The Early Ethology of Wild Grey Seal (Halichoerus grypus) Neonates over the Period of Maternal Dependency: An Assessment of the Nature and Function of Behaviour

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The Early Ethology of Wild Grey Seal (*Halichoerus grypus*) Neonates over the Period of Maternal Dependency:

An Assessment of the Nature and Function of Behaviour

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ABSTRACT

With the purpose of quantifying the behavioural patterns of pre-weaned grey seal (*Halichoerus grypus*) neonates, detailed neonatal behavioural records were used to provide an overview of typical behaviour budgets during the brief period of maternal dependency, or lactation period. Neonates were shown to spend the majority of their time inactive, with the assumption that such immobility maximises energy retention and mass gain during the short nursing period. Investigation at an individual-level revealed that neonates show consistent differences in the expression of some behavioural traits relative to one another over time. However, behavioural repeatability estimates for neonates were found to be lower than those so far reported in adult behavioural traits, suggesting that individual behavioural consistency may increase with age in this species. To current knowledge, this study represents the first attempt to quantify individual behavioural consistency in the neonatal age-class in this species.

This study provides a rare exploration of neonatal play behaviour in grey seals. There is weak suggestion that different types of play show different developmental trajectories over time, inferring that play function(s) could be dynamic. However, this area of study requires further systematic research to confirm these ideas. Maternal nutritional investment was not found to have a direct relationship with neonatal play. Interestingly, when the independent play behaviour of male and female siblings was compared in order to control for maternal and genetic differences, a sex difference was indicated in relation to maternal condition. Sons of poorer condition mothers (estimated by post-partum mass) were found to play more, whilst daughters of better condition mothers played more by contrast. An intriguing relationship was also indicated to suggest that the offspring of mothers in poorer condition (based on morphometric estimations) engage in more social play, predominantly with the maternal figure. Validation of estimations of maternal body condition is essential for enhancing confidence in these initial results. Increasing sample size in complementary future research would also be highly advantageous.

Declaration of Copyright

I confirm that no part of the material presented in this thesis has previously been submitted by me or any other person for a degree in this or any other university. In all cases, where it is relevant, material from the work of others has been acknowledged.

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CHAPTER 1

This intention of this introductory chapter is to introduce the rationale behind this thesis, followed by an overview of key ecological information regarding the study species: the grey seal. Finally, a synopsis of the general methodology used to collect the behavioural data used for the thesis will be given.

1.1. Overview of research rationale.

Perhaps a common perception is encapsulated in Bateson (1981): "*In many respects, the behaviour of the young animal is less complete, less complicated and less competent than that of an adult*". Although it is true that the behavioural repertoire of a young organism is unlikely to contain the full complement expressed by an adult, with behavioural development likely to continue throughout an individual's lifetime (Bateson, 1981; Stamps, 2003), it is arguably no less complicated. Timing is undeniably important, with early life crucial from many developmental perspectives (e.g. Musculo-skeletal: Freide and Weinstock, 1984; Nunes et al. 2004; Neurological: Hutchinson et al. 1984). Indeed, the period of early development is a critical time for the integration of environmental and social information into the behavioural phenotype of an organism (Stamps and Groothius, 2010a,b; Reddon, 2012). It has been widely observed that an individual's characteristics can be more strongly influenced during early stages of development than others, in what may be considered as 'sensitive phases' (e.g. Bateson, 1981; Mann and Fraser, 1996; Stamps, 2003). Moreover, it has generally been assumed that early experiences are more important in terms of their effects on later (adult) behaviour than those occurring at other stages of the life cycle (Bateson, 1981). A further reason to investigate behaviour in early life is that indications of species-specific patterns of behaviour may emerge during this stage in ontogeny. For example, defined sex differences in the behaviour of male and female infants in many mammalian species have been reported (e.g. Wedge-capped capuchins: Fragaszy, 1990; Gorillas: Maestriperi and Ross, 2004; Yellow-bellied marmots:

Jamieson and Armitage, 1979; Ground squirrels: Waterman, 1988). Such findings are intriguing and demonstrate the importance of behavioural studies in young age-classes, since this may ultimately help to inform us about the origins and characteristics of adult social behaviour.

1.1.1. Why study the early behaviour of grey seals?

The ecology of grey seals (*Halichoerus grypus*) has been widely studied, yet comparatively little has been published about the early ethology of neonates, nor how behavioural patterns develop during post-natal life. There is therefore a need in this species for an ethologically-focussed study of individuals in this earliest ontogenetic stage. An 'ontogenetic stage' simply describes a convenient means of drawing a line across a continuum; and in this thesis the term 'neonatal period' denotes the time between birth and weaning (termed the 'lactation period'). This 16-18 day period is the only time in the lifespan that individual grey seals are dependent upon their mothers for nutrition and represents a time of major body reorganisation and change. The defining of this period as a particular 'stage' can therefore be justified by its unique nature. Whilst a major thrust of research on the ontogeny of behaviour has been to show the large variety of ways in which a particular pattern of behaviour can be influenced, an important first step in understanding the developmental process is to document species-typical behavioural patterns, and any broad-scale changes that occur as a function of age. A further important reason for assessing the behaviour of animals in early life is that the information and skills acquired through these actions are likely to have long-term effects and may even influence adult behaviour. To use an analogy from Bateson (1981): "*When a house is built, the roof is not put on until the walls have been raised. Similarly, one kind of behavioural process may have to precede another if the second is to function properly*".

Play is a particularly interesting example of 'behaviour in development' (Bateson, 1981). This behaviour typically occurs more commonly during the earlier stages of ontogeny, but is speculated to have a long-term influence on adult behaviour as a result of information and skills gained during the performance of this behaviour.

There has however been relatively little documented research on the play behaviour of young grey seals, nor how its nature and function may change over time. Since play is a potentially important behavioural axis in young age-classes, there is need for a detailed exploration of neonatal play in order to evaluate its nature and functional significance.

Over recent years there has been increasing interest in the concept of 'animal personalities', whereby individuals consistently behave differently relative to one another over time. This phenomenon has been considered widely from a functional and evolutionary viewpoint, however little attention has been given to the ontogeny of personality until very recently. In turn, relatively few studies of inter-individual behavioural differences ('personality') in non-human species have concerned young individuals, with the majority of empirical work conducted on adults. Indeed, the only work conducted on the existence of behavioural consistency in wild grey seals has pertained to adults, and nothing is known about individual variation or behavioural repeatability in pups. Investigation is therefore required to address these issues, presenting a first step by which the development of behavioural consistency over ontogeny can be explored in this species.

The primary aims of this thesis are three-fold:

1. *To provide a quantitative description of typical neonatal behavioural budgets over the lactation period, including any broad-scale changes over this time.*
2. *To provide a preliminary investigation of individual behavioural consistency over the lactation period; providing a means of comparing behavioural repeatability estimates between neonatal and adult age-classes.*
3. *To assess the nature and function of neonatal play, through complementary assessment from two UK grey seal breeding colonies: North Rona and Donna Nook.*

1.2. Geographical distribution of grey seals.

Grey seals occur in the eastern and western Atlantic and in the Baltic sea. Boness and James (1979) give a detailed account of Canadian colonies, and accounts of the Baltic colonies can be found in Karlsson et al. (2005) and Jussi et al. (2008).

There are two main populations of grey seal in the western and eastern-atlantic (Boskovic, *et al*., 1996). Individuals of the eastern-atlantic population have several breeding colonies in Great Britain and Ireland. Typically, breeding colonies are formed on remote or uninhabited beaches or islands, although over recent years there has been an expansion of onshore breeding colonies in England. One such colony is Donna Nook nature reserve in Lincolnshire, where pup production has risen from 618 in 2000 to 1,520 in 2012.

In the British Isles, grey seal breeding colonies include large aggregations at: the Farne Islands in Northumberland (e.g. McConnell *et al*., 1999); the coasts of Ireland (see Lockley, 1966); the Scottish Isles of Orkney (Fogden, 1991), the Isle of May (e.g. Pomeroy et al. 2000a) and North Rona (e.g. Anderson et al. 1975, Twiss et al. 2000, 2006, 2010, 2012a,b), and Donna Nook in Lincolnshire (McConnell et al. 1992). Indeed, approximately 36% of the world's grey seals breed in the UK and about 90% of these breed at colonies in Scotland, with the main concentrations in the Inner and Outer Hebrides and in Orkney[2]. For reference, figure 1 shows a distribution map of grey seal breeding colonies in the UK, with primary breeding sites that are surveyed annually encircled.

For information, the data analysed and presented in this thesis was derived from the Hebridean offshore colony of North Rona, and the onshore colony of Donna Nook (see further descriptions of these colonies in section *1.5.1*).

(*figure 1, below*)

Figure 1: Grey seal breeding colonies in Britain. Colonies asterisked are potential Special Areas of Conservation, major colonies encircled are surveyed annually. Source: [1]

1.3. Studying the behavioural ecology of neonatal grey seals.

The grey seal has a number of characteristics that have facilitated its wide study. The formation of accessible colonies during the breeding season in the majority of its distribution makes the species an excellent model system within which to conduct ecological research, including behaviourally-based studies. Critically, breeding occurs on land - (see further details in *section 1.4*) - meaning that individuals are relatively easily observable during the autumnal breeding season. Female grey seals have characteristic pelage markings (Pomeroy et al. 1994; Twiss et al. 1994; Redman et al. 2001), which allows individuals to be reliably identified both intra and inter-seasonally. Further to this, mothers generally maintain close-proximity to their offspring during lactation (Redman et al. 2001; Twiss et al. 2006). By consequence of these virtues, individual neonates (figure 2c) can be reliably identified, thereby facilitating the repeated observation of individuals' behaviour during their respective lactation periods. It is acknowledged that observing pup behaviour during the lactation period alone means that assessment of more long-term ontogenetic changes is not possible. However since this period represents a unique time in the life history of this species, the study of neonatal behaviour in this brief but critical ontogenetic phase is warranted.

1.4. An overview of grey seal breeding ecology.

Grey seals (figures 2a, b, c) are a large, colonial pinniped and assemble in social groups (colonies) for one season a year for the birth of offspring and to reproduce (Caudron, 1998). In the UK, grey seals typically breed in the autumn months, with the start of the breeding season becoming later with clockwise movement from Ireland to the east of England around the coast (Caudron, 1998; McConnell et al. 1999). Breeding seasons can extend over 8 to 10 weeks, but individual female stays at breeding colonies are shorter than this (Pomeroy et al. 1994), generally coming ashore for 18-20 days. During this time, females give birth and nurture their pup to weaning (see *section 1.4.1*), when they will mate shortly before returning to the sea to forage.

Figure 2. Adult male grey seal from North Rona (a), Adult female from North Rona showing characteristic pelage patterns that vary from one individual to another (b), Grey seal neonate from the North Rona breeding colony, showing characteristic white lanugo (c). *Source of photos: CLC.*

Grey seals generally have a polygynous mating system (Anderson and Fedak, 1985; Twiss, 1991; Twiss et al. 1998), with males competing with one another to maintain loose territories (~home ranges) amongst groups of females for mating access (Twiss et al. 1998; see figure 3). Female grey seals tend to have limited space use on a daily basis (e.g. North Rona: Pomeroy et al. 2005), whereas males show a greater degree of mobility, but are still confined within an area comparable to a home range.

Figure 3: Intra-sexual aggression between two adult males at the grey seal breeding colony of North Rona. *Source of picture: CLC.*

Long-term studies have shown that females generally show inter-annual site fidelity and return within a short distance of their previous pupping site to give birth the following breeding season (Pomeroy et al. 1994; Pomeroy et al. 2000b). Although, recent analyses suggest that site-fidelity may not be as characteristic as once thought, with some females in fact giving birth at quite different localities over successive seasons (North Rona: unpublished MSc thesis; Amelia Saxby, 2012; University of St Andrews).

1.4.1. Nursing strategy and maternal behaviour.

Within the phocids there are different nursing strategies. The pattern displayed by grey seals, harp seals (*Phoca groenlandica*) and hooded seals (*Cystophora crjstata*) seals involves a short lactation period, during which a large amount of energy is transferred from the mother to the pup through extremely energy-rich milk (Lydersen and Kovacs, 1999; figure 4). Generally, the pups of these species are very inactive and consequently a high proportion of the received energy can be stored as body tissue, mainly in the form of subcutaneous blubber. Mothers generally feed little or not at all during the nursing period and the pups are weaned abruptly, having not yet entered the water in most cases (Lydersen and Kovacs, 1999). Grey seals are therefore known as 'capital breeders', since females fast during their stay on the breeding site and rely on fat reserves to provision their young.

Figure 4. Illustration of maternal mass loss and pup (foreground, showing white lanugo) growth: note that the hip bone of the female (encircled) is becoming more visible, an indication of decreasing body condition as subcutaneous fat stores are depleted.

Indeed, female phocid seals can lose 40-50% of their body mass during lactation (Hall et al. 2002). In terms of energy transfer, maternal investment in pups is therefore high, with lactation milk containing up to 40-60% fat depending on the individual female and the pup's age (Lydersen et al. 1995). Milk composition varies through lactation, with fat content low initially and water high, but as lactation proceeds, fat content increases as water decreases. As a consequence, pups can easily triple their mass during the lactation period (typical birth mass: 16kg). There is however individual variation in the amount of energy expended on rearing pups, with some mothers losing much more mass than others during lactation and vice versa (see Pomeroy et al. 1999). Interestingly, even when body size is taken into account, some mothers still produce bigger pups than others (Pomeroy et al. 1999).

The composition of pups' bodies changes dramatically over lactation. Pups are born without an insulating subcutaneous blubber layer and their body composition consists of only 3-6% fat (Lydersen and Kovacs, 1999). As lactation progresses, pups rapidly accumulate a layer of subcutaneous blubber, which takes on the primary role of insulation, diminishing the thermoregulatory importance of the lanugo (~white fur; see figure 5). These reserves sustain pups from weaning until they attain nutritional independence, and the size of these reserves are supposed to be critical in determining subsequent chances of survival (Hall et al. 2002). In addition to internal compositional changes, the external appearance of pups also changes over lactation, with the moulting of white lanugo in the later stages of development (figure 5; see *section 1.52* for more detailed descriptions of pup developmental stages).

Figure 5. Grey seal pup in process of moulting white lanugo on North Rona. *Source of photos: LOC.*

Parental care in pinnipeds is exclusively the task of females (Lydersen and Kovacs, 1999) and a key aspect of grey seal breeding ecology is therefore the relationship between mothers and pups during the short lactation period. Maternal care consists primarily of milk transfer to the offspring, but also includes protection and social interaction. Mothers will generally stay in close proximity to their pup on land or close to the shore, depending on the topography (e.g. Twiss et al. 2000). At colonies where seals breed inland, or with long difficult access to the sea, mothers tend to remain at their birth location (median daily movement of occurs within a 10m radius of pupping sites: Pomeroy *et al.*, 2005). Regarding social interaction, there is reported variation in the time dedicated to this amongst females, ranging between 1-12% of activity budgets (Caudron, 1998). Indeed, there is indication that maternal behaviour may consistently differ amongst individual females (see Twiss et al. 2012a), with indication more ‘proactive’ and ‘reactive’ personality types. Recent research suggests that such personality differences may affect fitness parameters in pups, such as daily growth rates (Twiss et al. 2012b).

1.4.2. The early behaviour of neonates.

A general characteristic of grey seals pups is that they are precocial, since they are born with their eyes open, able to vocalise, move around and suckle often within minutes after birth (Lydersen and Kovacs, 1999). Another general feature of pup behaviour in this species is the solitary nature of early development; indeed pups interact almost exclusively with their mothers during the nursing period, despite the proximity of conspecifics (Lydersen and Kovacs, 1999). However, although grey seals have been widely studied throughout their distribution, there remain few detailed empirical studies of neonatal behaviour.

In the past, research has focussed on the perinatal behaviour of pups; with particular interest in behaviours associated with the correct formation of the mother-offspring bond (Fogden, 1971; Burton et al. 1975). A study by Kovacs (1987) provides a rarer more detailed assessment of the early behavioural patterns of grey seal neonates from the Isle of May breeding colony, Scotland. This study documents the ontogeny of pup behaviour during the lactation period, following known pups to beyond weaning in some cases (to age 25 days+). The results of this study are discussed in more detail in chapter 2, however to summarise: neonates were found to spend most of their time '*prone and immobile*'. Pup behaviour patterns were found to be dynamic and changed with age, with some behaviours increasing in prevalence over time and others decreasing. Interestingly, females were found to invest more resources in male offspring, and mothers of sons were also found to be more aggressive: both of which have been found to be important for pup survival (Boness et al. 1982). This study therefore provides a platform to build from, with the potential to consider behavioural variation amongst neonates and how repeatable neonatal behaviour is over the lactation period. These areas will be addressed further in chapter 2.

1.4.3. Idle behavior is also characteristic of ice breeding grey seals.

A comparative review of five ice-breeding phocid species in the North Atlantic by Lyderssen and Kovacs (1999) offers useful comparison with terrestrially breeding populations of grey seals. Ice-breeding grey seals give birth in January, unlike UK populations (Duck, 2010). The colonies within which pups are born have densities similar to land-breeding populations of this species, and, like pups born in terrestrial colonies, grey seal pups born on ice do not normally enter the water during the nursing period (Haller et al. 1996). Lactation is a little more ‘intense’ for these pups, being shorter in duration compared to terrestrially breeding seals (e.g. Kovacs 1987, Haller et al. 1996). Ice-breeding grey seal pups spend more than 70% of their time idle or asleep and only about 4% of their time moving around on the ice. Therefore, broadly-speaking, the most prominent part of neonatal behaviour budgets (‘resting’) seems to be comparable amongst ice and terrestrially breeding populations, assumed to be a strategy for maximum energy retention and therefore maximum mass gain during the lactation period (Kovacs, 1987).

1.5. General methodology.

1.5.1. Study sites.

North Rona (figure 6a,b) is an uninhabited rocky island, located in the Northern Atlantic at a bearing of 59^{o} 06’ N, 05^{o} 50’ W (taken from Twiss and Franklin, 2010). The island covers an approximate area of 1.2km^2, and rises to 108m above sea level (Pomeroy et al. 2005). The site consists of undulating grassy terrain with slopes ranging from 0^{o} to 40^{o} (Twiss et al. 2012). Other notable topographical features of the breeding site are rocky outcrops, stone wall remnants, irregularly spaced boulders and pools of water of varying sizes (Twiss et al. 2012). When pregnant females arrive at North Rona they will initially occupy sites around pools of water and access gullies, however, subsequently seals spread up to 300m in land (Pomeroy et al. 2005).

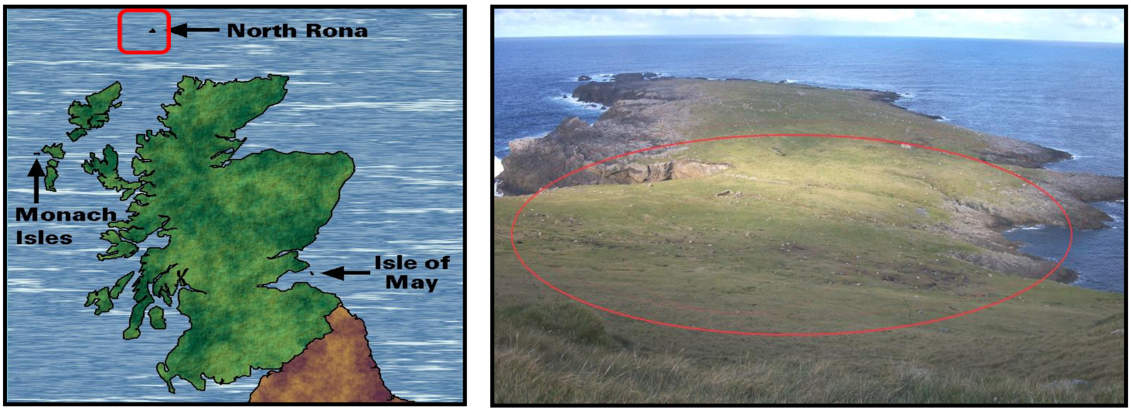


Figure 6. Location of the island of North Rona in relation to the Scottish mainland (a); Photograph demonstrating the approximate perimeter (in red) of the ‘Study Area’, leading into ‘Fianius South’ (b). *Source of picture (a): http://www.dur.ac.uk/s.d.twiss/studsites.html; photo (b): CLC.*

This offshore breeding colony has been the site of grey seal research for several decades (e.g. Boyd and Campbell, 1971; Anderson et al. 1975; Pomeroy et al. 1994; Twiss et al. 1994, 2000, 2006, 2007, 2010, 2012). However, the breeding colony is now in decline, meaning that insights gained from contemporary research are increasingly valuable. The breeding season here lasts from late September to November, with the majority of pups born located on the "Finanius peninsula". This area has subsequently been split up into three areas, largely based on topographical features and natural seal distribution: the 'Study Area', 'Fianius South' and 'Fianius North'. Almost all of more recent research has been focussed within the 'Study Area' (see figure 6b).

By contrast, Donna Nook nature reserve (figure 7) on the east-Lincolnshire coast is a relatively new and increasing onshore grey seal breeding colony. Year on year pup production has been increasing, rising from 618 in 2000 to 1,520 in 2012. Here, formal grey seal research has only begun in very recent years, with no published results as yet. However some new research relating to novel male behaviour is currently under peer review (Amy Bishop, 2012; Durham University). The reserve covers more than 10km of Lincolnshire coastline (figure 7a), and is characterised by a topography of dunes, slacks and inter-tidal areas. The largest part of the reserve consists of sandflats, and raised sand bars provide a hauling-out point for grey and common seals (GR: TF 422998). Every November and December, grey seals give birth to their pups near the sand dunes (figure 7b), attracting a large degree of public interest. Indeed, in 2011 almost 70,000 people visited this seal colony[2]. It is notable that the

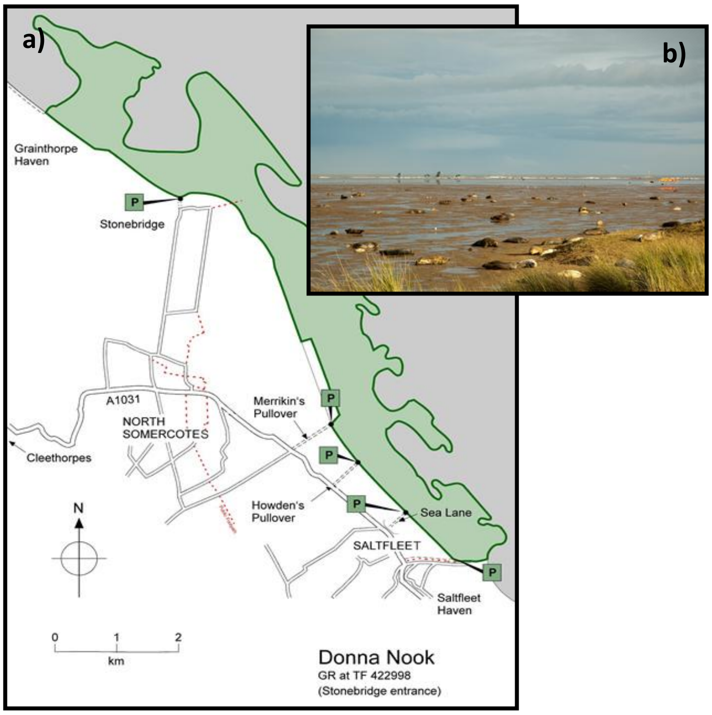


Figure 7a,b. Map of Donna Nook Nature Reserve (a). Breeding grey seals on sand flats at Donna Nook (b). *Source: [1]*

breeding colony is subject to different levels of disturbance, as a result of public access regulations: one site is accessible to the public (with the breeding seals separated from the public via wooden posting), whilst a second site along the breeding beach is used by the Ministry of Defence, and is therefore generally off-limits to the general public[2]. This colony therefore contrasts with North Rona not only in terms of its increasing breeding populations and onshore location, but also in terms of levels of disturbance.

1.5.2. Behavioural data collection at North Rona.

The behavioural data analysed in this thesis from the North Rona breeding colony were collected over three successive breeding seasons: 2008, 2009 and 2010. In these seasons, behavioural observations were made over the following dates: 2008: 4th-30th October; 2009: 1st -31st October; 2010: 29th September-1st November. All data were collected on British Summer Time (BST). To minimise disturbance to the breeding seals and to facilitate prolonged observation of individuals, hides were erected in all three seasons at a vantage point overlooking the colony. Behavioural observations were carried out by SDT (Dr Sean Twiss, Durham University) from the hide from dawn to dusk on all days, with the exception of when other field studies required assistance from all members of the field team or during unsafe weather conditions (e.g. storm force winds). Behavioural records were gathered for individually identified males, females and pups, however only pup data will be analysed and presented in this thesis. The sampling method used to obtain behavioural records for individuals is described in more detail below.

Sampling method:

From the hide, instantaneous scan samples (Altmann, 1974) were performed to record the behaviour of identified pups at five minute intervals (see table 1 for descriptions of behavioural categories). To do this, the primary observer (SDT) made a fast and informed decision as to the behavioural state of each focal animal at the designated sampling points. Scan samples were always completed within a minute and every effort was made to keep the order in which individuals were scanned invariable so that the interval between subsequent scans was kept as consistent as possible. Previous studies investigating grey seal behaviour have also used instantaneous scan sampling techniques, with intervals ranging from 30 seconds to 20 minutes (e.g. Kovacs, 1987; Twiss, 1991; Smiseth and Lorentsen, 1995; Boness, 1984). There is a trade-off when selecting the appropriate interval between sample points (Altmann, 1974), but shorter intervals were preferred in the field in order to maximise the amount of data collected on individuals. This decision was based on Twiss' (1991) suggestion that a minimum of 200 scan samples is required to give an accurate representation of male grey seals' behaviour.

Table 1. Description of the behavioural states used to quantify neonatal behaviour when in sight.

	Description
Alert	The pup is aware and may be looking all around themselves or in the direction of a perceived threat; typically the head will be up and the neck extended.
Comfort Move	The pup makes adjustments to its position and/or shuffles its body on the spot, but remains in the same location. The pup may scratch itself with its flippers.
Explore	The pup investigates or manipulates an object or another pup using its vibrissae, nose, mouth or fore flippers.
Locomotion	The pup uses its fore-flippers to change geographical location, in a directed fashion.
Nurse/Nipple-nosing*	The pup is considered to be 'nursing' when it makes oral contact with a nipple. The term 'Nipple-nosing' is used when direct contact with nipple and sucking is uncertain.
Maternal Interaction	The pup physically interacts with its mother, this includes but is not limited to nosing (touching its mother with its nose) and flippering (using its flipper to 'stroke' the mother).This category represents time spent in behaviours that are associated with social interactions with the maternal figure, but excludes nursing behaviours.
Solitary Play	Individual locomotor play: typified by repeated, exaggerated, jerky or wriggling body movements by the pup. *(See chapter 4 for illustrated examples).*
Rest	The pup is in a non-active state, head on the ground, its eyes may be open or closed.

**Note: 'nursing' and 'nipple nosing' behaviours are combined into a joint category describing behaviours associated with attempted or successful attainment of milk, and is here-on termed 'milk'.*

1.5.3. Individual time-activity budgets.

In order to convert the collated raw scan-sampling data for individual neonates into a more usable form for analyses, time-activity budgets were derived (note that these may be referred to as 'behaviour budgets' elsewhere in this thesis). The time-activity budgets of individual neonates were calculated by deriving the proportion of scans that pups spent in respective behavioural states, using the statistical software 'R' (see *appendix 1* for annotated R-code used to derive time-activity budgets from raw scan-sampling data). At the end of this process, the percentage time spent in key behavioural states by individual pups was available for further analyses.

Using the same method, individual time-activity budgets were also derived for 'early' and 'late' lactation phases separately, in order to assess behavioural changes and individual behavioural repeatability over this time. For reference, in this thesis the 'early lactation' phase describes the period from birth to the end of the first week of life. This categorisation is intended to encapsulate pup developmental stages 1 and 2 (see table 2 for descriptions). By contrast the 'late lactation' phase defines the period following the first 7 days of life and is intended to cover developmental stages 3+ (see table 2). Behavioural observations were therefore classified as occurring in early or late lactation depending on individual birth dates, which were known. It is acknowledged and accepted that there is individual variation amongst pups regarding the amount of time taken to reach these defined developmental stages. However this coarse segregation between 'early' and 'late' lactation phases is intended to provide a convenient means of enabling a broad comparison of pup behaviour over the lactation period, and is a consistent means of comparing behaviour in this time.

(*table 2, below*)

Table 2: Descriptions of diagnostic pup characteristics for each developmental stage. *Adapted from Culloch (2012).*

	Approx. Age (days)	Description
Stage 1	**1-3**	Pup lacks co-ordination and the contours of the ribs, hips and shoulders are clearly visible. The umbilicus looks pink and fresh and the skin forms loose folds around the neck and shoulders; there may be a yellowish tint to the pelage.
Stage 2	**4-7**	Pup shows improved co-ordination and the ribs become less prominent as a layer of blubber is deposited. The umbilicus is dried and shrivelled (it is lost in the early part of this stage) and the pelage is white.
Stage 3	**7-14**	Pup shows good co-ordination and the body is barrel-shaped. The pelage is white, although the muzzle and flippers may show a slight loss of natal coat. There are no signs, on the body, of moulting to the juvenile pelage.
Stage 4	**14-17**	Pup starts moulting to the juvenile pelage, although the white coat is still present in places.
Stage 5	**18+**	Pup has completely moulted white lanugo to the juvenile pelage.

1.5.4. Filtering of time-activity budget data.

Preliminary plots of individual time-activity budgets for respective behavioural states against total number of scans per individual (one scan= one behavioural record) suggested that the '200 scan minimum' (Twiss, 1991) rule is also applicable to pups. Therefore, to increase confidence in the representativeness of the behavioural data used for further analyses, a filtering process was conducted whereby individuals with fewer than 200 scans were excluded. In addition, the proportion of observation time spent "out of sight" was used in combination with this, again to increase confidence in the reliability of remaining behavioural budgets. Pups that were recorded as being out of sight for more than 50% of the time were excluded. From this process, an eventual overall sample size of 72 neonates from the three breeding seasons was reached (2008: 21 individuals, 2009: 28 individuals, 2010: 23 individuals). Some pups had to be excluded from 'early' and 'late' lactation analyses since some individuals did not meet the 200 minimum requirement in either one or both lactation phases. Therefore, the overall sample size of individual neonates for study across the two lactation phases was 44 individuals in total (2008: 14 individuals; 2009: 17 individuals; 2010:13 individuals).

1.5.5. Behavioural data collection at Donna Nook.

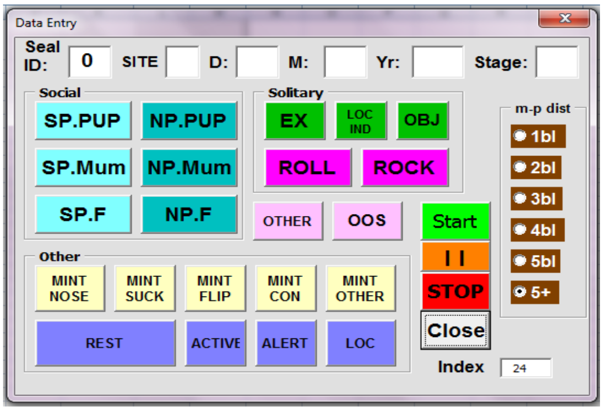
The behaviour of known neonates from the Donna Nook breeding colony was documented via focal-follow videos of individually identified mother-pup pairs. Behavioural footage was collected by Hani Castle-James (HCJ) at the colony during the 2011 breeding season; between 10th November and 10th December. Focal-follow videos (focals), typically around 30 minutes in duration, were collected for individually identified mother-pup pairs during their lactation period. Two types of focals were collected in the field: 'nursing' focals and 'random' focals. The former were restricted to nursing contexts only, whereas the former were not restricted to any particular behavioural context. The behavioural data analysed in chapter 4 of this thesis is derived from 'random' focals, since the aim of the aforementioned chapter is to investigate play behaviour and therefore non-contextually restricted focals were preferred so as not to bias observations.

Repeat random focals were collected where possible for known mother-pup pairs at different points in their lactation period; with the aim to obtain a focal-follow in early and late lactation phases. The number of days between repeat focals is however not equal amongst focal pairs due to logistical restrictions in-field. Regarding the random focals used for analyses in chapter 4 of this thesis, the time between first and repeat focals ranges between 3 and 13 days (see *appendix 2* for full details pertaining to the focal data collected at Donna Nook). For fuller details of data-collection methodology, refer to MSc thesis by Hani James; Durham University, 2012.

1.5.6. Focal decoding and behavioural state definitions.

The behaviour of eighteen neonatal pups was decoded from the aforementioned focal-follow videos. Observation footage (whilst pups were fully in-sight) ranged from 32.3 minutes to 95.2 minutes per individual; with total observation time for all subjects being 20.6 hours (see table 1; *appendix 2*). The developmental stage (table 2) was known for individual pups in each focal (see *appendix 2*). Pup sex was also recorded by HCJ in the field via a completely hands-off approach relying upon opportunistic and unambiguous observation of genital openings. Females have two genital openings in their anal region, whereas males have one enlarged hole in this area and a further hole ventrally-located. The sex of 10 of the 18 individuals in this study was identified in this way in the field by HCJ (*appendix 2*).

A focal sampling method (Altmann, 1974) was used to decode the behaviour of individual neonates, whereby all occurrences of specified actions (see *section 1.5.7*) of one individual are recorded during the sample period. Records of the length of the sample period and the amount of time each focal animal was in view were also recorded. An Excel program (see figure 8) was used to



Seal ID: 0

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Figure 8. Excel program used for recording behavioural states of focal pups in real time. *Designed by SDT.*

simultaneously record behavioural and temporal records; thereby building behavioural sequence data and allowing the duration of each behavioural action to be calculated by deriving the time difference between adjacent behavioural records.

1.5.7. Descriptions of pup behavioural categories for focal-sampling.

Neonatal behavioural actions were broadly classified within two main categories to facilitate subsequent analyses of independent and social play: 'Social' and 'Solitary'. Other behaviours are detailed within the "Additional" category. See full behavioural descriptions below:

SOCIAL BEHAVIOUR = Performed during social interactions in which there is a decrease in social distance between the interactants, and no evidence of social investigation or of agonistic (offensive or defensive) or passive-submissive behaviours on the part of the members of a dyad. Actions may include: 'flippering' between individuals, exaggerated sequences of physical contact, faux-fighting and so on.

Social-Pup (SP) = social behaviour, initiated by the focal pup, may include (extended) nosing/chasing/leaping on top of one another.

SP_Pup= with another pup(s)

SP_Mum= with focal pup's mum.

SP_F = with another female (i.e. not maternal figure).

Social-Not Pup (NP) = social behaviour, initiated by the interaction partner, may include (extended) nosing/chasing/leaping on top of one another.

NP_Pup= with another pup(s)

NP_Mum= with focal pup's mum.

NP_F = with another female (i.e. not maternal figure).

SOLITARY BEHAVIOUR = behaviour involving the focal individual only.

Exploratory Behaviour (EX) = the pup may remain stationary or move location during the behavioural sequence, showing inquisitive behaviours at the same time, such as 'sniffing' the air or nosing the ground.

Individual Locomotor Behaviour (INDLOC) = localised self-oriented behaviour, including: ‘wriggling’, ‘flippering head’, ‘biting flippers’, or exaggerated and repeated body movements.

Object Manipulation (OBJ) = Behaviour involving manipulation of an *inanimate* object, such as the ground, fence or grass, including ‘lunging’ at the object, flippering the object, forceful nosing and biting of the object.

ADDITIONAL BEHAVIOUR CATEGORIES:

Alert= The pup is aware and may be looking all around themselves, or in the direction of a perceived threat, or in the direction of another individual; typically the head will be raised.

Directed Locomotion = locomotion has a purpose, with relatively direct movement from location A to location B, with no investigative behaviours performed simultaneously, such as sniffing or nosing the ground.

Resting= describing periods of behavioural inactivity.

Out of Sight (OOS) = the pup cannot be seen (e.g. view may be obstructed by the mother).

Mum Interaction (MINT) = This behavioural category represents time spent in behaviours that are associated with social interactions with the maternal figure, but interaction is unreciprocal and can be brief.

MINT_NOS = nosing (when the pup is in the nursing position, but oral contact with the nipple is not certain).

MINT_SUCK= sucking (the pup is considered to be suckling when it makes oral contact with the nipple).

MINT_FLIP = pup flippers mum (if repeated for long duration/mum reciprocates, will be classed as social play).

MINT_CON = to describe the pup making nasal contact with the mother, but not in a nursing context, nosing occurring at part of mother’s body besides nipple region.

MINT_Other = to describe any other brief physical interaction with mum.

Roll = lateral locomotive movement by the pup, with 180 degree minimum-generally would expect movement in single direction.

Rock = pup may either rock back and forth in 90^0 movements, or may describe one 90^0 movement of pup from one position to another, whilst predominantly remaining in the same location.

Other = to describe behaviour that cannot be categorised in any of the above.

1.5.8. Use of behavioural data in chapter 4 analyses.

For the play analyses presented in chapter 4 of this thesis, behavioural records within the “Social” and “Solitary” categories as described in section *1.5.7* were focussed upon. Preliminary frequency-distribution analysis of ‘individual locomotor behaviour’ (INDLOC) showed that approximately 74% of these records lasted for 5 seconds or less, and so it was reasoned that these were likely to represent briefer ‘comfort move’ actions (see table 1 for description). Therefore, to increase confidence that recorded incidences of this behaviour represented ‘individual locomotor *play’*, only single records with durations of 10 seconds or more were included in analyses. The ‘object manipulation’ (OBJ) category is used to represent ‘object play’, since this describes interest in inanimate objects in the environment. Finally, behaviours in the “social” category were used to investigate ‘social *play*’. This category is fundamentally different to the ‘maternal interaction’ (MINT) categories, since the classification of social behaviour during observations required a degree of *reciprocity* in the behaviour between the interactants.

1.6. Chapter summary.

Grey seals are a temporally gregarious marine mammal species and have been widely studied. However, there is a relative lack of ethological work relating to the neonatal age-class, yet this would add greatly to the holistic understanding of this species. It is possible to successfully study the behaviour of individually identified pups owing to the characteristic pelage of their mothers; the close proximity maintained between mother-pup pairs, and relative site-faithfulness during the lactation period. Neonatal behavioural records have been collected from two contrasting breeding colonies in the UK, using differing sampling techniques, and will be analysed in the following chapters to address the following research aims:

1. *To provide a quantitative description of typical neonatal behavioural budgets over the lactation period, including any broad-scale changes over this time.*
2. *To provide a preliminary investigation of intra-individual behavioural consistency over the lactation period; providing a means of comparing behavioural repeatability estimates between neonatal and adult age-classes.*
3. *To assess the nature and function of neonatal play, including consideration of maternal effects, through complementary assessment from two UK grey seal breeding colonies: North Rona and Donna Nook.*

1.6.1. Overall thesis hypotheses:

Chapter 2:

Hypothesis 1: Neonatal grey seals will spend the majority of their time inactive/resting during the lactation period, with the assumed benefit of maximising energy retention and therefore the building of fat reserves.

Hypothesis 2: Sex differences will be present in behavioural budgets, reflecting known differences in adult roles.

Hypothesis 3: Behavioural consistency, as reflected by average trait repeatability scores, will be lower in neonates compared to those reported in adult traits.

Chapter 3:

Hypothesis 1: There will be evidence of a direct link between levels of maternal nutritional investment and neonatal play behaviour, with increased nutrition leading to enhanced play behaviour.

Hypothesis 2: In-line with differential maternal investment theory, male offspring of mothers in better condition will play more than female offspring, with the opposite pattern observed in pups of poorer condition mothers.

Chapter 4:

Hypothesis 1: The prevalence of s*ocial and object play will increase over lactation, peaking later than the peak in individual locomotor play.*

Hypothesis 2: Male pups will conduct relatively more individual locomotor play than females, who by contrast will spend more time in social and object play.

Hypothesis 3: A sex difference will be found in the expression of independent types of play in relation to maternal condition. Pups belonging to mothers in poorer condition will engage in more social play than those of mothers in better condition.

CHAPTER 2

An Assessment of Typical Behaviour Budgets and Behavioural Repeatability in Pre-Weaned Grey Seal Neonates

Chapter Introduction:

The first aim of this chapter is to provide an overview of typical neonatal behaviour budgets during the lactation period and to explore how behavioural patterns change over this time. Secondarily, a preliminary investigation of neonatal behavioural consistency over the lactation period will be presented, thereby contributing to a more holistic understanding of the ecologically important phenomenon of 'animal personality'. This will be particularly insightful since there remains little research either on wild marine mammals, or young individuals in this field. Indeed, currently nothing is known of behavioural consistency in grey seal neonates, with all existing research in this area pertaining to adults of the species.

1. INTRODUCTION

1.1. The early ethology of grey seals.

As reported in chapter 1, there have been relatively few quantitative studies of the behaviour of phocid neonates (Lydersen and Kovacs, 1999). Although there is much literature on the social behaviour of grey seals, it has almost entirely focussed on the breeding behaviour of adults. By contrast, the behaviour of mothers and pups has received relatively little attention and indeed, where aspects of maternal behaviour or pup development have been reported, it has tended to be of a more descriptive nature (e.g. Fogden, 1971; Wilson, 1974; Burton et al. 1975).

