01	1242.82
	(1155.7 - 1608.3)	(1478.7 - 3413.5)	(965.73 - 1612.18)	(1393.37 - 3483.6)	(508.37 - 903.87)	(831.91 - 1401.21)	(505.6 - 1114.11)	(898.31 - 1803.01)
DDT Total	288.59	465.74	244.94	381.39	167.24	230.14	195.67	265.69
	(227.49 - 371.41)	(315.18 - 538.08)	(204.25 - 286.74)	(228.3 - 545.48)	(144.16 - 196.04)	(181.8 - 269.38)	(144.94 - 258.57)	(201.92 - 318.78)
Total	1618.49	2868.84	1452.54	2569.22	850.71	1295.44	1017.77	1511.33
	(1383.19 - 2011.43)	(1785.81 - 4059.82)	(1169.98 - 1928.57)	(1611.46 - 4048.38)	(641.37 - 1099.91)	(1049.5 - 1627.19)	(648.16 - 1340.42)	(1117.56 - 2162.62)

Appendix 11. Geometric mean and quantile ranges of OC concentrations in blood (ng/ml serum) from grey seal (*Halichoerus grypus*) females at early, mid and late lactation. A: Isle of May. B: North Rona.

A	2005			2006		
	Early	Mid	Late	Early	Mid	Late
101	0.16	0.14	0.17	0.21	0.2	0.3
	0.11 - 0.28	0.08 - 0.2	0.09 - 0.27	0.2 - 0.31	0.17 - 0.28	0.22 - 0.41
149	0.08	0.12	0.2	0.14	0.16	0.22
	0.04 - 0.15	0.04 - 0.17	0.07 - 0.28	0.12 - 0.17	0.1 - 0.18	0.14 - 0.34
118	0.03	0.04	0.04	0.16	0.17	0.17
	0 - 0.01	0 - 0.03	0 - 0.03	0.11 - 0.18	0.09 - 0.2	0.12 - 0.24
153	1.43	1.13	2.79	1.61	1.28	2.78
	0.97 - 2.33	0.89 - 1.88	1.5 - 4.04	1.09 - 2.21	0.85 - 2.33	1.38 - 3.69
138	0.99	1	1.73	1.13	1.06	1.89
	0.76 - 1.46	0.78 - 1.07	1.18 - 2.45	0.82 - 1.51	0.71 - 1.44	0.99 - 2.1
187	0.17	0.14	0.39	0.31	0.3	0.71
	0.11 - 0.27	0.09 - 0.22	0.19 - 0.77	0.21 - 0.43	0.19 - 0.55	0.28 - 1.01
183	0.08	0.06	0.19	0.12	0.16	0.33
	0.01 - 0.14	0.01 - 0.12	0.07 - 0.52	0.08 - 0.19	0.07 - 0.24	0.14 - 0.63
128	0.08	0.04	0.18	0.05	0.02	0.07
	0 - 0	0 - 0.01	0 - 0.22	0 - 0.04	0 - 0.01	0 - 0.05
156	0.12	0.03	0.15	0.06	0.06	0.12
	0 - 0	0 - 0	0 - 0.11	0.04 - 0.1	0.01 - 0.08	0.06 - 0.19
180	0.56	0.45	1.4	0.71	0.55	1.61
	0.31 - 1.1	0.21 - 0.73	0.83 - 2.76	0.45 - 1.09	0.34 - 1.18	0.62 - 2.71
195	0.09	0.02	0.19	0.04	0.04	0.14
	0 - 0	0 - 0.01	0 - 0.18	0 - 0.04	0 - 0.07	0.02 - 0.48
194	0.14	0.06	0.51	0.12	0.11	0.28
	0 - 0	0 - 0	0 - 0.41	0.02 - 0.12	0.02 - 0.15	0.06 - 0.85
206	0.1	0	0.61	0.06	0.05	0.15
	0 - 0	0 - 0	0 - 0.18	0 - 0.06	0 - 0.07	0.02 - 0.5
209	0	0	0	0.07	0.05	0.13
	0 - 0	0 - 0	0 - 0	0 - 0.06	0 - 0.06	0.01 - 0.48
DDE	0.75	0.53	1.22	0.94	0.7	1.3
	0.55 - 1.15	0.46 - 0.93	0.71 - 1.89	0.62 - 1.3	0.56 - 1.35	0.72 - 1.63
DDD	0.04	0	0.09	0.03	0.04	0.03
	0 - 0	0 - 0	0 - 0	0.02 - 0.04	0.02 - 0.06	0.02 - 0.06
DDT	0.04	0.01	0.12	0.05	0.06	0.08
	0 - 0.01	0 - 0	0 - 0.03	0.04 - 0.08	0.04 - 0.07	0.06 - 0.14
PCB Total	3.59	2.48	7.39	4.74	4.19	9.08
	2.5 - 5.54	2.45 - 4.52	4.39 - 11.68	3.29 - 6.4	2.52 - 7.04	3.9 - 13.33
DDT Total	0.76	0.53	1.25	1.03	0.89	1.43
	0.55 - 1.17	0.46 - 0.93	0.71 - 1.97	0.73 - 1.4	0.62 - 1.46	0.77 - 1.78
Total	4.36	3.02	8.68	5.79	5.11	10.59
	3 - 6.72	3.04 - 5.46	5.04 - 13.43	4.03 - 7.87	3.14 - 8.39	4.67 - 14.68