Some studies have documented the behaviour of perinatal pups, with particular focus on factors affecting the successful formation of the mother-pup bond in early life (Fogden, 1971; Burton et al. 1975). However, behavioural studies of neonatal pups over a greater proportion of the lactation period are rarer. Smiseth and Lorentsen (1995) documented the behaviour of grey seal pups during the breeding season at Froan in Norway, however a large focus here was on the behaviour of pups in water and terrestrial behaviour was only coarsely categorised. Since neither mothers nor infants in the UK are generally known to enter the sea during lactation, there is limited comparison of UK colonies with this study. To current knowledge, Kovacs (1987) presents the most detailed account of neonatal behaviour from a UK colony (the Isle of May), quantifying the ontogeny of neonatal behaviour alongside maternal behaviour. Neonates were reported to spend most of their time 'prone and immobile', assumed to maximise energy retention and therefore maximise the development of fat reserves (Kovacs, 1987). This is also supported in ice-breeding grey seals, where neonates have been reported to spend more than 70% of their time idle or asleep and only about 4% of their time moving around on the ice (Lydersen and Kovacs, 1999). Other notable findings from Kovacs (1987) relating to the ontogeny of behaviour and sex differences are outlined below in table 1. To summarise, the prevalence of several behavioural states in activity budgets was found to be affected by age, but significant sex differences were rare. It is notable however that this study continued behavioural observations into the post-weaning phase (day 25+post-birth), and therefore the results described are not wholly comparable with behavioural assessment of neonates during the period of maternal dependency alone.

Table 1: Summary of findings reported in Kovacs (1987), detailing whether the expression of behaviours in activity budgets was influenced by pup age, and whether significant differences between male and female pups were reported.

Behaviour	Influenced by age?	Sex Difference found?	Comments
Alert	Yes	X	Time spent alert increased with age.
Agonistic	X	Yes	Male pups were more aggressive than females, however overall only a very small proportion of time spent in this behaviour (0.5%).
Comfort	X	X	Quite high proportion of behaviour budgets (13-22%)

Moves			
Exploratory	Yes	n/a	Behaviour increased with age.
Total locomotion	Yes/No	X	Only saw effect of time at one of study sites.
Nosing	X	Yes	Males performed this behaviour more on mothers.
Solitary play	Yes.	X	Increased over time.
Resting (Idle)	Yes.	X	Decreased over time.

1.2. Consistent individual differences in behaviour.

Increasing interest over recent years has provided growing evidence that there are distinct behavioural differences between individuals of non-human species, from mammals to cnidaria (e.g. Budaev and Zworykin, 2002; Bell et al. 2009; Briffa and Weiss, 2010; Biro and Stamps, 2010, Briffa et al. 2011a,b; Dall et al. 2004, Kralj-Fiser et al. 2007; Sih and Bell, 2008). Individual behavioural consistency over time has been accepted as adequate evidence for 'personality' (e.g. Dall et al. 2004), but other authors have argued that consistency should be expressed both over time and contexts (e.g. Stamps and Groothius 2010a,b). However, Twiss et al. (2012a) notes that the two are inevitably linked as an individual can only be in one context at any one time. In this paper, the term 'consistent individual differences' is used in preference as this carries fewer connotations, and merely describes observed patterns in behavioural data. This is in-line with the view of Groothius and Trillmich (2011) who argue that the behavioural patterns observed are the outward manifestations of underlying neurobiological characteristics, which arguably provide a more appropriate basis for classification of personalities. With this body of evidence growing, it is therefore important not just to describe the behaviour of populations via general trends, but to also assess behaviour from an individually-based approach.

There remain few studies of individual behavioural consistency in marine mammal species, possibly owing to the logistical difficulties of repeatedly observing the same individuals in wild species. Where studies have occurred, they are often based on captive individuals and the use of human observer judgements to assess the temporal consistency of behavioural characteristics (e.g. Bottlenose dolphins: Highfill and Kuczaj, 2007). 'Behavioural individuality' has also been alluded to from studies of some wild marine mammals, including individual foraging specialisations (Dolphin *spp*: Duffy-Echevarria et al. 2008, Mann et al. 2008); individual differences in early social development (Bottlenose dolphins: Gibson and Mann, 2008) and multiple studies of individual vocalisations in bottlenose dolphins (e.g. Janik et al. 2006). Investigations of long term patterns of consistency are not common, although there are some rare examples relating to long term trends in movement patterns (e.g. West Indian Manatees: Deutsch et al 2003).

Within the pinnipeds, literature is even scarcer. There is evidence that individual vocalisations in male *Mirounga spp.* are repeatable (Sanvito and Galimbetti, 2003), and individual reproductive performance was found to be repeatable in grey seals (Lang et al. 2009). However, Twiss et al. (2010, 2012a, 2012b) are the only researchers who have explicitly tested for evidence of behavioural consistency in wild grey seals in their natural environment. These studies have provided evidence to suggest that personality or individual 'behavioural types' exist in both males and females, with individuals behaving differently relative to one another, and consistently so across time. However all that is currently known about the existence of personality in this species pertains to adults, and the ontogeny of behavioural consistency in this species is unknown.

This gap is also reflected in the wider literature. Indeed, animal personality has been extensively studied from evolutionary and functional viewpoints (e.g. Schuett et al. 2010; Wolf et al. 2007; Reale et al. 2007; McElreath et al. 2007; Dingemanse and Wolf, 2010), but its developmental origins have been neglected with the majority of studies being conducted on adults. It is however becoming increasingly apparent that such investigation is important (Trillmich and Hudson, 2011; Groothius and Trillmich, 2011) and there is consequently growing interest in the developmental underpinnings of individual behavioural variation (e.g. Stamps and Groothius, 2010a,b; Rodel and Meyer, 2011; Gracceva et al. 2011; Reddon et al. 2012). Assessment of the behavioural consistency of young individuals is therefore required as a first step in such investigations; with a view to increasing the number of neonatal/juvenile-adult comparisons that can be made as a means of exploring the ontogeny of individual behavioural repeatability. Within the human literature, it is generally accepted that personality stability increases with age, as does rank-order stability (see review by Roberts and DelVecchio, 2000). Indeed, this meta-analysis of 152 longitudinal studies found that trait consistency increased with age (0.31 in childhood and 0.74 between age 50-70 years). This suggests an age-related increase in the consistency of personality traits. Such findings may guide predictions regarding the trajectory that trait consistency may take in grey seals over a life history.

1.3. Study hypotheses.

Hypothesis 1: Neonatal grey seals will spend the majority of their time inactive/resting during the lactation period, with the assumed benefit of maximising energy retention and therefore the building of fat reserves.

Hypothesis 2: Sex differences will be present in behavioural budgets, reflecting known differences in adult roles.

Hypothesis 3: Behavioural consistency, as reflected by average trait repeatability scores, will be lower in neonates compared to those reported in adult traits.

2. METHODS

2.1. Study colony and behavioural data.

The behavioural data presented and analysed in this chapter were collected by SDT from the breeding colony of North Rona over three successive breeding seasons: 2008, 2009 and 2010. As described in chapter 1, behavioural data were collected from a hide by SDT from dawn until dusk on all field days (see *section 1.5.2*, chapter 1 for field dates), with the exception of when other field studies required assistance from all members of the field team or during unsafe weather conditions (e.g. storm force winds). An instantaneous scan sampling (Altmann 1974) approach was used in the field to record focal pup's behavioural state at 5 minute intervals during observation hours (0700 to 1800 h BST) using the following broad behavioural categories: *resting*, *alert*, *comfort move*, *explore*, *locomotion*, *maternal interaction*, nipple-nosing and nursing (combined to *milk-attaining* behaviours), and *play* (see chapter 1; table 1 for descriptions).

From this raw scan sampling data, individual time-activity budgets were derived by the author post-hoc (as described in chapter 1). These behaviour budgets provide an estimate of the percentage time that individual neonates spent in these different behavioural states, whilst in sight. All neonates included in the analyses in this chapter were scanned more than 200 times (range: 228-1299 scans per pup).

2.2. Preliminary inter-seasonal comparisons of neonatal behaviour budgets.

For the analyses presented in this chapter, neonatal behavioural data were combined from the 2008, 2009 and 2010 breeding seasons. Preliminary analysis was however first conducted in order to compare the prevalence of *alert behaviour, comfort moves, exploratory behaviour, locomotion, mum interactions, milk-attaining behaviour, play* and *resting* behaviour in neonatal time-activity budgets between the breeding seasons. The results of this analysis are presented in *section 3.1* of this chapter.

2.3. Statistical approach.

The Kruskal-Wallis test was used to compare the prevalence of different behaviours in time-activity budgets between the 2008, 2009 and 2010 seasons, owing to non-normal distribution of data. Sample sizes for the respective seasons were as follows: 2008= 21, 2009= 28, 2010= 23 neonates.

For the following analyses in this chapter, non-parametric tests were used to avoid validation of fundamental assumptions, due to the non-normal distribution of some behavioural traits and the desire to have consistent tests between behaviours. Statistical significance was 0.05 in all tests. In cases of repeated statistical testing, the Bonferroni correction was applied post-hoc to lower the *alpha*-level for tests, in order to control for the possibility of type 1 errors (rejecting the null hypothesis when it is true). The Bonferroni adjustment tests each of the individual tests at a significance level of α/n, *where* α is the desired significance level for the whole family of tests (0.05) and n is the number of individual hypotheses being tested [2].

Sex differences in behavioural traits and changes in behaviour budgets over lactation were investigated using the Mann-Whitney U test; this was chosen over a two-way randomisation procedure (Design 5a from Todman and Dugard, 2001) which is a more conserved alternative (see chapter 3), since this latter test is specifically designed for small sample sizes (<10) and here the number of males and females being compared was at least 10 or more. The Wilcoxon-signed rank test was used to compare behaviour budgets between early and late lactation phases (see chapter 1 for definition). This test is designed to compare two paired samples. Since the behaviour of the *same* individuals was being compared between lactation phases the samples were regarded as matched or paired. There are two tests that can serve this purpose: the sign test and the Wilcoxon signed rank test. The former compares the number of cases where the first sample is greater than the second sample to the number of cases where the second sample is greater that the first sample. However, it does not take into account the size of the difference between each pair, data which is often available. For this study therefore, the Wilcoxon signed-rank test was chosen as a preference, as this takes the size of the difference within pairs into account.

A variety of statistics have been used to estimate behavioural consistency such as the "product moment correlation" or the "Spearman rank correlation", however the most widely used statistic is the "intraclass correlation coefficient", ICC (Bell et al., 2009). This estimates repeatability, which is the "*fraction of behavioural variation that is due to differences between individuals*". That is, behaviours that show relatively low *within-individual* variance compared to high *among-individual* variation are more repeatable. The ICC is a therefore an appropriate means of assessing the consistency of a behavioural trait over time (Bell et al. 2009, Twiss et al. 2012a). A single measure two-way random effects model was used, since both individuals and sampling intervals were regarded as random effects (Twiss et al. 2012a). For all ICCs *no= 2* (Bell et al. 2009). Statistical analyses were conducted using 'R**'** version 2.14.1 (R Development Core Team 2011). Of note, a combined behavioural category named *general activity* was derived for this analysis by summing individual activity budgets for all behavioural states bar *resting* and those relating directly to mum (*mum interaction* and *milk-attaining* behaviours), in order to give an idea of how much time pups spent being behaviourally active. As detailed in a full report in appendix 3, principal components analysis (PCA) was attempted to reduce the dimensionality of the data and describe the behaviour of individual pups more concisely. That is, PCA was used in an attempt to reduce multiple behavioural traits into broader descriptors which encapsulate the individual behavioural variation exhibited by an individual. However this method proved unsuccessful (see appendix 3 for report and summary of reasons why PCA proved ineffective for these data), and consequently the behavioural repeatability estimates presented in this chapter were calculated for single behavioural traits.

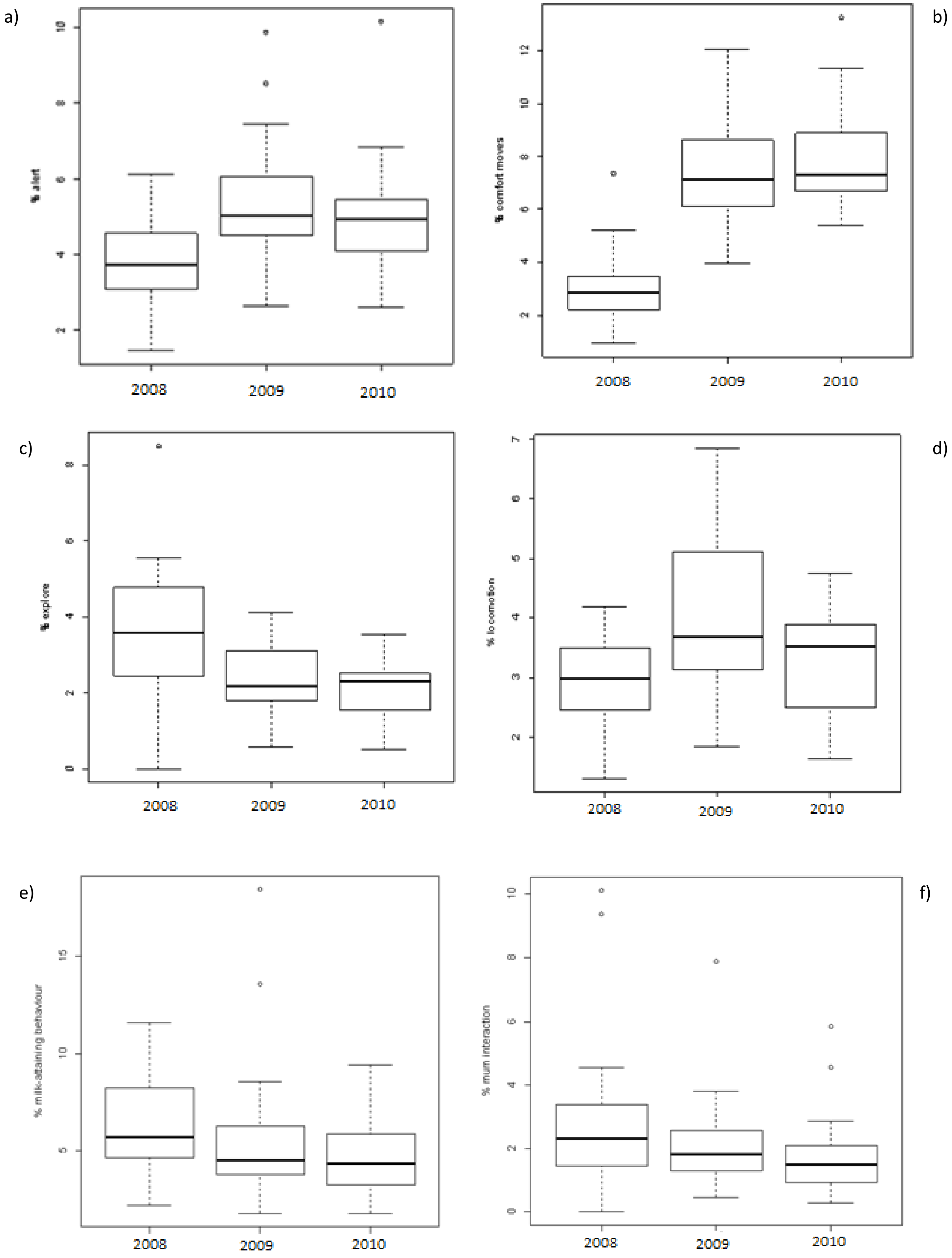
3. RESULTS

3.1. Preliminary inter-seasonal comparisons of neonatal behaviour budgets.

For analyses, neonatal behaviour budgets were combined from the 2008, 2009 and 2010 breeding seasons. However, there are some notable inter-seasonal differences that ought to be acknowledged. Overall, pups in 2008 spent less time *alert* than pups in 2009 and 2010 ($H_{(2)}$= 13.18, P= 0.001; figure 2a). Pups performed significantly less *comfort moves* in 2008 compared to 2009 and 2010 ($H_{(2)}$ = 38.14, P= <0.001; figure 2b). Pups in the 2009 season were more *locomotory* than in 2008 and 2010 ($H_{(2)}$ = 8.86, P= 0.01; figure 2d). The percent time *exploring* was found to be greater in 2008 than the other breeding seasons ($H_{(2)}$ = 8.74, P= 0.01; figure 1c), as was also true of *play* behaviour ($H_{(2)}$ = 43.51, P= <0.001; figure 1g). Pups also *rested* more in 2010 compared to 2008 and 2009 ($H_{(2)}$ = 6.26, P= 0.04; figure 1h). The amount of time spent in *milk-attaining* behaviour was found to be marginally higher in 2008 compared to 2009 and 2010 *(*$H_{(2)}$ = 6.68, P= 0.04; figure 1e), but the proportion of time spent in *maternal interactions* did not differ between years ($H_{(2)}$ = 4.06, P= 0.13; figure 1f). As figures 1b and 1g illustrate, the biggest difference between breeding seasons was regarding the proportion of time spent expressing *comfort moves* and individual *play* behaviours by pups in the 2008 breeding season compared to the other seasons. It is likely that these differences are the result of a higher frequency of miss-categorisations in the field in 2008, with more *comfort moves* being classified as solitary *play* compared to the 2009 or 2010 seasons; possibly the result of a more ‘trained eye’ in these seasons. This is supported by a lack of significant difference between years when *comfort moves* and *solitary play* behaviour budgets are combined ($H_{(2)}$ = 3.47, P= 0.18).

There is a risk of type 1 errors in this analysis owing to the use of multiple statistical testing. The Bonferroni correction was therefore applied to lower the *alpha* level for tests accordingly (*alpha*= 0.05/8= 0.006 3d.p). Following this adjustment, a significant difference remains in the amount of *alert* behaviour, *comfort moves* and *play* behaviour expressed by neonates between the seasons. The differences in these traits between years remain as described previously.

(*Figures 1a-h, below*)


a)
% alert
2
4
6
8
10
2008
2009
2010
b)
% comfort moves
2
4
6
8
10
12
2008
2009
2010
c)
% explore
0
2
4
6
8
2008
2009
2010
d)
% locomotion
2
3
4
5
6
7
2008
2009
2010
e)
% milk-attaining behaviour
5
10
15
2008
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f)
% mum interaction
0
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2009
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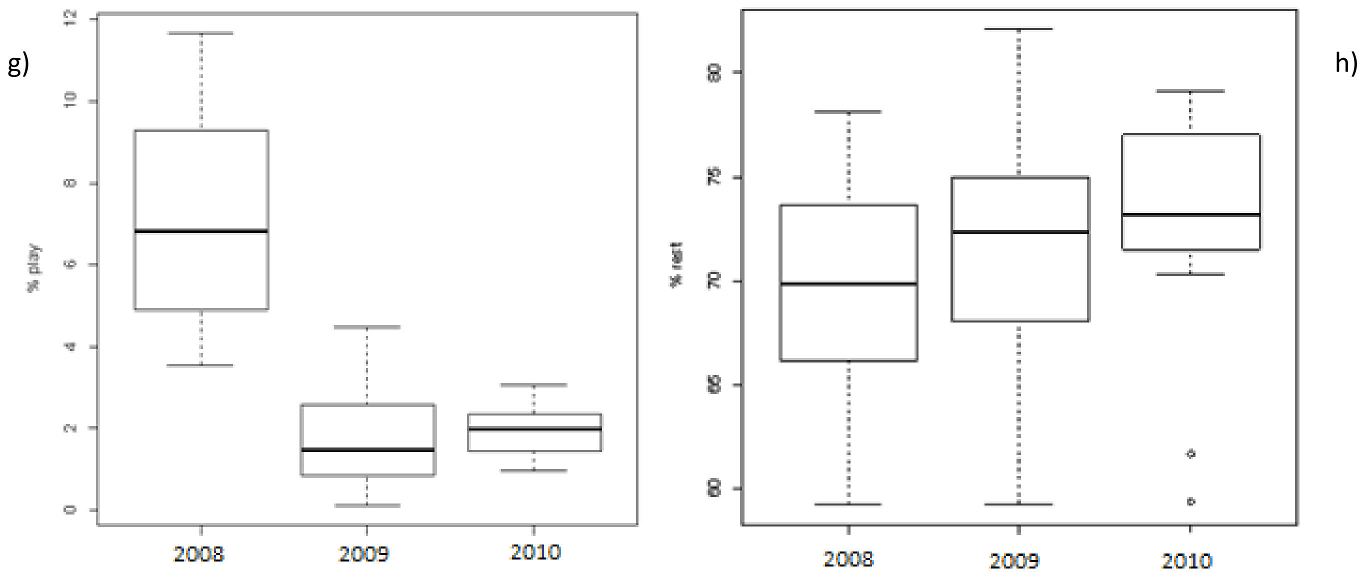


Figure 1(a-h). Behaviour budgets (percent time spent in each category) during lactation for neonatal pups during the 2008 (n=21), 2009 (n=28) and 2010 (n= 23) breeding seasons on North Rona: (a) Alert, (b) Comfort moves, (c) Explore, (d) Locomotion,(e) Milk-attaining behaviour, (f) Maternal interaction, (g) Solitary Play, (h) Resting. *For behavioural descriptions, see chapter 1: table. 1.*

Due to probable miss-categorisation of *comfort moves* and individual *play* behaviour more often in the 2008 breeding season (see figures 1b; 1g), only data relating to these behaviours from the 2009 and 2010 breeding seasons have been used in analyses pertaining to these behaviours in this chapter. Despite inter-seasonal differences in other behaviours being inferred (although these were found to be non-significant following Bonferroni adjustment- except for *alert* behaviour), this was considered as natural variation expected within a wild setting, and so data from all three breeding seasons were still combined for analyses of other behavioural traits.

3.2. Neonatal behaviour budgets.

Grey seal neonates were found on average to spend the greatest proportion of time inactive ("*rest*") during the lactation period (over 70% of the time, see figure 2). '*Comfort moves'* was the next most prevalent behaviour amongst pups, occupying on average 7.8% of behaviour budgets. Time spent in *milk-attaining* behaviours (nipple-nosing and nursing) occupied the next greatest proportion of behavioural budgets (~6%, see figure 2). Solitary *play* and *maternal interaction* represent the behaviours that neonates spent the least amount of time expressing during lactation, relative to other behaviours (1.8% and 2.2% respectively; figure 2).

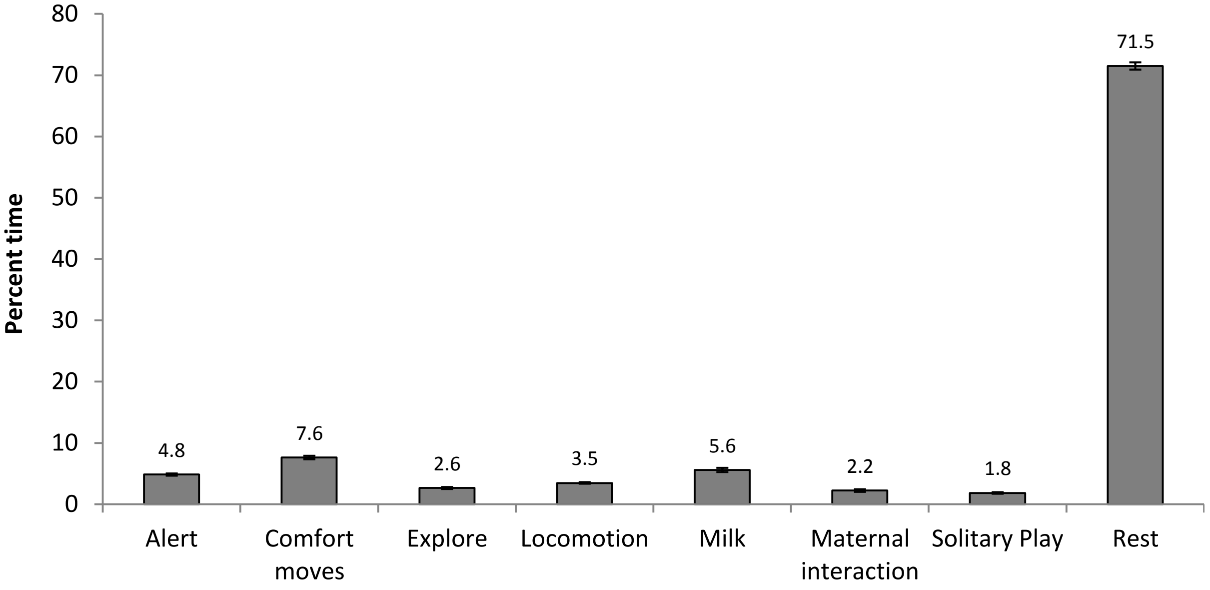


Figure 2: Behavioural budgets of neonatal grey seal pups from the North Rona colony during lactation. Values are mean ±SEM. *n=72, except for comfort moves and solitary play where n= 51.*

3.3. Sex differences in behavioural budgets.

Female pups were found to spend a greater proportion of time *alert* (*U*= 348.0, *P*= 0.01; figure 3) during lactation compared to male pups. Females were also found to express more *exploratory* behaviour than male pups (*U*= 377.0, *P*= 0.03; figure 3) and to spend more time in directed *locomotion* (*U*= 402.0, *P*= 0.05). By contrast male pups were found to spend a greater time in *milk-attaining* behaviours (U= 393.0, *P*= 0.001; figure 3) and also in *maternal interactions* (*U*= 367.0, *P*= 0.02; figure 4). No sex differences were found in the proportion of *comfort moves* expressed by males and females (*U*= 238.0, *P*= 0.78), nor the time spent *playing* (U= 207.0, *P*= 0.33), or *resting* (*U*= 454.0, *P*= 0.25). Since male and female behavioural data were compared multiple times in order to test several hypotheses (to compare 8 behavioural traits), the Bonferroni correction was applied post-hoc. The adjusted *alpha*-value (0.05/8= 0.006, 3d.p), renders only *milk-attaining* behaviour as significantly different between the sexes. Cautious interpretation of these results will therefore be considered in the discussion.

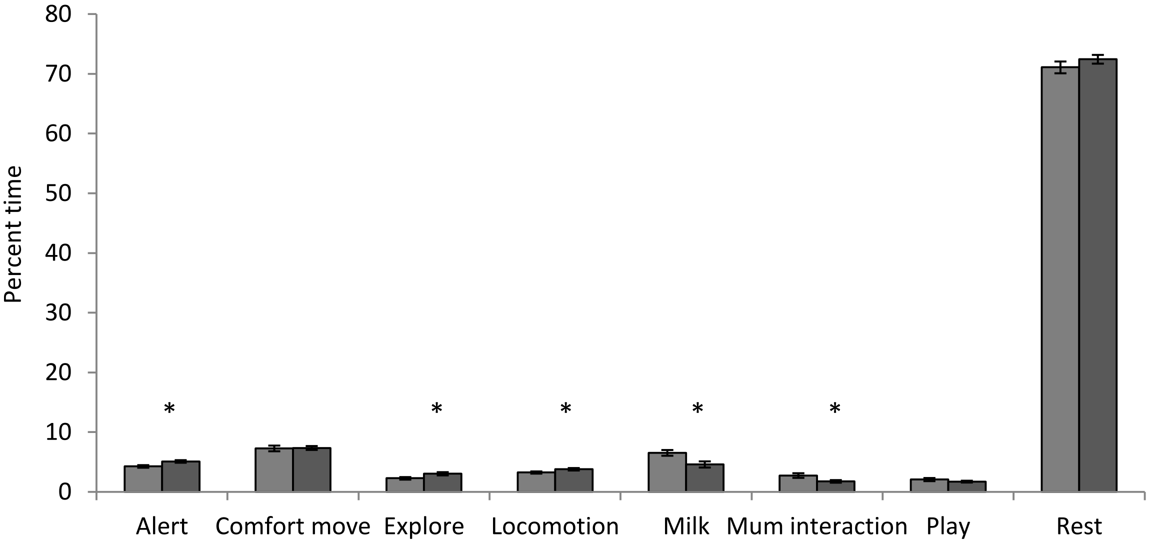
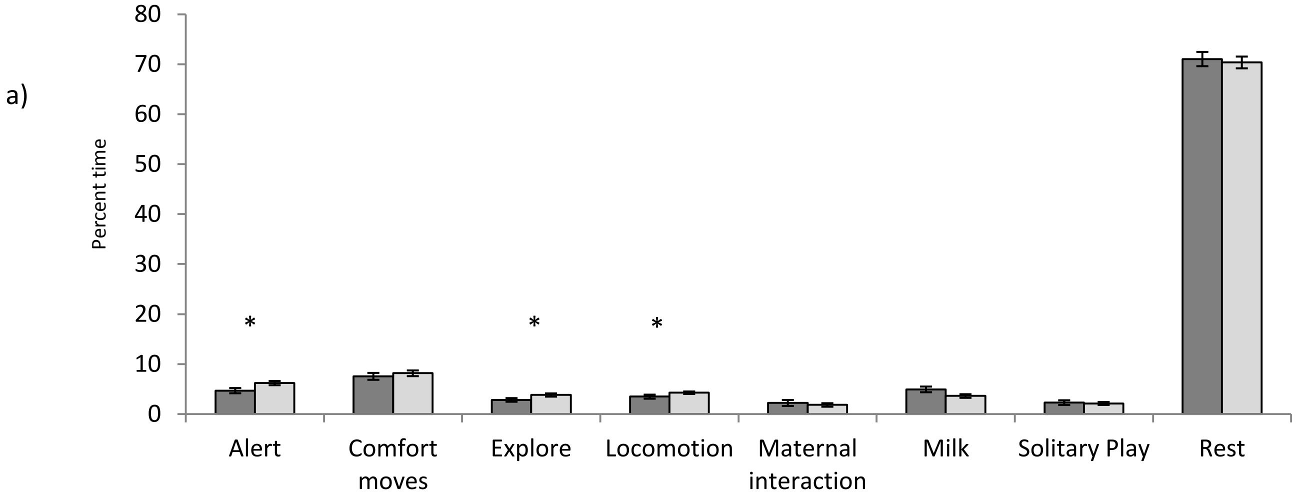


Figure 3. Behaviour budgets of male (light grey) and female (dark grey) neonatal pups from North Rona during lactation. Values are mean ±SEM, numbers show mean vales. Males, n=34, Females, n=32 *except for comfort moves and solitary play where males, n=20, females, n=25.* *Significant at 0.05 level. **Note: only *milk-attaining* behaviour remains significant in light of Bonferroni adjustment, *alpha*= 0.006, 3d.p

When sex differences in behaviour were explored in early and late lactation phases separately, *alert*, *exploratory* and *locomotive* behaviour were found to differ significantly in early lactation (figure 4a). However, following Bonferroni adjustment (*alpha*: 0.05/8= 0.006, 3d.p), these results are no longer significant. Caution will therefore be taken when considering these results in the discussion of this chapter. No significant sex differences in behaviour were identified in the late lactation phase.

Table 2: Summary results of Mann Whitney U tests to compare the behaviour of male and female neonates in early and late lactation phases respectively. Males, n=17, Females, n= 24[*]

	Early lactation		Late lactation	
	***U*-value**	**Two-tailed significance value**	***U*-value**	**Two-tailed significance value**
Alert	125.0	0.04	164.0	0.29
Comfort Moves	88.0	0.94	54.0	0.09
Explore	128.0	0.04	138.0	0.08
Locomotion	118.0	0.02	188.0	0.67
Maternal Interaction	178.0	0.49	139.5	0.09
Milk	137.0	0.08	134.0	0.06
Play	87.5	0.91	80.0	0.65
Rest	194.0	0.79	172.0	0.40



**Comfort move and play comparisons: Males, n=10, Females, n= 18.*

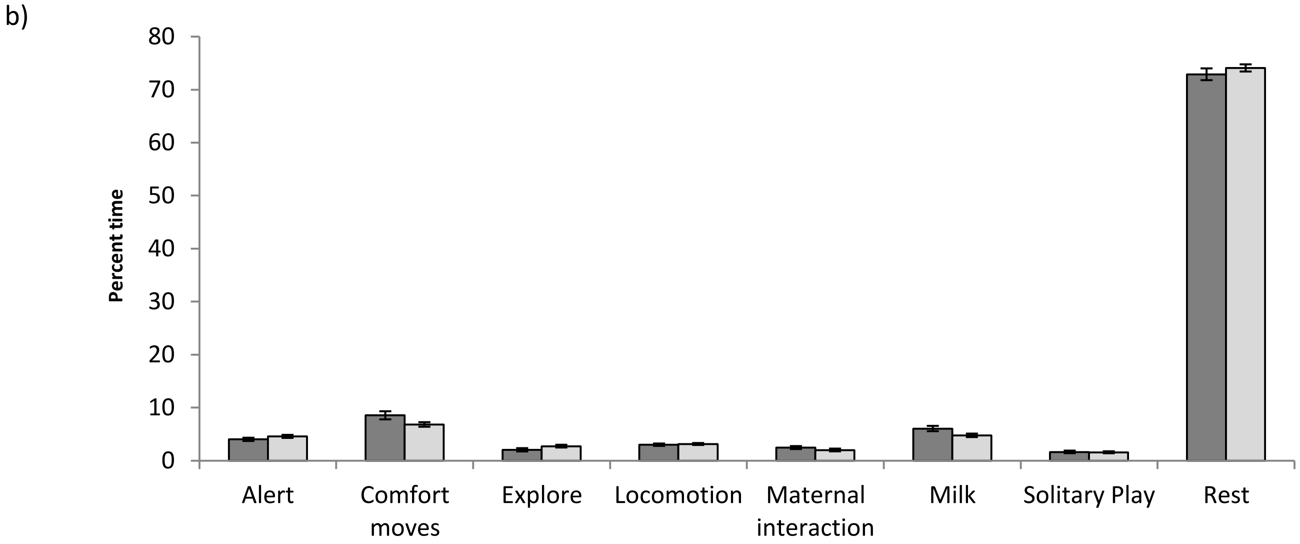


Figure 4a, b: Behavioural budgets of neonatal grey seal pups from North Rona in the early lactation phase (a) and the late lactation phase (b). Values are mean ± SEM. *Males, n=17, females, n=24, except for comfort moves and solitary play where males, n=10, females, n=18. *Significant at 0.05 level. **Note: significant gender differences presented in fig.4a not apparent when Bonferroni adjustment applied, alpha= 0.006, 3d.p.*

3.4. Changes in behaviour budgets over lactation.

A comparison of the behaviour budgets of individual neonates in early and late lactation phases revealed a significant difference in *alert* behaviour between the two phases, with neonates decreasing their time spent in this behaviour over the lactation period on average. Significant negative changes were also found for *exploratory* behaviour, *locomotion* and *play* behaviour (table 3). By contrast, some behaviours were found to increase in prominence in behavioural budgets over time, with *milk-attaining* behaviour, *maternal interactions* and *resting* all showing this pattern (table 3). The percent time that pups spent expressing *comfort moves* was not found to differ significantly between lactation phases, although a slight overall decline was identified (table 3).

Table 3: Summary of Wilcoxon-Signed rank tests comparing the behaviour budgets of individual neonates during early and late lactation phases, and the mean percent change (showing its direction) over this time. *N= 44.*

Behaviour	Z	2-tailed Sig.	Mean % Change
Alert	-3.57	<0.001	-1.25
Comfort Moves	-1.08	0.28	-0.59
Explore	-3.58	<0.001	-0.97
Locomotion	-3.30	0.001	-0.78
Milk	-3.23	0.001	+1.19
Maternal Interaction	-1.59	0.01	+0.24
Play	-2.21	0.03	-0.57
Rest	-2.79	0.005	+2.81

To counter the effects of multiple testing in this analysis, the Bonferroni correction was applied (adjusted *alpha*: 0.05/8= 0.006, 3d.p). Significant results remain in *alert*, *exploratory*, *locomotion*, *milk-attaining* and *resting* behaviours. The overall patterns in these behaviours over lactation remain as described.

3.5. Sex differences in behavioural budget changes over time.

The mean overall change in male and female behaviour budgets between early and late lactation phases were compared and analyses revealed that by enlarge the pattern of behavioural change did not significantly differ between the sexes. In the majority of cases, both male and female pups showed the same direction of change in behaviours and to extents that did not differ significantly (table 4, figure 5). However, a difference was identified in the degree of change of *comfort move* expression between the sexes, with male pups increasing the time spent in this behaviour over lactation on average, whilst females showed an average decrease in this behaviour (table 4, figure 5). However, this was no longer significant when Bonferroni adjustment was applied to the results to account for multiple testing (adjusted *alpha*: 0.05/8 = 0.006, 3d.p). This result must therefore be viewed with due caution.

Table 4: Summary results of *Mann Whitney U*-tests to compare the change in behaviour budgets of male and female neonates between early and late lactation phases, and the mean percent change in behaviour over this time for male and female pups. Males, n=17, Females, n= 24*.

	U-Value	Two-tailed Sig.	Mean male % change	Mean female % change
Alert	139.0	0.09	-0.68	-1.63
Comfort Moves	40.0	0.02	+1.01	-1.34
Explore	190.0	0.71	-0.79	-1.09
Locomotion	141.1	0.10	-0.48	-1.14
Milk	197.0	0.85	+1.11	+1.15
Maternal Interaction	180.0	0.53	+0.23	+0.17
Play	85.0	0.83	-0.65	-0.55
Rest	153.0	0.17	+1.85	+3.72

**Except for comfort move and play comparisons where: male, n= 10; female, n=18.*

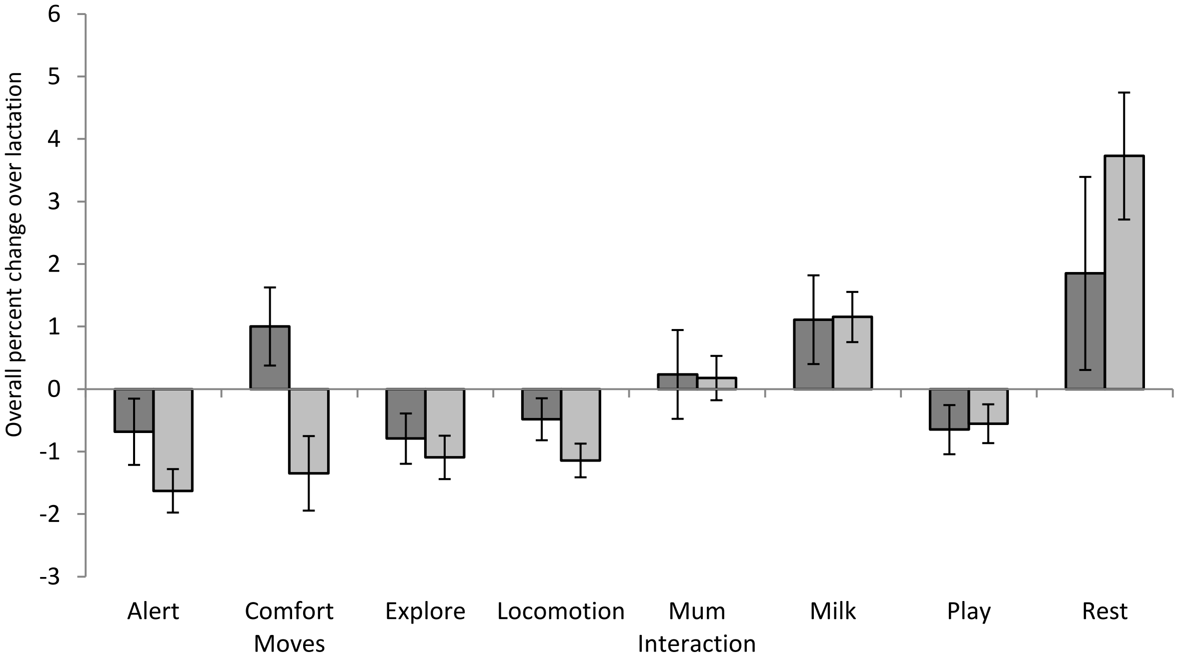


Figure 5: Change in male (dark grey) and female (light grey) behavioural budgets over lactation. Values represent mean changes in behaviour budgets (%) ± SEM between early and late lactation. Males, n 17; Females, n= 24*, except for comfort move and solitary play changes where males, n= 10; females, n=18.*

3.6. Intra-individual behavioural consistency over lactation.

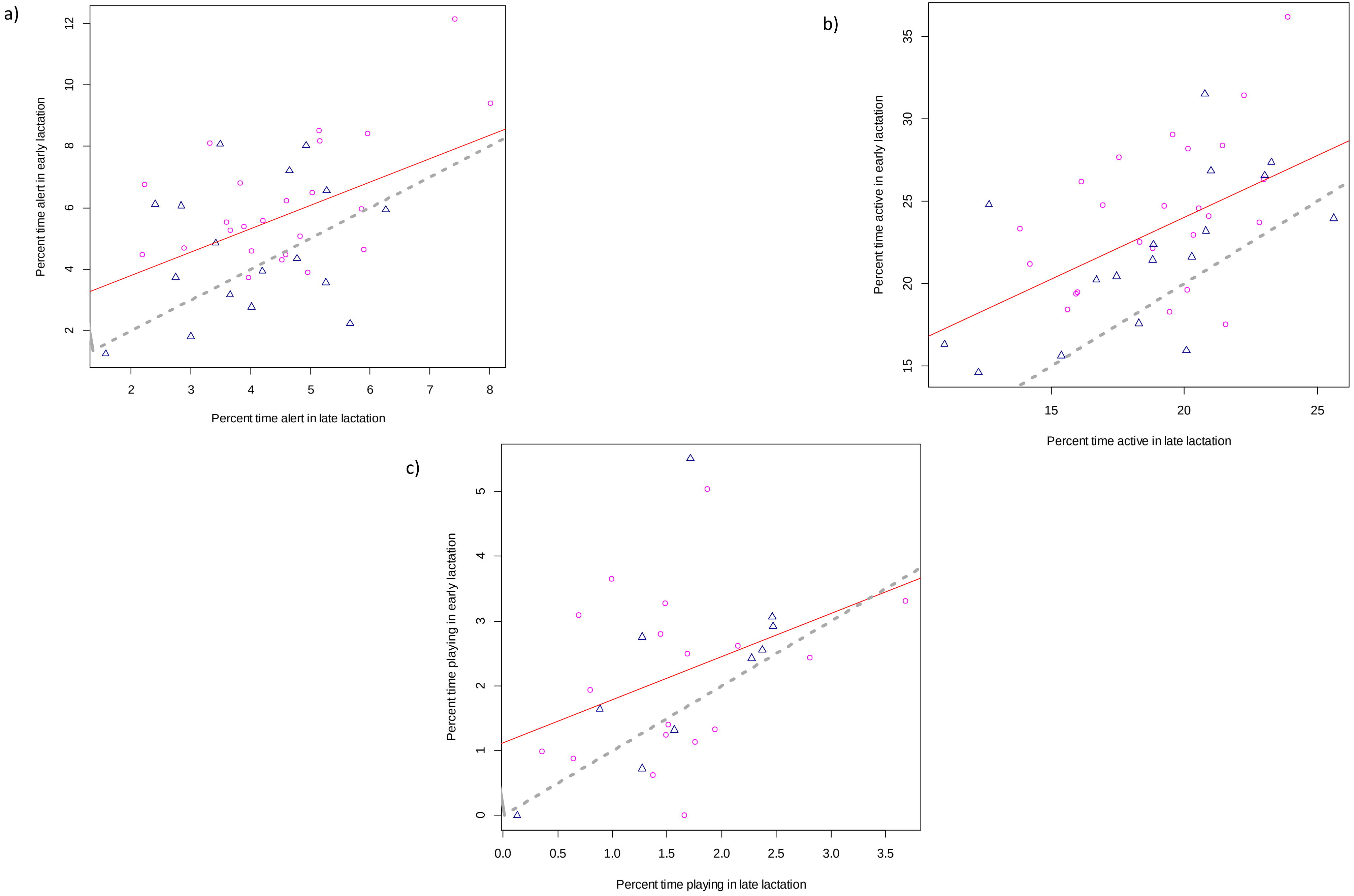
The repeatability of single behavioural traits was found to be significantly different from zero for the majority of behaviours investigated; with ICC scores ranging from 0.11 to 0.49 (see figures 6a-c for depictions inter-individual variation in *alert*, *play* and *general activity* behaviours). Table 5 summarises the findings, showing that all individual behavioural traits, except maternal interactions, were found to be significantly repeatable over time. Some apparent sex differences were also found, with individual females showing significant repeatability in *alert, milk-attaining* and *resting* behaviours, but males not. By contrast, only male pups showed consistent individual differences in *comfort moves* and *locomotory* behaviour. Following Bonferroni adjustment of the *alpha*-level for each dataset (*alpha*: 0.05/9 = 0.0056, 4 d.p), the repeatability of *alert behaviour*, *milk-attaining* behaviour and *general activity* remains significantly different to zero for all pups. Apparent sex differences in repeatability remain in *alert* behaviour and *general activity* (see table 5). It should be noted that when outlying data were removed and analysis repeated for *play* behaviour (see footnote under table 5), a significant difference between males and females was revealed.