Appendix 11. Continued

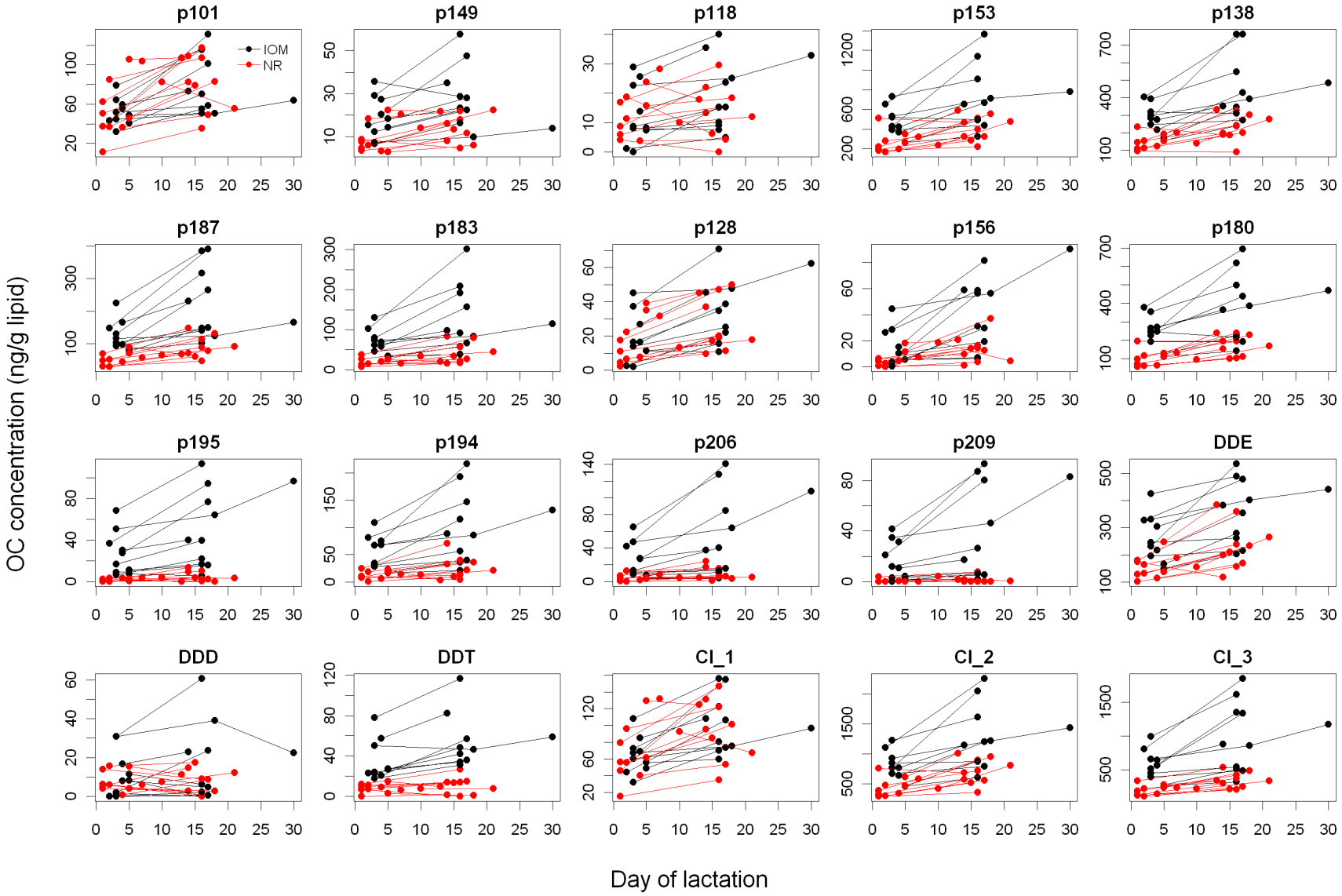
B	2005			2006		
	Early	Mid	Late	Early	Mid	Late
101	0.13	0.15	0.22	0.34	0.22	0.31
	0.13 - 0.21	0.1 - 0.22	0.15 - 0.31	0.19 - 0.6	0.15 - 0.38	0.18 - 0.49
149	0.05	0.05	0.11	0.21	0.1	0.16
	0.02 - 0.12	0.02 - 0.09	0.08 - 0.21	0.11 - 0.37	0.03 - 0.18	0.1 - 0.36
118	0.07	0.08	0.1	0.26	0.13	0.14
	0.04 - 0.08	0.06 - 0.09	0.07 - 0.14	0.1 - 0.36	0.08 - 0.23	0.08 - 0.21
153	0.83	0.9	1.13	2.28	1.02	1.75
	0.65 - 1.15	0.59 - 1.49	0.78 - 2.44	1.05 - 4.8	0.6 - 1.56	0.86 - 3.01
138	0.55	0.59	0.88	0.98	0.88	1.17
	0.46 - 0.76	0.39 - 0.86	0.56 - 1.49	0.6 - 2.96	0.52 - 1.86	0.62 - 2.33
187	0.15	0.14	0.29	0.38	0.25	0.35
	0.11 - 0.23	0.07 - 0.22	0.18 - 0.69	0.17 - 0.76	0.12 - 0.34	0.18 - 0.82
183	0.05	0.09	0.13	0.05	0.05	0.09
	0.01 - 0.08	0.04 - 0.11	0.08 - 0.19	0 - 0.07	0 - 0.03	0.02 - 0.22
128	0.02	0.02	0.04	0.14	0.08	0.09
	0.02 - 0.02	0.01 - 0.03	0.02 - 0.06	0.01 - 0.26	0 - 0.17	0.01 - 0.27
156	0.03	0.04	0.06	0.04	0.02	0.05
	0.02 - 0.05	0.02 - 0.06	0.03 - 0.11	0 - 0.07	0 - 0.01	0.01 - 0.11
180	0.23	0.29	0.57	0.79	0.43	0.63
	0.16 - 0.36	0.17 - 0.51	0.33 - 1.18	0.38 - 1.77	0.27 - 0.5	0.29 - 1.73
195	0.02	0.02	0.03	0.03	0.02	0.04
	0 - 0.01	0 - 0.02	0.01 - 0.05	0 - 0.01	0 - 0.02	0 - 0.05
194	0.03	0.03	0.07	0.05	0.04	0.08
	0 - 0.02	0 - 0.04	0.02 - 0.19	0 - 0.07	0 - 0.01	0.01 - 0.16
206	0.01	0.02	0.04	0.03	0.04	0.09
	0 - 0.01	0 - 0.03	0.02 - 0.09	0 - 0.02	0 - 0.02	0 - 0.08
209	0	0	0	0.02	0.03	0.06
	0 - 0	0 - 0	0 - 0	0 - 0.01	0 - 0	0 - 0.01
DDE	0.51	0.52	0.69	1.13	0.65	0.74
	0.49 - 0.56	0.41 - 0.62	0.51 - 0.98	0.55 - 2.13	0.37 - 1.01	0.43 - 1.24
DDD	0.02	0.03	0.03	0.03	0.01	0.03
	0.02 - 0.03	0.01 - 0.04	0.02 - 0.05	0 - 0.03	0 - 0.01	0.02 - 0.04
DDT	0.03	0.04	0.05	0.04	0.03	0.03
	0.02 - 0.04	0.03 - 0.04	0.03 - 0.08	0.01 - 0.07	0.01 - 0.03	0.02 - 0.04
PCB Total	2.16	2.39	3.85	6.07	3.19	5.06
	1.84 - 3.05	1.67 - 3.65	2.4 - 7.66	3.01 - 12.64	1.95 - 5.17	2.38 - 11.14
DDT Total	0.57	0.59	0.77	1.21	0.68	0.78
	0.54 - 0.61	0.47 - 0.69	0.56 - 1.08	0.59 - 2.25	0.4 - 1.02	0.47 - 1.31
Total	2.75	3.02	4.66	7.38	3.88	5.91
	2.4 - 3.66	2.13 - 4.25	2.98 - 8.77	3.45 - 15.28	2.35 - 6.18	2.86 - 12.75

Appendix 12. Geometric mean and quantile ranges of OC concentrations in milk (ng/g lipid) from grey seal females at early, mid and late lactation. A: Isle of May. B: North Rona.