Table 5. Summary results of two-way mixed effects model for assessment of individual consistency over time. Intraclass correlation coefficient scores and significance values shown. *All pups: n= 44, Males: n=17, Females: n= 24.*

	ICC Score			***P*-Value**		
	All	***Males***	***Females***	***All***	***Males***	***Females***
Alert	0.41	0.23	0.53	0.003	0.183	0.003
Comfort Moves	0.38	0.64	0.31	0.02	0.02	0.10
Explore	0.37	0.29	0.30	0.007	0.12	0.07
Locomotion	0.36	0.49	0.27	0.008	0.02	0.09
Maternal Interaction	0.11	-0.14	0.35	0.24	0.71	0.04
Milk	0.39	0.12	0.33	0.004	0.31	0.05
Play**	0.33	0.47	0.24	0.03	0.07	0.16
Rest	0.37	0.27	0.44	0.007	0.14	0.02
General Activity	0.49	0.59	0.42	<0.001	0.005	0.02

**Comfort move and play correlations: All: n= 30, Males: n= 10, Females: n=* 24.

**Note: when analyses were repeated for play behaviour, following the removal of two outliers (see figure 6c), the results were affected as follows: All pups: ICC= 0.43, *P*= 0.01, n=28; Males: ICC= 0.80, *P*= 0.003, n=9; Females: ICC= 0.25, *P*= 0.16, n=23.



Figures 6a-c. Consistency in percent time spent in alert behaviour (a), general activity (b), and individual play behaviour (c) between the early and late lactation phases by neonatal grey seal pups on North Rona. *Graphs show 1:1 line (----) and line of best fit (red). Blue triangles= male pups, pink circles = female pups. Data combined from 2008, 2009 and 2010 breeding seasons, except for solitary play where data from 2008 excluded.*

Table 6: Summary data for Spearman's correlation tests between percent time spent in multiple behavioural states by neonatal pups and maternal physiological and behavioural parameters; Rho = Spearman's correlations and *p* = two-tailed significance. *Sample size is 72 in all tests except for tests involving birth date, transfer efficiency and maternal post-partum mass (n= 66, 43**, 62 respectively).*

	Pup Behavioural Category															
Variable	**Alert**		**Comfort Moves**		**Explore**		**Locomotion**		**Milk**		**Mum Interaction**		**Individual Play**		**Rest**	
	rho	***p***	**rho**	***p***	**rho**	***p***	**rho**	***p***	**rho**	***p***	**rho**	***p***	**rho**	***p***	**rho**	***p***
Birth Date	0.11	0.36	0.17	0.27	0.14	0.25	0.12	0.35	-0.18	0.14	-0.23	0.06	-0.08	0.59	-0.06	0.63
Maternal Transfer Efficiency	-0.02	0.88	-0.08	0.73	-0.05	0.76	0.08	0.62	-0.23	0.14	0.04	0.81	-0.02	0.92	0.29	0.06
Maternal Postpartum Mass	0.26	0.04	-0.04	0.79	0.23	0.07	0.14	0.28	-0.34	0.007	-0.28	0.03	0.05	0.77	0.17	0.18
Maternal Behaviour Budgets:																
Aggression	-0.08	0.53	-0.15	0.31	-0.20	0.09	-0.07	0.58	0.20	0.10	0.11	0.37	-0.17	0.24	-0.06	0.60
Alertness	0.04	0.71	-0.11	0.44	-0.13	0.29	-0.02	0.85	0.16	0.17	0.33	0.005	-0.08	0.58	-0.09	0.43
Comfort moves	0.11	0.37	-0.01	0.97	-0.26	0.03	-0.02	0.89	-0.17	0.16	0.04	0.73	-0.07	0.65	0.26	0.03
Exploratory behaviour	0.02	0.90	-0.11	0.46	-0.22	0.06	-0.22	0.06	0.05	0.68	0.18	0.14	-0.07	0.65	0.11	0.37
Locomotion	0.26	0.03	0.16	0.27	0.12	0.32	0.24	0.04	0.02	0.88	0.08	0.51	0.03	0.82	-0.13	0.29
Nursing	-0.16	0.18	0.03	0.86	0.02	0.85	-0.11	0.36	0.83	**<0.0006**	0.37	0.001	-0.25	0.07	-0.47	**<0.0006**
Pup-interaction	-0.07	0.54	-0.14	0.33	-0.27	0.02	-0.11	0.36	0.29	0.01	0.71	**<0.0006**	-0.13	0.36	-0.20	0.10
Resting	0.01	0.93	0.09	0.55	0.32	0.006	0.12	0.32	-0.53	**<0.0006**	-0.65	**<0.0006**	0.21	0.14	0.27	0.02

Note: for 'comfort move' and 'play' correlations, only data from the 2009 and 2010 breeding seasons were used owing to suspected miss-categorisation of these behavioural states in the 2008 breeding season. Note: for maternal transfer correlations with 'comfort move' and 'play' categories, 2009 data used solely. General note: Bonferroni correction used due to multiple testing; adjusted significance value= P= 0.0006 (4dp). Significant correlations in bold.*

4. DISCUSSION

4.1. Neonatal behaviour budgets.

Within the phocid seals there are two prominent strategies for nursing pups to weaning: 1) Mothers feed little or not at all during the nursing period and pups are weaned abruptly, having not yet entered the water. Pups are generally very inactive during the lactation period and consequently a high proportion of the received energy can be stored as body tissue, mainly in the form of subcutaneous blubber. 2) Lactation takes place over a longer time frame, and less-energy-rich milk is transferred to very active pups. Pups learn to swim and dive during the nursing period, and are weaned with body compositions that are similar to the adults. The mothers feed during lactation and weaning is less abrupt, with pups feeding independently, while still receiving milk from their mothers (Lydersen and Kovacs, 1999).

As predicted by hypothesis 1 (*section 1.3*), neonates were found to spend the majority of their time resting (inactive); grey seals therefore adopt the first strategy described and the high proportion of time spent immobile by neonates supports this (see figure 3). The dominance of inactivity in neonatal behaviour budgets is consistent with other findings both from North Rona and different grey seal colonies (North Rona: Harwood, 1976; Anderson and Harwood, 1985; Isle of May: Kovacs, 1987; Froan, Norway: Smiseth and Lorentsen, 1995), and highlights that the early post-natal period is dominated by the need for the rapid transfer and retention of energy. The sedentary nature of pups during the lactation period minimises energy expenditure and metabolic costs (Worthy, 1987), thereby maximising the amount of milk energy that can be used to build muscle and blubber. In turn, it has been shown that increased body condition (mass/length, as a proxy for total body fat) at weaning has a positive effect on the first year survival of grey seal pups (Isle of May and Farne Island colonies: Hall et al. 2001, 2002). By spending a large proportion of time idle, grey seal pups also minimise the risk of premature separation from their mothers and the risk of maternal confusion in milk delivery (Kovacs, 1987).

Early neonatal development in this species also has a solitary nature, with pups interacting almost exclusively with their mother. Maternal interactions were found to comprise only a relatively small proportion of behaviour budgets on average (<3%; figure 3), with the greatest amount of time in contact with the maternal figure revolving around acquiring milk (5.6%; figure 3). Of the solitary behaviours recorded (excluding *resting* behaviour), *comfort moves* were found to be the most prominent in neonatal activity budgets (>7%; figure 3). These movements generally consist of small adjustments to position or brief scratching actions and therefore are likely to occur when pups are showing '*idle'* (resting) behaviour when not asleep. Therefore, a relatively high proportion of *comfort moves* is not unexpected considering the dominance of *resting* behaviour in activity budgets. Kovacs (1987) also reported a high and constant prevalence of *comfort moves* in neonates on the Isle of May; in fact reporting a higher proportion of this behaviour (13-22%).

4.1.1. Sex differences in behaviour budgets.

In-line with hypothesis 2 (*section 1.3*), some sex differences in pup behavioural budgets were found. Female pups were found to spend a greater proportion of their activity budgets *exploring*, *locomoting* and *alert* compared to male counterparts (*section 3.2*). It may be speculated that such differences are reflective of the different interactions with the environment that males and females have as they grow older. Female grey seals have been shown to express fine-scale philopatry and site-fidelity, returning to their natal colony within a short distance of previous pupping sites (Pomeroy, 1994; Pomeroy et al. 2000b); and therefore may be assumed to have detailed knowledge and memory of the surrounding environment. It is possible that such knowledge is acquired through investigation of the area surrounding their natal site during early life, with the simultaneous development of cognitive systems enabling retention of this information. Interestingly, significant sex differences in these behavioural traits were only identified during the early lactation phase, giving intriguing suggestion of a possible 'sensitive phase' in which cognitive-retention following from such environmental awareness and exploration is most susceptible. There is support for this within the literature where it has been widely observed that an individual's characteristics can be more strongly influenced during one stage of development than others, so-called in *sensitive phases* or *critical periods* (e.g. Bateson, 1981; Mann and Fraser, 1996; Stamps, 2003). Further, it has generally been assumed that *early* experiences are more important in terms of their effects on later (adult) behaviour than those occurring at other stages of the life cycle (Bateson, 1981). However, it must be acknowledged that when a lowered *alpha* value was employed to counter the possible effects of multiple testing, these results were no longer significant. The Bonferroni test is conservative, and whilst the differences are viable in terms of biological reasoning, we should view these results with due caution. In this way, the results discussed may offer weak inference only about possible gender behavioural differences in this species, with larger sample sizes and more directed hypotheses required in future to confirm their existence.

By contrast, male pups were found to spend more time in *milk-attaining* behaviours (*nipple-nosing* and *nursing*) than females (*note*: this result was still robust following Bonferroni adjustment). This therefore leads to speculation of differential maternal investment, whereby mothers invest more heavily in males due to the asymmetry in reproductive success between the sexes. Male grey seals do not contribute to the care of the pups, but monopolise access to groups of females. Males have higher variance in their reproductive success than females (Anderson et al. 1975), and their reproductive success is highly influenced by adult body size (Anderson and Fedak 1985). That is, a successful male is likely to sire multiple offspring, with females parenting fewer pups owing to the single-pup nature and long duration of the gestation period. By contrast, a successful male can impregnate multiple females within a single season. Evidence for such differential investment has been found before in grey seals, with mothers found to invest more resources in males (Isle of May: Kovacs, 1987), although equal maternal investment has also been reported elsewhere (Froan, Norway: Smiseth and Lorentsen, 1995). Male pups were also found to partake in more *maternal interactions* than female pups, which may speculatively be thought to relate to the greater amount of time spent in close proximity to the maternal figure for nursing by males. In Kovacs (1987), male pups were also found to perform more ‘nosing’ behaviour (“*non-nursing physical contact*”) towards the maternal figure. These combined findings therefore suggest that differential maternal investment may exist in this population, consistent with theoretical predictions for maternal investment among dimorphic polygynous species.

4.1.2. A comparison of Kovacs (1987) and present study.

There are some similarities between Kovacs' 1987 study of neonatal behaviour on the Isle of May and the findings of the present study (see table 6 for summary), including the prominence of *resting/idle* behaviour in activity budgets. It is likely that the differences observed (table 7) between the two studies result from the longer observation period of pups into the post-weaning phase in Kovacs' study. Indeed, inclusion of post-weaning observations seems to impact upon the behaviour patterns reported in the study; with some trends present before weaning not continuing into the post-weaning phase. For example, *locomotory* activity declined to a low immediately after weaning but subsequently climbed to its highest level. Similarly, the reported overall decrease in time spent 'idle' with age is also likely to have been affected by observation during the post-weaning phase, when pups tend to move greater distances around the colony compared to when they are still dependent on their mother for milk (*personal observation of author*). This therefore reflects a limitation of the present study and demonstrates the importance of continuing neonatal observations into the post-weaning phase. Comparisons with this study are also limited by slight differences in behavioural definitions. For example, *exploratory* behaviour in Kovacs' paper included interaction with both inanimate objects and other individuals, including pups. This, in combination with observation into the post-weaning phase, therefore limits comparison with the present study where only interaction with *inanimate* objects was included in this definition, and observations were limited to the lactation period only.

Interestingly, Kovacs (1987) reports that *play* was "rare" in young pups, yet *comfort moves* occupied a relatively constant and high proportion of pups' time (13-22%), which is much higher than found in the present study (~7%). This could indicate a potential miss-categorisation of *play* for *comfort moves* in Kovacs' study, resulting in the conclusion of play being rare in young pups. Play may become more exaggerated with age, owing to greater physical capability, thereby making miss-categorisation less likely; this could suggest why individual play was noted to become more prevalent in activity budgets from approximately 15-19 days after birth. This discussion is however merely speculative. The results of these two studies may reflect true inter-colony differences, possibly owing to different demographic and topographical attributes. However, methodological differences have been noted between the studies and so an independent, complementary study would be beneficial, using the same methodology and behavioural definitions at both sites, to encourage consistency and confidence in comparisons.

Table 7: Comparison of findings reported in Kovacs (1987) and present study, in relation to neonatal behavioural patterns.

Behaviour	**Influenced by age? (Kovacs/Present study)**	**Sex Difference found? (Kovacs/Present study)**	**Is the present study consistent with Kovacs (1987)?**
Rest ('Idle')	Yes/Yes	No/No	No. Kovacs reported a decrease with age, whereas the present study found an overall increase.
Comfort moves	No/No	No/No	Yes.
Alert	Yes/Yes	No/Yes	No. Kovacs found an increase with age, whereas the present study found a decrease. Behaviour was also greater in females in the present study.
Mum Interaction ("Nosing" in Kovacs)	No/Yes	Yes/Yes	Mixed. Present study noted an increase over lactation, whilst Kovacs found no difference with age. Higher prevalence in males in both studies.
Locomotion	Yes/Yes	No/Yes	Mixed. Present study found decrease over lactation and higher prevalence in females. Kovacs found age effect only at one study site, and no sex difference was found.
Exploratory	Yes/Yes	Na/Yes	No. An increase in behaviour with age found in Kovacs; whilst a decrease with age found in present study. Higher prevalence in female pups in present study, no information in Kovacs.

Play	Yes/Yes.	No/No	Mixed. Kovacs reports an increase with age; a decrease was found over lactation in the present study. No sex difference was found in either study.
Nursing (Milk)	Na/Yes	Yes/Yes	Yes. Increase in time spent nursing over lactation found in present study. Male pups found to spend more time nursing in both studies.

**Na: information not available within paper.*

4.2. Changes in neonatal behaviour budgets over the lactation period.

In general, pups became less active over lactation. On average, all behaviours associated with independent, metabolically-costly behaviour showed a negative change between early and late lactation phases (*alertness*, *comfort moves*, *exploratory behaviour*, *locomotion* and *solitary play*). The change in the amount of time expressing *comfort moves* was found to decrease, but not significantly so between lactation phases. By contrast, positive changes in activity budgets were seen in *milk-attaining* and *resting* behaviours. These overall changes are therefore consistent with what has been discussed already, showing that broadly-speaking, behaviours associated with energy transfer and retention increased over lactation. Even after lowering the *alpha* level for these tests using Bonferroni adjustment to account for multiple testing, significant differences between early and late lactation phases were still present in *alertness*, *exploratory behaviour*, *locomotion*, *milk-attaining* behaviour and *resting*; thereby supporting the discussion given by enlarge.

Speculatively, pups may benefit from being more behaviourally active in the early lactation phase, as a means of building up muscle and physicality before the deposition of subcutaneous fat peaks and the body is less supple. Indeed, there is suggestion by Fagen (1976), based on a review of exercise physiology, that there may be a '*physiological sensitive period'* during mammalian infancy when exercise is most effective. If this were true, then musculo-skeletal development of young neonates may be encouraged through enhanced physical activity in early lactation, reaping benefits to individuals once at sea when muscular strength and agility is required. The observed decrease in *solitary play* over lactation is particularly interesting. Many hypotheses have been suggested to explain the functionality of this behaviour, with one of the most prominent being the ‘motor-training’ hypothesis. This suggests that play enhances the development of bone remodelling, muscle hypertrophy, cardiopulmonary capacity and neuromuscular coordination (Jamieson and Armitage, 1987), with proposed benefits including increased strength and endurance. The relative increased occurrence of play in early lactation could therefore be an indication that this period is important for physical training and development. If play functions as described, we may predict that enhanced play during early sensitive phases would be most beneficial to males, since size and strength are ultimately important indicators of reproductive success in this sex, and early physical developmental trajectories may influence adult attributes. A more detailed study of play, with consideration of differences between the sexes and influencing factors would be beneficial for considering the function of play in neonates (see chapters 3 and 4).

4.3. Consistent individual behaviour over the lactation period.

Intraclass correlation (ICC) analysis indicated that most neonatal behavioural traits had repeatability measures significantly different to zero. Simply, this means that individuals behave differently relative to one another, and behave consistently so over time (across lactation). Bell et al. (2009) conducted a meta-analysis of the repeatability of animal behaviour and found that the average repeatability across all estimates was 0.37 (see figure 7). In this study, the average repeatability score from all behavioural traits was also 0.36, and when broken down by pup sex, the average repeatability of males was found to be 0.33 and females 0.35. The red circle on figure 7 depicts where the average repeatability of grey seal neonates (based on the findings from this study) is found in the distribution of repeatability. This shows that the average repeatability of neonates is toward the higher end of the distribution. However, it should be noted that the majority of study subjects were adults (Bell et al. 2009).

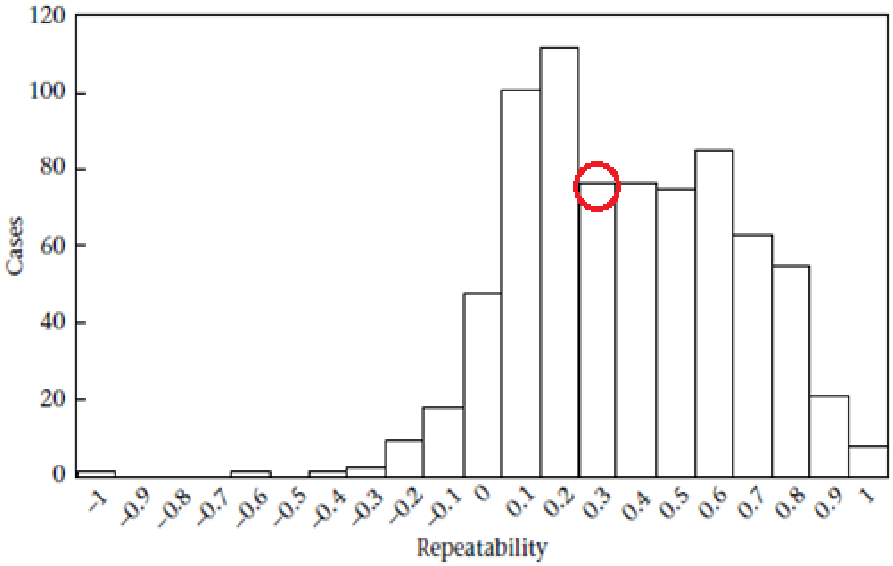


Figure 7. The distribution of repeatability from published studies, identified from 759 estimates of repeatability. *Most estimates came from vertebrates, with repeat measures taken within a year. The majority of behaviours were studied in adult individuals. Source: Bell et al (2009).* Red circle represents average repeatability estimate for grey seal neonates, derived from estimates from all behavioural traits studied.

Whilst some authors have reported relatively stable traits over time (e.g. Three-Spined Sticklebacks: Bell and Stamps, 2004; Rabbits: Rodel and von Holst, 2009; Cote et al. 2010; Firebugs: Gyuris et al. 2012), others have suggested that personality traits are unlikely to be stable over the course of a lifetime (e.g. see review by Groothius and Trillmich, 2011; Koolhaas et al. 2007; Biro and Stamps, 2008). There is some particularly interesting suggestion that changes in the primary food source may lead to behavioural/personality changes. In mammals, including grey seals, milk is the primary source of nutrition during the early stages of life. It is suggested that *specific* food contents may be important for methylation and that this may consequently lead to changes in personality if relevant promoters or binding sites are changed though this process (Groothius and Trillmich, 2011). That is, once the lactation period has ended and grey seals become independent at sea; a wider more varied diet may elicit proximate changes that impact upon any previously existing personality axes. Therefore, whilst consistent individual variability in behavioural traits may exist in early life, it cannot be certain how stable such individual variation is.

4.3.1. A comparison of neonatal and adult behavioural repeatability estimates.

Of the studies reviewed for Bell et al. (2009) there were only a few examples of repeatability comparisons between juveniles and adults of the same species, and these did not suggest a strong pattern. In some species, repeatability estimates were lower in juveniles compared to adults (Sticklebacks, *Gasterosteus aculeatus*; Big brown bats, *Eptesicus fuscus*; Godwits, *Limosa limosa baueri*), although this pattern was not found in the invertebrate species (Scorpionfly, *Panorpa vulgaris;* see Bell et al. 2009). Therefore, comparing the repeatability of behaviour in young individuals versus adults within the same species is an important, interesting and relatively unexplored area. Whether trait consistency maximises and stabilises at a specific period in the life course in non-human species is largely unknown. Much theory has been put forward on this subject. On one hand, we might expect young individuals to be undergoing dramatic developmental change and as a consequence not show very repeatable behaviour. On the other hand, we might expect juveniles to be more repeatable because the costs of straying from a developmental trajectory are higher for young individuals (Biro and Stamps, 2008). Bell et al. (2009) suggest that changes in repeatability with age could reflect the action of selection on phenotypic variance. If there is directional or stabilising selection on a particular behaviour, then phenotypic variance will decrease after selection. This could cause repeatability to decrease with age (if there is less variation among adults compared to juveniles). Alternatively, if traits expressed early in life are subject to stronger selection pressures than traits expressed later in life, then overall repeatability might increase with age (because there is more variation among adults compared to juveniles).

Study hypothesis 3 (*section 1.3*) predicted that behavioural repeatability scores would be lower in neonates compared to those found in adult behavioural traits; and there is some preliminary support for this from the results. Namely, behavioural trait repeatability estimates have been found to be much higher in studies of adult grey seals compared to those reported in this study in neonates for any trait. Individual adult males from North Rona were found to show strong inter-annual consistency in their percentage time devoted to *alert* behaviour, with a high repeatability measure significantly greater than zero (ICC = 0.83, 95% CI = 0.42 to 0.96, $F_{7,8}$ = 10.95, P = 0.002; see Twiss and Franklin, 2010). Adult males on North Rona have also been shown to be consistent in their behavioural responses towards novel auditory stimuli (delivered by a remote control vehicle, *RCV*) in the field, with some males showing overt aggressive responses and others expressing lower levels of response (ICC = 0.68, 95% CI = 0.19–0.90, $F_{11,11}$= 5.0, P= 0.007; see Twiss et al. 2012a). Further, adult females on North Rona have been shown to be highly consistent in their pup-checking behaviour during the same RCV tests (ICC = 0.80, 95% CI = 0.56–0.92, $F_{19,19}$= 8.6, P < 0.001; see Twiss et al. 2012a). These estimates therefore lie within the upper range of repeatability estimates presented in Bell et al (2009), see figure 7. In consideration of these findings, there is some preliminary suggestion that trait consistency maximises and stabilises later in ontogeny in this species. *Alert* behaviour may be particularly useful singular example, since behavioural data for this trait has been collected for both adults and pups on North Rona. As already shown, in Twiss and Franklin (2010) the ICC score for this trait in adult males was 0.83, whereas in neonates the repeatability estimate for *alert* behaviour was 0.41, and lower still for male neonates alone at 0.23 (see table 5). These estimates therefore support the suggestion that behavioural consistency in this trait increases with age in this species. However, these repeatability estimates were calculated over different time spans (adults: inter-annually, neonates: intra-seasonally), and it would perhaps be more insightful to assess the repeatability of this trait in the *same* individuals over a greater proportion of their life history.

Support from the wider literature:

Preliminary conjecture that behavioural consistency may increase with age in grey seals is supported in the wider literature. Within the human literature, it is generally accepted that personality stability increases with age, as does rank-order stability (see review by Roberts and DelVecchio, 2000). Indeed, this meta-analysis of 152 longitudinal studies found that personality trait consistency increased with age (0.31 in childhood and 0.74 between age 50-70 years). This suggests an age-related increase in the consistency of personality traits, and that major changes are more likely to occur in young age rather than later life. However, the question of when in the life course personality traits reach their peak consistency has received little systematic attention in non-human species. There is some scarce empirical support that behavioural consistency also increases with age in non-human species, where 'individuality' has explicitly been studied (Black-tailed prairie dogs: Loughry and Lazari, 1994; Fish species: Budaev and Zworykin, 2002). However, these studies are rare examples and further investigation in a wider range of taxa is required. Figure 8 illustrates two potential ontogenetic trajectories for the development of inter-individual behavioural consistency. Note that the term "discriminability" is synonymous with 'personality'. Figure 8a shows a trajectory whereby inter-individual variation increases over time alongside intra-individual behavioural consistency (therefore individual 'discriminability' increases over time). This trajectory is perhaps more in-line with what is found in humans, with individual personality differences becoming more stable with age. By contrast, f8b depicts a scenario where individuals develop distinct behavioural phenotypes in early life, which then gradually reduce over time as individuals are buffered by external factors and become more alike. The preliminary indication of the results of this chapter suggest that the trajectory over ontogeny in grey seals is more likely to be similar to figure 8a, akin to what is seen in humans.

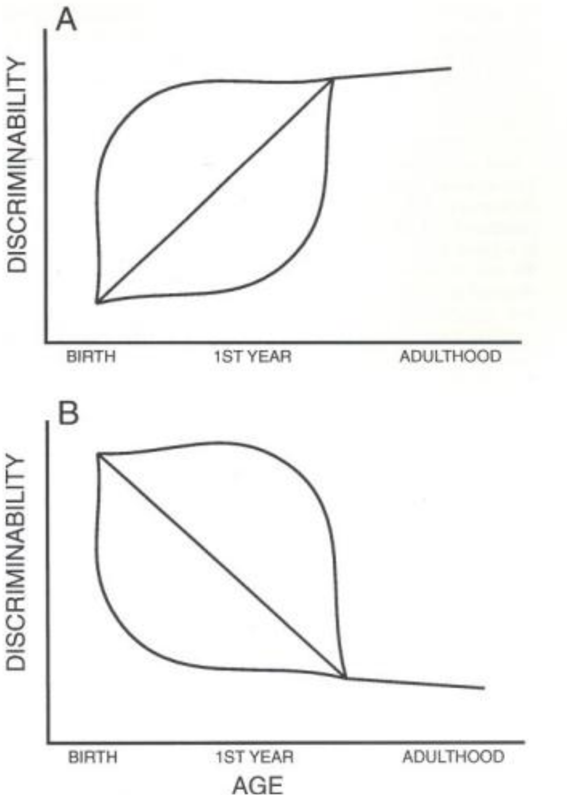


Figure 8.a,b: Theoretical ontogenetic trajectories in the development of individual differences. A) Individual discriminability increases over time; B) Individual discriminability decreases over time. (*Source: Loughry and Lazari, 1994*).

4.3.2. Sex differences in trait repeatability.

The findings of Bell et al. (2009) were inconclusive regarding sex differences in repeatability. Males were generally found to be more repeatable in their behaviour than females, however this sex difference was only observed in adults, not in juveniles. Further, when mate preference was omitted from the data set, the pattern was reversed and females were found to be more repeatable than males. Sex differences in the consistency of behaviour are also rarely observed in humans. However, again, these conjectures are primarily based on work with adult individuals. In the present study, it is interesting to note that only female pups were found to express significantly repeatable variation in *alert* behaviour, with a moderately high repeatability score (ICC score= 0.53). This significant result also remained following Bonferroni adjustment of the alpha-level to counter possible type 1 errors from multiple testing (see table 5). This is interesting as consistent variation in this trait has also been identified in adult male grey seals (Twiss and Franklin, 2010) and also in adult females (Culloch, 2012). The fact that there appears to be a sex difference in the repeatability of this behaviour in the neonatal age-range is interesting and suggests that perhaps behavioural consistency in this trait develops at different stages in ontogeny amongst the sexes. However, longer-term observation of the same cohort male and females over a greater proportion of their lifetime would be required to substantiate such theory. There would however be many problems to overcome in order to conduct long-term observation of individuals from the neonatal period onwards, including obtaining a large enough sample size to account for high first year mortality and measures to aid identification of individuals in the future, such as tagging and photo-ID efforts. A high investment of time and resources would be needed for such work.

4.3.3. Caveat of repeatability estimates.

A point to consider is that repeatability statistics say little about whether individuals themselves are repeatable, instead, the statistic is a property of the population of individuals (Bell et al. 2009). That is, even when a repeatability statistic is significantly different from zero, it does not necessarily mean that all of the individuals within the population behaved equally consistently over time. Indeed, some individuals may have been more consistent than others. Consistency itself seems to be a trait that varies amongst individuals. For example, the behaviour of *proactive* individuals, which tends to be more rigid and "routinised" (see Twiss et al. 2012b), is more repeatable than the behaviour of *reactive* individuals, which tend to be more responsive to cues in the environment (see Bell et al., 2009; Twiss et al. 2012b). There is some evidence to support this in adult female grey seals, with *proactive* individuals performing consistent levels of pup-checking behaviour between different contexts, and *reactive* individuals tending to modulate their behaviour more between contexts (Twiss et al. 2012b). It would be interesting to investigate whether pups may also be classified as *proactive* or *reactive* in a similar way, perhaps by assessing a particular behavioural trait (such as *alert* behaviour) behaviour between different contexts; such as "relaxed" and "alarmed" (see Twiss et al. 2012a, b for methodology details). This would be possible to do using focal footage of pups collected from North Rona during the 2012 breeding season (collected by author in-field).

As a further point, it is possible that significant repeatability in individual behavioural traits may have been enhanced by the short time frame over which behavioural consistency was assessed (intra-seasonally). Indeed, Bell et al. (2009) report that repeatability estimates were higher for behaviours measured close together in time. It would therefore be beneficial to assess behavioural consistency over a greater temporal span, certainly between early lactation and the post-weaning phase. Ideally though, it would be insightful to assess behavioural repeatability on an inter-annual basis to gain greater insights into the ontogeny of behavioural consistency, although the difficulties of achieving this are acknowledged. It is also interesting to consider that behavioural traits were found to be more repeatable in field studies compared to those conducted in the laboratory in Bell et al. (2009). Initially, the authors reasoned that greater environmental variance in the field would increase within-individual variation and thereby decrease repeatability. However, greater environmental variance in the field might allow the expression of more behavioural variation among individuals, by creating micro-niches, and thereby actually increase repeatability (Bell et al. 2009). These findings therefore again reinforce the importance of conducting more studies in natural environments with wild animals.

4.3.4. General implications of behavioural trait consistency.

Significant repeatability was found in the *general activity* levels (excluding behaviours directly related to the maternal figure) of neonates (figure 6b), and this result remained robust following Bonferroni adjustment for multiple testing (see table 5). This finding raises intriguing questions as to why such individual variability would exist, since if reserving energy and building up body fat stores (~body condition) increases the chance of first year survival (Hall et al. 2001, 2002) then for an individual to consistently be more active relative to another during the lactation period seems a little odd. Active behaviours require energy, thereby reducing that which can be used to for growth and the development of reserves. For example, Miller and Byers (1991) report that fawns that eschewed play behaviour and 'shifted energy savings to growth' could expect to weigh 7% more in post-natal time than fawns that played more. Consistent individual differences in activity levels therefore may suggest the existence of different behavioural 'strategies' amongst pups that have as yet been unidentified due to the description of behaviour via "general trends". Each 'strategy' may have equal fitness consequences, thereby enabling both to exist in the population. Perhaps more active pups gain benefits such as increased muscular development, strength and coordination, which in turn may aid success in the post-weaning phase. By contrast, a less active 'behavioural type' may benefit from accruing fat reserves and increasing body condition and therefore have longer term sustenance post-weaning. In order to test these hypotheses, individual pups would need to be followed on a more long-term basis and their first-year survival monitored in order to assess the fitness consequences of different behavioural strategies during the neonatal phase.

Consistent individual differences in the expression of play behaviour (table 5; figure 6c) were also reported amongst neonates, again raising questions as to why some pups would play more relative to others and vice versa. However, it must be acknowledged that when the *alpha*-level was lowered to counter the risk of type 1 errors, this result was no longer found to be significant. We must therefore treat this outcome with due caution. A more focussed study of the repeatability of individual neonatal play from the North Rona colony, with a greater sample size and directed research objective would be beneficial to confirm or deny this result. Despite questions surrounding individual consistency over time, it is still apparent from the results that variation in the expression of play exists amongst neonates, which remains a point of interest.

Play is a particularly interesting behaviour, owing to debate surrounding its adaptive significance. It would therefore be interesting to assess the fitness consequences of individual variation in neonatal play; this would help to decipher the potential function of play in this age class. Interestingly, within the literature there is suggestion that variation in play behaviour may be linked to levels of nutrition received by young individuals (e.g. Belding's Ground squirrels: Nunes et al. 1999; Meerkats: Sharpe et al. 2002). This is highly relevant for grey seal pups, where variation in maternal investment has already been identified (Pomeroy et al. 1999). Therefore, variation in play behaviour may be hypothesised to be related to patterns of maternal nutritional investment and maternal condition. This will be investigated in more detail in chapter 3.

4.4. Maternal effects and individual behavioural variation.

There is substantial evidence that maternal effects can influence behavioural variation in offspring. These include heritable components (e.g. Rodents: Sluyter et al. 1996; Koolhaas et al. 1999; Birds: Groothius and Carere, 2005; van Hierden et al. 2002; Fish: Overli et al. 2005), however, genetic effects do not account for all of the behavioural variation observed amongst individuals (see Stamps and Groothius, 2010a; Aphids: Schuett et al. 2011). A range of maternal effects have been implicated, including differences in maternal provisioning. Differences in maternal provisioning have been suggested to influence behavioural variation amongst offspring through differences in post-natal growth and physical development, which subsequently influences the personality traits adopted by offspring. (e.g. Rats: Rodel and Meyer, 2011, Rabbits: Rodel and von Holst, 2009; Rodel and Monclus, 2011). As Rodel and Meyer (2011) succinctly articulate: *"State-dependent constraints in behaviour during early life may lead to the adoption and manifestation of distinct personality types by positive feedback loops".* Therefore there is reason to suppose that differences in maternal mass and available energy stores could indirectly have implications for behavioural variation amongst young individuals. Such individual differences in female performance have already been found in female grey seals (Iverson et al. 1992; Mellish et al. 1999; Pomeroy et al. 1999). Furthermore, similar temperaments and behaviour patterns between mothers and offspring have been reported in the literature (e.g. Rhesus Monkeys: Stevenson-Hinde and Simpson, 1981 ; Sullivan et al. 2011 ; Humans: Cohen et al.1998; Dolphins: Mann and Smuts. 1999; Manatees: Deutsch et al. 2003), which might be expected between individuals that share similar environments and shared genes. It is also possible that young individuals may adopt certain ways of behaving by observing their mother.

The dependence of grey seal pups on their mothers for nutrition during the lactation period and the proximity maintained between the pair during this time means that maternal physiological and behavioural attributes are an important consideration in terms of neonatal variation. See further exploration of this below.

4.4.1. Exploration of the effect of maternal parameters on neonatal behavioural variation.

Maternal behavioural and physiological parameters were correlated with neonatal behavioural budgets using Spearman rank correlations (see table 6 for summary of analyses). Note that for these analyses, maternal behavioural budgets were derived using the same method as described in chapter 1 for neonates, using from raw scan-sampling data. Maternal physiological parameters were provided by Dr. Patrick Pomeroy of the Sea Mammal Research Unit, University of St Andrews.

Neither *pupping date* nor the physiological parameters of *maternal energy transfer efficiency* and *postpartum mass* had a significant correlation with any neonatal behavioural trait. However, significant correlations were found between some maternal and neonatal behavioural parameters. The percent time spent *nursing by mothers* positively correlated with pup *milk-attaining* behaviours and *maternal interactions*, as would be expected. A correlation was also found between maternal *nursing* and pup *resting* behaviour, with pups that nursed more resting less and vice versa. Pups that spend less of their activity budget nursing may be able to spend relatively more time resting, rather than energetics playing a role in influencing behaviour patterns (and this is reinforced by the lack of correlation with any behaviours and maternal transfer efficiency).

These results therefore suggest that some of the variation in neonatal behaviour patterns can be at least in-part attributable to variation in maternal behavioural characteristics or influences. Regardless of how neonatal behavioural variation may be influenced and shaped, what is perhaps more interesting is whether the individual variation observed amongst neonates is *canalised* during the neonatal period in a way that is indicative of the future behaviour of individuals. Unfortunately such questions cannot be answered with the current data, but would prove an interesting line of study for the future.

4.5. A note regarding the limitation of sample size and possible sampling effects.

The interpretation given in this discussion should be considered in light of limitations relating to sample size and sampling effects. Firstly, the behavioural data analysed in this chapter were recorded only for a sub-set of the total pups born on North Rona over the three successive breeding seasons considered. Hundreds of pups are born during a breeding season on North Rona, and therefore a sample size of 72 for three seasons combined has obvious limitations regarding the application of findings more generally. Another notable point is that sample size was decreased substantially when the dataset was explored at finer levels, such as comparing temporally between early and late lactation phases; or between males and females separately. The statistical power of tests was therefore decreased for these analyses. Future work would benefit from efforts to maximise the number of individuals for which 200 or more scan samples is achieved, with this being a minimum target level for early and late lactation phases respectively.

Further, it is clear that inactive behaviour (*resting*) constitutes the majority of neonatal behavioural repertoires. As a consequence of this, the sample size for other behaviours is very small. Perhaps *resting* behaviour could be excluded in future when deriving time-activity budgets; that is, to give the percentage of time spent in different behavioural traits when neonates are ‘active’. This would however require a larger number of scans to be conducted per individual in order to account for the high proportion of *resting* behaviour and still obtain representative results.

As a final point, the behavioural data used for analyses in this chapter were collected primarily from neonates that were born in a research locality of North Rona known as the 'Study Area' (see figure 6, chapter 1). The island is however constituted by three research localities; including two domains known as 'Fianius North' and 'Fianius South' (see chapter 1, *section 1.5.1* for more details). Therefore, the data analysed in this chapter may be reasoned to come from a biased sample in this respect. Future descriptive studies of this colony would benefit from including a sample of individuals from across the island as a whole, if this were logistically possible and the number of behavioural data collected per individual was not compromised.

4.6. Chapter summary.

As seen in other phocid species and previous studies of grey seals, neonates were found to spend the greatest proportion of time during the lactation period *resting/idle*. This is assumed to be an evolutionary behavioural mechanism for minimising energy expenditure and maximising mass gain during this period. In-line with this, the time spent in behaviours associated with energy transfer (*nursing*) and energy retention (*resting*) were found to increase in behaviour budgets over lactation overall. Whilst these general trends confirmed established biological theory, individuality was also found to exist in some neonatal behavioural traits, with pups expressing certain behaviours to higher or lower degrees relative to one another and consistently so over time. Repeatability estimates were found to be lower for neonates than have been found in adult behavioural traits so far, thus providing preliminary suggestion that behavioural consistency may increase with age in this species. Play has emerged as an interesting behaviour to focus on in further chapters. This is an example of a seemingly 'purposeless', energetically-costly behaviour that is nevertheless apparent in neonatal activity budgets, with variation in its expression found between individuals. The nature and function of play in neonates will be the focus of the following chapters.

CHAPTER 3

An Investigation of Maternal Nutritional Investment and Neonatal Solitary Play Behaviour

Chapter Introduction:

In this chapter, the solitary play behaviour of neonatal pups will be investigated in relation to maternal nutritional investment and condition. Play has been suggested to vary between individuals in response to food availability and differences in diet, suggesting that there is potentially a direct relationship between play behaviour and nutrition. The breeding system in grey seals is closed, meaning that females do not leave the breeding site to forage during this time, allowing the nutritional transfer from mother to pup to be quantified via repeated measurements of individuals during this time. Since maternal milk is the only source of nutrition for pre-weaned neonatal pups, there is a good opportunity to test whether there is a link between nutrition and neonatal play in this species. Further, since the grey seal is a sexually dimorphic, polygynous species, it might be expected that mothers will differentially invest between the sexes, in relation to their own condition and the future reproductive potential of their offspring. If there is a link between nutrition and play, we may therefore expect to see differences in the play behaviour of male and female offspring in relation to the relative condition of their mother. This chapter aims to test these hypotheses and discuss the potential function of solitary play in neonates.