A	2005			2006		
	Early	Mid	Late	Early	Mid	Late
101	39.76	37.24	53.56	35.7	37.11	55.77
	34.03 ± 49.07	35.12 ± 39.12	42.3 ± 65.4	30.4 ± 44.14	31.49 ± 43.44	43.47 ± 73.91
149	16.28	9.76	24.85	20.55	20.57	37.55
	12.46 ± 22.09	7.79 ± 14.61	20.64 ± 32.54	14.56 ± 33.33	14.56 ± 31.13	28.76 ± 50.09
118	8.69	6.72	14.67	12.65	12.59	19.49
	5.6 ± 14.13	4.71 ± 9.05	10.52 ± 19.54	10.94 ± 19.46	12.22 ± 16.7	13.73 ± 25.88
153	213.77	182.27	290.29	137.44	124.17	207.47
	159.64 ± 270.65	137.41 ± 217.01	213.97 ± 389.56	87.91 ± 181.52	94.44 ± 147.2	130.19 ± 339.08
138	143.62	123.2	191.97	147.11	131.59	203.59
	107.09 ± 180.68	97.14 ± 149.96	148.31 ± 256.38	97.06 ± 181.05	99.69 ± 160.65	124.34 ± 308.19
187	36.46	20.21	54.1	39.32	33.47	64.49
	28.11 ± 50.04	16.66 ± 33.64	36.84 ± 74.8	22.26 ± 52.11	24.24 ± 44.45	39.09 ± 117.09
183	6.85	4.34	14.28	22.02	18.34	36.86
	3.73 ± 13.76	2.26 ± 10.05	8.36 ± 21.44	11.88 ± 28.45	12.6 ± 22.16	19.98 ± 64.53
128	16.48	10.58	24.7	11.95	10.5	22.56
	10.88 ± 20.98	4.38 ± 16.12	13.2 ± 39.43	7.07 ± 15.19	7.29 ± 14.07	11.25 ± 37.29
156	4.63	2.86	5.48	5.09	7.02	20.91
	0 ± 7.57	0 ± 2.88	3.56 ± 10.21	3.01 ± 13.57	4.67 ± 12.78	9.66 ± 24.67
180	66.45	46.6	101.36	80.04	70.5	121.72
	48.66 ± 102.8	35.88 ± 76.65	62.72 ± 148.52	49.47 ± 105.13	48.66 ± 86.56	56.83 ± 209.12
195	4.94	11.81	8.36	3.53	4.24	7.35
	0 ± 1.41	0 ± 0	0 ± 7.99	0 ± 4.14	0 ± 4.18	0.41 ± 14.09
194	6.82	5.34	8.3	6.12	5.43	12.24
	2.02 ± 10.68	0 ± 4.56	0.71 ± 19.84	1.75 ± 11.43	0.17 ± 7.37	3.15 ± 30.27
206	3	3.74	5.28	5.25	3.04	28.11
	0 ± 1.08	0 ± 0	0 ± 3.44	0 ± 0	0 ± 0.28	0 ± 3.68
209	2.01	0	0	10.48	4.28	15.02
	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
DDE	162.42	147.77	225.13	132.72	129.81	178.27
	117.66 ± 198.06	115.24 ± 164.97	162.18 ± 284.12	103.04 ± 166.23	109.87 ± 154.88	122.78 ± 253.44
DDD	4.49	3.94	5.28	6.71	7.8	13.17
	0 ± 1.48	0 ± 2.64	0 ± 5.68	5 ± 9.84	5.29 ± 11.35	8.22 ± 16.91
DDT	20.28	15.06	31.72	14.82	15.4	25.66
	11.17 ± 31.66	11.86 ± 17.46	22.73 ± 45.73	10.64 ± 20.6	12.78 ± 21.43	17.79 ± 39.2
PCB Total	563.82	455.33	798.52	481.76	483.64	828.03
	417.02 ± 692.93	348.61 ± 557.79	561.03 ± 1128.02	326.92 ± 654.58	357.44 ± 571.02	483 ± 1308.41
DDT Total	182.92	165.52	260.32	153.51	154.36	219.01
	133.49 ± 231.92	129.77 ± 183.16	184.99 ± 345.12	114.79 ± 207.14	125.84 ± 188.55	147.35 ± 310.95
Total	747.66	621.62	1060.69	638.07	640.03	1052.06
	550.51 ± 887.21	478.69 ± 740.96	747.81 ± 1461.32	439.15 ± 861.73	475.74 ± 773.33	628.4 ± 1614.25

Appendix 12. Continued

B	2005			2006		
	Early	Mid	Late	Early	Mid	Late
101	47.45	42.12	75.38	44.89	40.94	51.79
	40.6 ± 56.45	33.09 ± 52	57.33 ± 107.38	36.36 ± 43.16	30.16 ± 49.19	41.6 ± 62.69
149	13.61	13.42	19.27	17.95	16.38	20.97
	13.15 ± 13.66	10.84 ± 12.95	14.38 ± 25.38	14.42 ± 20.9	9.75 ± 20.05	11.12 ± 22.78
118	7.26	6.26	14.33	15.29	12.71	18.74
	2.42 ± 7.98	4.24 ± 11.34	9.31 ± 24.7	10.79 ± 22.65	9.03 ± 19.32	14.1 ± 29.23
153	109.15	92.67	180.4	103.43	81.38	81.84
	85.35 ± 129.3	69.78 ± 129.57	122.47 ± 333.47	77.73 ± 127.73	56.32 ± 106.05	57.58 ± 117.76
138	75.05	68.09	126.65	89.16	71.92	112.04
	60.44 ± 88.53	53.2 ± 93.28	90.7 ± 218.94	65.62 ± 127.97	50.04 ± 87.72	76.25 ± 178.32
187	14.34	10.46	35.91	24.98	18.02	31.49
	10.06 ± 18.73	5.96 ± 18.44	20.52 ± 74.33	16.36 ± 36.07	10.87 ± 24.38	21.27 ± 53.8
183	4.14	2.55	9.99	15.63	11.24	19.73
	1.12 ± 5.17	1.64 ± 5.27	6.78 ± 15.72	9.62 ± 23.94	7.26 ± 15.41	12.6 ± 35.01
128	7.68	5	18.49	7.3	5.61	9.77
	0 ± 5.12	0 ± 6.49	8.4 ± 27.11	3.68 ± 12.76	3.14 ± 11.1	6.37 ± 12.92
156	5.91	6.44	8.52	6.65	3.66	8.89
	0.37 ± 8.25	2.01 ± 7.95	5.08 ± 15.09	2.51 ± 11.91	1.68 ± 10.77	6.08 ± 15.15
180	26.29	21.93	59.27	44.76	32.55	50.07
	17.72 ± 38.73	16.03 ± 32.97	37.84 ± 128.74	33.36 ± 59.9	21.49 ± 43.81	34.84 ± 73.94
195	0	0	7.07	2.39	0.5	3.31
	0 ± 0	0 ± 0	0 ± 2.46	0 ± 1.16	0 ± 0.04	0 ± 4.55
194	0	0.48	6.79	3.57	1.71	3.55
	0 ± 0	0 ± 0	0 ± 14.14	0.67 ± 5.07	0 ± 2.56	0.99 ± 10.49
206	0	0	2.76	0.49	0	4.59
	0 ± 0	0 ± 0	0 ± 0.96	0 ± 0	0 ± 0	0 ± 0
209	0	0	0	0.09	0	0
	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
DDE	106.1	99.43	159.52	117.7	95.65	124.9
	83.84 ± 125.58	77.65 ± 113.61	126.52 ± 206.55	79.66 ± 160.22	74.03 ± 106.32	100.62 ± 160.59
DDD	5.55	4.05	5.04	8.53	8.69	9.28
	0 ± 4.14	0.18 ± 7.73	0.62 ± 7.4	4.14 ± 12.73	5.4 ± 10.32	6.04 ± 14.16
DDT	4.27	5.01	15.19	6.61	8.47	11.74
	4.36 ± 7.06	4 ± 8.73	11.39 ± 20.66	4.04 ± 9.42	6.26 ± 9.16	8.85 ± 14.5
PCB Total	305.94	271.67	555.36	379.23	297.05	430.78
	231.69 ± 356.03	206.5 ± 371.98	378.57 ± 1036.73	280.68 ± 494.12	201.59 ± 377.34	302.83 ± 587.08
DDT Total	113.47	109.21	179.61	133.17	111.91	147.82
	85.42 ± 136.74	87.31 ± 127.78	146.97 ± 230.38	88.6 ± 177.84	85.48 ± 125.79	115.62 ± 188.76
Total	420.1	382.75	740.69	513.81	410.59	581.24
	316.18 ± 492.19	293.81 ± 491.34	525.62 ± 1292.91	386.49 ± 678.61	280.83 ± 496.7	413.64 ± 795.25