1. INTRODUCTION

1.1. Play: a behavioural enigma.

Play behaviour may be considered as easily recognisable and observable, yet its objective definition has been long debated. A concise working definition from Bekoff (1984) is useful for reference: *"Play is all motor activity performed post-natally that appears to be purposeless, in which motor patterns from other contexts may often be used in modified forms and altered temporal sequencing".* Such behaviour has been shown to be characteristic of young mammals and birds in particular (e.g. Ortega and Bekoff, 1987; Barber, 1991; Power, 2000). Play comprises significant proportions of time-activity budgets in mammalian species (e.g. Stumptail Macaques: Bernstein, 1980; Sea Lions: Gentry, 1974). Due to its prominent role in the behaviour of mammalian young and to its presence across a wide range of species, play has long attracted attention from researchers (Gomendio, 1988). However, it has widely been viewed as a biological enigma, owing to the associated costs of the behaviour to the individual (e.g. Energetic-costs: Miller and Byers, 1991; Sharpe et al. 2002). In order for play to exist so prominently across taxa, its benefits are reasoned to outweigh such associated costs. Smith (1982) considers the potential functions of animal play and speculates that whilst play may not always have direct or obvious benefits, it may have delayed-benefits in the future life of the animal. As Smith (1982) articulates, animals may "*…incur the cost of energy expenditure in active play in order to gain the future benefits of well-exercised muscles and superior general physical capacity*". This point of view is reiterated by Spinka et al. (2001) who note that functional explanations of play have tended to focus on ways in which play during early development could benefit animals as in later life, on the basis that animals are usually more playful when young.

The function(s) of play have been long-debated (Bekoff, 1984; Spinka et al. 2001), with more than 30 hypotheses postulated so far (Sharpe et al. 2002; Cameron et al. 2008). Play has been suggested to enhance motor skills and physical fitness (Bekoff, 1988; Nunes et al. 2004); build social relationships and dominances (Fagen, 1981, Rats: Panksepp, 1981), and provide opportunity to 'train for the unexpected' (Spinka et al. 2001). As yet, no one hypothesis has gained overwhelming support, which may in part be due to the difficulty of detecting benefits that are delayed (Sharpe et al. 2002), and there has been suggestion that non-human play may be multifunctional (Power, 2000). Some short-term benefits have been identified in the literature (e.g. Fagen and Fagen, 2004; Chimpanzees: Palagi et al. 2006), however long-term or delayed benefits, as predicted by Smith (1982), have so far proven difficult to assess with only a few attempts to investigate this (e.g. Chimpanzees: Palagi et al. 2004). Indeed, the time lapse between early development and sexual maturation exacerbates the difficulty of relating variations in early behavioural ontogeny to differential reproductive success.

1.2. Play, nutrition and differential maternal investment theory.

Play has been found to vary between individuals in response to food availability (e.g. Rats: Smith, 1991; Loranca et al. 1999; Review: Martin and Caro, 1985, Mammals: Barber, 1991). Further, correlations between diet and play behaviour have been found across taxa, with increases in nutrition leading to increases in the amount of play (e.g. Gelada baboons, *Theropithecus gelada*: Barrett et al. 1992; White tailed deer, *Odocoileus virginiarus*: Muller-Schwarze et al., 1982; Rodents: Smith, 1991; Belding's ground squirrels, *Spermophilus beldingi*: Nunes et al. 1999). There is also complementary evidence from experimental studies of effects on play behaviour, where nutrition was deliberately manipulated (e.g. Belding's Ground squirrels: Nunes et al. 1999; Meerkats: Sharpe et al. 2002). Similarly, other studies have found increased expression of play in offspring in conditions associated with food deprivation (Rats: Smith, 1991; Domestic Kittens: Bateson et al. 1990). These studies therefore suggest that in some species play behaviour can be directly influenced by nutrition levels and factors of diet.

The young of many mammalian species initially depend entirely on milk from their mother to attain the energy necessary for maintenance, activity and growth. Further, the strong link between mother and young leads to the expectation that a mother's activity pattern and investment may directly influence the behaviour of her young (Arnold and Trillmich, 1985). Nutritional provisioning is an example of how differing levels of maternal investment can alter resource availability to offspring. Interestingly, maternal investment has been shown to vary between mothers of the same species, with evidence that reproductive effort and investment can show intraspecific variation (e.g. Horses: Cameron and Linklater, 2000; Grey seals: Pomeroy et al. 1999).

According to theory, mothers should alter their level of investment in their offspring in relation to their own body condition and the potential reproductive success of their offspring (Trivers and Willard, 1973). As a consequence of this differential investment between the sexes may be expected, since mothers in good condition would gain more fitness from investing more in the more reproductively variable sex, which is usually males in polygynous mammals (Cameron et al. 2008).

In such a system, a highly successful son would be more successful than a successful daughter. However, females in poor condition should invest more in female offspring since an unsuccessful daughter is likely to breed at least once, whereas an unsuccessful son may gain no successful mating.

Differences in maternal investment and nutritional transfer may be postulated to influence the play behaviour expressed by offspring, owing to variation in energy levels (‘*surplus energy hypothesis*’, Spinka et al. 2001). There is support for this in feral horses (Cameron et al. 2008). This study found evidence to suggest that maternal condition influenced the play behaviour of foals, with sons of mothers in good condition playing more. In support of this, when the play behaviour of sons and daughters of an individual mother were compared, to control for maternal effects, the daughters of mothers in poor condition played more than their sons, whereas the sons of mothers in good condition played more than their daughters. Offspring are suggested to benefit from enhanced condition as a result of increased play (Cameron et al. 2008). These findings therefore suggest that foals responded to an increase in energy with an increase in play behaviour. The author speculates that play both burned “excess energy” and enhanced physical development, including tendon strength and flexibility and skeletal and muscular growth, proving a form of early exercise. Further, foals that played more were found to be in better condition as yearlings. This study therefore provided support for the hypothesis that enhanced neuromuscular performance may be one benefit of play (see Fagen and Fagen, 2004), and therefore that maternal investment can enhance both the social and physical development of offspring (Cameron et al. 2008).

1.3. Nutrition and play behaviour in grey seals.

Being ‘capital breeders’, female grey seals fast during their stay at breeding colonies and rely on their fat reserves to sustain themselves and provision their young during lactation. Pups rely entirely on maternal milk to provision them through the 16-18 day lactation period, using this to fuel rapid growth and the development of subcutaneous fat stores. Maternal investment in pups is high, with lactation milk containing up to 40-60% fat depending on the individual female and the pup’s age (Lydersen et al. 1995). As a consequence of this high investment, pups put on mass at about 2kg/day, whilst mothers may simultaneously lose mass at approximately 4kg/day. There is however individual variation in the amount of energy expended on rearing the pup, with some mothers losing much more mass than others and vice versa and pups growing at different rates (see Pomeroy et al. 1999). Interestingly, even when body size is taken into account, some mothers still produce bigger pups than others (Pomeroy et al. 1999). Whilst there is evidence from other phocid species of differential maternal investment (Northern Elephant Seals: Reiter, 1978, 1981), there has been mixed findings in grey seals, with some authors supporting this (Isle of May: Kovacs, 1987) and others disputing such findings (Froan, Norway: Smiseth and Lorentsen, 1995). Regarding the colony of North Rona, preliminary indication of possible differential investment was found in chapter 2, with male pups spending more time nursing and in *maternal interactions* compared to female pups (see chapter 2, *section 4.1.1*). The grey seal study system therefore lends itself well to the investigation of nutrition and play, with particular focus on the possible influence of maternal condition and investment.

There are limited studies documenting the play behaviour of young grey seals. Wilson (1974) conducted a comparative study between the play behaviour of grey and common seals. Aquatic and terrestrial displays of play were observed, with the latter generally taking place on the beach and consisting of two animals lying beside one another, each lunging gently at the other's head in between head-over-back signals (see figure 2, chapter 5). From their observations, dyadic (social) play was the only kind of play observed, usually occurring between two males or a male and a female, although play between two females occurred occasionally. However, this study was focussed on grey seals between 6 months and 5 years old and therefore the findings are not generalisable to pre-weaned neonates. Kovacs (1987) reports a comparative lack of dyadic or social play in neonatal pups from the Isle of May breeding colony. It was noted in this study that play behaviour was "rare" in young neonates and increased towards the end of lactation and into the post-weaning phase. Interestingly, when play was observed, it was recorded as always being either self or object-directed. To current knowledge, there have been no studies specifically addressing how maternal investment or condition may affect pup play behaviour during the lactation period in this species.

1.4. Study hypotheses.

<u>Hypothesis 1</u>: There will be evidence of a direct link between levels of maternal nutritional investment and neonatal play behaviour, with increased nutrition leading to enhanced play behaviour.

<u>Hypothesis 2</u>: In-line with differential maternal investment theory, male offspring of mothers in better condition will play more than female offspring, with the opposite pattern observed in pups of poorer condition mothers.

2. METHODS

2.1 Study colony and play definition.

The neonatal behavioural data analysed in this chapter was collected by SDT on the breeding colony of North Rona over the successive breeding seasons of 2008, 2009 and 2010. A scan-sampling method was employed by SDT to collect behavioural data for individually identified neonates in the field (as described in detail in *section 1.5* of chapter 1). From this raw behavioural data, individual time-activity budgets were derived by the author post-hoc (see chapter 2 for more details). These behaviour budgets detail the percentage of time that pups spent in a variety of behavioural categories, including *play*.

In chapter 2, 'play' was considered as a unitary behaviour, describing *individual locomotor play* only; characterised by localised jerky, exaggerated or repeated actions (see chapter 1; table 1). However, convincing evidence has been collected to indicate that play is in fact a heterogeneous behaviour, with distinctly different forms being identified (e.g. Barret and Bateson, 1978; Chalmers, 1980; Martin and Caro, 1985). Consequently, in this chapter the behaviour budgets for '*exploratory*' behaviour and '*play'* have been combined so that the newly-derived 'solitary play' budgets incorporate two prominent types of independent play: *individual locomotor play* and *object play* (since '*exploratory*' behaviour describes instances of pups physically investigating inanimate objects in their environment; chapter 1, table 1). The definition of play in this chapter is therefore broadened, incorporating self and object-directed play behaviour. It is necessary to briefly consider the boundaries of such behavioural categories. For example, it may be questioned whether small, localised movements may instead be related to issues such as thermal regulation, insect disturbance or gastric discomfort, rather than an expression of solitary play. However, actions attributed to such stimuli (due to feelings of discomfort) would typically be classed as *comfort movements* due to their briefer nature. Self play is potentially more ambiguous than object-play in this regard, since object play requires repeated interaction with an inanimate object, such as grass, and is therefore more easily identifiable in a field context. To correctly identify self play however, the use of contextual cues is important. For example, if individual movement was followed by direct locomotory behaviour to a pool, it would be assumed that the prior behaviour was linked to a thermoregulatory motivation and therefore be recorded as a *comfort movement* rather than individual *play*. It is also common for self play to occur whilst the individual is lying on its back, and is typically characterised by extensive wriggling and 'flippering' behaviour whereby the pup repeatedly appears to "scratch" itself and/or exhibit erratic flipper movements for prolonged periods (see figure 1a in chapter 4 for visual depiction of such actions). Such knowledge, in combination with contextual cues, facilitates the identification of boundaries for individual-based behaviours to be classed as 'play'.

2.2. Proxy data for maternal investment and condition.

Since female grey seals fast during the breeding season, relying on stored reserves to provision their pup, they present a 'closed' system for the monitoring of reproductive expenditure. Such investigation has been carried out at North Rona on a long-term basis (lead researcher: Dr Paddy Pomeroy, Sea Mammal Research Unit)**.** To briefly summarise the methodology of such investigation, females and their pups are captured twice during their lactation period, in order to determine a range of phenotypic measures of reproductive performance (for further details see Pomeroy et al. 1999). These reproductive metrics are used in this study as best proxies for maternal nutritional investment in young and maternal condition. Descriptions of these measures are given below (taken from Twiss et al. 2012b):

Maternal postpartum mass (kg): represents a standard reference point for the mother's mass and is an index of somatic growth and prior foraging success.

Maternal daily mass loss rate (kg/day): describes maternal mass loss over the lactation period, providing a time-averaged index of rate of maternal expenditure.

Daily rate of pup mass gain (kg/day): represents time-averaged pup growth rate over lactation.

Maternal mass transfer efficiency: estimated proportion of maternal expenditure converted into pup mass gain (calculated by: pup mass gain/ maternal mass loss).

2.2.1. Maternal nutritional investment.

To quantify the nutritional investment of mothers to their offspring, the 'maternal mass transfer efficiency' metric was used. As described above, this metric uses the estimated daily mass loss of females and mass gain of their pups to approximate the proportion of maternal expenditure converted into mass gain in the pup. This can be an accurate measure in species such as the grey seal where females do not forage during the lactation period (Lydersen and Kovacs, 1999). Data from the 2008, 2009 and 2010 breeding seasons were combined for analyses involving this metric.

Estimated maternal condition loss (mass in kg) over lactation was used as a further means of assessing the potential relationship between maternal investment and offspring play, with the assumption of a positive correlation between initial maternal body condition and energetic investment in offspring. Measures of condition loss for females from the 2008 and 2009 breeding seasons were derived using the following equation: *Maternal post-partum mass - (Maternal daily mass loss x Duration of lactation).* Parameters for these calculations for the 2010 season were not available at the time of thesis-writing and so data from this year was not included in these analyses. The proportion of time spent in *milk-attaining* behaviours was used as a purely observational means of estimating the degree of maternal nutritional investment received by pups.

2.2.2. Maternal condition.

Maternal condition was indexed as ‘maternal post-partum mass’ (MPART), giving a description of somatic growth and prior foraging success (*see section 2.2*). The use of maternal post-partum mass to reflect relative body condition is therefore based on the assumption that an animal in good condition has more energy reserves (positive energy balance) than an animal in poorer condition (Schulte-Holstedde et al. 2005). In order to increase the statistical power of tests by increasing *N* in each group, mothers were segregated into two condition categories for analyses: ‘*Below-average’* (females with below-average post-partum masses) and ‘*Above-average’* (females with above-average post-partum masses). To aid clarity in analytic presentation, these respective groups will be denoted as either “*above*” or “*below*”, and mothers may be referred to as being in “better” (above-average) or “poorer” (below-average) condition within the text.

2.3. Controlling for maternal and genetic effects.

In order to control for the possible influence of maternal and genetic effects on analyses of maternal condition, the play behaviour of male and female siblings (born to the same mother in different breeding seasons) was compared. Although the genetic connectedness of siblings was not empirically assessed, it is known that siblings are on average 50% related. However, it is thought that in fact genetic relatedness may be more due to the site-fidelity of males and females over pairs of years (SDT, personal observation). Therefore, there is good reason to suppose that genetic differences are controlled for to a reasonable extent through sibling comparisons.

In order to conduct sibling play comparisons, mothers who had given birth to a son and a daughter in different reproductive bouts (breeding seasons) were first identified and categorised into pairwise comparisons (2008-2009: n= 4, 2008-2010: n=5, 2009-2010: n=6). The relative condition (MPART) of these females was found to be consistent between reproductive bouts: that is, a female found to be in below-average condition in one breeding season was also found to be so in the pairwise season. Relative maternal condition in each breeding season was calculated based on the group average for that year. In this way, all that varied in these play comparisons was the sex of the siblings, with relative maternal condition held constant. The *solitary play* behaviour budgets of sons and daughters over lactation were then compared by deriving a 'play index' for each sibling pair using the following equation: **son – daughter / son+ daughter** (taken from Cameron et al. 2008). For reference, the nature of this index is that positive index values indicate that the son played more than the daughter, and vice versa. Average play indices were then derived for the two maternal condition groups, thereby attaining a mean play index for siblings of *below* and *above-average* condition mothers respectively.

2.4. Statistical approach.

Data were tested for normality and equality of variance before analyses were conducted, and where transformation was unsuccessful or sample sizes were low, non-parametric tests were performed. The statistical significance level used for all tests was 0.05 and all tests were two-tailed. The Bonferroni correction was applied post-hoc where appropriate, to address the risk of type 1 errors resulting from multiple statistical testing. This correction assesses each of the individual tests at an *alpha*-level of α/n, where α is the desired significance level for the whole family of tests (0.05) and n is the number of individual tests [**2**].

Potential sex differences in play behaviour over the entire lactation period were assessed using an independent *t*-test, and sex differences within early and late developmental phases explored using the Mann-Whitney *U* test. An independent *t*-test was used to compare the play behaviour of pups in relation to maternal condition over the whole of lactation; and the Mann-Whitney *U* test used early and late lactation phases separately. A randomisation procedure for small samples was used to compare male and female play respectively in relation to maternal condition in early and late lactation. An MS-Office Excel based randomisation procedure for small samples was used for this (Design 5a from Todman & Dugard 2001). This test computes the difference between the mean values for each group (above and below) and then repeats this for 1000 randomly chosen arrangements of the data that conform to the number of cases in each group. The probability (for two-tailed tests) is given by the proportion of permuted iterations that result in an absolute mean difference at least as large as the statistic derived from the actual data. This approach was preferred over the Mann-Whitney *U* test as it is more conservative, given the relatively small sample sizes (Todman and Dugard, 2001). This test was also used to compare the play indices obtained from sibling pairs of mothers in above or below-average condition. Maternal investment was investigated using Spearman rank correlations to assess the relationship between play behaviour budgets and a range of parameters indicating levels of maternal investment: proportion of time in milk-attaining behaviour, maternal transfer efficiency and estimated maternal condition loss over lactation.

3. RESULTS

***A note regarding maternal investment and condition analyses*:**

Data from the 2008, 2009 and 2010 breeding seasons were combined for analyses involving the 'maternal mass transfer efficiency' (*section 2.2.1*), since there was no significant difference in either parameters used to derive this index between years (*Pup daily growth rate*: $F_{(2,58)}$= 1.95, *P*=0.15; *Maternal daily mass loss*: $F_{(2,58)}$= 0.18, *P*= 0.84). The mean (±SD) level of estimated condition loss over lactation, using data from the 2008 and 2009 breeding seasons, was 73.8kg ± 12.5 (n= 35). There was no significant difference between the estimates of condition loss from the 2008 and 2009 breeding seasons (mean ± SD: 2008= 72.4kg ± 13.7, 2009= 75.1kg ± 11.6; *t*-test: t_{33}= -0.63, *P*= 0.53). As mentioned previously, in order to increase the statistical power of tests concerning maternal condition, mothers were segregated into two categories relating to their relative body condition for analyses: '*Below-average'* (females with below-average post-partum masses) and '*Above-average'* (females with above-average post-partum masses). The mean (±SE) post-partum mass of females from all three breeding seasons was 193.2kg ± 22.87; thus rendering 26 focal females in the *above-average* condition category (mean±SE= 216.49kg ± 3.02), and 36 females in the *below-average* category (mean±SE= 178.07kg ± 1.70). For the sibling analyses presented (*section 3.1.4*), the relative condition of mothers in pairwise breeding seasons was deduced from the group average for each respective season (Mean ± S.E. *2008*: 192.7kg ±4.46, *n*= 21; *2009*: 194.34kg ± 4.9, *n*= 22; *2010*: 195.6kg ± 5.79, *n*= 19).

3.1. Sex differences in play behaviour.

During lactation, pups were found to express solitary play for an average ± S.E. of 6.03% ± 0.44 of the time (n=72). There was no difference in the overall play expressed by male or female pups over lactation (*males*: mean ± S.E. = 6.41% ± 0.67; *females* = 5.96% ± 0.69; *t*-test: t_{64}= -.05, *P* = 0.96; figure 1). This was also true when male and female play behaviour was compared during the early (*males*, n=18: mean ± S.E = 7.97 ± 1.09, *females*, n=24 = 6.92 ± 0.65; *U*= 187.0, *P* = 0.46; figure 1) and late (*males*: mean ± S.E = 5.37 ± 0.83, *females* = 5.66 ± 0.72; *U*=201.0, *P*= 0.70; males, n=18, females, n=24; figure 1) lactation phases separately.

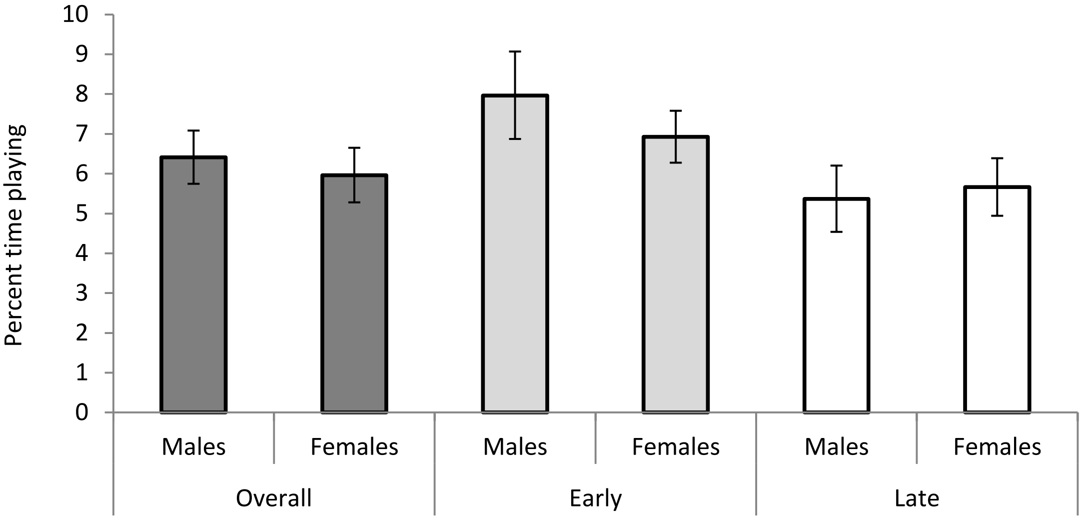


Figure 1: Percent time spent playing by male and female neonatal pups from North Rona during the lactation period. Early and late lactation phases are represented separately. Values are mean ± SEM. *Overall: males, n= 34; females, n= 32; Early: males, n= 18; females, n= 24; Late: males, n=18, females, n=24.*

3.1.2. Intra-sex comparisons in relation to maternal condition.

There was no difference in the proportion of time spent playing by pups in relation to maternal condition (mean ± SE: *Above* = 6.86% ± 0.90; *Below* = 5.98% ± 0.56; *t*-test: t_{60}=0.38, *P*= 0.71). This was also found to be true for both early (mean ± SE: *Above* =7.94% ± 1.04, *Below* =7.01% ± 0.71; *U*= 178.0, *P*= 0.42) and late (mean ± SE: *Above* =6.54% ±0.95, *Below* = 4.81% ± 0.57; *U*= 170.0, *P*= 0.31) lactation phases. Male pups did not vary their quantity of play behaviour in relation to their mother's condition (mean ± SE, *Above*: 6.07% ±1.69, *Below*: 6.85% ± 0.74; Two-way randomisation test statistic= 0.78, *P*= 0.62; figure 2). However female pups of mothers in better condition were found to play more than female pups of mothers in poorer condition over lactation (mean ± SE, *Above*: 7.28% ± 1.06, *Below*: 4.60% ±

0.76, Two-way randomisation statistic = 2.68 , *P*= 0.04; figure 2). However, these analyses were repeated for each lactation phase separately, this difference was no longer found (Early: mean ± SE: females; *Above* = 7.75% ± 0.88, *Below* = 6.08% ± 1.01; Two-way randomisation test statistic= 1.67, *P*= 0.22; Late: *Above*: 6.57% ± 1.04, *Below* = 4.79% ± 1.01; Two-way randomisation test statistic=1.78, *P*= 0.24). This was also true of male pups. (Early: mean ± SE, Males: *Above*= 8.34% ± 2.86, *Below*= 7.78% ± 0.97; Two-way randomisation test statistic= 0.56, *P*= 0.83; Late: Males: mean ± SE: Above = 6.46% ± 2.17, Below= 4.82% ± 0.67; Two-way randomisation test statistic=1.65, *P*=0.38). The Bonferroni adjustment was applied to these results post-hoc to account for multiple testing. Since three tests were conducted on male neonate data, and the same number for female data, the adjusted *alpha*-value is 0.017 for the tests (0.05/3). Doubt is therefore cast upon the previously significant difference between the amount of solitary play expressed by female pups belonging to mothers in above and below-average condition. This result must therefore be considered with due caution.

3.1.3. Inter-sex comparisons in relation to maternal condition.

No significant difference was found when the play behaviour of male and female pups belonging to mothers in above-average condition were compared (mean ± SE, Males: 6.07% ± 1.69, Females: 7.28% ± 1.06; figure 2; *U*= 57.0, *P*= 0.29; figure 2). This result was also echoed when early (mean ± SE, Males= 8.34% ± 2.86, Females= 7.75 ± 0.88; Two way randomisation test statistic=0.59, *P*= 0.78) and late (mean ± SE, Males: 6.47% ± 2.17, Females: 6.57% ± 1.04; Two way randomisation test statistic=0.10, *P*= 0.96) lactation phases were investigated. By contrast, over lactation as a whole, male pups were found to play significantly more than female pups when their mothers were in below-average condition (mean ± SE, Males: 6.85% ± 0.74, Females: 4.60% ± 0.76; figure 2; *U*= 80.0, *P*= 0.02; figure 2). Although, this significance was not repeated when lactation phases were studied separately (Early: *mean ± SE*: males= 7.78% ± 0.97, females= 6.08% ± 1.01; Two way randomisation test statistic= 1.70, *P*= 0.24. Late: *mean ± SE*, Males=4.82% ± 0.67, Females: 4.79% ± 1.01; Two way randomisation test statistic= 0.02, *P*= 0.99). Since three sex comparisons were made in each maternal condition group (“above” and “below”), the Bonferroni adjustment was applied post-hoc to account for multiple testing. The adjusted *alpha*-value was lowered to 0.017 for these respective tests (0.05/3). In light of this, the significant difference between the amount of play expressed by male and female pups of mothers in below-average condition (*P*= 0.02) falls just out of significance.

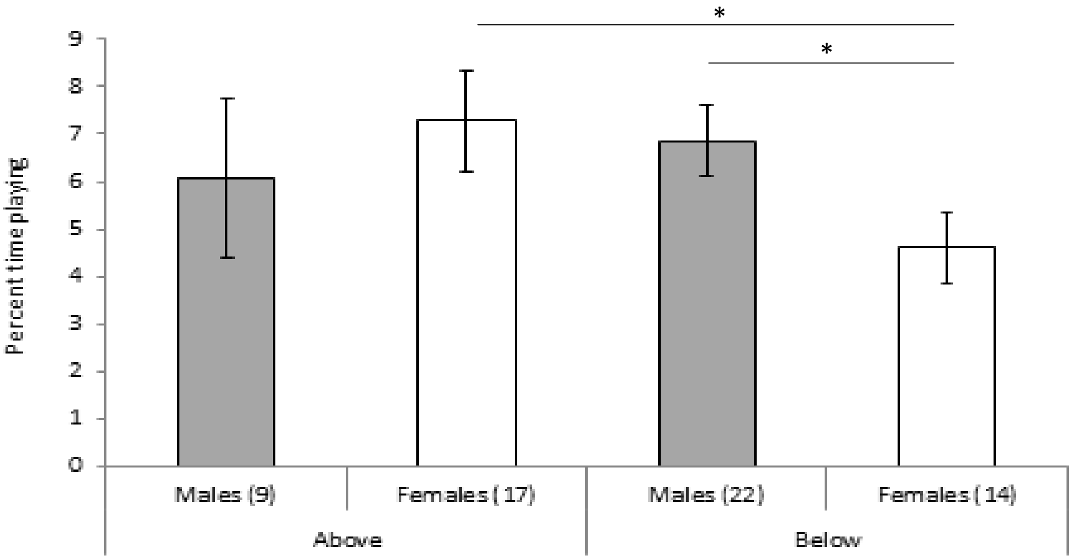


Figure 2: Percent time playing by male and female neonatal pups during lactation in relation to their mother's relative condition. Values of bars are mean ± SE, values in parentheses represent sample size of group. ****Signifies significant differences at p=0.05*. ****Note: results not significant following Bonferroni adjustment of alpha-value (0.017).*

3.1.4. Sibling play analyses in relation to maternal condition.

A significant difference was found in the play behaviour of sons and daughters in relation to the relative body condition of the mother. Daughters of mothers in better (above-average) condition played more than their sons, whereas the sons of mothers in poorer (below-average) condition played more than their daughters (Mean play index value ± SE, *Above*: -0.251 ± 0.16, n= 9; *B*elow: 0.253 ± 0.10, n=6: Two-way randomisation test statistic= 0.51, *P*= 0.03; figure 3).

(figure 3, below)

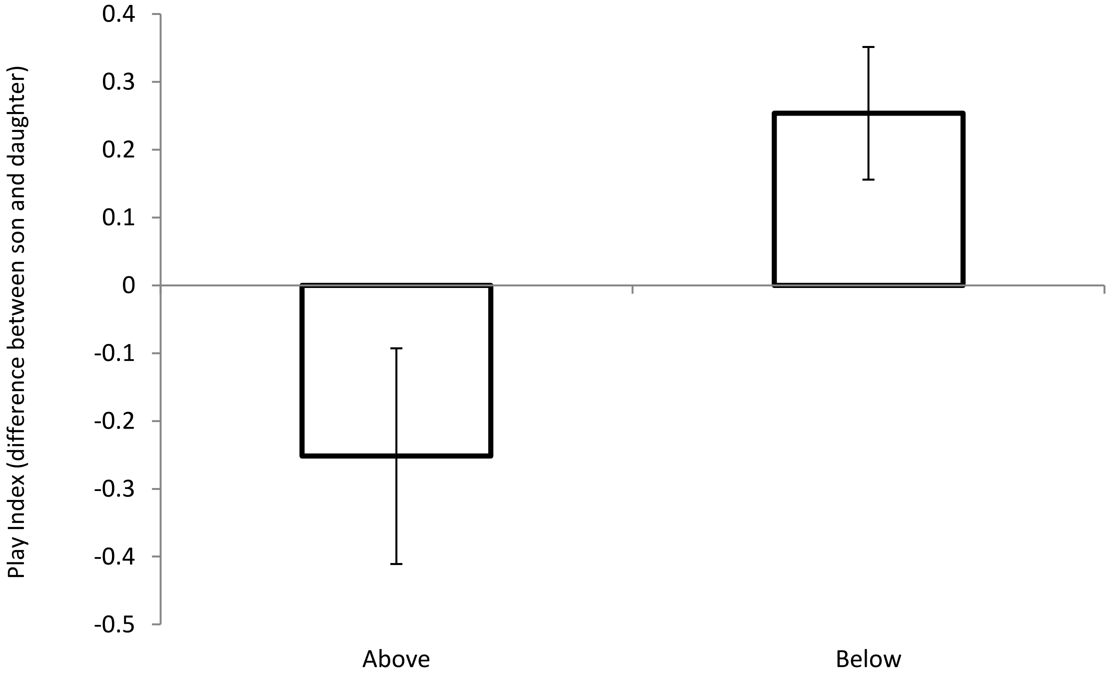


Figure 3: Differences in play behaviour of male and female pups born in different years to the same mother in relation to maternal condition (represented by maternal post-partum mass relative to the average). Index calculated as (son - daughter)/(son + daughter). Positive values show that male pups played more whereas negative values indicate that female pups played more. Values are mean ± 1S.E.

3.1.5. An exploration of independent play in male and female neonates.

When the two elements of solitary play (*individual locomotor* and *exploratory* play) were investigated separately, it was found that a higher proportion of female solitary play behaviour budgets were constituted by '*exploratory*' behaviour- which describes investigative behaviour directed at inanimate objects in the environment (mean proportion ± SE: *Males*, n=34: 0.41 ± 0.03, *Females*, n=32: 0.57± 0.03; *U*=290.5, *P*= 0.001; figure 4). Therefore, by contrast, male pups on average have a higher proportion of '*individual locomotor play'* in their solitary play budgets.

(figure 4 below)

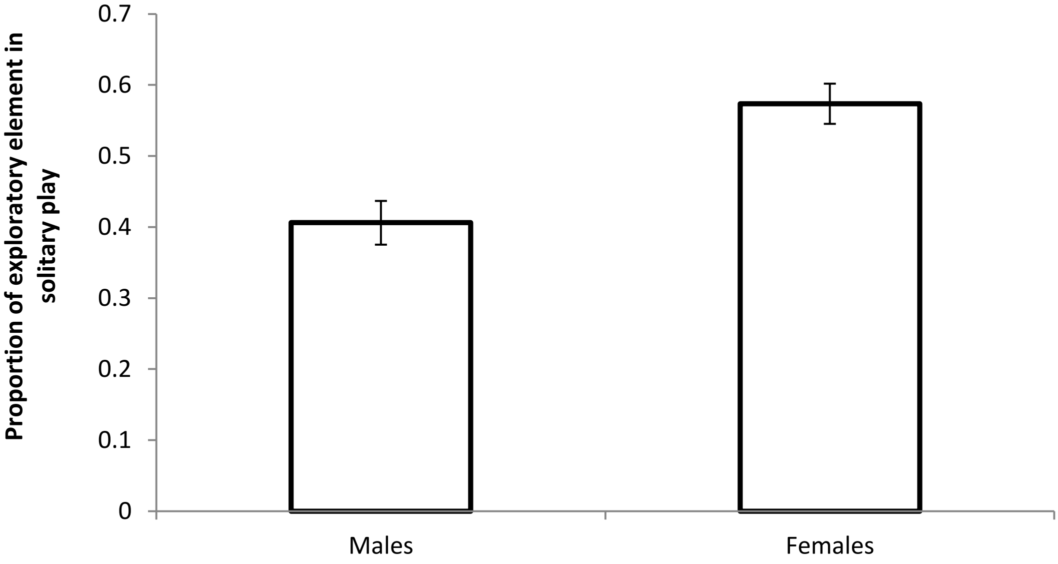


Figure 4. Proportion of exploratory behaviour (object-oriented) in the solitary play budget of male and female pups. Males= 34, Females =32. *Values are mean ± SEM.*

3.1.6. Investigating the possible effects of miss-categorisation on analyses.

A concern raised in chapter 2 was the potential miss-categorisation of pup *comfort moves* for *play* behaviour in the 2008 breeding season, leading to significantly higher records of play in this season compared to either 2009 or 2010. The inclusion of 2008 behavioural data in the analyses presented in this chapter may therefore have resulted in unreliable results, since it is not certain that the solitary play activity budget data for neonates in this season is representative of true levels. However, separate analyses of the 2009 and 2010 breeding seasons, where miss-categorisations are thought to have occurred less frequently, reveal similar results. In these seasons, no significant difference was found in the solitary play behaviour of male and female pups (2009, *males*: 4.00% ± 0.75, *female*s: 4.28% ± 0.35, *t*-test: $t_{13.4}$ = -0.33, *P*= 0.74; 2010, *males*: 4.00% ± 0.33; *females*: 4.09% ± 0.27, t test: t_{17}= -0.05, *P*=0 .96). Nor was a correlation found between milk-attaining behaviour and play in either of these seasons (2009: r_s = -0.20, *P*= 0.30; *males*: r_s = 0.19, *P*= 0.60; *females*: r_s = -0.35, *P*= 0.19; 2010: r_s = -0.01, n= 23, *P*= 0.96; *males*: r_s = -0.26, n= 10, *P*= 0.43; *female*s: r_s = -0.42, n=9, *P*= 0.27); or maternal mass transfer efficiency and play (*2009*: r_s = -0.25, n= 22, *P*= 0.26; *2010*: r_s =- 0.18, n=18, *P*= 0.46). Further, female pups were still found to have a higher proportion of *exploratory* behaviour in their solitary play budgets compared to males (Mean *female* proportion: 0.57; mean *male* proportion: 0.51; *U*= 160.5, *P*= 0.04).

Implications of miss-categorisation for sibling play analyses:

Possible miss-categorisation between *comfort moves* and *play* in the 2008 breeding season is likely to have affected the sibling analyses previously presented (s*ection 3.1.4*), since this breeding season was present in two of the pair-wise comparisons used for analysis (2008-2009 and 2008-2010). As a means of coarsely addressing this problem, sibling analyses were repeated but individual *comfort move* and *solitary play* behaviour budgets were combined to rebalance the variance between years (referred to as ”*independent locomotor behaviour*”; figure 5). Although the same pattern is seen, statistical testing of this combined behavioural category revealed no significant difference between the independent locomotor behaviour of sons and daughters belonging to mothers of either above or below-average condition (Mean combined-play index value ± SE, *Above*: -0.05 ± 0.06, n= 9; *B*elow: 0.03 ± 0.06, n=6; Two-way randomisation test statistic= 0.08, *P*=0.35).

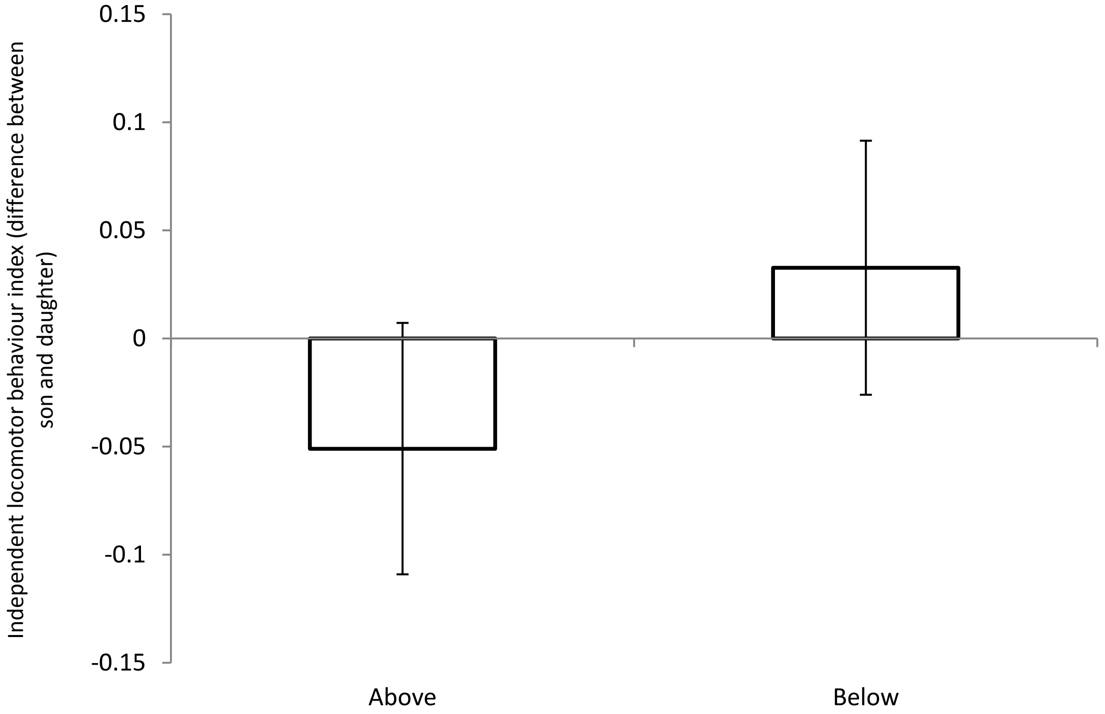


Figure 5: Results of repeated sibling analyses when *comfort move and solitary play* behaviours were combined (“independent locomotor behaviour”). Index calculated as (son - daughter)/(son + daughter). Positive values show that male pups expressed more independent locomotor behaviour whereas negative values indicate that female pups expressed more of this behaviour. *Values are mean ± 1S.E.*

3.2. Maternal investment.

3.2.1. Behavioural correlates of milk-transfer and maternal transfer efficiency.

There is little evidence that increased maternal investment is correlated with increased play in neonates. Over the lactation period as a whole, no significant correlation was found between the percentage time spent playing by pups and in milk-attaining behaviours (*All pups*: r_s= 0.10, n= 72, *P*= 0.42, *Males*: r_s= 0.19, n=34, *P*= 0.28; *Females*: r_s= -0.13, n=32, *P*= 0.47). Nor was a significant correlation found between neonatal play behaviour and maternal transfer efficiency (*All pups*: r_s = 0.02, n=61, P= 0.89; *Males:* r_s= - 0.29, n= 30, P= 0.12; *Females:* r_s = 0.28, n=31, *P*= 0.13). Since milk quality and transfer efficiency are likely to be affected by the condition of a female, correlative analyses were repeated, taking the relative condition of mothers into account. Still no evidence was found to suggest a statistically significant correlation between neonatal play and milk-attaining behaviour (table 1) or maternal transfer efficiency (table 2).

Table 1: Results of Spearman rank correlations between the solitary play behaviour of neonates belonging to mothers of above and below-average condition and percentage time spent in milk-attaining behaviours.

Relative Maternal Condition	**All Pups**			**Males**			**Females**		
	rho	**n**	***P***	**rho**	**n**	***P***	**rho**	**n**	***P***
Below	0.19	36	0.28	0.16	22	0.47	-0.19	14	0.51
Above	0.15	26	0.46	0.33	9	0.38	0.16	17	0.54

Table 2: Results of Spearman rank correlations between the play behaviour of neonates belonging to mothers of "above" and "below" average condition over lactation and estimated maternal transfer efficiency.

Relative Maternal Condition	**All Pups**			**Males**			**Females**		
	rho	**n**	***P***	**rho**	**n**	***P***	**rho**	**n**	***P***
Below	0.04	35	0.81	-0.13	21	0.56	0.10	14	0.75
Above	-0.07	26	0.75	-0.45	9	0.22	0.29	17	0.25

3.2.2. Maternal condition loss.

Mothers that were estimated to lose more condition over their lactation period did not have pups that spent more time expressing solitary play, indicating that increased maternal investment does not correlate with increased play in offspring (r_s = -0.11, n = 35, P= 0.55). Further, no correlation between these parameters was found when males (r_S = 0.00, n= 17, *P*= 1.0) and females (r_s = -0.12, n=18, 0.63) were assessed separately, reinforcing these findings.

4. DISCUSSION

4.1. Maternal investment and neonatal solitary play.

No strong evidence was found in support hypothesis 1 of this study (*section 1.4*); namely there is no suggestion of a direct link between maternal nutritional investment and neonatal solitary play. This was true in both male and female neonates, suggesting that, if differential investment does occur in this population, it does not directly lead to a sex difference in play behaviour. This is also reinforced by the finding of no overall difference in the amount of time spent playing by males and females. It is interesting to note that although Kovacs (1987) reports evidence of differential maternal investment on the Isle of May, with grey seal mothers allocating more resources to sons than daughters, male and female pups were found to spend similar proportions of time engaged in play. This therefore supports the findings of the present study, namely that in grey seals, there does not seem to be a direct link between nutritional investment and neonatal play behaviour.

As a point of caution, the assumption that the rate of milk transfer is positively correlated with time spent suckling has been reported to have inadequate empirical foundation (Cameron, 1998). Therefore, using percentage time spent in *milk-attaining* behaviours (‘nosing’ and ‘sucking’) as a proxy for milk transfer rate is potentially inaccurate and the results pertaining to such correlations must be considered as best estimations only. A further consideration is that non-filial nursing could confound the accuracy of maternal transfer efficiency estimations, since some mothers may lose energy reserves as milk to a non-biological pup.

However, whilst there is evidence for this behaviour in grey seals at other colonies (e.g. Sable Island, Farray Island and Ramsey Island populations: Perry et al. 1998), observations from North Rona suggest that non-filial nursing is low and so this is unlikely to have affected the reliability of this parameter to a large degree. Also, if non-filial nursing had been observed in the field, these individuals would have been excluded from analyses.

4.2. Maternal condition and pup play behaviour.

It is important to note that analyses relating to maternal condition in this chapter were based on proxy data, namely estimated maternal post-partum mass. Whilst this gives a generalised impression of available maternal resources, there remains uncertainty as to the suitability of this metric to represent maternal condition. This problem is discussed further in chapter 5 (*section 2.2*), highlighting that a more direct means of assessing maternal condition would be preferential for validating these analyses. For example, one of the most commonly derived indices for body condition is the 'mass-size residual'. The basic premise with this method is to determine the mass of an individual relative to its body size, thereby gaining residuals from a regression of body mass on a linear measure of body size, such as body length (Schulte-Holstedde et al. 2005). Body composition (fat-free mass and fat mass) can be calculated from body mass and total body water with an assumption as to the hydration of the fat-free mass, and thereby can give an accurate description of body condition (fat reserves). The water turnover technique can be used to assess body composition, using 'doubly labelled water' (Westerterp, 1999). Until such techniques are used to validate maternal post-partum mass as a proxy for body condition, the results relating to maternal condition discussed in this section should be treated with appropriate caution.

The second study hypothesis (*section 1.4*) postulated that mothers in better condition would preferentially invest in their male offspring, since successful males are the more reproductively viable sex; and further that this increased energy would lead to enhanced play in males and vice versa. However, in fact, the opposite pattern was inferred from the results, with male pups found to play more than female pups when their mother was in below-average condition (see figure 2).

When the *alpha*-level was lowered through Bonferroni adjustment to correct for multiple testing, this result fell just out of significance (*P*=0.02, adjusted *alpha*= 0.017). This result does nonetheless remain very close to significance and may therefore provide inference still. Repeated investigation in future with larger sample sizes would therefore be instructive.

These inter-sex comparisons did not account for potential genetic and maternal differences amongst pups; and therefore the play behaviour of male and female siblings was compared. The results of these analyses will now be discussed.

4.2.1. Sibling play analyses.

Assessment of play behaviour between male and female siblings revealed that sons of mothers in poorer condition (below-average) played more than their female siblings, and daughters of mothers in better condition played more than their male siblings (*section 3.1.4*). Higher nutritional investment in female pups by mothers in better condition does not seem to provide explanation for these findings, since maternal transfer indices (derived for the same sibling pairs, using the equation: *son-daughter/son+daughter*) indicate that in fact maternal efficiency was higher for sons than daughters (denoted by positive mean maternal efficiency index values) regardless of the relative condition of the maternal figure (Mean transfer efficiency index: *Below*: n=6, 0.04± 0.04, *Above*: n= 9, 0.03 ± 0.02, Two-way randomisation test statistic: 0.006, *P*= 0.88). This therefore supports the results of maternal investment analyses by suggesting that play behaviour is not directly influenced by nutrition levels. Alternative theory is therefore required for this intriguing sex difference in play with regards to relative maternal condition. We may gain insight by considering play behaviour in terms of potential future benefits, relating to known adult social roles and characteristics. Discussion of this follows below.

4.2.2. Could the function of play differ between males and females?

Smith (1982) states that: "*…although by definition play should confer no clear, immediate benefit on the animal, species-typical play should nevertheless be explicable in terms of survival and reproductive success*". The author goes on to say: "*The primary benefits of playful behaviour for the individual should reflect the* *functions for which behaviour has been selected in the evolutionary history of the species*". With this in mind, we may become more informed as to why a condition-dependent sex difference in play behaviour may exist by considering known sex roles and reproductive behaviour of *adult* individuals, an approach that has been used for other species (e.g. Gorillas: Maestripieri and Ross, 2004).

Males:

Male neonates were found to play more than females when their mothers were in poorer condition. Since physical strength and size are important in grey seal males for reproductive success, it may be speculated that 'poorer' males compensate for a poorer start to life through enhanced individual play in order to support neuromuscular development and performance. This may have potential benefits such as facilitating the development of motor skills and physical development (e.g. muscle: Byers and Walker, 1995; Cameron et al. 2008) which may in turn increase competitive ability later on. Play has been suggested to be important in the development of strength, endurance and physicality. Indeed, Bateson et al. (1990) suggested that kittens in their study perceived cues that their mother was experiencing a food shortage, and subsequently "accelerated their development" through enhanced play.

There is some support for this conjecture from neonatal energetic data (collated by Dr Patrick Pomeroy, St Andrews University). Namely, sons of mothers in poorer condition have a significantly lower mean daily growth rate during lactation than sons of mothers in better condition (Mean kg/day ± SE: *Below,* n=22: 2.04kg ± 0.08; *Above*, n=9: 2.50kg ± 0.09, Two-way randomisation test statistic: 0.45, *P*= 0.003). This therefore reinforces the proposal of play as a developmental compensatory mechanism. In support of this, there is suggestion by Fagen (1976), following a review of exercise physiology, of a 'physiological sensitive period' during mammalian infancy in which exercise is most effective. If this were true also of grey seals, then this would provide support for a 'motor-training' function of play in this context.

However, it is notable that the difference in mean growth rates in relation to maternal condition is also mirrored in female pups (*Below,* n=14: 1.91kg ±0.15; *Above*, n=17: 2.33kg ± 0.07, Two-way randomisation test statistic: 0.41, *P*= 0.002). Why sons of mothers in poorer condition would play more than daughters is therefore unclear. Hall et al*.* (2001) found that the odds of survival for female pups was higher than for males, and further, that the effect of condition at weaning on survival was significantly greater for male pups than females. Therefore, it could be that physical compensation for a poorer developmental start is 'more important' to males in the pre-independence stage.

The energetic implications of the speculated relationship between play and enhanced physical development are interesting. Indeed, it may be reasoned that increased energy expenditure resulting from enhanced play would then have to be paid for with enhanced milk resource from the maternal figure. However, this is of course a contradictory concept since enhanced play is suggested in pups of mothers in poorer relative condition, who have fewer reserves to support this. It seems that, as discussed, there is no direct link between maternal milk provisioning and play, as supported by specific investigation of maternal investment on the expression of this behaviour (*section 3.2.1*). Indeed, other studies have suggested that, as seen here, play can increase even in situations associated with food deprivation (Rats: Smith, 1991; Domestic Kittens: Bateson et al. 1990). In such scenarios, the assumed benefits of enhanced playful activity outweigh sacrifices made in relation to energy depletion. An alternative explanation may be approached if we reverse the cause and effect relationship being considered here. That is, perhaps we may postulate that mothers in poorer condition reject their pups more often due to lower milk reserves, and as a by-product of this, pups may spend a greater relative proportion of their activity budgets playing, and/or in other behaviours not associated with the mother. Directed future research to address these ideas would be instructive.

Females:

By contrast, female grey seals have been shown to exhibit natal site fidelity (Pomeroy et al. 2000b) and breeding site faithfulness as adults (Anderson, 1975; Pomeroy et al. 1994, 2000b; Twiss et al*.* 1994). We may conclude therefore that females require certain cognitive skills, including the ability to recognise environmental features. It may be speculated that daughters receiving better nutrition from their mothers play more and in doing so enhance their environmental perception. In support of this, it has been suggested that juvenile humans use play to sample their environment and that innovative behaviours may be developed during play in response to environmental novelty (Pelegrinni et al. 2007). Further, Kuczaj and Makecha (2008) suggest that the significance of solitary play behaviour may rest in its role in the ontogeny of 'flexible thought', and further that an animal's interaction with the environment during solitary play could facilitate cognitive growth and flexibility since the individual has control over the play activity without the outside influence of other animals. Interestingly, Arnold and Trillmich (1985) noted that during play Galapagos fur seals will often look at objects in front of them by turning the head around the longitudinal axis. They speculate that such behaviour must provide considerable practice in recognising objects irrespective of their orientation on the retina, a useful ability later on when young seals must learn to find and identify prey while diving in the three-dimensional marine environment. Similar benefits may also be attributed to grey seal young.

In support of the above conjecture, it was found that a higher proportion of female solitary play behaviour budgets were constituted by '*exploratory*' behaviour, which describes investigative behaviour directed at inanimate objects in the environment (see *section 3.1.5*; figure 4). This in turn means that male pups on average have a higher proportion of '*individual locomotor play'* in their solitary play budgets. These findings therefore support the preliminary suggestion that independent play in disadvantaged neonatal males may function as a compensatory mechanism for physical development, whereas in female neonates play may have a more cognitive role; possibly training information processing systems.

4.3. Implications of potential miss-categorisation of behaviours in the field.

A concern raised in chapter 2 was the potential miss-categorisation of *comfort moves* for individual *play* behaviour in the 2008 breeding season, leading to significantly higher records of play compared to either the 2009 or 2010 breeding seasons. As presented in *section 3.1.6*, separate analyses of the 2009 and 2010 breeding seasons, where miss-categorisations are thought to have occurred less frequently, revealed similar results to those previously presented. It therefore seems that the general conclusions regarding maternal investment previously discussed in this chapter are unaffected. It is also interesting to note that female pups were still found to have a higher proportion of *exploratory* behaviour in their solitary play budgets when 2008 data were excluded (*see section 3.1.6*). This therefore gives support to the previously discussed suggestion of differential play function between male and female neonates, but further testing with a larger sample size and over a longer temporal span would be instructive to increase confidence in this speculation.

However, it was reasoned that possible miss-categorisation was likely to have affected the 'sibling analyses' presented in *section 3.1.4* to a greater degree, since the 2008 breeding season was included in two of the three pair-wise comparisons used for this (2008-2009 and 2008-2010). Sibling analyses were therefore repeated but individual *comfort move* and *solitary play* behaviour budgets were combined (="*independent locomotor behaviour*") to rebalance the variance between years (see *section 3.1.6*). The same general results were obtained from this new analysis: namely that daughters of mothers in better condition expressed more *independent locomotor behaviour* than sons, and sons of mothers in poorer condition expressed more *independent locomotor behaviour* than daughters (see figure 5). However, this difference was no longer found to be significant (Two-way randomisation test statistic= 0.08, P=0.35). This re-analysis therefore reduces confidence in the previously discussed condition-dependent sex difference in play. The biological reasoning previously discussed is still relevant, but this must be considered with caution. It is noted that this re-analysis was not ideal; it is not possible to draw conclusions regarding *play* behaviour from these results, as desired in the original investigation. Future comparison of play behaviour between siblings from different breeding seasons would be instructive, to limit concerns regarding behavioural miss-categorisation and increase confidence in the results outlined.

4.4. A note on the limitations of sample size and possible sampling effects.

The solitary play data analysed in this chapter was collated from a relatively small sub-set of the total pups born on North Rona over the three breeding seasons. Indeed, the maximum sample size presented here was 72 neonates, yet in the majority of analyses, sample size was 30 or less. Sample size for analyses was decreased further when finer levels of investigation were pursued, such as comparing play behaviour between the sexes, and in relation to relative maternal condition and physiological attributes. The statistical power of tests was therefore decreased for these analyses. The interpretation given in this chapter should therefore be considered within this framework. Future work would benefit from a more systematic approach to subject selection and sampling regime (to maximise the number of individuals for which 200 or more scan samples is achieved). As summarised in chapter 2, it is clear that the majority of neonatal activity budgets is constituted by inactive behaviour (*resting*), and so the sample size of play behaviour was relatively small. Further work investigating play at this colony would therefore benefit from a concentrated effort to investigate this behaviour in finer detail, perhaps through the collection of focal videos where possible.

Finally, the behavioural and energetic data analysed in this chapter were collected primarily from focal mothers and pups located in the research locality of North Rona known as the ‘Study Area’ (as shown in figure 6b, chapter 1). As a consequence of this, pups born in ‘Fianius North’ and ‘Fianius South’ (chapter 1, *section 1.5.1*), were not equally sampled. In this regard, description of solitary play behaviour may not generalise to the colony as a whole, since each locality has its own topological and demographic features that may influence behaviour differently. Further exploration of the relationship between play and maternal investment at this colony would therefore benefit from the inclusion of individuals from across the island as a whole, should this be logistically possible.

4.5. Future work.

Different types of play behaviour have been identified within the literature (Smith, 1982; Gomendio, 1988), with three main forms typically described:

- *Locomotor play*: the predominant form of solitary play, involving exaggerated individual motor actions.
- *Object play*: involving the manipulation of an inanimate object in the environment.
- S*ocial play*: play that involves contact or interaction with another individual/s.

Whilst *individual locomotor* and *object* play have been addressed in this chapter, it would be insightful to conduct detailed focal observations of neonatal play behaviour in order to gain more insight into the nature and function of play. Smith (1982) highlights that different types of play often have different developmental profiles, for example this has his been found for *social* versus *object* play in kittens (Barret and Bateson, 1978) and *contac*t versus *non-contact* social play in other species (e.g. Chalmers, 1980). Therefore, it would be interesting to investigate the developmental trajectories of different types of play in neonates, as this could give a more informed indication of its adaptive significance.

Further reason to expand study to include both *independent* and *social* forms of play is that, as noted in Smith (1982), if play serves to promote 'amicable' social behaviour, then it would be expected to occur in species with social groups- where social recognition seems important. This is certainly true of grey seal breeding aggregations, particularly for females for which evidence of 'protosociality' has been described (see Pomeroy et al., 2005). Although notably, enhanced social play has not been found to reduce aggressive behaviour towards conspecifics in all species (e.g. Meerkats: Sharpe and Cherry, 2003). Social play may help to develop such simple social skills. Indeed, in the stellar sea lion, the development of social behaviour was found to be aided by play behaviour (Gentry, 1974). Further, Wilson and Kleiman (1974) argued that body-odour sniffing is enhanced during social play which may aid individual recognition. Since social interactions during the lactation period are likely to be predominantly with the maternal figure, an exploration of interactions with the maternal figure is informative when considering social play. It would therefore be insightful to investigate play behaviour, both independent and social forms, in relation to maternal condition in more detail using focal observations to see if such results are supported.

4.5. Chapter summary.

This study has provided no strong evidence to suggest a direct link between maternal nutritional investment and solitary play behaviour in neonatal grey seals. Preliminary suggestion that maternal condition may influence the play behaviour of males and females has been identified, with sons of poorer condition mothers found to play more than daughters, and daughters belonging to better condition mothers playing more by contrast. Solitary play in female pups could be important for training information-processing systems and developing environmental recognition. By contrast, play in nutritionally-disadvantaged males may be more important from a physical perspective, contributing towards a compensatory mechanism by which to improve physicality and motor skills. However, it is acknowledged that validation of the quantification of maternal condition using estimations of post-partum mass needs to be addressed before confidence can be given to this discussion. It is also apparent that miss-categorisation of play behaviour may have affected the sibling analyses of play, and therefore focussed future research in this area would be beneficial, with efforts taken to maximise sample size over successive breeding seasons. More detailed investigation of both independent and social forms of play is recommended for a more holistic understanding of the adaptive significance of this behaviour, including exploration of the developmental trajectories of different play types over lactation, and further investigation of the role of maternal condition.

CHAPTER 4

An Integrative Exploration of Play Behaviour in Pre-Weaned Grey Seal Neonates at an Increasing Onshore Colony

Chapter Introduction.

In chapter 2, play behaviour was regarded as unitary, describing *individual locomotor* play. Within the literature there is general consensus that animal play is a heterogeneous behaviour, consisting of different forms or ‘types’, and in-line with this, *exploratory* (object-oriented) behaviour was included as an element of solitary play in chapter 3. However, thus far *social play* has been excluded from discussions. To gain more accurate insight into the function of play, a more holistic approach is required with consideration of both independent and social forms of this behaviour. This chapter aims to explore neonatal play in more detail, using focal observations of pups from the onshore breeding colony of Donna Nook in Lincolnshire to assess the developmental trajectories of different types of play; investigate any sex differences in the expression of independent and social play; and to explore the expression of different types of play in relation to maternal condition.

1. INTRODUCTION

1.1. The development of play.

Due to its prominent role in the behaviour of mammalian young and to its presence across a wide range of species, play has long attracted attention from researchers (Gomendio, 1988). There is much debate within the literature regarding the possible function(s) of play, yet understanding how play develops and peaks over ontogeny will help us to achieve a more comprehensive insight into this biological enigma. Broadly speaking, play is predicted to be the most frequent and/or intense during periods of rapid allometric growth, since rapid changes in body proportions make the control of movements less precise, thus resulting in more ‘frequent misjudged actions’ (Spinka et al. 2001). In support of this, play is found to be prominent in younger age-classes where periods of rapid growth generally occur. Interestingly though, neonatal individuals are forecasted to play relatively less than juveniles due to a comparative lack of physical and neurological development at this stage (Spinka et al. 2001). Research into the development of play in non-human species has detailed some interesting patterns amongst species, for example amongst the primates (Rhesus Monkeys: Harlow and Harlow, 1961; Feral Olive Baboons: Chalmers, 1980; Chimpanzees: Mendoza-Granados and Sommer, 1995). For instance, in a study of rhesus macaques by Singh (1986) it was reported that mature and old age-classes partook in play rarely, and that play frequencies decreased linearly with age. Within the wider literature, there is further support for this pattern, with play documented to increase with age, peak in the juvenile phase and decline thereafter (Biben, 1983; Gomendio, 1988; Prescott, 1985; Newberry, 1988; Renouf, 1993).

In the past, play has been considered to be an 'homogenous' behaviour; a single behavioural entity (Gomendio, 1988). However, play is now accepted as a heterogeneous behavioural category, with the observation of distinctly different forms with different developmental trends (e.g. Barret and Bateson, 1978; Chalmers, 1980; Martin and Caro, 1985). Therefore, the assumption that play is homogenous in terms of its causation, function and evolution is no longer tenable (Bateson, 1981). Differences in the developmental trajectories of different types of play have been reported in the literature. For example, Barrett and Bateson (1978) found age-related changes in the play of domestic cats. The authors reported peaks in individual play actions such as 'rearing' and 'arching' occurring around 6 weeks after birth, with more object and social-orientated (e.g. 'wrestling') behaviours peaking later on. The identification of 'peaks' in the expression of certain play actions have also been documented in other mammals (e.g. Rodents, Panksepp, 1981). In this study, incidences of 'pinning' behaviour were found to peak between 32-40 days old and decline thereafter. Singh (1986) reported that '*self play'* was most prominent in Rhesus Macaques during infancy and mainly directed towards the environment, suggesting that this type of play may facilitate the exploration of environmental features by youngsters. These examples therefore indicate that play is a dynamic behaviour, changing in prevalence over ontogeny, with peaks in different types of play typically occurring at different times. This is particularly important since different forms of play may have different functions; and so the primary function of play may also change depending on the form most prevalent in the behavioural repertoire. For instance, different types of play are known to have different effects on motor development (e.g. Nunes et al. 2004), just as different types of exercise have been shown to differentially affect skeletal, muscular and neurological development (e.g. Horses: Firth, 2006).

1.2. Sex differences in play behaviour.

There are examples of pronounced, moderate and non-existent sex differences in play behaviour within the literature (e.g. Bekoff, 1974; Olioff et al. 1978; Biben, 1983; Prescott, 1985; Caro, 1988). Where differences have been found, both the expression and intensity of play has been found to vary between the sexes. For example, significantly more object play in male kittens was found compared to females of the same age, although interestingly, females were more likely to play with objects *if* they had a male in their litter (Barrett and Bateson, 1978). Smith (1982) states that: "*although by definition play should confer no clear, immediate benefit on the animal, species-typical play should nevertheless be explicable in terms of survival and reproductive success*". The author goes on to say: "*The primary benefits of playful behaviour for the individual should reflect the functions for which behaviour has been selected in the evolutionary history of the species*". There is evidence for this across species, with differences in play reflecting differences in adult behaviour: for example crab-eating fox cubs *(Cerdocyon thous)* engage in extensive solitary object play and hunt individually as adults, whereas bush dog pups *(Speothus venaticus)* share rather than compete for objects and as adults hunt in social groups (Biben, 1982). **I**nsights into sex differences in play behaviour can therefore be gained using knowledge of characteristic adult behaviours.

Maestripieri and Ross (2004) suggest that sex differences in play are expected to occur whenever males and females differ in their physical characteristics (e.g. body size), behavioural characteristics (e.g. infant care-taking) or social preferences (e.g. formation of bonds with same-sex vs. opposite-sex individuals). This has been supported within the literature, for example in cercopithecene monkeys. In such species, males are generally larger than females and compete aggressively for mating partners. Further, males are also typically the dispersing sex whilst females are the philopatric sex. This thereby implies that males generally establish dominance relationships with unrelated males in the groups to which they immigrate, whilst females typically form strong social bonds and alliances with their female relatives. In agreement with these observed adult roles, infant and juvenile males typically play more often and more vigorously than females (e.g. Macaques: Koyama, 1985; Yellow Baboons: Pereira, 1984). Further, immatures have been found to typically play with individuals of the same sex and age (e.g. Ehardt and Bernstein, 1987), therefore suggesting that play in males may act as a way of practicing skills necessary for intra-sexual competition later in life. These differences in cercopithecene species are therefore largely consistent with the *motor-training* hypothesis of play, detailed further below.

Two prominent hypotheses of mammalian play are the *motor-training* and *social-cohesion* hypotheses. The motor-training hypothesis suggests that play enhances development of bone remodelling, muscle hypertrophy, cardiopulmonary capacity and neuromuscular coordination (Jamieson and Armitage, 1987), with proposed benefits including increased strength, endurance and socially competitive skill levels (Fagen, 1981, Smith, 1982). By contrast, the social-cohesion hypothesis suggests that play enhances the formation, strengthening and maintenance of social bonds (Fagen, 1981, Smith, 1982). Indeed, Harlow and Harlow (1966) noted that 'interactive play' is an essential element in the development of affectionate behaviour towards age-mates in primates. Jamieson and Armitage (1987) investigated social play in yellow-bellied Prairie dogs in an observational field study. They tested these two major functional hypotheses to explain the function of play in this species, ultimately finding support for the motor-training hypothesis. Males preferred to play with other males, and females avoided initiating play with males. Females also terminated more bouts of play than males. It was therefore suggested that individuals prefer to play with partners of similar ability, and that females engage in less play and in a less 'dominating' way than males because the fitness costs associated with an adult losing a fight are lower for females compared to males. Cameron et al. (2008) also found support for the motor-training hypothesis in young feral horses, showing that males initiated more play bouts and tended to spend more time involved in *play-fighting* than females, supporting the hypothesis that play functions as practice to refine motor skills and physical skills needed later in life. These studies demonstrate that insights into play function can be gained through investigation in sex differences in play behaviour.

1.3. Maternal condition and offspring play behaviour.

To current knowledge, there has been little investigation of the effect of maternal condition on offspring play behaviour, with more studies concerning the ways that variation in maternal behaviour/care can influence infant behaviour (e.g. Guinea Pigs: Albers, 1999; Rats: Parent and Meaney, 2008; Macaques: Bardi and Huffman, 2002). Cameron et al. (2008) present a rare example of investigation into maternal condition and offspring play, finding evidence to support the theory that mothers in better condition should invest preferentially in the more reproductively viable sex, which in polygynous systems is generally males. Differences in maternal investment were found to influence the play behaviour of offspring, with sons of mothers in better condition playing more. Enhanced play was also found to have positive fitness benefits, with higher survival in foals that played more (see Cameron et al. 2008). However, exploration of behavioural and reproductive data from the colony of North Rona did not provide support for this theory, finding no evidence to support a direct link between nutritional investment and play behaviour (see chapter 3). Interesting results were however inferred from male-female sibling analyses; with sons playing more when their mothers were in poorer condition and daughters playing more when their mothers were in better condition. These intriguing results therefore stimulate the need for further investigation of the interaction between maternal condition and neonatal play, in both independent and social types of play.

1.4. Study species and research predictions.

As considered previously, the behavioural ecology of the grey seal has been widely studied; however the play behaviour of free-living pups has not been reported in great detail. As discussed in chapter 3, there has been some work describing the play behaviour of juveniles (between 6 months to 5 years old: Wilson, 1974), and some limited study of neonatal play (Isle of May: Kovacs, 1987). In the latter study, when play was observed it was always recorded as being either *self* or *object*-directed. These types of play have also been observed in neonates from the colony of North Rona (see chapter 3). However, personal observations of the author suggest that social play also occurs in pre-weaned pups, often with the maternal figure. Therefore there is reason to study all three main types of play in this age-class: *individual locomotor*, *object* and *social* play.

Developmental trajectories:

Grey seal pups undergo rapid development during their 16-18 day lactation period. When first born, pups are largely defenceless and not very coordinated. It may be hypothesised therefore that types of play associated with greater cognitive development and awareness, such as *social* and *object* play, will increase in relative proportion with age, with relatively more *individual locomotor* play during early lactation. Such aspersions are consistent with those related by Spinka et al. (2001), noting that the peak in *independent locomotor* play typically peaks before *social* play for reasons including the relatively high cognitive demands of play with others.

Sex differences:

If play evolved in this species as a mechanism by which to increase strength, endurance and fighting skills (motor-training hypothesis) during early ontogenetic stages, then both males and females should play frequently, but males should engage in more *individual locomotor play* as a means of developing motor skills and strength since sexually mature male fitness is much more strongly linked to territorial defence than female fitness (Twiss, 1991). If play functions to develop, strengthen and maintain social bonds then females should engage in more social play relative to males, as females have been shown to express ‘protosociality’ as adults, actively interacting with certain individuals over successive breeding seasons (North Rona: Pomeroy et al. 2005). If play functions to facilitate learning about the environment, then we may hypothesise that females would express more object-oriented play, since females have been shown to express philopatry and site-fidelity (Anderson, 1975; Pomeroy et al. 1994, 2000b; Twiss et al. 1994), and are therefore expected to have knowledge of the colony and natal site. Therefore, whilst sex differences in the overall expression of play (including all types together) may not be apparent, sex differences may be expected when independent and social forms are assessed separately.

Maternal Condition

As discussed, investigation of behavioural data from North Rona provided interesting inference that male pups of mothers in poorer condition engaged in more solitary play than females, whilst female pups of mothers in better condition played more than males (although these results should not be overstated due to non-significant results when analysed; see chapter 3). When solitary play was looked at in more detail, it was found that a significantly higher proportion of solitary play budgets was constituted in by *individual locomotor play* in males, and *object-oriented* (exploratory) behaviour in females (see chapter 3 for details). We may therefore hypothesise that similar differences between the sexes in *individual locomotor* and *object play* will be repeated in the present study. We may also predict to find increased mother-pup *social* play when the mother is in poorer condition, as a means of enhancing pup development, from either a cognitive or physical perspective.

1.5. Study hypotheses.

Hypothesis 1: The prevalence of s*ocial and object play will increase over lactation, peaking later than the peak in individual locomotor play.*

Hypothesis 2*: Male pups will conduct relatively more individual locomotor play than females, who by contrast will spend more time in social and object play.*

Hypothesis 3*: A sex difference will be found in the expression of independent types of play in relation to maternal condition (see above). Pups belonging to mothers in poorer condition will engage in more social play than those of mothers in better condition.*

2. METHODS

2.1. Study site and behavioural decoding of focal videos.

The data analysed and presented in this chapter were collected from the Donna Nook grey seal breeding colony on the east-Lincolnshire coast during the 2011 breeding season (for a more detailed description of this breeding colony, refer to chapter 1: *section 1.5.1*). Behavioural data were collected using a focal-sampling approach (Altmann, 1974) from focal video footage. In total, behavioural observations were made for eighteen individual neonates, with observation footage (with pups fully in-sight) per infant ranging from 32.3 minutes to 95.2 minutes (mean ± SD: 68.65 minutes ± 19.73) and a total observation time for all subjects of 20.60 hours (see chapter 1, *section 1.5.4* for further details of focal video collection methodology and appendix 2, *table 1* for summary details of focal videos, including focal date, pup stage and focal video durations). In order to assess changes in play over time, behavioural observations for individuals were classified, depending on pup developmental stage, as occurring in either 'early' or 'late' lactation. Pups in developmental stages 1 or 2 were considered as being in the 'early' lactation phase, with pups at developmental stage 3 or more were classified as being in 'late' lactation (see chapter 1; table 2 for detailed descriptions of pup developmental stages). For information, 14 individuals in this study were eligible for investigation over early and late lactation phases, but 4 individuals were not (due to early and/or late lactation footage not being available).

Complete behavioural sequence data were collected during each focal, with associated records of the time that the animal changed from one behavioural state to another (see chapter 1, *section 1.5.6* for full ethogram description). It was therefore possible to calculate the duration of each distinct behavioural state, and the *total* amount of observation time spent in behavioural categories of interest-specifically those representing play behaviours (see *section 2.2*). Since the durations of individual focals were not consistent (see *appendix 2*), the amount of time spent in each type of play was derived as a *proportion of total observation time* (whilst the pup was in-sight).

2.2. Definition and types of play.

The classification of different types of play was aided by preliminary observation of video footage to record typical behavioural elements associated with independent and social play behaviours. Descriptions of different types of play were then refined using the following definition from Caro (1988) as guidance: *"Play is all locomotor activity performed postnatally that appears to an observer to have no obvious immediate benefits for the player…. The motor acts constituting play have some or all of the following structural features: exaggeration of movements, repetition of motor acts, and fragmentation or disordering of sequences of motor acts*". Neonatal play was thus divided into three different *types*: *individual locomotor*, *object* and *social* play, since behavioural elements pertaining to each of these tended to occur in temporal association. Table 1 gives descriptions of each type of play referred to in this study, with illustrated examples in figures 1a-d.

Table 1: Descriptions of extracted behaviours of interest, believed to be important elements of play behaviour in pre-weaned grey seal neonates.

Play Type	Description
Individual Locomotor Play (INDLOC)- Figure 1a.	Localised and self-oriented movement, including extended 'wriggling', 'flippering' and 'biting', and exaggerated actions.
Object Play - Figure 1b.	Behaviour directed towards an inanimate object, such as grass, sand and fence posts. Includes 'lunging at', 'flippering', 'nosing' and 'biting' of the object.
Social Play- Figures 1c,d.	Performed during social interactions in which there is a decrease in social distance between the interactants, and no evidence of social investigation or of agonistic (offensive or defensive) or passive-submissive behaviours on the part of the members of a dyad. May include 'flippering' between individuals, exaggerated sequences of physical contact, faux-fighting between individuals and so on.

Figure 1a: Typical individual locomotor play behaviour, wriggling, undulatory and exaggerated actions whilst lying on back. *Donna Nook 2011.*

Figure 1b: Object play. Pup is showing typical investigative behaviours including nosing, lunging and biting actions towards object (grass). *Donna Nook 2011.*

Figure 1c: Typical social play behaviour between mother and pup, including lunging, faux-open mouth aggression and reciprocal’ flippering’ behaviours. *Donna Nook 2011.*

Figure 1d: Rare example of pup-pup social play, including naso-naso initiation (far left) of interaction, ‘chasing’ behaviour between pups (middle) and naso-anal sniffing (far right).

2.3. Maternal body condition.

Appendix 4 (*pg. 160*) should be referred to for a more in-depth explanation of how body condition index values were derived for these analyses. Maternal condition was estimated using a ‘morphometric method’, whereby body measurements of individual females were derived from photographs or stills from video footage (collected by HCJ). Images were firstly filtered by various criteria. Firstly, images were chosen so that females were lying in a relaxed stance parallel to the camera and adjacent to the ground, where possible. Further, so as to facilitate fair comparison amongst individuals and assess likely maternal condition shortly following birth, images were filtered on the basis of the developmental stage of the pup at the time of the photograph. Images for 17 mothers were suitable for these analyses, with fourteen of these taken within the early lactation phase (pup development stages 1 or 2) and three during the late lactation phase (pup stage 3+). From these images, measures of maximal body length (nose-end of hind flippers) and body depth (ground-maximal point of height) were made and used to calculate an index of maternal condition (length/depth). The nature of this index assumes that larger index values (i.e. larger depth: length ratio) convey fatter individuals in better body condition (greater fat stores), and vice versa. Table 2 summarises these results.

Table 2: Individual morphometric measurements and body condition index values. The developmental stage of the pup at the time of the photograph, pup sex ("M","F") and the type of image used for measurements are also displayed.

Female ID	PUP STAGE	PUP SEX	Method (P/S)*	LENGTH (cm)**	DEPTH (cm)**	INDEX VALUE (L/D)
LINDA	**2**	M	S	11.0	2.4	**4.58**
P16	**2**	F	P	10.4	2.1	**4.95**
P20	**3**	M	S	11.6	2.3	**5.04**
P21	**2**	-	P	8.5	1.8	**4.72**
P22	**1**	-	P	8.4	1.9	**4.42**
P26	**1**	F	S	8.9	2	**4.45**
P36	**3**	F	S	8.1	1.6	**5.06**
R1	**1**	-	P	11.2	2.1	**5.33**
R5	**1**	-	P	8.6	1.8	**4.78**
R7	**3**	M	P	11.1	2.1	**5.29**
R9	**1**	F	P	9.6	2.1	**4.57**
R11	**2**	-	S	10.1	2	**5.05**
R12	**2**	-	P	8.1	1.7	**4.76**
R13	**2**	-	P	8.7	1.7	**5.12**
R14	**1**	M	P	8.2	1.7	**4.82**
R26	**2**	F	S	8.2	1.8	**4.56**
RES2M	**2**	M	S	8.5	2	**4.25**

*P= photograph taken in field, S= Still from focal video footage. ** Measurements given to the nearest millimetre.

2.4. Statistical methods.

Development of play behaviour:

The development of play was investigated via comparisons of the proportion of observation time spent in each type of play in early and late lactation phases. Fourteen individuals were included in these analyses. Owing to the non-normality of the data and the matched nature of the sample (repeat observations of the same individuals), a Wilcoxon signed rank test was used to assess differences between early and late lactation phases. There are two tests that could serve this purpose: the 'sign test' and the 'Wilcoxon signed rank' test. The former compares the number of cases where the first sample is greater than the second sample to the number of cases where the second sample is greater that the first sample. However, it does not take into account the size of the difference between each pair, data which are often available. Therefore, the Wilcoxon signed rank test was chosen as a preference, as this takes the size of the difference within pairs into account. Output of this statistical test also provides "ranks" data; describing whether behaviour was found to decrease between early and late focals ("negative ranks"); increase between focals ("positive ranks"), or show no difference ("ties").

Sex differences in play:

A randomisation procedure (Design 5a from Todman and Dugard, 2001) was used to compare the time spent in each play type by male and female pups. This test computes *P*-values from the proportion of randomised iterations generating differences in group mean values as large as, or larger, than the observed difference. This test was chosen in preference over the Mann Whitney *U* test since it is more conserved for small sample sizes (n=5 in both groups). This method was also used to compare the proportion of time spent in each play type by males and females in early and late lactation phases.

Maternal condition and play:

A randomisation procedure was used to compare the play behaviour of male and female pups in relation to maternal condition, necessitated by very low sample sizes. Maternal condition was treated as a categorical variable for these analyses, with mothers classified as either: “above” or “below” average condition (as judged in relation to the mean condition index value: 4.81, n=17). Notably, male and female offspring sample sizes in each group were very low: *Above*: females= 3, males= 2; *Below*: females= 2, males =3.

A linear regression that incorporated a randomisation test was performed to investigate the relationship between maternal condition and the proportion of time spent in each type of play by pups. Randomisation was necessary to account for the non-normal distribution of residuals about the fitted regression line. The test statistic was the absolute value of the slope of the fitted line, and we performed 2000 randomisations of each analysis to calculate levels of significance.

3. RESULTS

3.1. Development of play behaviour over lactation.

The majority of individuals (n=9) showed a relatively higher proportion of *individual locomotor* play in early lactation, with 5 individuals expressing higher levels in late lactation by contrast. However, the difference between lactation phases was found to be non-significant (Z= -0.910, n=14, *P*= 0.36; figure 2). This was also true when male and female pups were assessed separately (*Females, n=3*: Z= 0.000, *P*=1.0; *Males, n=5*: Z= -0.674, *P*= 0.50). Most pups showed more *social play* in the late lactation phase (n=7); with 5 pups showing no difference between lactation phases (no records of social play in either), and 2 individuals showing greater proportions of this type of play in early lactation. Despite a trend for more of this play behaviour in late lactation (figure 2), this was not found to be significant (Z= -1.362, n=14, *P*= 0.17) and this was true for both male and female pups (*Females, n=3*: Z= -1.0, *P*= 0.32; *Males, n=5*: Z= 0.00, *P*= 1.0). Most pups expressed higher proportions of *object play* in late lactation (n=6), with 5 individuals showing no change (no records of this behaviour in either phase), and 3 individuals spending more time engaged in this play type in early lactation. The change over lactation was found to be non-significant (Z= -1.599, n=14, *P*= 0.11; figure 2), in either males or females (*Females, n=3:* Z= -1.0, *P*= 0.32; *Males, n=5*: Z= 0.00, *P*=1.0).

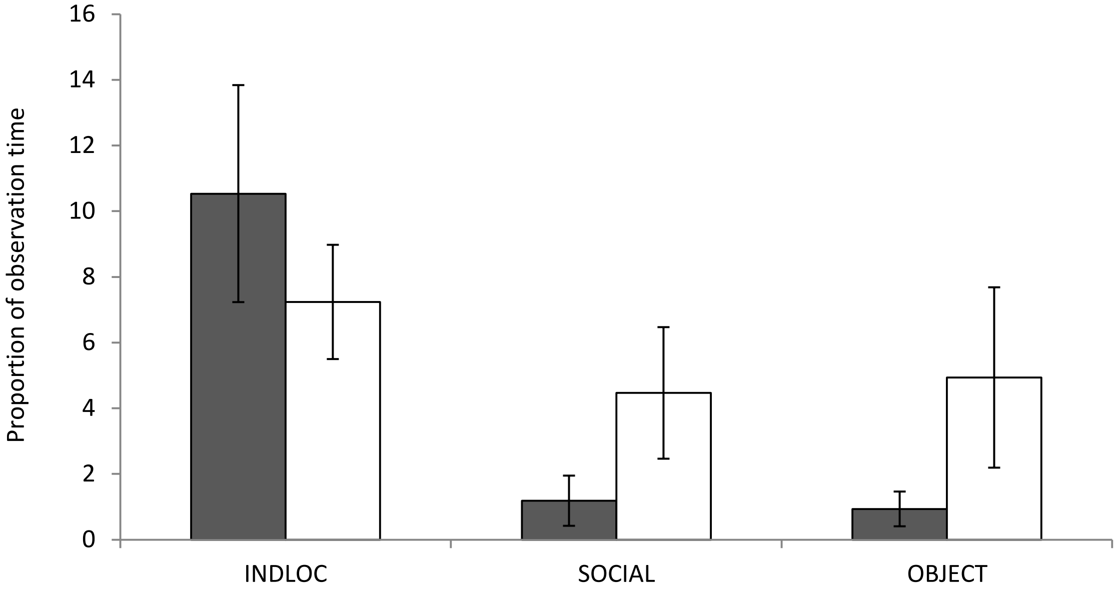


Figure 2: The proportion of total observation time spent expressing *individual locomotor* (INDLOC), *social* and *object* play by neonatal pups at Donna Nook in early and late lactation. Grey bars= early lactation phase, White bars= late lactation phase. Values are mean ± SEM, n=14.

3.2. Comparison of play behaviour between male and female neonates.

As figure 3 illustrates, an overall trend was found for female pups to express higher mean levels of all types of play compared to male pups. However, despite this, table 3 shows that no significant differences were found between male (n=5) and female (n=5) pups for any play type, or play overall (all types combined) when conserved randomisation tests were conducted.