Appendix 13 A. Line plots showing the development of OC concentrations in blubber during lactation period in grey seal females. Each line represents and individual sampled in Isle of May (IOM) and North Rona (NR) colonies in 2005.

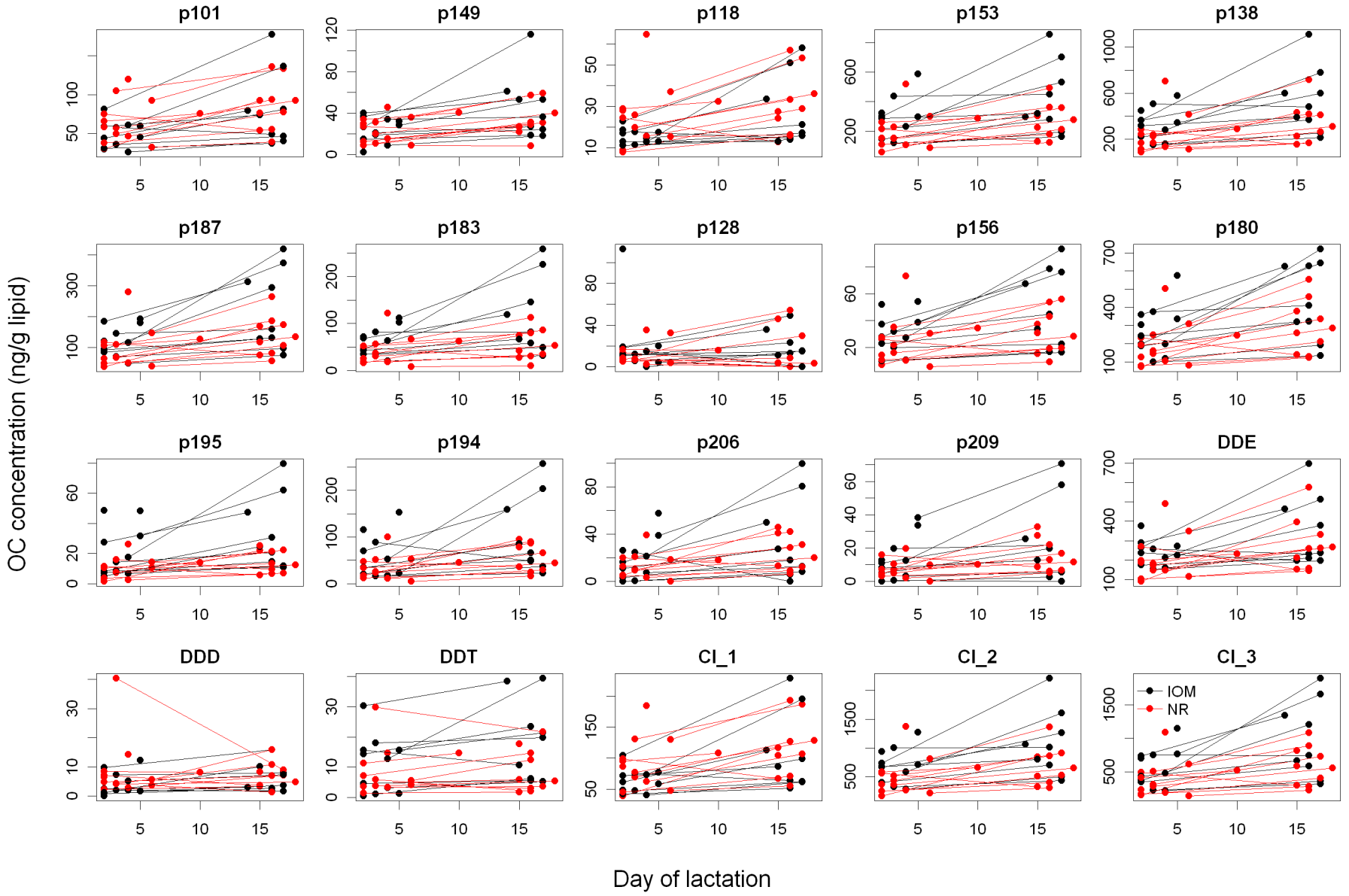


Figure 13 B. Line plots showing the development of OC concentreations in blubber during lactation period in grey seal females. Each line represents and individual sampled in Isle of May (IOM) and North Rona (NR) colonies in 2006.

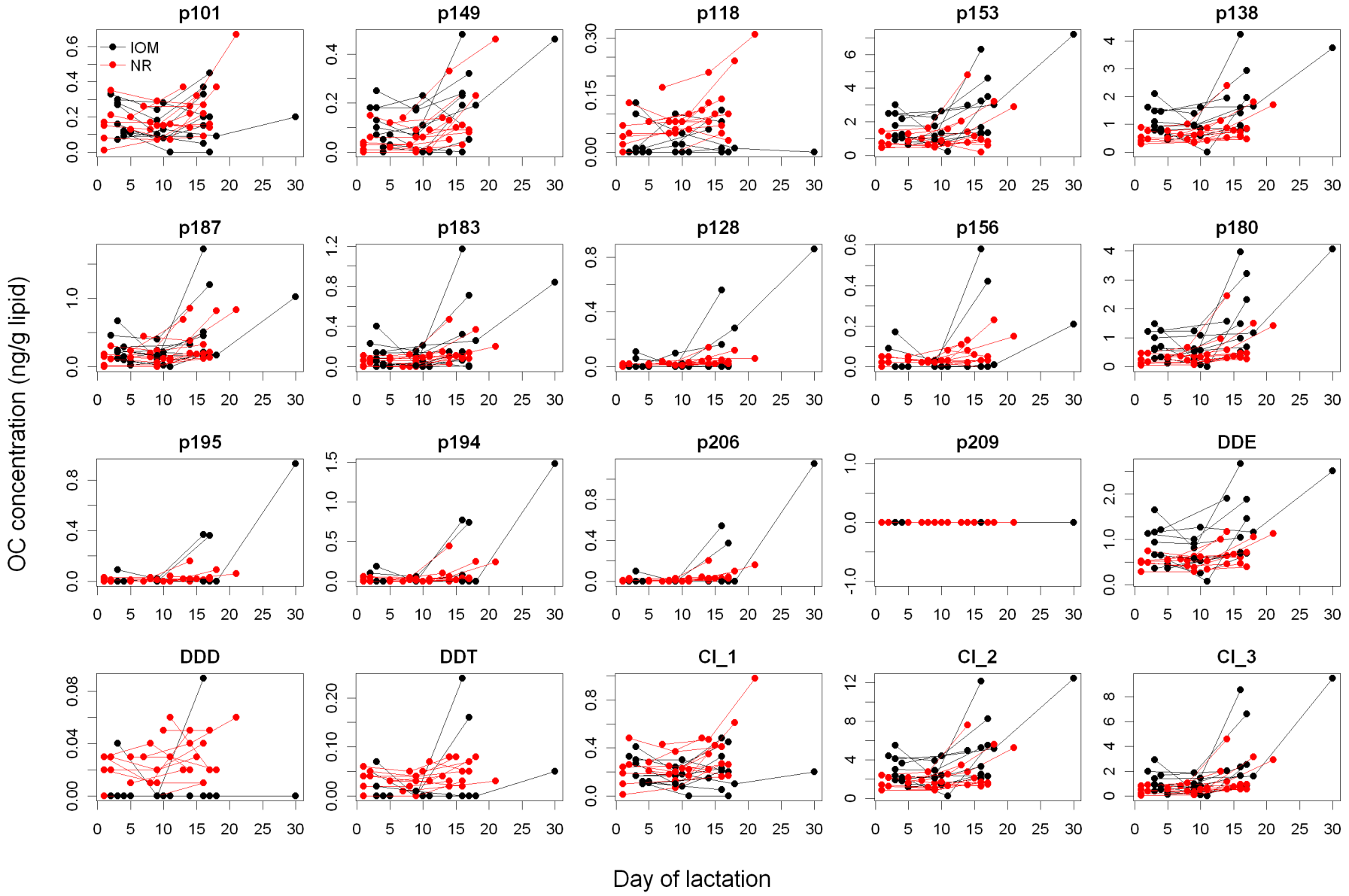


Figure 14 A. Line plots showing the development of OC concentrations in serum during lactation period in grey seal females. Each line represents and individual sampled in Isle of May (IOM) and North Rona (NR) colonies in 2005.

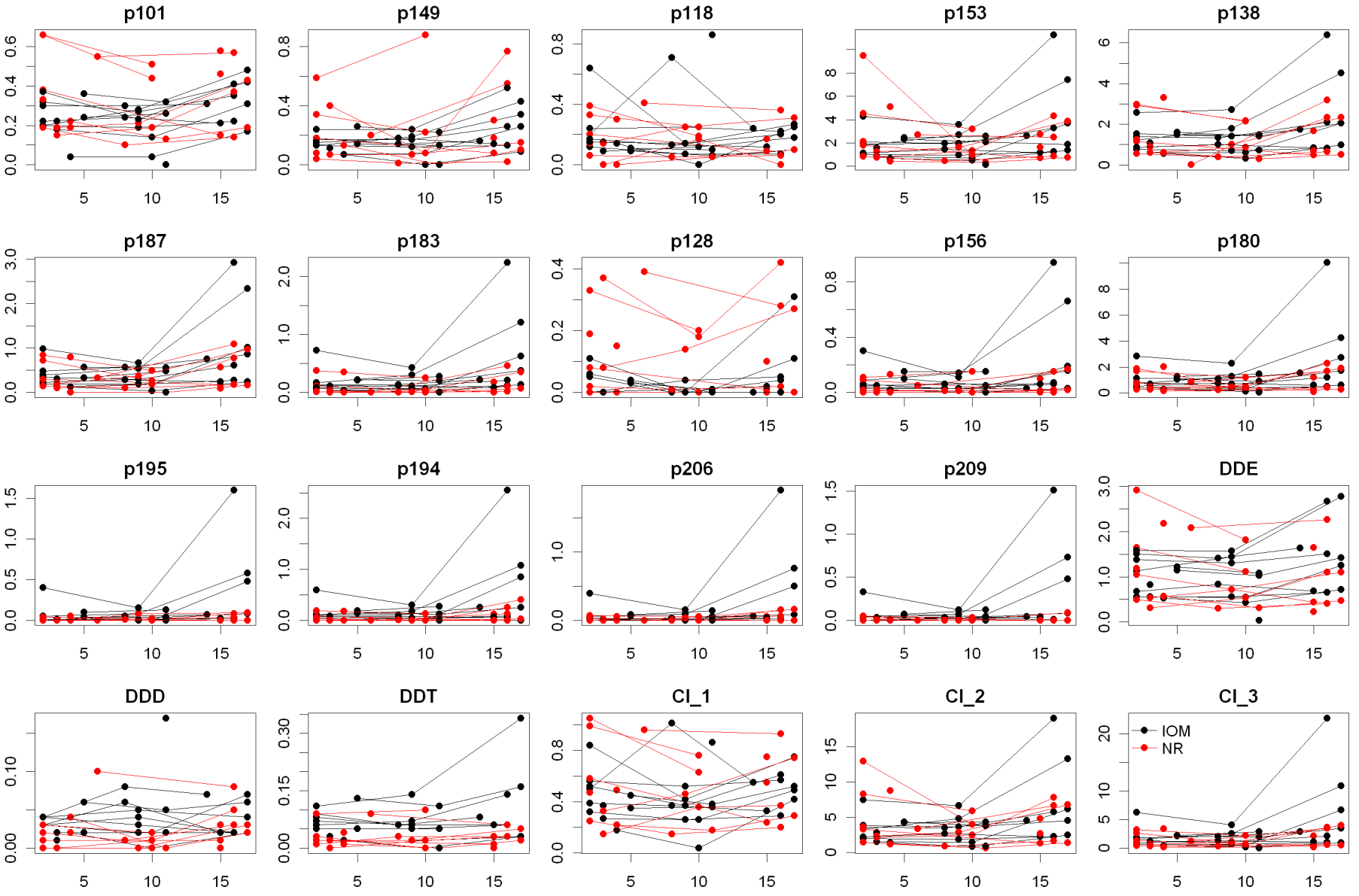


Figure 14 B. Line plots showing the development of OC concentrations in serum during lactation period in grey seal females. Each line represents and individual sampled in Isle of May (IOM) and North Rona (NR) colonies in 2006.