Table 3: Results of two-tailed randomisation tests comparing male and female play behaviour during the 2011 breeding season at Donna Nook. *P-values computed from the proportion of randomised iterations generating differences in group mean values as large as, or larger, than the observed difference.*

	Test Statistic	***P*-value**
Overall Play	9.68	0.08
Individual Locomotor	5.02	0.18
Social	3.01	0.37
Object	1.65	0.65

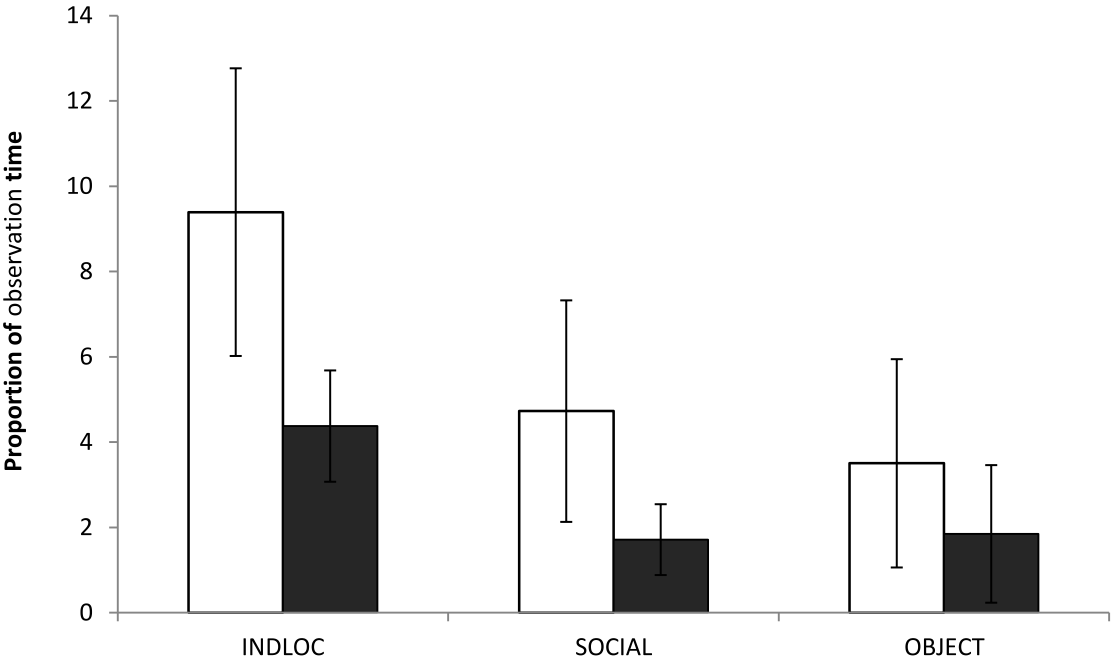


Figure 3: Proportion of total observation time spent in *individual locomotor* (INDLOC), *social* and *object* play by female (white bars, n=5) and male (dark grey bars, n=5) pups during the 2011 breeding season at Donna Nook. Values are mean ± SEM.

To address the potential confounding effect of pup age on these comparisons, the play behaviour of males and females was then compared in early and late lactation stages separately. Table 4 shows the results of these tests, again revealing no significant differences in the proportion of time spent in any play category between the sexes in either developmental stage. Although, female pups were found to spend just a significantly greater amount of time expressing *individual locomotor play* in late lactation (table 4; figure 4).

Table 4: Results of two-tailed randomisation tests comparing male and female play behaviour during early and late developmental stages. *P-values computed from the proportion of randomised iterations generating differences in group mean values as large as, or larger, than the observed difference.*

	EARLY		LATE	
	Test statistic	***P*-value**	**Test Statistic**	***P*-value**
Individual Locomotor	8.66	0.43	7.21	0.05
Social	1.32	0.47	3.07	0.73
Object	0.60	1	0.66	0.27

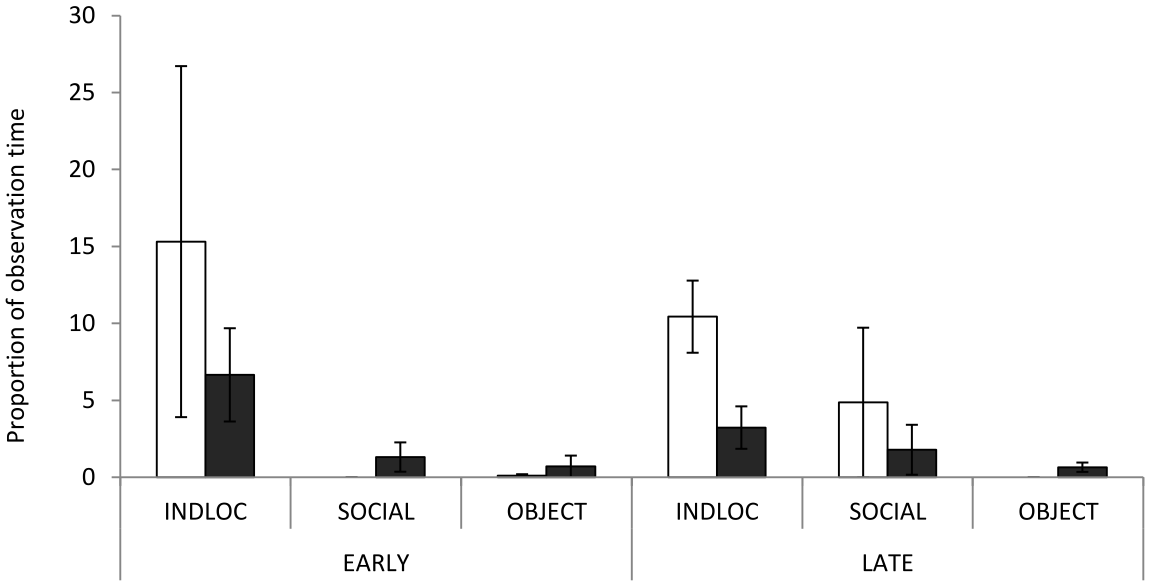


Figure 4: Proportion of observation time spent expressing *individual locomotor* (INDLOC), *social* and *object* play by female (white bars, n=3) and male (dark grey, n=5) pups in early and late lactation phases. Values are mean ± SEM.

3.3. Maternal condition and neonatal play.

As figure 5a shows, there was an apparent mean trend in the results for female pups of mothers in above-average condition to spend a greater proportion of time in *individual locomotor* and *object play.* However, no statistically significant differences between males or females were found when a conserved randomisation procedure was conducted. Female pups of mothers in below-average condition also expressed more *individual locomotor* play on average compared to males (figure 5b), whilst male pups showed more *object* play. Again however, no significant differences were found. This was also true when social play was compared between the sexes for both maternal condition groups (see table 5).

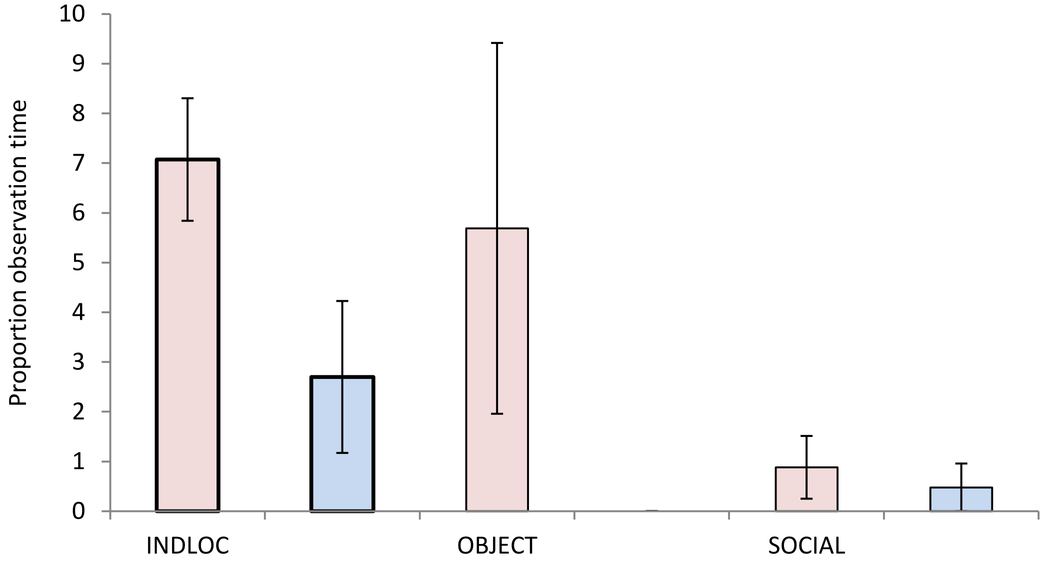
(*Table 5 and figures 5a,b below*)

Table 5. Results of two-tailed randomisation tests comparing male and female play behaviour in relation to maternal condition. *P*-values computed from the proportion of randomised iterations generating differences in group mean values as large as, or larger, than the observed difference.

	Above-average		**Below-average**	
	Test statistic	***P*-value**	**Test statistic**	***P*-value**
Individual Locomotor	4.37	0.10	7.38	0.43
Social	5.69	0.43	2.85	0.61
Object	0.40	0.89	7.96	0.10

**Females: Above-average, n=3, Below-average, n=2; Males: Above-average, n=2, Below-average,,n=3.*

a)



b)

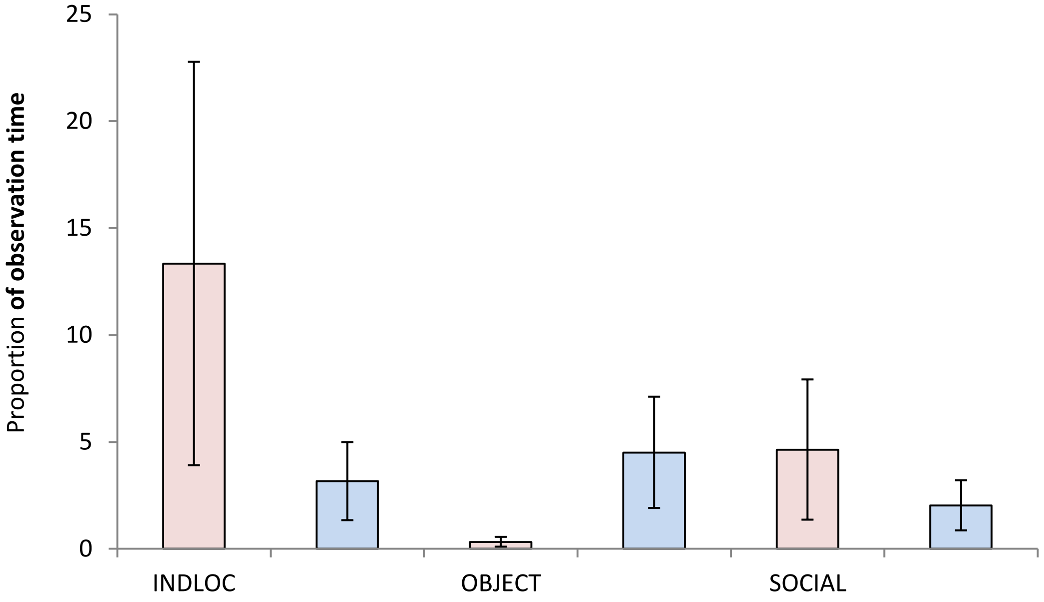


Figure 5a, b: Proportion of total observation time spent expressing *individual locomotor* (INDLOC), *social* and *object* play by pre-weaned female (pink) and male (blue) pups of above-average (a) and below-average (b) condition mothers. Values are mean± SEM.

3.3. 1. Maternal condition and the occurrence of different types of play.

Linear regression analysis revealed no significant relationship between maternal condition and independent play behaviours (*individual locomotor* or *object-play*). However, a significant positive relationship was found between maternal condition and pup *social play* with pups of mothers in poorer condition engaging in more social play, and vice versa (table 6; figure 6).

Table 6. Results of randomisation regression tests examining the significance of the relationship between a mother's position on the body condition continuum and proportion of time spent expressing *individual locomotor*, *object* and *social* play by their offspring.

	b	***P*-value**
Individual Locomotor	9.89	0.183
Social	9.21	0.028
Object	-1.32	0.69

The *p* value is computed using an approach that combines linear regression with a randomisation test. Sample size = 14. Significant results ($P< 0.05$) are in bold, ***b*** represents slope of the relationship.

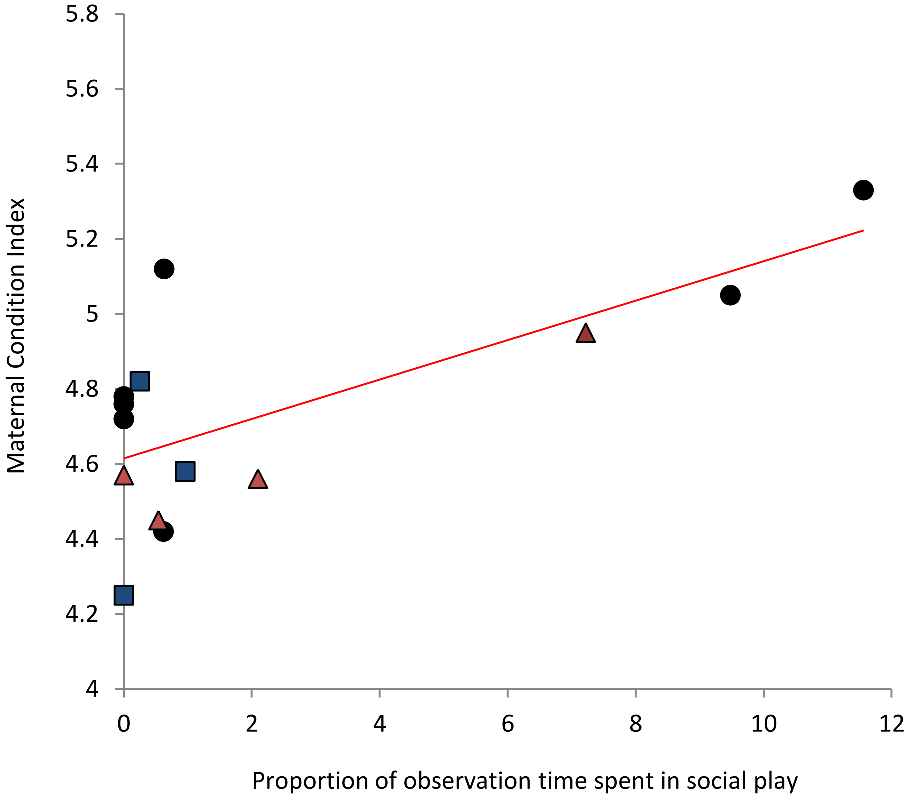


Figure 6. Scatterplot of the proportion (%) of observation time that pre-weaned grey seal pups spent in social play in relation to the condition of their mother (determined by morphometric ratio measures). *Red line represents the line of best fit*. Larger maternal condition index values indicate poorer condition and vice versa. Red triangles= female pups, Blue squares= male pups, Black circles= sex unknown, (n=14).

4. DISCUSSION

4.1. Development of neonatal play behaviour over lactation.

When considered as a unitary behaviour (all types of play combined), the time spent playing by pups was found to be remarkably similar in early and late lactation phases (Mean ± SEM: *Early*: 15.84% ± 3.89; *Late*: 16.52% ± 3.66, n=14). However, when separate types of play were investigated, some interesting developmental trends emerged. The mean proportion of time spent in i*ndividual locomotor* play was found to decline with age (figure 2), whilst time spent in both *object* and *social* play increased over lactation (figure 2). These trends in the results are therefore broadly in-line with the *first hypothesis* of this chapter (*section 1.5*). However, these differences were found to be non-significant, and so study hypothesis 1 must be rejected. It is important to remember that the analyses in this chapter were limited by a small sample size, and future work in this area would benefit from study of a greater number of individuals to more reliably assess the development of play in this species. The following discussion must therefore be considered within this framework, and is supported by inference from the findings of independent studies.

Within the literature, there is evidence that different types of play wax and wane at different ages within the same species, specifically that *locomotor* play precedes *social* and *sexual* play during ontogeny (Bighorn Sheep: Berger, 1980; American Bison: Prescott, 1985; Gazelles: Gomendio, 1988; Domestic Cattle: Haynes, 1997; Stellar Sea Lions: Gentry, 1974; Olive Baboons: Chalmers, 1980). Such findings support theory presented in Spinka et al. (2001), who note that the peak in independent *locomotor* play is typically expected to peak before *social* within species play for reasons including the relatively high cognitive demands of play with others and the developmental 'lag-phase' for internal machinery to reach this level of sophistication/ability. Based on such findings, it may be predicted that the developmental trajectories of play types in grey seals will become more pronounced with age, beyond the lactation period. See further discussion of this concept in chapter 5.

This study has shown that different types of play can be reliably identified in young grey seals. However, whether differential developmental trajectories exist in these different play types is less clear, since no significant differences between lactation phases were found. If future research is able to test the ideas raised in this chapter more robustly, and evidence is found in support of differential developmental trajectories, then this would support the concept that different types of play have different functions that wax and wane over ontogeny (Bateson, 1981; Gomendio, 1988). Namely, a greater prevalence of *individual locomotor* play in early lactation may indicate that this type of play functions to facilitate the development of physicality and motor skills. Whereas by contrast, relative increases in *social* and *object* play over lactation may suggest that as pups grow older, play takes on a more cognitive function, facilitating the development of social skills in individuals and training information processing systems to recognise environmental features. Increases in these types of play may be associated with the development of "internal machinery" to higher levels of ability (Spinka et al. 2001). Indeed, differential play trajectories might be suited to the most appropriate stages in ontogeny when different skills or information can be gathered from the social or ecological environment (Gomendio, 1988).

4.1.1. Future insights.

This study was not able to offer any long-term ontogenetic indication of play behaviour, since the age-class investigated was restricted to pre-weaned neonates. However, within the literature there is support that play increases with age, peaking in the juvenile phase and declining thereafter (Biben, 1983; Gomendio, 1988; Prescott, 1985; Newberry, 1988; Renouf, 1993). However, such conjecture is currently unexplored in this species, with little comparative data regarding the amount of play expressed by individuals in older ontogenetic stages. Play has certainly been identified in juvenile individuals (Wilson, 1974), but the proportion of time spent in play was not documented in this study. To current knowledge, there is no literature detailing play in the adults of this species, therefore suggesting that a general decline in play after the juvenile stage may be correct. However, play has been recorded in the adults of some species (e.g. Marsupials: Watson, 1998;

Rodents: Pellis et al. 1993; Primates: Zucker et al. 1986), and indeed, has been documented in captive adult common seals (Renouf, 1993). Further investigation of a cohort of identified individuals over a greater temporal range (as done elsewhere: Gomendio, 1988), would therefore be insightful for gaining a more holistic interpretation of the developmental trajectory of play and would provide greater understanding of its function and consequences.

4.2. Sex differences in play behaviour.

No significant sex difference was found in the overall proportion of play (all types combined) expressed by male and female pups over lactation (Mean ± SEM: *Females*: 21.93% ± 4.72, n=5; *Males*: 10.04% ± 3.23, n=5; Two-way randomisation: test statistic= 11.89, *P*= 0.08). Similarly, no significant sex differences were found in any of the respective play types. The statistical results of this investigation therefore give no support to the second study hypothesis (*section 1.5*), which predicted that female pups would spend more time in *social* and *object* play. These analyses were however severely hindered by a very low sample size of male and female pups (males=5, females=3). Confidence in these results would be increased by study of a greater number of neonates in the future, to more reliably assess whether any true differences in play behaviour are apparent.

As stated, no significant inter-sex differences were found between any type of play behaviour. However, at a general level, the female pups in the study played more on average than males in each play category (see figure 3). Whilst this finding cannot be overstated, owing to the limitation of small sample size and non-significant result, it does provide footing from which to make predictions to guide complementary future research. The following discussion considers the biological reasoning behind such predictions.

We may expect female pups to express more *social* and *object* types of play, since adult females have been found to show a simple form of sociality- actively associating with certain individuals during successive breeding seasons (North Rona: Pomeroy et al. 1999). We therefore may reason that females require basic social skills, and therefore could benefit from increased social training through play (“social-cohesion” hypothesis). Further, the ability of females to show breeding site fidelity to a high degree of accuracy (Pomeroy et al. 2000b) suggests that individuals require detailed knowledge of their environment, and therefore higher levels of object-play may provide a means of gaining environmental and site-specific knowledge. In this way, we may view play as being ‘multi-functional’, as suggested by other authors (Power, 2000).

It is important to consider that the growth trajectories of male and female grey seals are markedly different, as are their developing ‘social roles’. We may therefore propose that play may initially be slightly more important to females, but then take on a greater role for males at a later ontogenetic stage, with the sex difference becoming more pronounced. For example, play-fighting (*social play*) amongst juvenile males has previously been documented (Wilson, 1974). This type of play may be the primary means by which males can safely enhance their motor skills and obtain a sense of their own physical limits/competitive ability (Thompson, 1998). A peak in social play, specifically play-fighting with conspecifics, might be most appropriately suited to the juvenile phase in males, within the social/ecological environment provided. It is therefore conceivable that play functions very differently between the sexes at key points in their developmental trajectories. Studies within the literature support such aspersions, showing that sexual dimorphism in play increases with age (Sea lions: Gentry, 1974; Swine: Newberry et al. 1988; Chimpanzees: Spijkerman et al. 1996; Rodents: Smith et al. 1998). Further discussion of this concept will be presented in chapter 5.

It is also instructive to consider how changing contexts and environments may affect the play behaviour of males and females, since sex differences in play have been found to be context-specific in previous studies. For example, female spotted hyenas were found to conduct more *social* play when in all-female groups compared to same-sex male groups. When females were in mixed groups, they conducted more *locomotor* play, thus suggesting that the appearance of male partners suppressed the social play of females. In the pre-weaning neonatal phase, the social interaction partner for pups is largely restricted to the maternal figure. By contrast, in the juvenile phase individuals may find themselves in more mixed groups (such as at haul-outs), where the growing disparity between male and female size may suppress social play in females. In such contexts, it could be hypothesised that juvenile males would initiate a higher frequency of social play bouts and of a higher intensity, with females ending bouts more than males in male-female play scenarios (such findings would point towards the *motor-training* hypothesis being the primary function of play at this point in ontogeny). Further work is required to test these intriguing postulations, see further discussion in chapter 5.

4.3. Maternal condition and play behaviour.

It must be noted that the analyses presented in this chapter involving maternal condition were based on indices obtained via morphometric assessment of female body condition from in-field photographs (see appendix 4 for full report detailing methodology). However, until this method has been validated via a more direct, independent means, interpretation pertaining to these results must be considered with due caution. Examples of how such validation may be achieved in wild populations are considered in *section 4.2* of chapter 5.

It was predicted in the third study hypothesis (*section 1.5*) that male pups of mothers in poorer condition would engage in more *individual locomotor* play compared to females, whilst female pups of mothers in better condition would engage in more *object* play. These predictions were based on findings presented in chapter 3. However the results of the present study provide no strong support for this hypothesis, with no significant differences found between male and female pups in any type of play when compared in relation to maternal condition. This analysis was very limited by low sample sizes (with 3 or fewer male and female pups in each maternal condition group). It is therefore advised that no strong conclusions can be drawn from the presented condition-dependent, male-female comparisons of play. Analysis regarding the occurrence of different play types in relation to maternal condition offers a more robust line of investigation, which will now be discussed.

4.3.1. Maternal condition and the occurrence of different types of play.

Regression analyses revealed that maternal condition had no significant relationship with the expression of *independent* types of play. However, as predicted in study hypothesis 3 (*section 1.5*), a significant relationship was found between maternal condition and *social* play. Specifically, pups of mothers in *poorer* condition partook in higher proportions of social play during observations than pups of mothers in better condition (see table 6; figure 6).

This is an intriguing finding. The close social contact maintained as a by-product of this type of play gives opportunity for the training and development of simple social skills, including individual recognition. Indeed, in the stellar sea lion, the development of social behaviour was found to be aided by play behaviour (Gentry, 1974); and Wilson and Kleiman (1974) argued that body-odour sniffing is enhanced during social play, which may aid individual recognition. Whilst social play may provide such training, it is also tempting to speculate whether enhanced social play in pups of poorer mothers acts to facilitate development. Such pups may benefit from enhanced musculature and motor skills as from such repeated bouts of interaction. The enhancement of physiological attributes, such as self-awareness, social capabilities and motor training, are all traits that might help give an advantage to pups post-weaning. Interestingly, the concept of play as a 'compensatory' mechanism has been expressed elsewhere in the literature. Bateson et al. (1990) suggested that the kittens in their study perceived cues that their mother was experiencing a food shortage, and subsequently "accelerated their development" through play. It is also conceivable that pups may gain more from the more 'challenging' experience of social play with a larger, more dominant individual during such this early stage of life from a physical-enhancement perspective.

If play enhances physical development then it may be expected that poorer condition mothers would *initiate* social play more often; yet this was not found to be the case. Indeed, of the total social play bouts recorded from all focals, ~35% were constituted by mother-pup social play bouts that were initiated by the maternal figure, whereas ~63% were constituted by bouts initiated by the focal pup. As suggested by Spinka et al. (2001), amongst pairs of familiar conspecifics that differ widely in body size and/or experience (such as mothers and pups), play will most often be initiated by the smaller or less experienced partner, since they will gain a benefit from the experience of coping with being at a disadvantage in the interaction. This theory is interesting and could be applied to grey seal neonates, however since social play is a reciprocal act, question is raised as to the role that maternal behavioural tendencies may have on the occurrence of social play.

Variation amongst mothers in their maternal behaviour has been identified (Bardi and Huffman, 2002; Parent and Meaney, 2008; Twiss et al. 2012a), including tendencies to socially interact with their infants. We may therefore speculate that there may be a relationship between the relative body condition of a female and the maternal behaviour she expresses. Specifically, we may speculate that mothers in poorer condition are more likely to *reciprocate* social play with their pups once this is initiated, whereas mothers in better condition are less likely. Such mothers would be classified as being more ‘anti-social’, more often rejecting the attempts of pups for social interaction. Mothers in poorer condition by contrast may tend to be more ‘affiliative’ in their maternal tendencies, and be more likely to reciprocate in such playful behaviour. In this scenario, it may not be that social play is a compensatory mechanism for enhancing pup development, but simply that mothers in poorer condition show behavioural tendencies to socially interact with their pups more willingly, with pups potentially benefitting in the ways described as a by-product of this. Such speculation will be addressed in more detail in chapter 5 (*section 1.4*).

4.4. A note on the limitations of small sample size and possible sampling effects.

As previously discussed, sample size was very small for the analyses presented in this chapter (14 individuals), with this reducing substantially when finer levels were explored, such as those involving comparisons between the sexes (5 individuals or less in each group). The number of known male and female pups was limited primarily due to difficulties associated with the non-tactile, opportunistic approach adopted for sexing neonates in the field. The results may also have been affected by sampling effects, since behavioural data were collected from only a very small sub-set of pups. Indeed, hundreds of pups are born during the breeding season at Donna Nook each year (see chapter 1, *section 1.5.1*), and so generalising findings from analyses of just 14 or fewer neonates is obviously limited. Behavioural sampling was also restricted for each individual, with focal footage per individual ranging from 32.3 minutes to 95.2 minutes over lactation (see chapter 1, *section 1.5.6*). Therefore only a small proportion of study individual's total lactation period was captured by these focals, meaning that the behavioural records obtained for neonates may not have been entirely representative of individual behavioural repertoires. More discussion of possible sampling bias is presented in chapter 5, *section 1.2*. Such issues must therefore not be underestimated, and whilst the author feels that the analyses presented in this chapter represent a useful first step in this line of investigation, future research would benefit substantially from building from these foundations with more systematic study and objectives directed at specific research questions.

4.5. Chapter summary.

This chapter is based on sample sizes that are too small to provide sufficient power for the analyses presented, and thus the results presented and discussion there-of should be considered with due caution. On average, neonates spent more time expressing *individual locomotor* play in early lactation than late lactation, and *social* play was seen to show the opposite pattern. These results were however found to be non-significant, and are therefore only suggestive. The ideas discussed linking differential development trajectories to differences in play function therefore remain speculative until more robust testing is conducted to validate them with a larger sample size and efforts are taken to reduce possible sampling effects. Female pups played more on average than male counterparts, however no significant differences were found between the sexes. Again, these results were limited by small sample sizes and the addition of complementary future research would be highly beneficial. It is speculated that 'sexual dimorphism in play' will increase over time, as disparity between males and females in size and developing social roles increases over ontogeny. Finally, maternal condition was found to interact with neonatal social play behaviour, leading to the suggestion that social play may provide a means by which the development of pups with poorer nutritional starts can be enhanced. It is also postulated that mothers in poorer condition may simply tend to have more ‘affiliative’ maternal behaviour, engaging in social interactions more often with their pups when approached. Future research would benefit from investigation of the potential interaction between a mother’s body condition and her maternal behaviour to investigate this further and gain a more holistic understanding of factors influencing neonatal play.

CHAPTER 5

An Integrative Discussion of Key Concepts and Findings

Thesis overview.

The overriding aim of this thesis was to provide an ethological investigation of the early behaviour and development of pre-weaned grey seal neonates over the brief period of maternal dependency. In chapter 2, a summary of neonatal behaviour and inter-individual behavioural consistency was given, using data collected over three successive breeding seasons from the offshore colony of North Rona. In combination with these preliminary analyses and consideration of the age-class being studied, focus then turned to a more in-depth exploration of play behaviour in chapters 3 and 4. This chapter will provide an integrative discussion of the key results presented in this thesis relating to play behaviour, with the aim of providing a more holistic overview of the implications of these findings for the nature and function of play in neonates.

1.1. The developmental trajectory of play over lactation.

Analysis of behaviour budgets from the breeding colony of North Rona revealed that the overall proportion of time spent in solitary play (referring to *individual locomotor* play) by neonates decreased over lactation (see chapter 2). However, as discussed in chapter 4, it is not tenable to consider play as an homogenous category, since distinct types of play have been identified. It is therefore important to consider the developmental trajectories of all types of play. This approach was adopted in chapter 4. *Individual locomotor*, *object* and *social* play types were categorised, allowing the relative changes in independent and social types of play to be investigated. Despite broad trends in the average results, with a decrease in *individual locomotor* play and relative increases in *social* and *object* play over lactation, no significant results were found. However, these results were hindered by a low sample size and potential sampling bias. The author suggests that with targeted future research on a larger number of individuals and over a longer temporal span (including the post-weaning phase), more reliable support for differential developmental trajectories may be identified. Indeed, taking guidance from the wider literature, there is reason to believe that more robust indication will be found that peaks in *individual locomotor* play will be observed before peaks in *social* and *sexual* play during ontogeny, as observed in a number of species (Domestic Cats: Barrett and Bateson, 1978; Bighorn Sheep: Berger, 1980; American Bison: Prescott, 1985; Gazelles: Gomendio, 1988; Domestic Cattle: Haynes, 1997; Stellar Sea Lions: Gentry, 1974; Olive Baboons: Chalmers, 1980).

Should relative increases in *social* and *object* play be found with age, it may be proposed that these are reflective of simultaneous increases in (neuro)physiological development. As Kuczaj and Makecha (2008) suggest, perhaps play during this time has a role in the development of ‘flexible thought’, with the interaction between the individual and the environment during independent play facilitating cognitive growth and flexibility. This is supported by observation of pups manipulating inanimate objects in their immediate environment (including rocks, grass and sand) during *object* play (personal observation of the author). Infants may use such investigative play to sample their environment, and in doing so, develop behavioural responses to environmental novelty (Pelegrinni et al. 2007). The benefits of this to grey seal neonates as they approach independence are apparent as they will need to navigate the social and ecological environment alone. In previous discussion, it was therefore speculated that differential time courses of play may have evolved so that certain types of play are more prevalent at the most appropriate stages in ontogeny when certain skills or information can be best gathered from the social or ecological environment (Gomendio, 1988). These concepts will be discussed further in *section 1.2*, when considering the potential increase in the ‘sexual dimorphism’ of play over time.

It is important to consider that changes in the prevalence of different types of play may be heavily reliant on the presence of the maternal figure. Once the mother has abandoned the pup to return to sea, the primary interactant for social play is no longer there and so it may be reasoned that a decrease in this type of play would follow accordingly in the post-weaning phase. Personal observations of the author of post-weaned pups indicate that *individual locomotor play* can be highly prevalent in some pups during this phase (see figure 1a), thereby raising the possibility that the developmental trajectory of this type of play from birth to the end of the post-weaning phase may be "U-shaped", with a relative increase occurring post-weaning. Groups of weaned pups have also been observed in this phase, with pups settling in close proximity of one another (figure 1b). This therefore offers opportunity for *social play* with partners of a similar age and size; thereby presenting a very different social context for this type of play compared to the pre-weaning phase. The relative equality of pups during this period may give indication of the role that social play could have. If we assume that interactive play with a much larger, and dominant play-mate (maternal figure) is particularly beneficial in terms of physical training (Spinka et al. 2001), social play amongst similarly-matched individuals may function differently. Indeed, it may be hypothesised that *social play* in the post-weaning phase may adhere more to the 'social-cohesion' hypothesis of play. Namely, social play with other weaned individuals may allow pups to develop simple social skills, including individual recognition. In support of this, figure 1c shows naso-anal sniffing between two developmentally advanced pups at Donna Nook, a behaviour associated with individual recognition (Wilson and Kleiman, 1974). It would therefore be insightful to continue focus future efforts on recording the behaviour of neonates into the post-weaning phase to test such predictions.

Figure 1a,b: In-field observation of individual locomotor play by two weaned neonates during the 2012 breeding season on North Rona; also illustrating close-proximity between pups (a). 'Weaner gang' on North Rona, 2012 breeding season (b). Observation of naso-anal sniffing during a brief bout of social play between two developmentally-advanced pups during the 2011 breeding season at Donna Nook (c).

1.2. Sex differences in play behaviour.

In chapter 3, male and female pups were not found to spend significantly different amounts of time engaged in independent play behaviours at North Rona (Mean time playing ± SEM. *Males* = 6.41% ± 0.67; *Females* = 5.96% ± 0.69; *t* test: t_{64}= -0.05, *P* = 0.96). This was also true when overall play (all types combined) was compared between the sexes at Donna Nook, although the disparity between males and females at this colony was greater (Mean ± SEM: *Males*: 7.94% ± 2.62, n=5, *Females*: 17.62% ± 4.12, n=5; Two-way randomisation test statistic= 9.68, *P*= 0.08).

The greater disparity in play between male and female neonates from the Donna Nook colony may have been influenced by sampling effects. As described in chapter 1, focal videos of approximately 30 minute durations were collected for identified mother-pup pairs during their lactation period. Therefore only a small fraction of the total lactation period was captured by focals, meaning that the behaviour records attained for individual neonates may not have been entirely representative of pup behavioural repertoires. With this in mind, it is notable that for the five identified male pups in this study, four of these individuals were observed to spend a large proportion of observation time resting during at least one of their focals. Further, the remaining male pup was noted as having two ‘disturbed’ focals, meaning that the pup was not relaxed for the majority of the focal which may in turn have affected its natural behaviour. By chance, the five identified female pups tended to be more active during a greater proportion of sampled focals. This may therefore account in part as to why males on average were observed to play less than female pups during observations. To increase confidence in sex comparisons in the future, a more standardised sampling regime would be beneficial, in addition to increasing the number of focals made for individuals over time, in order to increase the reliability of derived behavioural data.

Interesting suggestion of a sex difference in *independent* play was found amongst the sexes from the North Rona colony. Although overall there was no significant difference in the amount of *solitary play* performed by male or female pups, the solitary play of female pups was found to be constituted by a significantly larger proportion of *exploratory* (object-oriented) behaviour compared to males (see chapter 3). In turn, the solitary play budgets of males were found to consist of a greater proportion of *individual locomotor play*. As discussed in chapter 3, the differing constituents of solitary play between the sexes leads to the intriguing suggestion that independent play in males and females in this age-class may have different roles. It was speculated that independent play in males may function to enhance physical development through exercise of muscles and the refinement of motor skills. This would be in-line with a review of exercise physiology by Fagen (1976) who suggested a 'physiological sensitive period' during mammalian infancy when exercise is most effective. Independent play in female pups by contrast may have a more important role from a cognitive perspective whereas in female neonates play may have a more cognitive role, possibly training information processing systems to recognise environmental features. Sampling the environment through play has also been recognised in human infants (Pelegrinni et al. 2007). Interaction with the environment during solitary play could facilitate cognitive growth and flexibility, since the individual has control over the play activity without the outside influence of other animals (Kuczaj and Makecha, 2008). Further, repeated investigation of objects during such play could provide valuable practice in recognising objects irrespective of their orientation on the retina (Arnold and Trillmich, 1985). It is uncertain whether the neonatal period represents a 'cognitive sensitive phase', but if this were so, increased object-oriented behaviour in female play budgets could indicate that this time is important for developing environmental knowledge before leaving the colony for independence at sea. In support of the described conjecture, it has generally been assumed in the literature that early experiences are more important in terms of their effects on later (adult) behaviour than those occurring at other stages of the life cycle (Bateson, 1981).

1.2.1. Increase in sexual dimorphism in play with age.

It is proposed that the difference in play, specifically *social play*, between males and females will increase over ontogeny. Put succinctly: "*sexual dimorphism in play increases with age*" (Spinka et al. 2001). We may expect a physical training role of play to become increasingly important and frequent in males as they grow older, with physical strength and the ability to assess competitive ability becoming increasingly important. Whilst females may successfully reproduce without much play experience, males have to develop their competitive abilities by other means, such as intensive play behaviour- particularly play-fighting (Arnold and Trillmich, 1985). Like many other polygamic species, including fur seals (Arnold, Trillmich, 1985), stellar sea lions (Gentry, 1974) and elephant seals (Reiter et al. 1978), 'wrestling' is one of the main elements of *social* play in grey seal juvenile and subadult males (Wilson, 1974: observation sites located in Shetland and around Pembrokeshire; Levetchuke, 2012; unpublished data: observation site in Abertay). In the former study, terrestrial play typically consisted of two animals lying beside one another, each lunging gently at the other's head in between head-over-back signals (as depicted in figures 2 and 3). Adolescent individuals were also noted to rear up and lunge at each other, in a manner similar to the fighting of adult males.

In light of this existing observation and research, the author suggests that social play in grey seals during the juvenile phase may provide a 'safe' means by which individuals can enhance their motor skills and gain a sense of their own physical limits, in preparation for intra-male competition as sexually mature adults. Arnold and Trillmich (1985) provide support for this in a study of play in Galapagos fur seals, where play-fighting bouts were found to be longer in male pups than females. The authors suggested that since adult fighting success is determined by size, strength and also fighting tactics, play-fighting may help to develop manoeuvring powers and fighting strategies, and therefore is "… *bound to be very beneficial in later life*". The juvenile stage may therefore provide a key period of 'self-assessment' (Thompson, 1998) for young males, facilitated through play. Studies in other mammalian species support this concept, for example, Palagi et al. (2004) noted that the juvenile period in chimpanzees is crucial for acquiring social role and hierarchical status, as well as developing physical skills. We may therefore expect a peak in social play (continuing along the trajectory indicated in this thesis) in the juvenile phase, owing to the contextual appropriateness at this period in the life history where skills necessary for later life can be gathered within the social/ecological environment provided.

(*figure 2, below*)

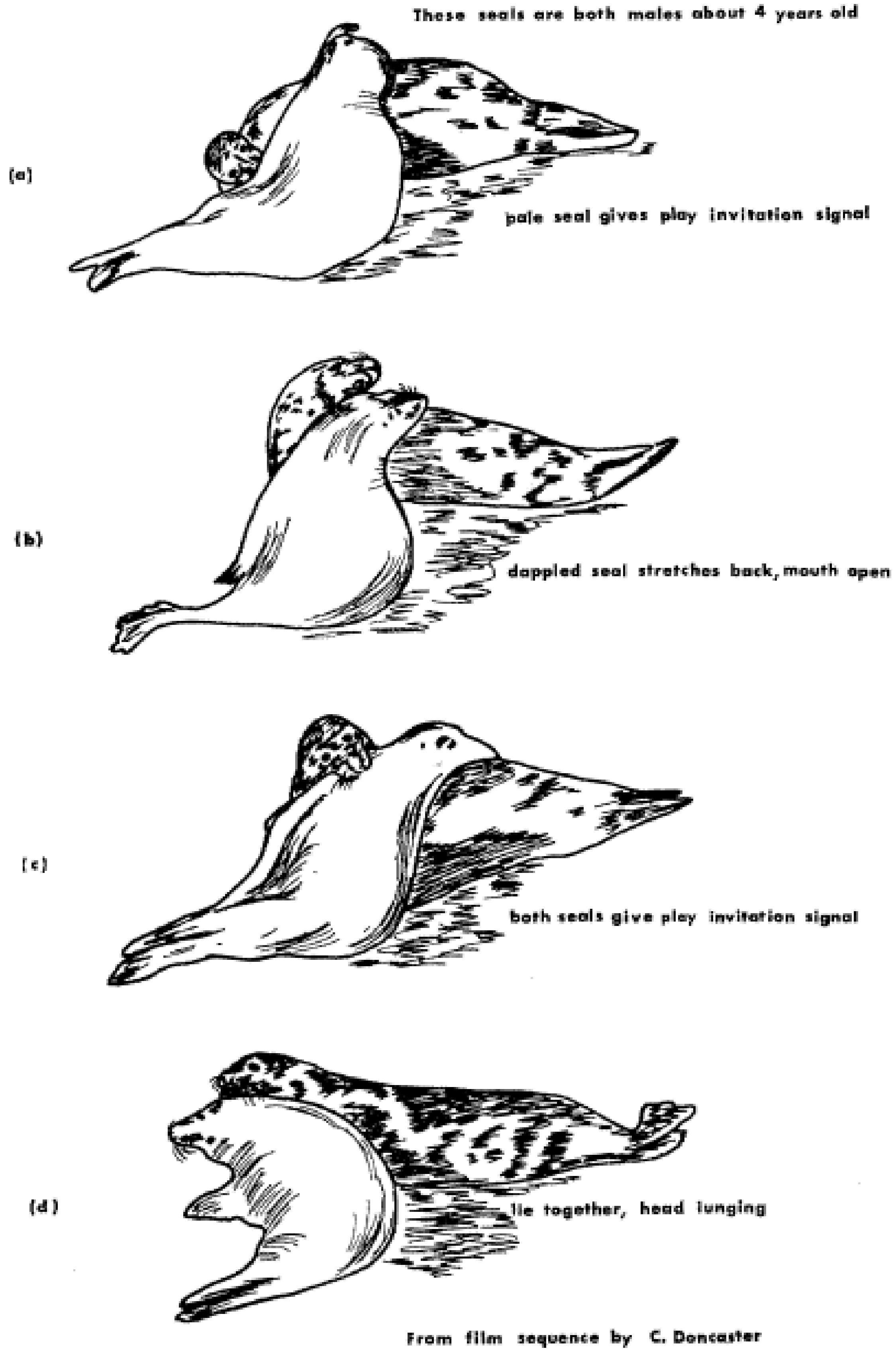


Figure 2: Typical terrestrial display of play behaviour between a dyadic pair of juvenile grey seals. Source: Wilson, 1974.