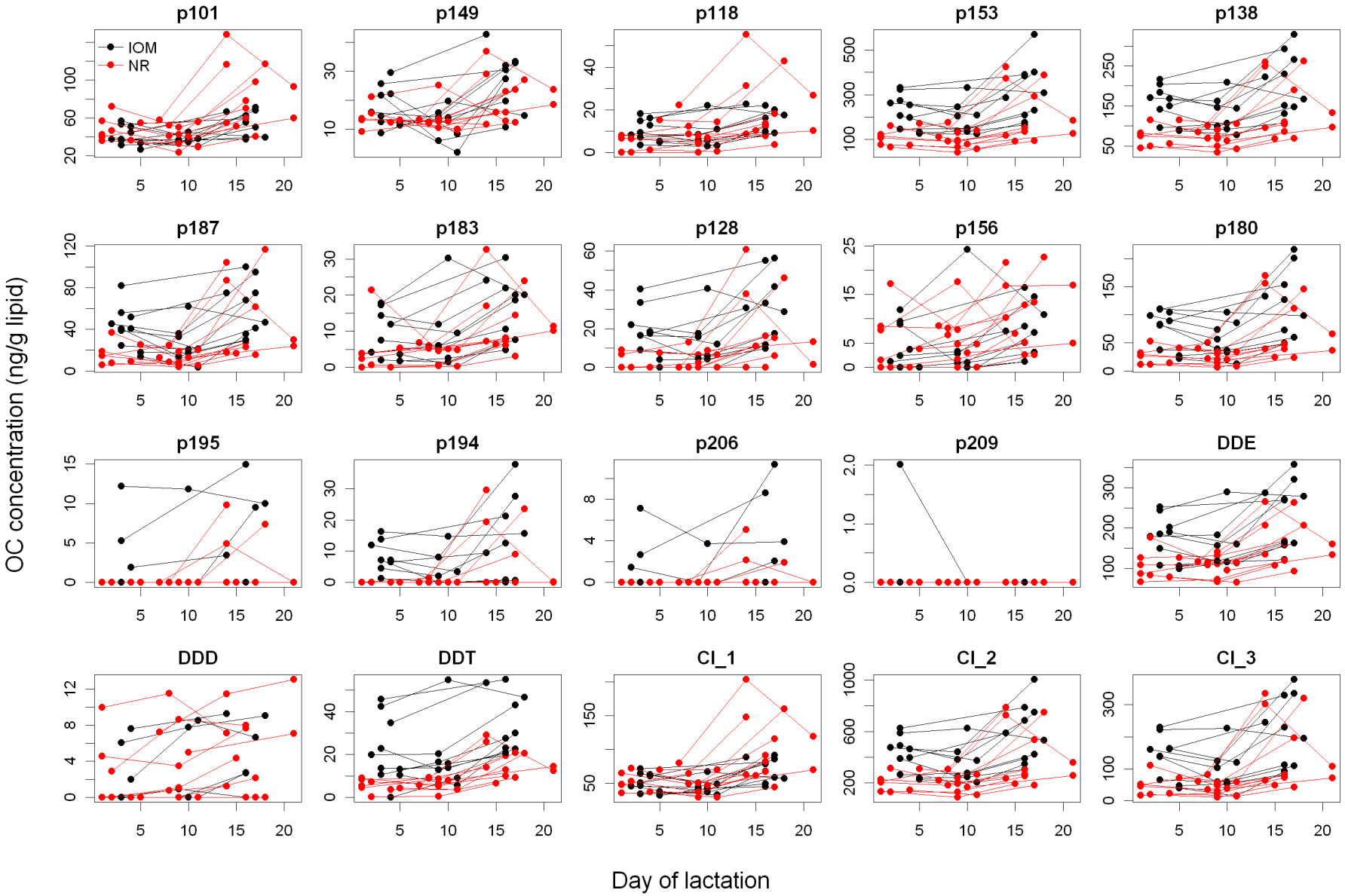


Figure 15 A. Line plots showing the development of OC concentreations in milk during lactation period in grey seal females. Each line represents and individual sampled in Isle of May (IOM) and North Rona (NR) colonies in 2005.

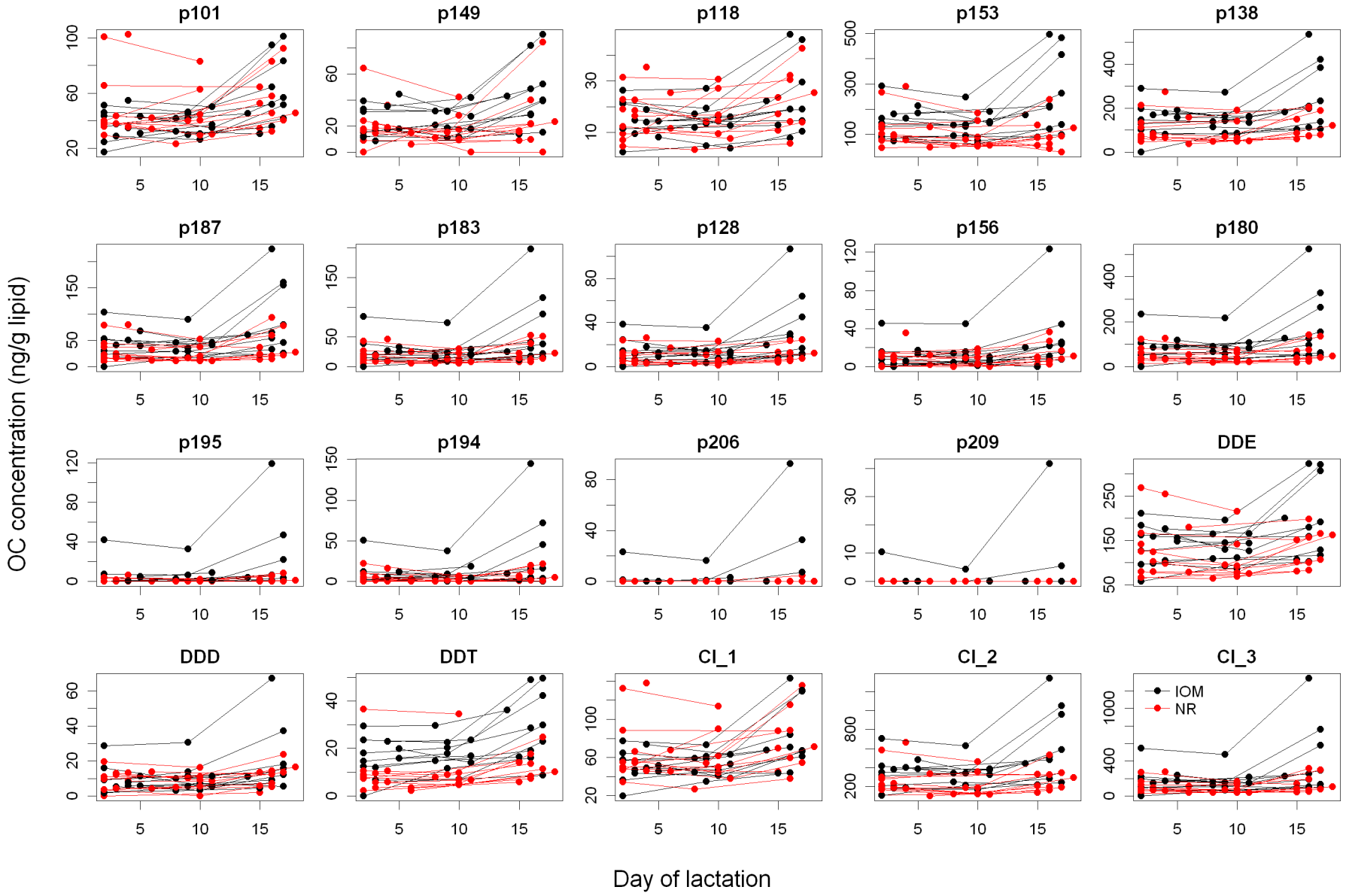


Figure 15 B. Line plots showing the development of OC concentreations in milk during lactation period in grey seal females. Each line represents and individual sampled in Isle of May (IOM) and North Rona (NR) colonies in 2006.

Appendix 16. Geometric mean and quantile ranges of OC concentrations in serum (ng/ml serum) from grey seal pups at early, mid and late lactation during two consecutive years. A: Isle of May. B: North Rona.

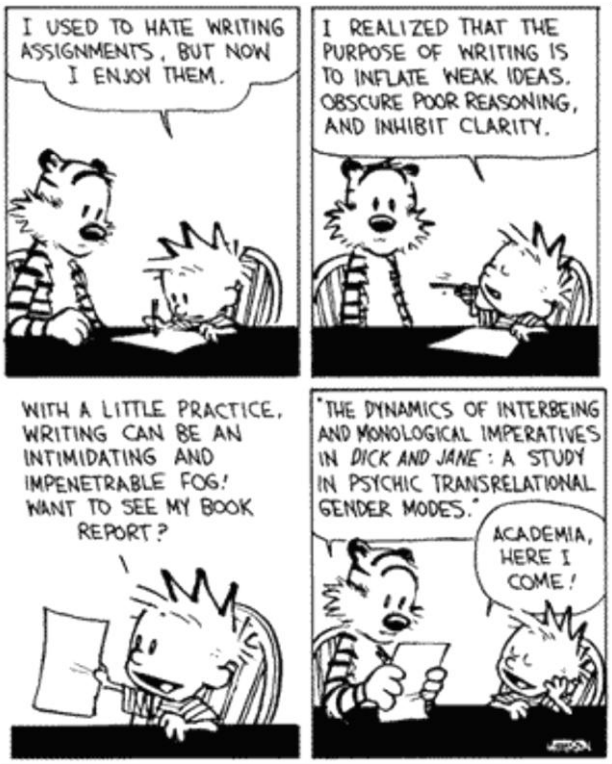
A	2005			2006		
	EL	ML	LL	EL	ML	LL
101	0.27	0.35	0.37	0.41	0.46	0.58
	0.2 - 0.4	0.37 - 0.49	0.28 - 0.65	0.33 - 0.53	0.41 - 0.55	0.39 - 0.77
149	0.16	0.23	0.36	0.22	0.26	0.4
	0.12 - 0.36	0.2 - 0.46	0.15 - 0.55	0.21 - 0.25	0.21 - 0.3	0.24 - 0.51
118	0.09	0.07	0.13	0.19	0.27	0.37
	0.04 - 0.1	0.05 - 0.12	0.12 - 0.15	0.16 - 0.29	0.2 - 0.33	0.24 - 0.54
153	3.17	3.16	5.66	3.01	3.04	4.32
	2.62 - 4	2.74 - 4.1	4.29 - 7.5	2.12 - 4.48	2.81 - 4.33	2.8 - 6.84
138	2.11	2.29	3.69	2.11	2.32	3.03
	1.77 - 2.59	1.9 - 3.01	3.16 - 4.88	1.46 - 3.03	1.96 - 2.92	2.17 - 4.74
187	0.6	0.57	1.01	0.5	0.73	1.34
	0.49 - 0.68	0.51 - 0.94	0.72 - 1.9	0.52 - 0.9	0.54 - 1.09	0.68 - 1.95
183	0.33	0.27	0.58	0.34	0.31	0.62
	0.27 - 0.43	0.26 - 0.43	0.37 - 1.25	0.19 - 0.47	0.23 - 0.46	0.29 - 0.88
128	0.1	0.08	0.49	0.09	0.07	0.29
	0.02 - 0.11	0.02 - 0.13	0.12 - 0.77	0.01 - 0.16	0 - 0.08	0 - 0.31
156	0.08	0.15	0.36	0.13	0.17	0.37
	0.04 - 0.12	0.04 - 0.17	0.1 - 0.63	0.08 - 0.28	0.11 - 0.22	0.21 - 0.51
180	1.68	1.58	3.07	1.48	1.51	1.98
	1.44 - 2.13	1.28 - 2.41	2.6 - 5.2	0.92 - 2.34	1.07 - 2.11	1.33 - 3.71
195	0.09	0.06	0.39	0.12	0.11	0.19
	0 - 0.07	0 - 0.03	0.11 - 0.69	0.04 - 0.17	0.04 - 0.16	0.04 - 0.3
194	0.16	0.18	0.8	0.31	0.25	0.46
	0.02 - 0.18	0 - 0.22	0.19 - 1.53	0.14 - 0.38	0.1 - 0.35	0.17 - 0.72
206	0.02	0.06	0.36	0.12	0.11	0.21
	0 - 0.01	0 - 0.01	0.01 - 0.62	0.04 - 0.18	0.03 - 0.16	0.05 - 0.29
209	0	0	0	0.1	0.09	0.15
	0 - 0	0 - 0	0 - 0	0.04 - 0.12	0.03 - 0.11	0.03 - 0.21
DDE	1.58	1.85	2.57	1.75	2.06	2.72
	1.22 - 1.96	1.72 - 2.31	2.1 - 3.44	1.15 - 2.41	1.96 - 2.47	1.82 - 3.83
DDD	0.01	0.11	0.11	0.05	0.04	0.06
	0 - 0	0 - 0	0 - 0.1	0.03 - 0.05	0.03 - 0.06	0.05 - 0.06
DDT	0.05	0.1	0.18	0.11	0.13	0.19
	0 - 0.05	0 - 0.2	0.01 - 0.37	0.07 - 0.2	0.08 - 0.23	0.1 - 0.34
PCBTotal	8.93	9.04	17.17	9.02	9.77	13.93
	7.45 - 11.11	8.09 - 12.43	14.4 - 25.59	6.29 - 13.81	7.98 - 13.42	8.83 - 21.34
DDTTotal	1.62	1.93	2.76	1.99	2.24	2.3
	1.27 - 2	1.74 - 2.51	2.31 - 3.75	1.45 - 2.66	2.06 - 2.62	2 - 4.26
AllTotal	10.58	11	20.01	11.1	12.05	16.4
	8.76 - 12.91	9.59 - 15.09	17.02 - 28.98	7.54 - 16.69	10.14 - 16.01	10.88 - 25.49

Appendix 16. Continued.