The suggested importance of social play to juvenile and sub-adult males is reinforced by the fact that although grey seal males become matured at a similar time to females, at around 3-5 years old, they generally do not start to breed until 9-12 years old. Such a reproductive delay may be because young matured males need to gain weight and social experience in order to be able to compete with adult males, therefore emphasising the importance of social play, particularly play-fighting (see figure 3 for in-field illustration) in males. By contrast, matured females need to save energy for their offspring (Levetchuke, 2012; unpublished manuscript).

Figure 3: In-field observation of juvenile play-fighting behaviour during the 2012 breeding season on the island of North Rona.

Interestingly, research with non-breeding grey seals near Abertay in Scotland reported a high male ratio in haul out groups, with adult and sub-adult males constituting 60% of the observed group for most of the time (Levetchuke, 2012; unpublished manuscript). Such findings have also been reported at other colonies (e.g. Celtic sea: Lenney et al. 2010), but opposite trends reported elsewhere (e.g. Ireland: Kiely et al. 2000). This raises the intriguing possibility of whether social interactions could determine sexual haul out patterns, and more specifically whether high male sex ratios may be related to the need for physical training through social play. However, more investigation is required to assess this.

To summarise, the author proposes that play functions differently in grey seal males and females at key points in their life histories, reflected by changes in the dominant types of play in the behavioural repertoire. The wider literature supports such suggestion, namely that sexual dimorphism in play increases with age (Sea lions: Gentry, 1974; Swine: Newberry et al. 1988; Chimpanzees: Spijkerman et al. 1996; Rodents: Smith et al. 1998). This is also in-line with theory reported in Spinka et al. (2001), namely that neonatal individuals are forecasted to play relatively less than juveniles due to a comparative lack of physical and neurological development at this stage. In order to test these propositions, more detailed study of play behaviour over a greater temporal life span of a cohort of individuals is essential.

1.2.1. Inter-annual comparisons of play in related individuals.

The results discussed so far in relation to male and female play behaviour are concerned with the comparison of *unrelated* neonates. Within such investigation there is the possibility for confounding effects from maternal and genetic differences between subjects. In order to control for such effects in chapter 3, the solitary play behaviour of male and female *siblings* born to the same mother in different breeding seasons was compared. It was predicted that mothers in good condition would invest more in their sons, since a successful son would out-compete a successful daughter, and that mothers in poorer condition would invest more in daughters since most females that survive to adulthood will breed at least once, whereas an unsuccessful male may never breed (Trivers and Willard, 1973). In turn, it was postulated that increased investment of energy would lead to greater expression of play in offspring, based on findings within the literature of a link between nutrition and play (e.g. Gelada baboons, *Theropithecus gelada*: Barrett et al. 1992; White tailed deer, *Odocoileus virginiarus*: Muller-Schwarze et al. 1982; Rodents: Smith, 1991; Belding's ground squirrels, *Spermophilus beldingi*: Nunes et al. 1999). However, the results did not support these predictions, and in fact sons of mothers in poorer condition were found to play more, whilst daughters of mothers in better condition played more by contrast (see chapter 3). As discussed previously, biological speculation based on known facts about adult behavioural characteristics suggests that enhanced independent play in female neonates may be particularly important for environmental exploration, which in turn may train information processing systems to retain information regarding environmental cues (see *section 1.2*). Such knowledge is assumed to be particularly useful for females, who have been shown to express accurate philopatry and site fidelity as adults (Pomeroy, 1994; Pomeroy et al. 2000b). It was posited in chapter 3 that play may be a compensatory mechanism for physical development in males when faced with poorer nutritional starts. This was supported by the finding that sons of mothers in poorer condition had lower average daily growth rates compared to male pups of mothers in better condition. This conjecture is further supported by the fact that a greater proportion of male independent play budgets were constituted by *individual locomotor play*, which is related to physical mobility and may be considered as 'exercise'. If play does provide a kind of physical nurturing role then this would help to explain why sons of better condition mothers did not play more than their sisters, since their growth and development was not compromised. Good early physical development is arguably more important for male neonates, as there is some indication that body condition at weaning may have more of an impact on male survival than female. Hall et al. (2001) investigated the probability of post-weaning survival to age 1 of a cohort of tagged pups at the Isle of May colony and found that the odds of survival for female pups was higher than males, and further that the effect of condition at weaning on survival was significantly greater for male pups than females.

This intriguing finding was however called into question owing to suspected miss-categorisation of *comfort moves* for individual *play* behaviour more frequently in the 2008 breeding season. In an attempt to account for this potential bias, sibling analyses were repeated but index values were derived from combined *comfort move* and *solitary* play behaviour budgets (referred to as '*independent locomotor behaviour'*). The results of this re-analysis resulted in the same pattern, however the difference between independent locomotor behaviour index values was not found to be significant (*Above*, n= 9: -0.05 ± 0.06; *Below*, n=6: 0.03 ± 0.06: Two-way randomisation test statistic= 0.08, *P*=0.35; see figure 4). This re-analysis is however obviously not without its own limitations, and certainly was not ideal. Indeed, it is not possible to draw conclusions regarding *play* behaviour from these results, as desired in the original investigation. The fact that a similar pattern was seen in the results is however encouraging, and the author feels that additional robust work in this area, with analyses of siblings across different seasons where possible (utilising newly collected data), may yield more reliable and interesting insight.

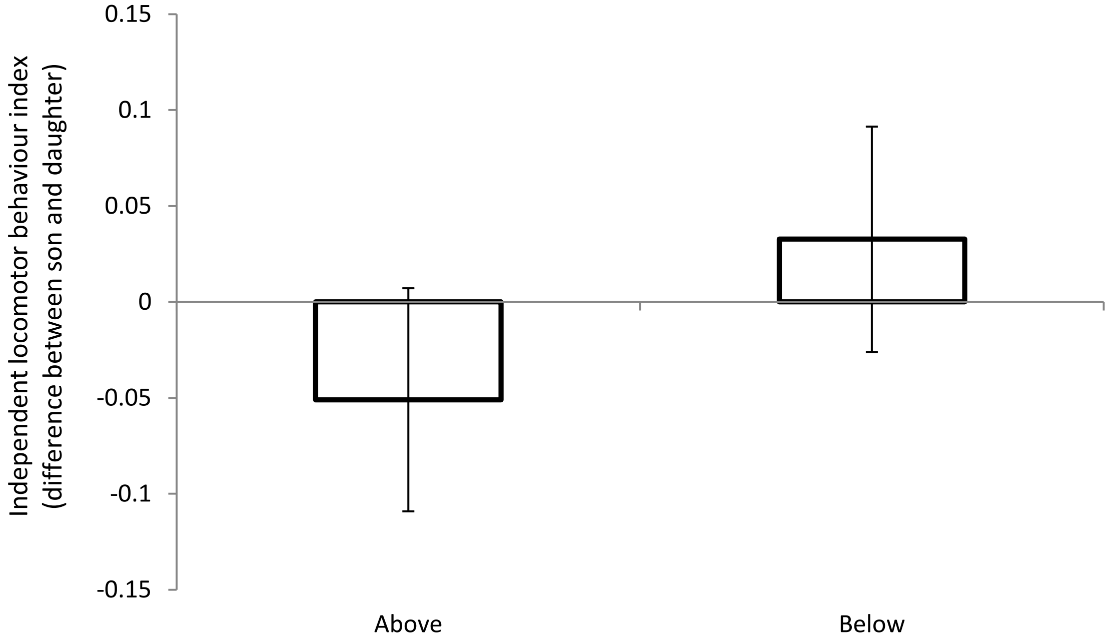


Figure 4: Differences in combined *independent locomotor behaviour* of male and female pups born in different years to the same mother, in relation to maternal condition (represented by maternal post-partum mass relative to the average). Index calculated as (son - daughter)/(son + daughter). Positive values show that male pups expressed more independent locomotor behaviour whereas negative values indicate that female pups expressed more independent locomotor behaviour. Values are mean ± 1S.E.

1.3. Maternal condition and social play.

In chapter 3, no significant correlation was found between maternal condition (indicated by post-partum mass) and *independent* neonatal play behaviour (excluding sibling analyses, as described above). However, when play was considered in its constituent forms in chapter 4, a significant relationship between maternal condition (as represented by morphometric estimation of body condition; see *appendix 4*) and neonatal *social* play behaviour was found. This again illustrates the importance of considering play as a heterogeneous behaviour. Specifically, infants of mothers in poorer condition were observed to partake in higher proportions of *social* play than pups of mothers in better condition (Randomised regression: b= 9.21, *P*= 0.028; figure 5). There is also some preliminary indication of such a relationship from the North Rona colony with pups of mothers in poorer condition (as judged in relation to average post-partum mass) spending more time in *maternal interactions* than those with mothers in better condition (mean ± SE: *Above*, n=26: 1.72% ± 0.22, *Below*, n=36: 2.54% ± 0.35). However this difference was found to be non-significant (*U*= 340.0, *P*= 0.07). The proportion of social play in these maternal interactions versus the proportion of briefer, unreciprocal bouts of contact cannot be certain, highlighting the need for more focussed study of social play at North Rona- possibly through the use of focal video collection. Despite this, the inference of such a relationship at two contrasting colonies suggests that this may be a particularly interesting line of enquiry for future investigations in this species. It is however important to again emphasise that, in both of these cases, there remains uncertainty surrounding the reliability of using proxies to represent true body condition, and validation using more direct means is required to increase confidence in these preliminary results (see *section 2.2* for more discussion of this).

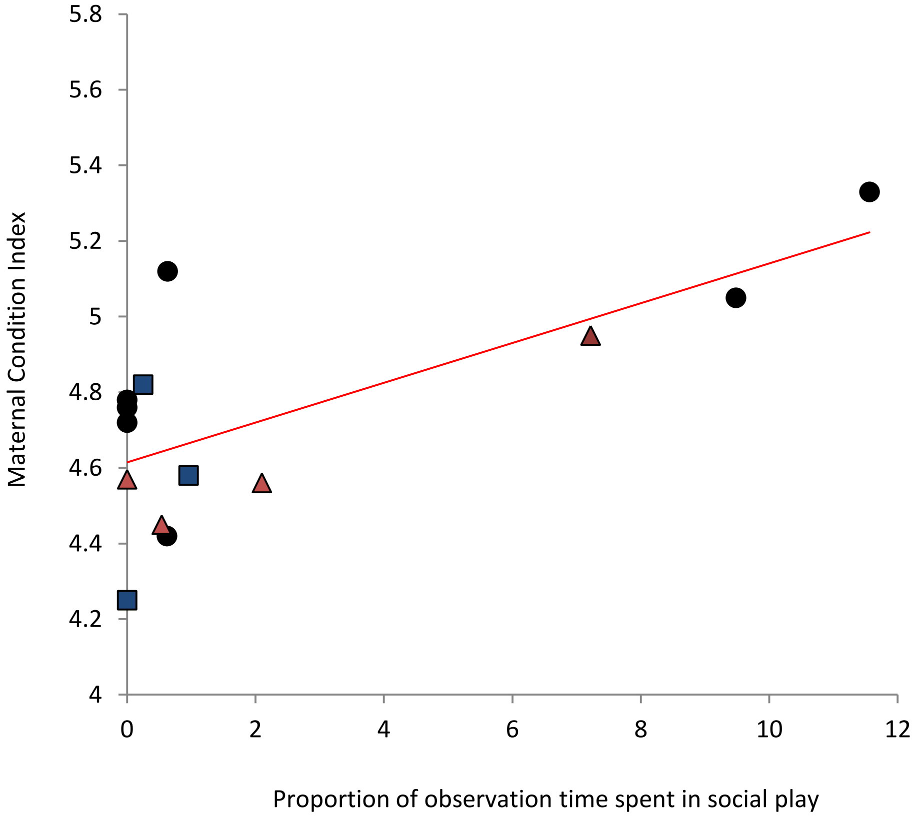


Figure 5. Scatterplot of the proportion (%) of observation time that pre-weaned grey seal pups spent in social play in relation to the condition of their mother (determined by morphometric ratio measures). ***Red line represents the line of best fit***. Larger maternal condition index values indicate poorer condition and vice versa. Red triangles= female pups, Blue squares= male pups, Black circles= sex unknown, (n=14).

In chapter 4 it was speculated that mothers who cannot provide nutritional investment much above the minimal level required to sustain their pup to weaning facilitate the development of their pups through increased *social* play with them.

Thereby, instead of giving pups an early advantage through enhanced provisioning, such pups may benefit from play enhanced development of bone remodelling, muscle hypertrophy, cardiopulmonary capacity and neuromuscular coordination (Jamieson and Armitage, 1987), with proposed benefits including increased strength, endurance and socially competitive skills levels (Fagen, 1981; Smith, 1982). As discussed previously, the finding that pups of mothers in poorer condition (below-average postpartum mass) had significantly lower mean daily growth rates than pups of mothers in better (above-average) condition (*Above*: 2.38kg/day ± 0.06, n= 26; *Below*: 1.99kg/day ± 0.08, n=35; *U*=187.5, *P*= <0.001, n= 61) at North Rona supports this conjecture. The effect of enhanced maternal interaction and play in such pups may also affect their future 'personality' type. Enhanced sociality is characteristic of more 'extroverted' personality types, and interesting recent work in captive gorillas (Weiss et al. 2012) has suggested that more extroverted personality types have a greater longevity than introverted individuals (living up to 30% longer). The authors postulate that being extrovert or outgoing could be a biomarker for differences in the functioning of the immune system. Therefore pups that interact socially with their mothers more often may be classified as more "extroverted", and could therefore potentially benefit from enhanced social play through better immune function. Longer-term study of the survival and longevity of individuals of known personality types is however needed to test such theory.

A secondary view of this relationship is that some mothers may have behavioural tendencies to socially interact with their pup more frequently, with enhanced social or physical development, as described above, a by-product of this. We are therefore compelled to consider whether variation in intrinsic physiological characteristics between mothers can influence their social interactive tendencies (~ 'mothering style'). This will now be discussed.

1.4. Maternal condition and 'mothering style'.

It has been recognised that individual mothers differ in their degree of maternal care (e.g. Guinea Pigs: Albers, 1999; Primates: Bardi and Huffman, 2002; Rodents: Parent and Meaney, 2008), including responses to their infants' demands for parental care (Fairbanks and McGuire, 1995). Because such individual differences remain consistent over time and across infants of the same mothers, common terminology has been introduced in recent decades to reject differences in 'mothering style'. As Bardi and Huffman (2002) succinctly describe: "*Rejecting mothers tend to frequently interrupt contact with their infants, stay longer off contact, and often prevent their infants from making contact with them. Protective mothers…tend to maintain contact longer…and when their infants are not in contact, tend also to watch them more often than rejecting mothers*".

There has been some exploration of the potential effect of maternal condition on this behavioural characteristic, with contrasting findings reported. Some authors have documented a decrease in maternal care (largely in terms of food-provisioning) with decreasing condition (e.g. Rodents: McClure, 1987, Seals: Trillmich: 1990, Monkeys: Lee, 1984; Reindeers: Weladji et al. 2003), whilst others have found a decrease in maternal care with increasing condition. For example, Altmann and Samuels (1992) showed that high ranking female baboons (likely to be in the best condition) spent less time carrying infants compared to low ranking (poorer condition) mothers. Further, Clutton-Brock (1982) showed that red deer mothers in best physical condition (fat reserves) spent less time nursing calves compared to poorer condition mothers. In captive settings this was shown likely to be because better condition mothers are able to produce higher quality milk and therefore can spend less time nursing their young (Loudon and Kay, 1984). The previous examples assume a linear relationship between maternal condition and maternal care. By contrast, Lee et al. (1991) suggest that the time and energy devoted to maternal care is an "inverted U-shaped function" of maternal condition and circumstances. That is, if a mother is maximising her own lifetime reproductive success by allocating parental energy according to fitness payoffs of parental care then we would expect that:

- *Mothers in extremely poor condition would do better by 'rejecting' their current infant and directing energy to self-maintenance and future reproduction.*
- *Mothers in very good condition, who are able to transfer energy to offspring more efficiently, should also show more rejective behaviours, since they are*

able to provide equal fitness benefits with less time and effort and can therefore can profit themselves (such as through earlier weaning).

- *By contrast, more average or 'intermediate' condition mothers would be expected to be the least rejective.*

There has been little empirical testing of the above theory, but a rare example is presented by Fairbanks and McGuire (1995) who analysed the mother-offspring interactions of over 100 capuchin monkey dyads in relation to reproductive maternal condition. There were three maternal condition categories in this study, based on age, weight and dominance rank. '*Prime*' mothers were high-ranking females of prime reproductive age; '*Marginal*' females were at demographic extremes in terms of age and body weight; and '*Average*' females were middle or low-ranking females. As predicted, it was found that mothers at the two extremes (very high and very low condition) showed the most rejective maternal behaviour, consistent with the theory that adult females balance their own needs for self maintenance with fitness benefits of maternal care for present and future offspring. The most nurturant, responsive mothering was characteristic of 'average' condition females. Average condition mothers spent less time rejecting offspring and more time in contact with them, compared to mothers in prime condition. Average mums also spent greater proportion of time in closer proximity to infants than prime condition mothers. These results therefore indicate that differences in mothering styles can arise along a continuum of 'normal maternal care' in response to demands for self-maintenance and opportunities for future reproduction (Fairbanks and McGuire, 1995).

1.4.1. Testing the theory: Donna Nook.

The theory outlined above was investigated using data from known mothers from the 2011 breeding season at Donna Nook. Due to a low number of females in the sample group (n=8), a linear relationship was assumed between maternal condition and mothering style, since 'extreme' examples of body condition would be difficult to gauge from this. Behavioural measures to describe the nature of mother-offspring relationships during lactation were made by HCJ from collected focal video footage. From these data, an empirical ratio of relative 'affiliative' to ‘agonistic’ behaviour was derived (“MORQ index”) for the focal females (for further details see MSc thesis by Hani James; Durham University, 2012). MORQ index values were calculated by HCJ for early and late lactation phases respectively, since the method relies on relative values. The MORQ index is based on measures of mother-offspring interactions, summarising a pair’s ratio of positive to negative interactions (methods are outlined in greater detail in Weaver and de Waal, 2002). As shown in figure 6, based on data from capuchin monkeys, MORQ index values above 1 indicate a secure/affiliative mother-offspring relationship, and values below 1 indicate ‘resistant’ and then ‘avoidant’ mothering styles.

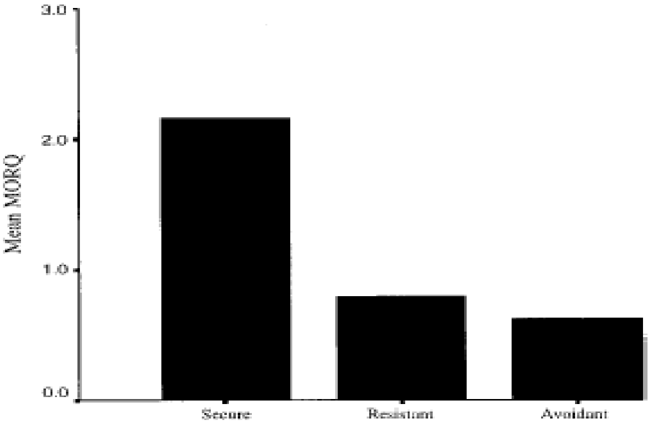


Figure 6: Empirical mother-offspring relationship quality (MORQ) values and quantitative attachment classifications in brown capuchin mother-offspring pairs. Source: Weaver and deWaal (2002).

For simplicity in the following analysis, values above 1 are taken to indicate more ‘affiliative’ maternal styles (more protective behaviours and social interaction), and values below 1 describe more ‘anti-social’ mothering styles (e.g. tendency to ignore their pups or spend more time doing other activities). Index values describing estimated maternal condition for the same sub-set of females were calculated by the author using the morphometric method (described in full detail in *appendix 4*). Table 1 summarises the maternal condition and mothering style index values derived for a sample of eight females from Donna Nook. For reference, three mothers in this sample were located at the publicly accessible site and five at the private RAF site (see chapter 1), thus reducing bias from only focussing on individuals from one kind of breeding habitat.

(table 1 below)

Table 1: Index values for maternal condition and mothering style (MORQ scores) for focal females from the 2011 breeding season at Donna Nook*. Higher maternal condition index values indicate poorer condition mothers (ẋ = 4.81, n=17). MORQ index values are shown for early and late lactation phases and were derived following observation of random video focals of identified females.

		MORQ Index Value	
Mum ID	**Condition Index Value**	**EARLY**	**LATE**
P16	**4.95**	0.18	2.29
R1	**5.33**	0.21	5.12
R13	**5.12**	0.22	0.04
P26	**4.45**	0.53	0.79
P21	**4.72**	0.99	0.58
R5	**4.78**	1.12	0.3
R7	**5.29**	2.01	0.96
R14	**4.82**	4	0.58

**MORQ values from MSc work conducted by HCJ, Durham University. Note: The condition of female 'R7' was derived from a photograph in late lactation (pup stage 3). The rest were derived from images taken in the early lactation phase (pup stages 1 or 2).*

From the index values shown in table 1, it was found that females in poorer condition had a mean MORQ index value of over 1 in both early and late lactation phases, indicating more affiliative maternal styles; whilst females in better condition had a mean MORQ index value below 1 in both lactation phases, indicative of more avoidant mothers (Mean MORQ ± SE. Early lactation: *Above*= 0.88 ± 0.18; *Below* = 1.32 ± 0.75. Late lactation: *Above*= 0.56 ± 0.14, *Below* = 1.80 ± 0.91; figure 5). Despite the trend for a higher mean MORQ index score for poorer condition mothers, no significant differences were identified between the two groups in either early or late lactation phases when a conserved two-way randomisation procedure was conducted- owing to small sample sizes (*Early*: Test statistic=0.44, *P*=0.78; *Late:* Test statistic= 1.24, *P*= 0.42; figure 7).

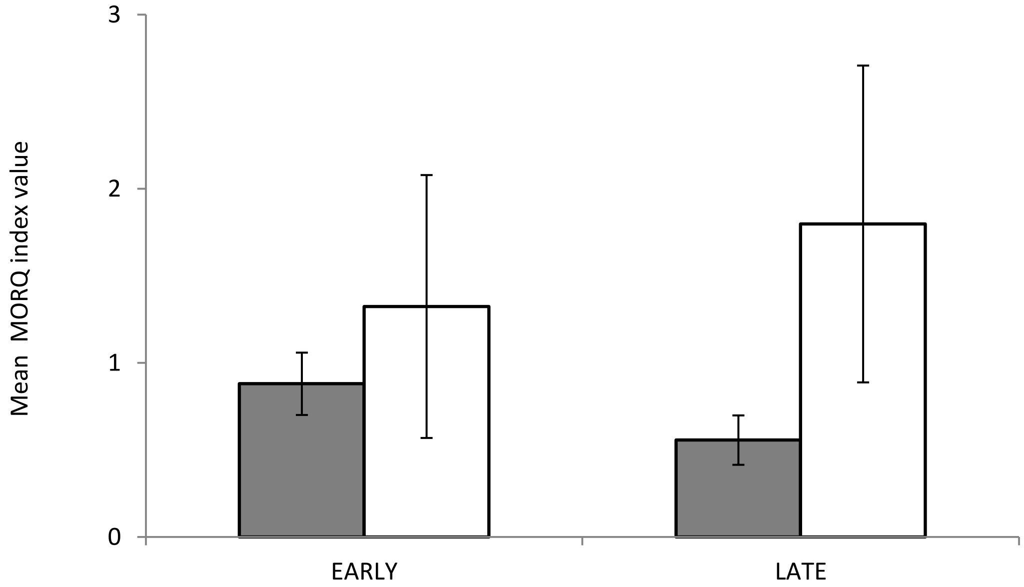


Figure 7: Mean (±SE) MORQ indexes of females (derived from *random* video focals) in relation to their relative body condition. Dark grey bars: *Above*-average condition (n=3), White bars: *Below*-average condition (n=5).

Individual MORQ scores were then averaged across early and late lactation phases (see table 2) and a randomised regression was conducted to investigate the potential relationship between mean MORQ scores and maternal condition index values. The test statistic is the absolute value of the slope of the fitted line, and the test performs 2000 randomisations to calculate levels of significance. This analysis did not reveal a strong association between the two, with more affiliative mothering styles (MORQ index values >1) not found to be significantly associated with decreased relative body condition and vice versa (Randomised regression: b= 1.26, *P*= 0.29; figure 8).

Table 2: Shows the maternal condition index values and mean MORQ (mothering style) index values, derived from averaging individual MORQ scores from early and late lactation. Green: above-average condition; Red: below-average condition.

Mum ID	Maternal Condition Index	Mothering Style Index
P26	4.45	0.66
P21	4.72	0.79
R5	4.78	0.71
R14	4.82	2.29
P16	4.95	1.24
R13	5.12	0.13
R7	5.29	1.49
R1	5.33	2.67

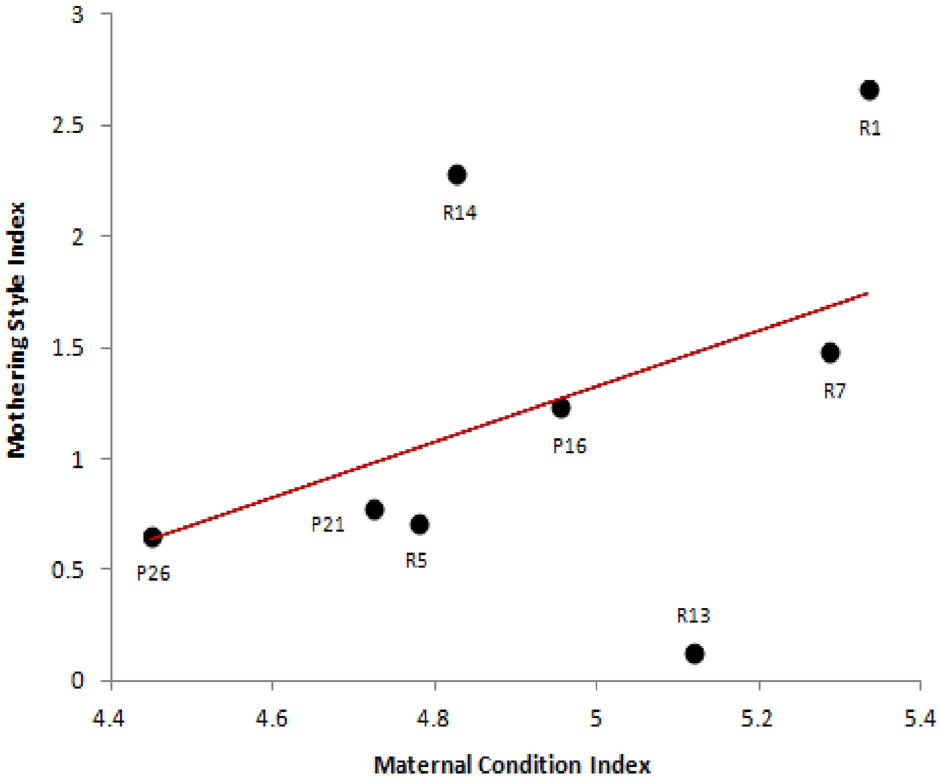


Figure 8. Scatterplot of estimated maternal body condition and mean mothering style index scores (MORQ scores averaged over early and late lactation). Red line represents line of best fit.

From these preliminary results there is consequently insufficient evidence to suggest a confident link between a female's body condition and her mothering style. However, this analysis is likely to have been hindered by low sample size and lack of statistical power. The author feels that this line of inquiry, based on sound biological reasoning (see *section 1.4*), may be worth pursuing in future with research based on a greater number of individuals.

As a further point, the methodology used to estimate maternal condition has not yet been validated with absolute measures of body condition in the field (see further discussion in *section 2.2*), and therefore future work in this area would also benefit from a more direct, independent assessment of female condition to improve reliability. It may also be reasoned that there were no 'extremely poor condition' females in this study, since mothers in such condition would be unlikely to attempt to breed, or if they had, their pups would have been more vulnerable to premature death during the lactation period and in which case would not have been included in the study. It therefore seems that the 'poorer condition' females in this study are more akin to the 'average' mothers as described in Fairbanks and McGuire (1995). Sampling a larger sample of females in future investigations would therefore facilitate the categorisation more finely, in order to investigate whether maternal care is an "inverted U-shaped function" of maternal condition as suggested by Lee *et al.* (1991).

3.1. Consistent individual differences in neonatal play.

3.1.1. North Rona.

Significant individual repeatability in independent types of play across lactation was found and reported in chapter 2 (*Exploratory/Object-0riented*: ICC= 0.37, 95% Cl= 0.08-0.60, $F_{43,43}$= 2.16, *P*= 0.007; *Individual locomotor play*: ICC= 0.43, 95% Cl= -0.07-0.69, $F_{27,27}$= 2.51, *P*= 0.01). However, it should be acknowledged that these results were no longer significant following the application of the Bonferroni correction (lowered *alpha*-level= 0.006, 3d.p), and further work pertaining to the North Rona colony would be instructive to confirm these results.

Some interesting results were also found from these analyses when the repeatability of male and female behavioural traits was assessed separately. Interestingly, a significant result was found regarding the repeatability of *individual locomotor play* ("solitary play", chapter 2) in male pups (ICC= 0.80, *P*=0.003; see chapter 2; table 5) compared to female pups (ICC= 0.25, *P*=0.16). In terms of play function, indication of consistent inter-individual differences in *individual locomotor* play in males may be seen as indirect support for previous conjecture: namely, that in male pups who consistently engage in more independent play, the primary function of play may be to enhance physicality and strength (*motor-training* hypothesis). However, if the neonatal stage represents a physical and/or cognitive sensitive phase, then it is unclear why some male and female pups would consistently express *less* independent play relative to others. We are then led to the concept of different "strategies" amongst pups, whereby pups that do not play as much in comparison to others presumably gain equal benefits from reserving energy and enhancing pre-weaning body condition levels (see chapter 2, *section 4.3.3* for discussion). Although this latter point assumes that time not spent playing is spent resting or acquiring milk, and not in another energy-requiring behaviour. To test this theory, it would be insightful to conduct more long-term studies exploring individual variation in neonatal play in relation to fitness cues.

3.1.2. Donna Nook.

When the individual consistency of different types of play was assessed between early and lactation phases at Donna Nook, significant repeatability was not found for either *independent* or *social* play types (Independent: *Individual locomotor*: ICC= 0.11, 95% Cl= -0.43-0.59, $F_{13,13}$= 1.25, *P*= 0.346; *Object*: ICC= 0.02, 95% Cl= -0.50-0.53, $F_{13,13}$= 1.05, *P*= 0.466; *Social*: ICC= 0.09, 95% Cl= -0.45-0.58, $F_{13,13}$= 1.2, *P*= 0.375). However, interestingly, when all types of play were combined for individuals, significant repeatability was found (ICC= 0.66, 95% Cl= 0.22-0.88, $F_{13,13}$= 4.89, *P*= 0.004; figure 9). One interpretation of these findings may be that: although pups tend to spend consistent amounts of time playing relative to one another, the relative proportions of different play types in the repertoire may change over time.

Indeed, this would be consistent with previous discussion of differential developmental trajectories of different play types.

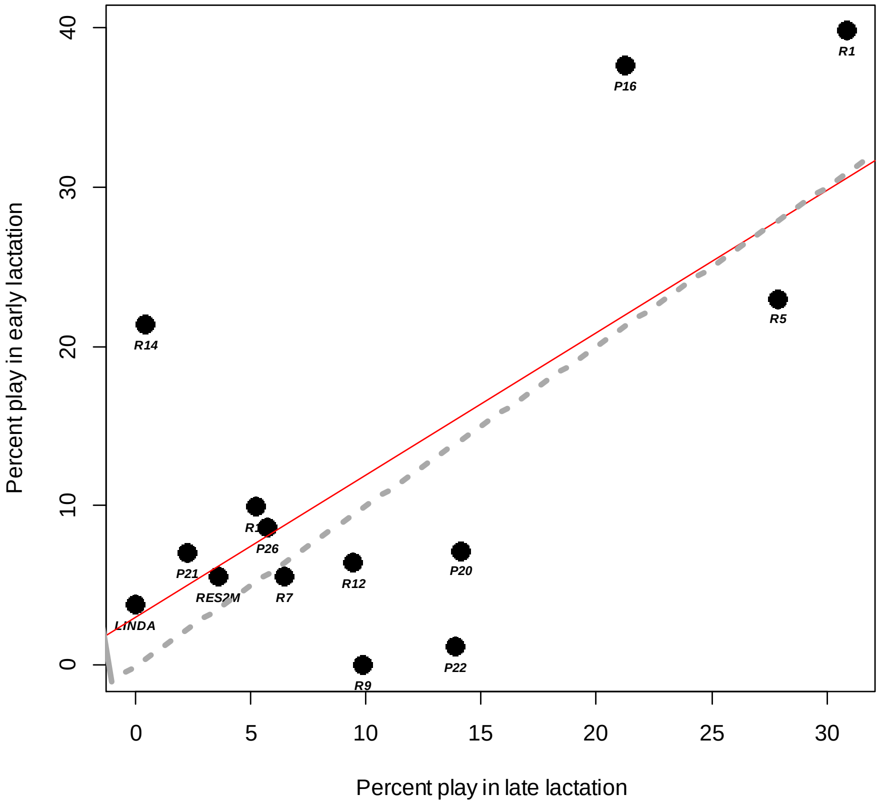


Figure 9. Percent time spent in 'combined play' (individual locomotor, object and social) **behaviour of grey seal pups in early and late lactation phases during the 2011 breeding season at Donna Nook.** Graph shows 1:1 line (----) and line of best fit (red).

The fact that indication of inter-individual behavioural consistency in play has been inferred from two contrasting colonies, using differing sampling methodologies, suggests that play may represent an intrinsic behavioural axis in this age-class. It is notable however that significant repeatability in independent play types was not found in neonates at the Donna Nook colony like at North Rona. Such differences may be the by-product of varying sample sizes and sampling methodologies between the two sites. Indeed, at North Rona, behavioural data from multiple breeding seasons was available for analysis thus increasing the sample size considerably compared to Donna Nook. By contrast, data from just one season was available from Donna Nook with a sample size of 14 neonates. In addition, neonates included in analysis from North Rona had over 200 behavioural scans (made using an instantaneous sampling regime in the field, with 5 minute intervals) conducted on them in both early and late lactation phases, compared to one focal observation video in each phase (restricted to approximately 30 minute durations) at Donna Nook. For future studies therefore, it would be advantageous if similar methods were adopted across colonies in order to increase confidence in analytical comparisons. Nonetheless, these preliminary results are still intriguing and suggest that variation in neonatal play behaviour is worthy of further investigation.

It is not possible to say whether individual differences in play behaviour persist into later ontogeny, since only data relating to pre-weaned neonates was collected and analysed. Indeed, there is evidence to suggest that levels of play in infancy may not indicate stable individual tendencies to later ages. For example, in a study of domestic cats, Barrett and Bateson (1978) found that kittens that played more in the 4-7 week period after birth were not those that played more in the 12 week period. The authors suggested that perhaps that the various facets of play come increasingly under different types of control over ontogeny, and that the *weaning event* may affect play behaviour. This illustrates the importance of further investigation over a larger temporal scale to investigate this fully in grey seal infants, post-weaning.

4. Recommendations for future research.

4.1. Scan sampling or focal sampling?

Behavioural data from the North Rona and Donna Nook breeding colonies were collected using contrasting sampling approaches: scan-sampling and focal-sampling, respectively (Altmann, 1974). It may be reasoned that the most accurate records of play are likely to be derived using a focal observational approach, since behavioural categorisation may be more reliable owing to full concentration on one animal at a time, in combination with complete contextual information. For example, whilst it would be easy to miss-classify a *comfort move* for *individual locomotor play* when using an instantaneous/scan sampling regime, this would be more difficult to do using a focal-sampling approach. The trade-off is that detailed observations (from focal sampling) can be made for a few individuals, but a large sample size is more difficult to achieve. If scan sampling were to be used in future to conduct further study of neonatal play, changes to the regime could be made to enhance the reliability of the collected data. For example, using a smaller interval time may help to reduce contextual ambiguity, thus reducing miss-categorisations and increasing the behavioural data collected per individual. Other studies of grey seals have adopted shorter interval periods (e.g. Kovacs, 1987: 30 seconds; Smiseth and Lorentzen, 1995: 30 seconds and 60 seconds).

If focal sampling were to be used in future investigations, it would be advisable to collect multiple focal videos per individual where possible. Indeed, focal footage used for analyses from the Donna Nook colony was limited in terms of the frequency that individual pups were filmed during their lactation period. The majority of pups in the study had two random focal videos collected on them during their lactation period, culminating in approximately one hour's worth of footage. Out of a 16-18 day lactation period, this is therefore a small amount of time from which to make conclusions about an individual pup's behaviour and may not be wholly representative. To increase confidence in results it would therefore be essential to systematically sample individuals more frequently during the lactation period, in order to get a more representative sample of their behaviour during this time.

4.2. Estimating maternal condition in wild grey seals.

As discussed in chapter 4 and this final chapter, there is indication that the pups of mothers in poorer condition engage in more social play. However, the main limitation of these intriguing preliminary results is that the morphometric method used to assess maternal condition from in-situ photographs and still images (see *appendix 4* for full methodology) has not been validated. Relative maternal condition was estimated using a morphometric technique, whereby the index values derived reflected the relative relationship between maximal body length and depth. This method needs to be validated to see if variation in these index values provides an accurate portrayal of true variation in relative body fat stores at the start of lactation (=true body condition). This would only be possible at breeding colonies where licences are held to handle animals, such as North Rona or the Isle of May. If a significant correlation was found between morphometric index values and true measures of body condition then this would give confidence to this 'hands-off' method, requiring only for photographs of appropriately-oriented females to be taken at consistent points in their lactation period.

In chapter 3, the proxy for maternal condition was estimated post-partum mass. However, there may be dispute as to the relevance of this measure to reflect this parameter. One of the most commonly derived indices for body condition is the 'mass-size residual'. The basic premise with this method is to determine the mass of an individual *relative* to its body size, thereby gaining residuals from a regression of body mass on a linear measure of body size, such as body length (Schulte-Holstedde et al. 2005). There has previously been criticism of this technique, owing to the suspected violation of assumptions, however a recent investigation by Schulte-Holstedde et al. (2005) found no reason to reject this method as a legitimate means of deriving indices of body condition. Therefore, a better way of estimating maternal condition may be to derive such mass-size residuals, if data regarding female body length is also available in conjunction with mass data. Mass-size residuals may be a more accurate description of body condition, but it would also be important to validate that such residuals accurately describe true body composition, namely fat stores.

Therefore, if further investigation of maternal body condition were to be undertaken at appropriate colonies, it would be advisable to calculate mass-size residuals *in conjunction* with body composition experiments as a means of estimating body composition. Body composition (fat-free mass and fat mass) can be calculated from body mass and total body water with an assumption as to the hydration of the fat-free mass, and thereby can give an accurate description of body condition (fat reserves). The water turnover technique can be used to assess body composition, using 'doubly labelled water' (Westerterp, 1999). Such experiments are already conducted at North Rona, and past data is available for known females, making this a possibility. The results of such experiments could be used to validate mass-size residuals, and if appropriate photographs were also available of the same individuals, the morphometric method could also be validated in turn.

There is also the possibility of using laser technology to obtain accurate measures of individual body length and depth in the field. In short, it is possible to fit a camera with a laser device so that photographs can be taken of focal animals with two laser points observable at a fixed, known distance apart. This technique could therefore be used to make accurate morphometric measures and obtain condition indices, as used in chapter 4 of this thesis. This also benefits from being a ‘hands off’ approach, thus causing minimal disturbance to the study animals. Some preliminary photographs incorporating laser technology have been collected on adult females on North Rona (2012 breeding season; Luke O’Connor and Dr. Paddy Pomeroy, Sea Mammal Research Unit) for which additional measures (including absolute mass and body length) were also taken. Therefore, an integrative study is possible to validate the described laser method, which could prove highly useful in future research.

4.3. Consideration of play in different contexts.

There is suggestion within the literature that the expression of play may differ between contexts. For example, Palagi et al. (2004, 2006) found that the frequency of *social* play in chimpanzees was highest in pre-feeding contexts than any other. In chapter 4 of this thesis, behavioural data were collected from ‘random focals’, and within these, *nursing* generally did not occur. It would therefore be instructive to conduct similar observations and analyses using nursing focals, particularly for investigation of the effect this may have on the occurrence of *social* play. It may be expected that social play would be enhanced in nursing contexts (as found by Palagi et al. 2004, 2006), since nursing requires the mother and pup to be in close proximity, which in turn may encourage bouts of *social* play. Aspects of *mothering style* may also have an indirect effect here; mothers who nurse more create ‘nursing contexts’ more often, which in turn may lead to greater expression of social play between the mother-pup pair. Indeed, MSc research by Hani James (Durham University, 2012) has investigated such conjecture from the Donna Nook colony. Further, as mentioned previously, it would be insightful to continue investigation of neonatal play into the post-weaning context, since the expression of certain types of play may be hypothesised to change in the absence of the maternal figure (see *section 1.1*).

4.4. The key to progression: long-term research.

A useful next-step would be to investigate the fitness consequences of variation in play behaviour, in order to test the theory presented in this thesis as to its potential function(s). This requires investigation of individuals beyond the lactation period. For example, Cameron et al. (2008) conducted a relatively long-term investigation of foals in relation to play behaviour and found that those that survived the first 110 days of life but died before the age of 1 year engaged in less running and play-fighting during their first 110 days compared to foals that survived beyond 1 year. Differences in play behaviour also translated into differences in condition as a yearling, with foals that played more during their first 110 days of life achieving better condition than those that played less. Therefore, it would be insightful to investigate the fitness consequences of play in grey seals; this could at first be achieved by assessing short-term fitness proxies such as mass at weaning and first year survival.