B	2005			2006		
	EL	ML	LL	EL	ML	LL
101	0.38	0.47	0.68	0.36	0.73	0.5
	0.29 - 0.43	0.45 - 0.62	0.56 - 0.93	0.25 - 0.55	0.46 - 0.85	0.33 - 0.74
149	0.15	0.2	0.29	0.14	0.24	0.25
	0.1 - 0.24	0.17 - 0.33	0.2 - 0.49	0.1 - 0.31	0.16 - 0.38	0.12 - 0.46
118	0.15	0.2	0.29	0.2	0.39	0.33
	0.12 - 0.2	0.15 - 0.29	0.23 - 0.38	0.17 - 0.33	0.34 - 0.44	0.15 - 0.74
153	1.85	2.43	3.49	2.95	3.22	3.85
	1.41 - 2.42	1.94 - 3.08	2.5 - 4.71	1.69 - 5.08	1.39 - 5.7	1.47 - 9
138	1.2	1.59	2.4	1.65	2.13	2.09
	0.92 - 1.61	1.34 - 1.99	1.61 - 3	0.9 - 2.98	0.99 - 3.88	0.95 - 5.73
187	0.38	0.46	0.81	0.47	0.5	0.76
	0.3 - 0.54	0.35 - 0.68	0.47 - 1.23	0.28 - 0.81	0.25 - 0.95	0.33 - 2.25
183	0.13	0.2	0.37	0.25	0.21	0.35
	0.09 - 0.21	0.15 - 0.3	0.22 - 0.54	0.1 - 0.41	0.11 - 0.33	0.14 - 1.16
128	0.06	0.08	0.15	0.11	0.27	0.18
	0.04 - 0.08	0.07 - 0.16	0.1 - 0.21	0.01 - 0.13	0.08 - 0.39	0.06 - 0.39
156	0.07	0.09	0.15	0.08	0.12	0.15
	0.04 - 0.09	0.06 - 0.2	0.09 - 0.3	0.04 - 0.2	0.06 - 0.24	0.07 - 0.42
180	0.74	0.96	1.6	1.07	1.15	1.54
	0.63 - 1.04	0.76 - 1.25	1 - 2.31	0.49 - 2.2	0.55 - 2.26	0.64 - 4.72
195	0.02	0.04	0.06	0.03	0.06	0.07
	0 - 0.04	0.02 - 0.06	0.02 - 0.16	0.01 - 0.06	0.02 - 0.09	0.01 - 0.26
194	0.07	0.13	0.24	0.11	0.12	0.21
	0.04 - 0.09	0.08 - 0.15	0.08 - 0.44	0.04 - 0.26	0.07 - 0.32	0.07 - 0.74
206	0.04	0.05	0.07	0.05	0.08	0.07
	0.02 - 0.05	0.01 - 0.07	0.03 - 0.19	0.01 - 0.08	0.05 - 0.11	0.03 - 0.22
209	0	0	0	0.03	0.03	0.03
	0 - 0	0 - 0	0 - 0	0 - 0.04	0 - 0.06	0.01 - 0.04
DDE	1.14	1.54	2.09	1.32	2.08	1.69
	0.92 - 1.28	1.41 - 2.02	1.65 - 2.34	0.89 - 2.5	1.23 - 3.26	0.94 - 3.68
DDD	0.04	0.04	0.06	0.04	0.07	0.04
	0.03 - 0.05	0.03 - 0.06	0.04 - 0.07	0 - 0.05	0.06 - 0.09	0.03 - 0.07
DDT	0.07	0.09	0.12	0.04	0.1	0.09
	0.04 - 0.1	0.07 - 0.11	0.1 - 0.17	0.03 - 0.1	0.08 - 0.12	0.04 - 0.18
PCBTotal	5.31	6.98	10.81	7.65	9.24	10.77
	3.97 - 6.84	5.88 - 9	7.02 - 15.3	3.87 - 14.62	4.22 - 16.43	4.57 - 27.57
DDTTotal	1.26	1.68	2.28	1.41	2.26	1.84
	1 - 1.38	1.52 - 2.17	1.78 - 2.56	0.93 - 2.61	1.35 - 3.52	1.03 - 3.91
AllTotal	6.59	8.69	13.22	9.12	11.56	12.74
	4.92 - 8.27	7.54 - 10.62	8.79 - 17.76	4.65 - 17.35	5.56 - 19.95	5.63 - 31.65

Appendix 17. Geometric mean and quantile ranges of OC concentrations in blubber (ng/g lipid) from grey seal pup at late lactation in Isle of May and North Rona during two consecutive years.

	Isle of May		North Rona	
	2005	2006	2005	2006
101	80.76	36.64	24.75	43.66
	62.25 - 95.71	29.93 - 45	22.69 - 26.17	35.33 - 51.23
149	28.59	12.97	8.76	15.46
	22.04 - 33.89	10.6 - 15.93	8.03 - 9.26	12.51 - 18.14
118	1.94	0.88	0.6	1.05
	1.5 - 2.3	0.72 - 1.08	0.55 - 0.63	0.85 - 1.23
153	1214.53	551	372.15	656.58
	936.13 - 1439.33	450.03 - 676.74	341.23 - 393.51	531.23 - 770.43
138	753.25	341.73	230.81	407.22
	580.59 - 892.68	279.11 - 419.72	211.63 - 244.06	329.47 - 477.82
187	274.84	124.69	84.22	148.58
	211.84 - 325.71	101.84 - 153.14	77.22 - 89.05	120.21 - 174.34
183	190.73	86.53	58.44	103.11
	147.01 - 226.03	70.67 - 106.28	53.59 - 61.8	83.42 - 120.99
128	5.07	2.3	1.55	2.74
	3.91 - 6.01	1.88 - 2.83	1.43 - 1.64	2.22 - 3.22
156	49.31	22.37	15.11	26.66
	38 - 58.43	18.27 - 27.47	13.85 - 15.98	21.57 - 31.28
180	701.8	318.39	215.04	379.4
	540.94 - 831.7	260.05 - 391.05	197.18 - 227.39	306.96 - 445.19
195	68.58	31.11	21.01	37.07
	52.86 - 81.27	25.41 - 38.21	19.27 - 22.22	29.99 - 43.5
194	151.79	68.86	46.51	82.06
	117 - 179.88	56.24 - 84.58	42.65 - 49.18	66.39 - 96.29
206	78.22	35.49	23.97	42.29
	60.29 - 92.7	28.98 - 43.58	21.98 - 25.34	34.21 - 49.62
209	38.92	17.66	11.93	21.04
	30 - 46.13	14.42 - 21.69	10.94 - 12.61	17.03 - 24.69
DDE	611.82	277.57	187.47	330.76
	471.58 - 725.06	226.7 - 340.91	171.9 - 198.23	267.61 - 388.1
DDD	0	0	0	0
	0 - 0	0 - 0	0 - 0	0 - 0
DDT	42.85	19.44	13.13	23.16
	33.03 - 50.78	15.88 - 23.87	12.04 - 13.88	18.74 - 27.18
PCBTotal	3638.34	1650.62	1114.84	1966.92
	2804.36 - 4311.78	1348.15 - 2027.31	1022.23 - 1178.85	1591.39 - 2307.97
DDTTotal	654.67	297	200.6	353.92
	504.6 - 775.84	242.58 - 364.79	183.93 - 212.12	286.35 - 415.28
AllTotal	4293.01	1947.62	1315.43	2320.84
	3308.97 - 5087.62	1590.73 - 2392.1	1206.16 - 1390.96	1877.73 - 2723.25



By Bill Watterson