Further, from an ontogenetic perspective this study was limited. Discussion of how the nature and function of play may change over time was largely speculative as a result, since only data pertaining to a small developmental window in the life history was investigated. However there is suggestion in the literature, mirroring the feeling of the author, that the prevalence of play (particularly *social* play) increases with age and peaks in the juvenile period before declining thereafter. Indeed, Palagi et al. (2004) notes that: "…*the juvenile phase is a period of rapid and profound changes involving physical, hormonal, and behavioural processes, and play has certainly a particular function during this crucial transitional stage*". It would therefore be interesting to investigate play in a cohort of individually identified grey seals through a greater proportion of their life history: from birth to beyond. This would necessitate a large effort of time and resources however, requiring tagging and photo-identification to be conducted at key sites over successive breeding seasons. Such a long-term comparative study of play would also be valuable for improving understanding of the true adaptive significance of this behaviour by assessing its functionality in a more holistic manner.

5. Preliminary inferences of thesis.

- Neonatal grey seal pups spend the majority of their time inactive during their lactation period, reflecting the short duration/high investment strategy adopted by this phocid species to raise offspring to weaning.

- On average, the proportion of time spent in energy-consuming behaviours decreases over lactation, whilst time spent acquiring milk and resting increases.

- Pre-weaned neonates show consistent inter-individual differences in some behavioural traits. However, average repeatability scores are lower than those found in adults, suggesting that behavioural consistency may increase with age in this species. It is also unknown as to how stable such variation is over time.

- There is no strong suggestion of a direct link between nutrition (milk-provisioning) and independent play behaviour in this species.

- When maternal and genetic effects are moderately controlled for, there is indication of a difference between the independent play behaviour of sons and daughters in relation to maternal condition. (Although sibling analyses over different breeding seasons is recommended for future research to limit concerns regarding behavioural miss-categorisation and increase confidence in results).

- Daughters of mothers in better condition were found to partake in more independent play than sons, which may reflect a cognitive role of play for training information processing systems in relation to environmental cues. This is reinforced by a higher proportion of *object*-oriented play in female independent play budgets.

- Sons of mothers in poorer condition were found to express more independent play than daughters, which may indicate a physical-enhancement role of play. This is reinforced by a higher proportion of *individual locomotor* play in male independent play budgets.

- It is proposed that '*sexual dimorphism in play*' will increase over time, and that play may increasingly become male-oriented and dominated by *social* play (specifically ‘play-fighting’) in training for intra-sexual competition in later life.

- In broad terms there is some weak inference that different types of play may have different developmental trajectories over the lactation period, with a decrease in *independent* play and relative increase in *social* play. However, these results were not supported statistically (with acknowledgement of limited sample size and possible sampling effects), and future work in the area would be instructive.
- In the initial post-weaning phase, *individual locomotor* play is speculated to show a secondary increase in wake of the mother’s absence, with an associated drop in *social* play.
- Pups of mothers in poorer condition engage in more *social* play compared to pups of mothers in better condition.
- Further investigation of a larger sample of females is required to assess the possible relationship between maternal condition and mothering style more reliably.
- It is essential that the ‘morphometric method’ used to estimate relative female body condition is validated using absolute body condition data, in order to increase confidence in using this metric in analyses.
- Longer-term research is essential for answering outstanding questions, including: how developmental trajectories change in the post-weaning phase; whether sex differences in play increase with age; how long individual behavioural variation persists; and associated fitness consequences of behavioural differences.

END

APPENDIX 1

R-code used to derive time-activity budgets from raw scan-sampling data:

1. Read scan-sampling data file into R:

```
rawscans<-read.csv(scansample_file.csv)
```

2. Explore data and attach so file headings recognised:

```
summary(rawscans)

head(rawscans)

View(rawscans)

attach(rawscans)
```

3. Create new data frame (“scansum”) detailing the number of scans that individual neonates spent in each behavioural state:

```
scansum <- tapply(Behaviour,list(IDCODE,Behaviour),length)
```

4. Remove last column (“OOS”= out of sight), so that derived activity budgets only describe time when neonates were in-sight and call new data frame “scansact”:

```
lastcol <- dim(scansum)[2]

scansact <- scansum[,-lastcol]
```

5. Calculate the number of scans (nscans) per individual neonates:

```
nscans <- rowSums(scansact)
```

6. Calculate the proportion of time spent in each behavioural state (scansprop):

```
scansprop <- as.data.frame(prop.table(scansact,1))
```

7. Convert this to a percentage value:

```
scansprop <- (scansprop[,1:(lastcol-1)]*100)
```

8. Add a column detailing the number of scans to this (scansfin):

```
scansfin <- cbind(scansprop,nscans)
```

9. Check time-activity budgets add up to one:

```
rowSums(scansprop)
```

10. Download the finalised dataframe so it is available for further analyses:

```
write.csv(scansfin, file= "activitybudgets.csv")
```

APPENDIX 2

Table 1: Summary of focal observations collected for neonatal grey seal pups at the Donna Nook breeding colony, Lincolnshire, in the 2011 breeding season, including pup sex where known, date focal collected on, pup stage and focal duration (hours: minutes: seconds**).**

		Focal 1			Focal 2			Focal 3		
Pup ID	**Pup sex**	**Date**	**Stage**	**Duration**	**Date**	**Stage**	**Duration**	**Date**	**Stage**	**Duration**
P16	2	10-Nov	2	00:38:36	16-Nov	3	00:38:06			
P20	1	12-Nov	1	00:38:11	18-Nov	3	00:37:52			
P21	na	12-Nov	2	00:37:39	15-Nov	3	00:37:58			
P22	na	14-Nov	1	00:38:40	23-Nov	3	00:37:05			
P26	2	16-Nov	1	00:38:23	29-Nov	3	00:20:11	05-Dec	4	00:36:04
LINDA	1				29-Nov	2	00:18:02	09-Dec	4	00:23:51
P36	2	29-Nov	3	00:30:32	03-Dec	4	00:37:34			
PR1	na	05-Dec	3	00:35:00						
R5	na	11-Nov	2	00:34:54	21-Nov	4	00:28:41			
R9	2	11-Nov	1	00:34:54	21-Nov	3	00:34:37			
R7	1	11-Nov	2	00:30:00	15-Nov	3	00:30:00			
R1	na	11-Nov	2	00:39:09	17-Nov	3	00:36:59			
R11	na	13-Nov	2	00:37:04	19-Nov	3	00:36:50	27-Nov	4	00:33:15
R12	na	13-Nov	2	00:36:39	17-Nov	3	00:30:00			
R14	1	13-Nov	1	00:34:00	19-Nov	3	00:30:00	27-Nov	4	00:36:06
R13	na				19-Nov	3	00:39:23	27-Nov	4	00:31:36
R26	2				04-Dec	3	00:38:04	08-Dec	3	00:39:42
RES2M	1	02-Dec	2	00:33:56	06-Dec	3	00:34:53	10-Dec	3	00:34:48

Nb. in some cases focal videos were terminated prematurely during decoding due to disturbances during the video or if pup behavioural state had not changed for > 30 minutes- i.e the focal pup was asleep. Pup sex: 1= male, 2= females*

APPENDIX 3

Exploring an Approach to Efficiently Describe Intraspecific Behavioural Variation

1. INTRODUCTION.

1.1. Principal Components Analysis in behavioural research.

Gosling (2001) highlights that predominantly in animal personality research only one or a few limited behaviours are studied to represent individual 'personality' (see chapter 2 for definition), and often in a specific context. It would therefore be beneficial to develop more robust measurements of individual personality from a larger range of behaviours, and combine these with analytical techniques to investigate broad personality factors, such as has been achieved with human research using factor analyses (e.g. Wilson et al.1990).

Principal Components Analysis (PCA) is the most common form of factor analysis and is used to assess the relationships within a single set of interdependent variables, regardless of any relationships they may have to variables outside of the data set (McGarigal, Cushman, Staffrord, 2000). The main purpose of PCA is to condense the information contained in a large number of original variables into a smaller set of new composite dimensions, with a minimum loss of information: it does this by reducing the original dimensions of the data set, where each dimension is defined by one variable, into fewer dimensions, where each new dimension is defined by a linear combination of the original variables (McGarigal, Cushman, Stafford, 2000). This thereby allows one to describe the variation in the data in a more economic and efficient way (Bardi and Huffman, 2006). The new dimensions derived are called 'principal components'. The principal components explain all the variance in any particular correlation matrix, with principal component 1 (PC1) accounting for the most variance, and subsequent components account for a decreasing proportion of the variance (Budaev, 2010).

In short, PCA takes a set of correlated behaviours and to find a linear combination of these to produce indices that are uncorrelated. The lack of correlation is a useful property of the new set of variables because it indicates that they are measuring different ‘dimensions’ in the data (Bardi and Huffman, 2006). As a consequence, PCA is frequently used in animal behaviour research as a means of reducing numerous measures to a small set of the most important summary scores (Budaev, 2010). By analysing multiple variables (behaviours) representing a given trait, this will lead to a more robust understanding of how the traits relate to other factors, for example, environmental elements. Also, by assessing a range of behaviours, it will be possible to assess what behaviours change within this factor and what stay the same as the individuals are influenced by experience and maturation (Putnam, 2011). Therefore potentially a useful tool for studying the ontogeny of behaviour. Putnam (2011) also points out that those aspects of behaviour that indicate the presence of an attribute in young of a species may not necessarily be the same as those that reflect the attribute in the adult. Therefore, cross-sectional, longitudinal approaches would be helpful in identifying ‘immature tendencies’ that shift in *form* over ontogeny in non-human studies.

1.2. Caveats of PCA in behavioural research.

There are a number of caveats to bear in mind when conducting PCA, summarised in a review by Budaev (2010). Firstly, there is debate regarding the appropriate sample size for this kind of analysis. There are arguments that the smaller the sample size, the larger the standard error of the correlations and therefore the more error in the factor analysis (Crawley, 2007), with some suggestion that sample sizes of less than 100 could produce misleading results. However, there is also argument that a minimum of a 3:1 ratio of *sample size: number of variables* is acceptable (Budaev, 2010). The author also highlights that it is essential to conduct measures of the ‘sampling adequacy’ of the correlation matrices used for factor analysis or PCA, something that is often either not conducted or not reported in papers using the technique. Two tests are suggested: the Bartlett Sphericity test, which tests whether all the correlations are zero, and the Kaiser-Meyer-Olkin test, which compares the observed correlations and partial correlations among original variables. In order to proceed with analysis, the null hypothesis of the Bartlett test must be rejected, and KMO value typically higher than 0.5. When considering which loadings are interpretable, again there is debate. Most textbooks recommend 0.3. or 0.4 as a cut-off, however there is argument that these are based on a large sample size and that when sample size is small, minimal interpretable loadings should be higher, possibly 0.5+. There are consequently many factors to consider before firm conclusions are drawn, and when reading the literature.

1.3. Uses of PCA to behavioural ecologists.

The main benefit of reductive techniques such as PCA to behavioural ecologists is arguably the ability to describe individuals more efficiently. For example, there is a well-established index within primatology, called the 'Gorilla Behavioural Index' which is a subjective assessment technique developed by Gold and Maple (1994). They conducted subjective assessment for 298 of 303 captive gorillas over 1 year of age, using over 20 behaviourally-based adjectives to describe individuals. The results were subjected to common factor analysis, which resulted in behaviours loading onto 4 'personality' factors: 'Extraversion', 'Dominant', 'Fearful', and 'Understanding'. This demonstrates the benefits in personality research of being able to reduce multiple behavioural traits into broader descriptors which encapsulate the individual behavioural variation exhibited by an animal. Bardi and Hoffman (2006) used PCA to cluster infant macaque behaviours into broader descriptive categories. Three categories of infant behaviour were derived in this way: 'anxiety', 'independence' and 'interaction'. The components were then used to assess the association of maternal attributes on infant behaviour. Bardi and Huffman (2002) used PCA to extract a smaller number of significant components from 8 measures of maternal-infant interactions. Data reducing techniques are also prominent outside of primate studies, with PCA used in studies of other taxa to derive personality descriptors from multiple behavioural traits. For example, Rodel and Meyer (2011) conducted a series of behavioural tests on rats and PCA was used to derive three new variables that could be interpreted as 'boldness', 'exploration' and 'anxiety' from the multiple behavioural responses recorded- specifically, these were derived from correlations between the different behavioural variables measured in the different tests. This study was able to show that distinct behavioural types existed that were found to be consistent within and across contexts, and further that features of early development affected the ontogeny of personality types in the rats. This study therefore demonstrates that PCA is a valid approach for studies of the ontogeny of personality. Lowe and Bradshaw (2001) conducted behavioural observations on domestic cats and recorded behaviours as frequencies. The authors conducted PCA on the correlation matrix of the square root transformed frequencies, and from doing so, were able to derive ‘unifying’ behavioural descriptors from the principal components (referred to as ‘elements’), which they termed ‘Staying Indoors’, ‘Rubbing’, ‘Investigative’ and ‘Boldness’. Like Rodel and Meyer (2011), consistency in individual scores on the identified elements was assessed over time, using Spearman rank correlations. Individual cats were found to score consistently on these elements between ages. Both of these studies also then used the derived individual scores on the principal components as response variables in further multivariate tests, in order to assess factors potentially affecting the development of personality in their respective study organisms. Rodel and Meyer (2011) used a multivariate linear mixed-effects model to conduct their analyses, using the R package Ime4.

These studies show that PCA is a very useful technique in behavioural research and could be used to derive more robust descriptions of personality differences in grey seal pups. Further, such analysis could provide fewer behavioural metrics to be used as response variables in mixed effect models, in order to assess the influence of socio-environmental factors on behavioural development during early ontogeny. The aim of this study was to explore the potential of PCA for reducing the dimensionality of time activity budget data in grey seal pups, in order to describe individuals more efficiently; that is combine several related behavioural measures into a single score.

2. METHODS

2.1. Behavioural Data.

Behavioural data for individual grey seal pups were collected in the way described in chapter 1 from the island of North Rona over three successive breeding seasons. The time activity budgets of individual pups were calculated for the 2008, 2009 and 2010 breeding seasons respectively. The individual activity budgets describe the percentage of time spent in each of the behavioural categories whilst in sight. The data were filtered so that pups with fewer than 200 scan samples conducted on them were dropped from analysis, as this was seen as the minimum requirement for accurate behavioural representation (based on Twiss, 1991).

2.2. Statistical approach.

We created new variables on the basis of different clusters of pup behaviour budgets, in order to reduce the number of behavioural variables used to describe pups and to get a more accurate measure of different individual ‘emotionality’ traits by taking multiple behaviours into account. All analyses were done using the software ‘R’ version 2.14.1. Single principal component analyses (PCA) were carried out on the correlation matrices of the activity budget data from all pups, for each breeding season respectively. R has two pre-installed functions for performing PCA: prcomp() and princomp(). Both of these commands expect data to be organised with variables in columns and observations in rows, and data is returned in this format too. Princomp() performs PCA using eigenvectors, whereas the prcomp() calculation is done by a singular value decomposition of the data matrix (Crawley, 2007). According to R help, this technique has slightly better numerical accuracy, and so this function was chosen in preference for these analyses. The prcomp() function returns the results as an object of prcomp, with information being returned via a selection of commands, including the ‘rotation’ command (which returns the matrix of variable loadings on the principal components), and the ‘x’ command (which returns the rotated data, the princomp() equivalent is ‘scores’) which retrieves the individuals’ scores on each principal component. Tables 1-9 in the appendices show the raw correlation matrices from which analyses were conducted.

In order to measure the adequacy of correlation matrices for the PCA analysis, two tests were performed using R: the Bartlett Sphericity Test, conducted using the cortest.bartlett function (package: Psych, R development Core Team 2008), and the Kaiser-Meyer-Olkin (KMO) measure, conducted using functions available with the R package: MASS. A scree plot approach was taken to decide how many factors to extract from analysis, and since the amount of variance explained by factors dropped significantly after the first two components in all cases, two factors were decided upon.

Within the literature, suggestions vary as to the minimum PCA variable-loading size appropriate for interpretation. For this study, the minimum acceptable loading value was 0.3+ (this being seen as a 'moderate' loading, Crawley, 2007), although it is noted that there are arguments that with low sample sizes only loadings of 0.5+ are appropriate (Budaev, 2010).

3. RESULTS

Tables of the raw correlation matrices used in analyses can be provided upon request, as can tables showing the full array of variable loadings on the first three principal components for each breeding season. Tables 1a-c show the results of PCA analysis for each breeding season and also summarise the results of tests performed to investigate the suitability of the data for these analyses.

Table 1a-c. Summary of PCA results for the 2008, 2009 and 2010 breeding seasons, including results of Bartlett Sphericity and Kaiser-Meyer-Olkin (KMO) tests: a) All behaviours, b) Rest behaviour excluded, c) Behaviours reflecting pup activity only.

(*continued below*)

a) Total variance explained by PC1 and PC2: 2008: 83.3%, 2009: 85.1%, 2010: 85.7%.

	2008		2009		2010	
	PC1	*PC2*	*PC1*	*PC2*	*PC1*	*PC2*
Alert						
Comfort Move				0.55		0.67
Explore		0.39				
Locomotion						
Mum interaction	-0.33	-0.42				
Milk	-0.39		0.45	-0.71	0.3	-0.68
Play		0.64				
Rest	0.84		-0.86		-0.9	
Proportion of total Variance	**58.10%**	**25.20%**	**68.70%**	**16.40%**	**74.69%**	**11%**
BARTLETT	<0.05		<0.05		<0.05	
KMO	0.13		0.09		0.08	

2008: N= 21, 2009: N= 28, 2010: N= 23.

b) Total variance explained by PC1 and PC2: 2008: 73.35%, 2009: 85.1%, 2010: 66.03%.

	2008		2009		2010	
	PC1	*PC2*	*PC1*	*PC2*	*PC1*	*PC2*
Alert				0.38		
Comfort Move				0.89	0.35	0.86
Explore	-0.37					
Locomotion						
Mum interaction	0.58					
Milk	0.51	0.62	-0.95		0.87	-0.38
Play	-0.43	0.75				
Proportion of total Variance	**55.72%**	**18.45%**	**51.80%**	**21.55%**	**39.20%**	**27%**
BARTLETT	<0.05		<0.05		0.62	
KMO	0.72		0.39		0.42	

2008: N= 21, 2009: N= 28, 2010: N= 23.

c) Total variance explained by PC1 and PC2: 2008: 79.3%, 2009: 72.77%, 2010: 80.08%.

	2008		2009		2010	
	PC1	*PC2*	*PC1*	*PC2*	*PC1*	*PC2*
Alert			0.42	0.53	0.33	0.9
Comfort Move	0.36		0.88	-0.34	0.92	-0.33
Explore	-0.45	-0.76				
Locomotion				-0.61		(-0.28)
Play	-0.8	0.57		-0.44		
Proportion of total Variance	**59.40%**	**19.90%**	**48.60%**	**24.17%**	**52.93%**	**27%**
BARTLETT	0.04		0.008		0.24	
KMO	0.72		0.44		0.36	

2008: n= 21, 2009: n= 28, 2010: n= 23.

4. DISCUSSION

4.1. Is PCA a successful technique for reducing the dimensionality of time-activity budget data?

4.1.1. Analysis including all behavioural traits.

For the analyses presented, there are problems associated with measures of sampling adequacy of the raw correlation matrices used. The Bartlett's sphericity test investigates whether all correlations in the matrix are zero (Budaev, 2010) and therefore tests the null hypothesis that the correlation matrix is an identity matrix (a matrix in which all of the diagonal elements are 1 and all off diagonal elements are 0). In order to validate PCA analysis, this null hypothesis needs to be rejected. However, Budaev (2010) points out that sometimes this test rejects the null hypothesis even when the correlation matrix is ill-conditioned and recommends therefore that this test is used as a lower bound to the quality of the matrix. Use of the Kaiser-Meyer-Olkin (KMO) test is therefore also strongly recommended to complement the former. This test of the adequacy of the correlation matrix compares the observed correlations and partial correlations among the original variables (Budaev, 2010), with values of 0.5+ required for satisfactory factor analysis to proceed. In summary, the correlation matrix is appropriate for PCA if the hypothesis of all 0 correlations is rejected and KMO significantly exceeds 0.5. Whilst the null hypothesis of the Bartlett test was rejected in all breeding seasons, KMO values for all years were however substantially less than 0.5 (table 1a). This therefore suggests that the correlation matrices used for these analyses were not appropriate, and thus reduces the confidence that can be had of the results and conclusions derived. With these caveats in mind, a brief discussion of the results will be given.

In all breeding seasons, it was found that ‘rest’ behaviour loaded highly onto the first principal component derived (>0.8 in all cases). In 2008, PC1 contrasts a high loading of ‘rest’ with loadings of behaviours associated with maternal interactions (including milk transfer), whereas PC2 contrasts high levels of play and exploratory behaviours (proxies of independent behaviour) with mum interaction (less independent). Similar behaviours also load onto PC1 in 2009 and 2010, with rest behaviour again contrasting with behaviours associated with milk acquisition. Regarding PC2, in both the 2009 and 2010 seasons this component contrasted ‘milk-attaining’ behaviour (high negative loadings) with ‘comfort moves’ (high positive loadings), suggesting that in these seasons this component was describing a similar behavioural trait in the pups. However, as stated, this component is not comparable with 2008 since different behaviours loaded onto this element. This would therefore make it difficult to use derived individual scores to compare pup behaviour across seasons.

- ***Sibling analysis across breeding seasons.***

The apparent similarity in the behaviours loaded onto the PC1 and PC2 elements in the 2009 and 2010 breeding seasons offered the opportunity to investigate the behavioural similarity of siblings born in these respective years (based on the reasoning that the derived factors described the same overriding ‘behavioural trait’ in each season). Therefore the PC1 and PC2 scores of siblings across these years were correlated using the Spearman Rank correlation. Results of Spearman rank tests revealed a significant correlation between the PC1 scores of siblings between these years (r_s= 0.599, n= 13, *P*= .03), whereas no significant correlation was found between the PC2 scores (r_s= 0.363, n= 13, *P*= .223). Perhaps this suggests that the behavioural trait described by PC1 (reflecting variation in resting behaviour predominantly) has a greater genetic basis, thereby promoting similarity, compared to the trait reflected by PC2.

Table 2: Results of PCA analyses for siblings, born in the 2009 and 2010 breeding seasons on North Rona, respectively. Scores for PC1 and PC2 scores are shown.

IDCODE	PC1_09	PC1_10	PC2_09	PC2_10
SA29	-4.32	-6.15	-2.08	0.30
SA30	-1.53	-4.57	-1.43	1.52
SA32	-4.19	-5.09	0.65	1.72
SA39	1.86	-5.40	1.55	-2.18
SA42	1.31	3.23	-1.39	0.63
SA45	-10.92	-1.00	-3.49	-3.68
SA1	-0.02	-3.97	-3.62	0.85
SA10	4.28	-0.30	1.50	-1.87
SA12	-7.95	-4.22	-3.32	0.59
SA14	3.04	0.06	6.95	2.75
SA15	-2.28	-1.60	1.06	2.25
SA5	3.52	1.35	1.92	1.41
SA9	15.18	12.57	-3.11	-1.64

4.1.2. Analysis excluding resting behaviour.

When the number of behavioural variables was reduced in order to improve the variable number: sample size ratio, it was found that there were again few similarities between the 2008 and other breeding seasons. In 2008, it was found that play and exploratory behaviour loaded highly onto PC1 and contrasted with behaviours associated with maternal contact (table 1b). However, in 2009 the only behavioural variable to load onto PC1 (at 0.3+) was milk-attaining behaviour, and in 2010 resting behaviour and comfort moves both loaded positively onto this component. Therefore, PCA does not seem useful here, since the dimensionality of the data is not particularly reduced by the process. Play and milk-attaining behaviours load positively onto PC2 in 2008; in 2009, alert behaviour and comfort moves load positively onto this factor, and in 2010, comfort moves and milk-attaining behaviours load onto this factor (notably, the same combination as loaded onto PC1). Therefore, again, comparisons could not be made between 2008 and the other seasons. It is also notable that whilst KMO values are better for these data (table 1b), the PCA results do not explain as much of the variation within the data, for any breeding season, compared to when all behaviours were included.

4.1.3. Analysis of active behaviours only.

In the third group of analyses, to enhance the variable: sample size ratio even further, only behaviours associated with independent pup activity (not directly related to the maternal figure) were included: 5 behaviours in total. Therefore, for each breeding season, the ratio was comfortably higher than the proposed 1: 3 minimum (Budaev, 2010).

The 2008 season PCA results were markedly different from the other breeding seasons. In 2008, play and exploratory behaviour were highly negatively correlated onto PC1, contrasted against comfort moves, whilst in 2009 and 2010, comfort moves and alert behaviour were positively loaded onto this factor. However, the results are increasingly uncertain, since in 2008 the same two behavioural variables are loaded on PC2 (play and explore) as were on PC1, suggesting that the analysis is not instructive. In 2009 and 2010, this is also true, with alert behaviour and comfort moves again being loaded on PC2, although in 2009, locomotion and play behaviour are also loaded on this factor. Overall, the results of these analyses, despite improved KMO scores (table 1c), lack reasonable biological interpretation.

4.2. Can we learn anything about behavioural ontogeny from these analyses?

From the initial PCA results, it was apparent that most behavioural variation was explained when all behavioural variables were included in the analysis. Therefore, in order to assess the potential for this approach to reveal information about the behavioural ontogeny of pups over the lactation period, it was decided to include all behavioural variables for subsequent analyses.

PCA was conducted in the same way as described in the methods for early and late lactation phases separately for each breeding season. This was conducted in order to assess whether the same behavioural variables loaded on principal components 1 and 2 in both lactation phases, since this would enable individual scores to be correlated between early and late lactation phases and therefore assess behavioural consistency over time. Tables 3a-b summarise the results of these analyses for each breeding season.

Table 3 (a-b). Comparison of variable loadings on PC1 and PC2 in a) early and b) late lactation phases from the 2008, 2009 and 2010 breeding seasons.

a)

	2008		**2009**		**2010**	
	PC1 (53.49%)	**PC2 (22.84%)**	**PC1 (76.56%)**	**PC2 (10.83%)**	**PC1 (66.58%)**	**PC2 (15.34%)**
Alert				-0.53		
Comfort Move		0.34	-0.34			-0.59
Explore						-0.53
Locomotion				0.47		
Mum Interaction		0.62				0.49
Milk	-0.30					
Play		-0.63		0.52		
Rest	0.91		0.90		-0.93	

Percentage of variance explained is shown in parentheses.

b)

	2008		**2009**		**2010**	
	PC1 (64.28%)	**PC2 (19.16%)**	**PC1 (64.39)**	**PC2 (14.24%)**	**PC1 (59.39%)**	**PC2 (23.83%)**
Alert				-0.32		
Comfort Move			0.40	0.67	0.37	-0.70
Explore						
Locomotion						
Mum Interaction						0.45
Milk		-0.59		-0.60	0.41	0.49

Play	0.34	0.72				
Rest	-0.90		-0.87		-0.80	

Percentage of variance explained is shown in parentheses.

Only in the 2009 breeding season were the same behavioural variables were found to load onto PC1 in both early and late lactation phases (tables 3a-b), these being ‘resting’ and ‘comfort moves’. However, this was not true of 2008 or 2010, where different behaviours loaded onto PC1 in each lactation phase, thereby suggesting different behavioural traits were described in each case. Therefore, it was only possible to assess the consistency of individual behaviour over time for the 2009 season, by correlating individual PC1 scores across lactation phases. It is notable that the same behaviours did not load onto PC2 across lactation in any breeding season (see tables 3a-b). When the repeatability of behaviour over time was assessed by correlating the PC1 scores of individuals between early and late lactation, no significant result was found for the 2009 season (N=17, ICC= -.493, 95% Cl= -.78- -.032, $F_{16,16}$= 0.34, *P*= .98). However, in light of the low number of behaviours loaded onto this factor, these results do not seem informative.

4.21. Inferring behavioural development from PCA loading changes.

In all breeding seasons, the behavioural variables loaded onto PC2 in early and late lactation were found to be different. Whilst this means that individual scores for this factor cannot be correlated across lactation phases, the results may still be insightful since it is possible to assess what behaviours change within this factor and what stay the same as the individuals are influenced by experience and maturation over the lactation period (Putnam, 2011).

In 2008, in early lactation play behaviour was found to load negatively onto PC2, contrasting with mum interaction and comfort moves; whereas in late lactation, whilst play behaviour remains loaded on this factor, now only milk-attaining behaviours contrast with it. In 2009, play behaviour and locomotion loaded onto PC2 and were contrasted with alert behaviour in early lactation, and in late lactation alert behaviour is still negatively loaded onto this factor, but in addition so is milk-attaining behaviour, with these contrasting comfort moves. In 2010, exploratory behaviour and comfort moves were contrasted against mum interactions in the early lactation phase, and in late lactation, mum interaction was again loaded on this factor, but now also negatively loaded with milk-attaining behaviour, and these were still contrasted by comfort moves. It is possible that such results indicate a general cline of more independent activity in early lactation to more dependent behaviour relating to attaining milk in late lactation, whilst still maintaining independent behaviour of a more sedentary nature, such as comfort moves.

Conclusions

PCA using all behavioural variables is more successful at describing the variation within the data and so may be seen as better at representing individual behavioural differences. However, measures of sampling adequacy suggested that when the number of behavioural variables included were reduced, these measures were better, potentially giving greater reliability to the results. Despite this results often lacked reasonable biological interpretation, suggesting that in order to gain instructive results, it is better to trade-off the variable number: sample size ratio and include all behavioural data. Overall, despite explaining more of the variation present in the data, the results of PCA when all behaviours were included do not seem wholly reliable regarding the description of individual behavioural variation, taking measures of sampling adequacy into account, and also do not reduce the dimensionality of the data to a large extent. Individual principal component scores will therefore not be used in further analyses as preference over behavioural budget data for single behavioural traits.

APPENDIX 4

Morphometric Method for Estimating Relative Body Condition of Wild Grey Seals

A 'Publicity Problem' at Donna Nook:

In previous studies, female grey seal body condition has been estimated from measures of maternal postpartum mass (kg), which is an index of somatic growth and prior foraging success (Twiss et al., in review). Such measurements have been calculated by repeatedly catching and subsequently weighing individuals in the field during the breeding season on isolated offshore island colonies (see Pomeroy et al., 1999). However, Donna Nook nature reserve is a relatively public site, attracting almost 70,000 people to the seal colony in 2011[2] and handling seals at this site is prohibited. The methods described previously for estimating female body condition are therefore not possible at this Donna Nook colony.

The Solution:

A novel approach was therefore developed for this thesis in order to estimate relative female body condition. Photographs taken in the field during the 2011 breeding season were available for individually identified females (these can be provided upon request) and so a method was developed to collect morphometric measurements from these in-field photographs to give an estimate of the relative body condition of individuals. The method uses the ratio of maximum body depth to body length from images to give a proxy for body condition. In this way, a high ratio index value indicates a 'fatter' individual (since the female is 'deeper' in proportion to her length) and vice versa. A beneficial aspect of this method is that the distance of the focal female to the camera does not need to be known for individuals, since it is the relative relationship between length and depth measurements that is important. Therefore it is possible to use this method to estimate female condition and then compare individuals as required.

The Method:

Photographs of individual females were collected and filtered to attain images taken as early in their lactation period as possible. The reasoning for this was that since breeding and foraging are temporally separated in this species, females lose significant levels of body fat through the lactation period and consequently body condition deteriorates over time. Therefore, it was desirable to assess photos from early lactation for each female and thereby increase the consistency amongst females and gain a more reliable indication maternal condition when individuals came ashore to give birth. Images of females were also filtered according to the position of the female relative to the camera, specifically, images in which individuals were lying parallel to the camera and in a relaxed stance (so as not to affect length measures) were selected. Figure 1 depicts a good example of such an image. However, since the photographs (taken in the field by HCJ) were not taken with these analyses in mind, this was not always possible to achieve- this will be considered in more detail later.

Figure 1: Example of a good photograph for analysis. The female is lying parallel to the camera and is in a relaxed stance. The pup in the foreground does not block the measure of maximal body depth.

Preliminary investigation revealed that the body condition index values (body length/ body depth) derived were not affected by the choice of length measurement used. It was considered that measuring body length from the nose to the end of the hind-flippers may not be as indicative of true body condition as a length measurement from the nose to the start of the tail (thereby just taking the main body into account). However, a significant positive correlation was found between ratios derived from using the two different length measurements (r_S= .909, n=35, P= <.0001; figure 2). Based on this, it was decided that absolute body length (nose-end of hind flippers) would be used.

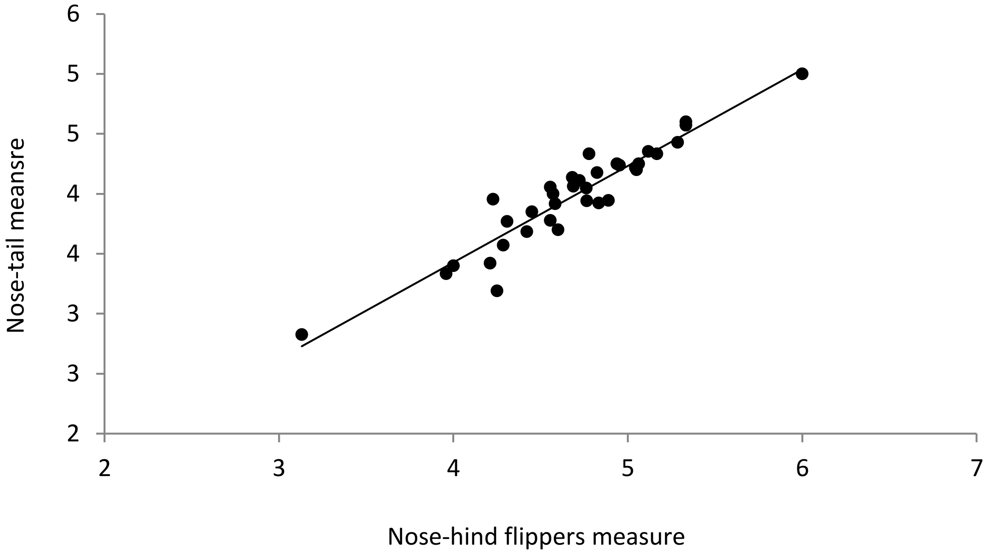


Figure 2: Relationship between the 'body condition ratios' (length/depth) calculated for individuals, derived from the two different length measurements.

When the photographs were collated and filtered, measures of maximal body length and depth were recorded for each individual (cm). Body length was measured from the nose to the tip of the hind-flipper and body depth as the distance between the ground adjacent to the individual's body and its maximal point of height. From these two measures, the length:depth ratio value for each individual could then be derived, by dividing length by depth.

The Results:

Multiple photographs were analysed per individual and graded as either "good" or "poor" quality based on a range of features, such as image clarity and the position of the individual relative to the camera. Where no "good" photographs were available for an individual, video footage of individuals collected in the field was analysed and, where possible, stills were taken using a snipping tool, thereby allowing morphometric measurements to be collected from these images instead. Using a combination of these methods, the best photograph or still was selected to represent each individual. Satisfactory images were collected for all females of interest, except one individual: "R5". In the photograph, this individual had her hind region slightly angled towards the camera and hind-flippers tucked in, making the length measure less reliable. Further, in focal videos, this individual was a relatively long distance from the camera and therefore stills taken were very blurry and it was difficult to take accurate measures. However, ratio values obtained from the three images for this female were all in a similar numerical region (4.3, 3.9, 4.2) and the index derived from the field photograph was used with relative confidence. Table 1 shows the results of these morphometric analyses.

Table 1: Individual morphometric measurements and body condition index values. The developmental stage of the pup at the time of the photograph, pup sex and the type of image used for measurements are also displayed.

Female ID	PUP STAGE	PUP SEX	Method (P/S)*	LENGTH (cm)**	DEPTH (cm)**	INDEX VALUE (L/D)
LINDA	**2**	M	S	11.0	2.4	**4.58**
P16	**2**	F	P	10.4	2.1	**4.95**
P20	**3**	M	S	11.6	2.3	**5.04**
P21	**2**	-	P	8.5	1.8	**4.72**
P22	**1**	-	P	8.4	1.9	**4.42**
P26	**1**	F	S	8.9	2	**4.45**
P36	**3**	F	S	8.1	1.6	**5.06**
R1	**1**	-	P	11.2	2.1	**5.33**
R5	**1**	-	P	8.6	1.8	**4.78**
R7	**3**	M	P	11.1	2.1	**5.29**
R9	**1**	F	P	9.6	2.1	**4.57**
R11	**2**	-	S	10.1	2	**5.05**

R12	**2**	-	P	8.1	1.7	**4.76**
R13	**2**	-	P	8.7	1.7	**5.12**
R14	**1**	M	P	8.2	1.7	**4.82**
R26	**2**	F	S	8.2	1.8	**4.56**
RES2M	**2**	M	S	8.5	2	**4.25**

*P= photograph taken in field, S= Still from focal video footage. ** Measurements given to the nearest millimetre.

The following graph shows the extent of derivation from the mean ratio value (4.81, n=17) for this sample of females, calculated by taking the average index value away from individual index values. Negative values therefore depict individuals with a condition index below the average and are consequently females in better condition (greater depth: length ratio), whereas positive values indicate ratio values above the average and therefore represent females in poorer condition (lower depth: length ratio).

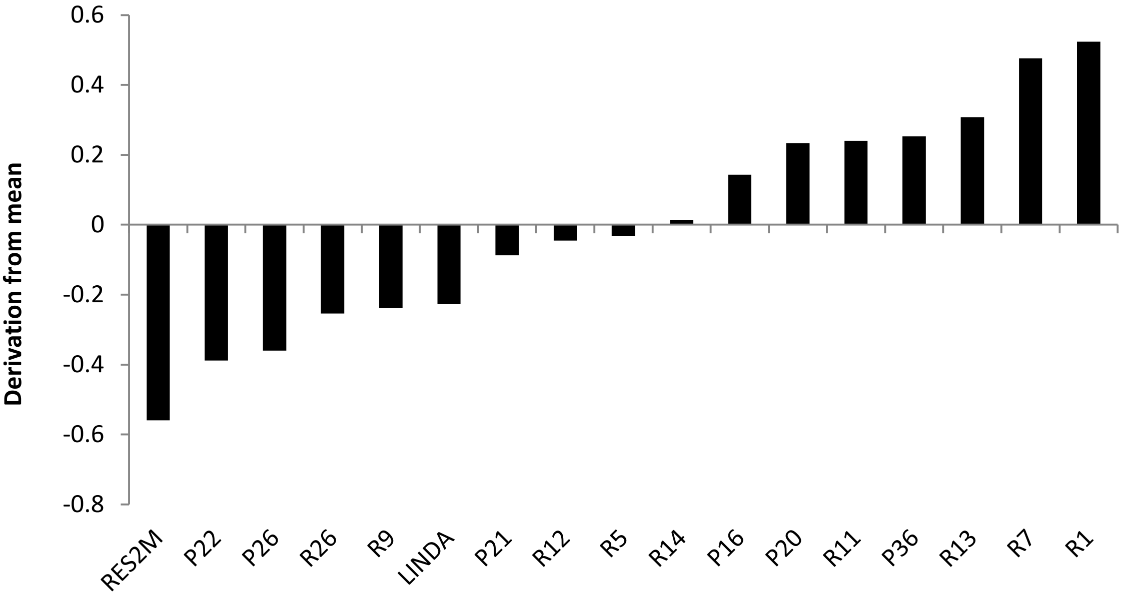


Figure 2: Individual residual scores, indicating the extent and direction of derivation from the mean ratio value. Negative residuals indicate females in better condition and positive values denote the opposite.

Disadvantages of method:

As a result of not being taken for the purpose of this study, photographs of individuals were not always consistent either in terms of female positioning or date of photograph within the lactation period. Each has implications for the accuracy and interpretation of body condition estimates from the derived morphometric ratios. The majority of photographs or stills were taken of females during the early lactation period (when pups were still in the early stages of development: stages 1 and 2), however three individuals had stage 3 pups when their images were taken (table 1; figure 3) and consequently had entered 'late' lactation. Their respective body conditions may therefore be lower as a consequence of having been lactating and present at the colony for longer at the point of the photograph. In support of this assumption, it is notable that the body condition ratios obtained for these females were all towards the higher end of the scale (table 1), inferring poorer condition. It is not possible to alter the measurements, however this inconsistency must be considered for any analyses using these data as a proxy for relative body condition.

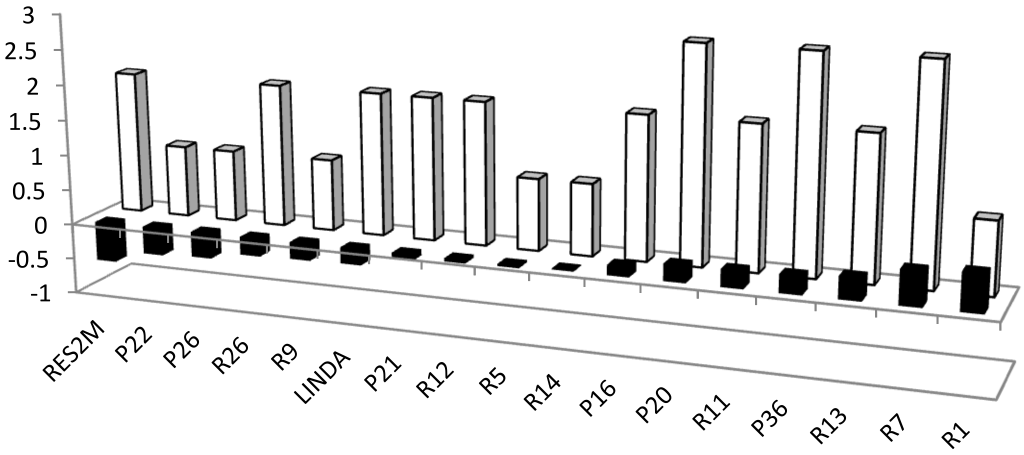


Figure 3: Relationship between individual residual values (indicating relative maternal condition) and pup stage at the time photographs were taken. Black bars= derivation from mean ratio index, White bars= pup stage at time of photograph.

Further, as acknowledged in chapter 5, this method has not been validated with in-field measures of actual body condition, either from relative maternal mass measurements (as used in chapter 3) or from more sophisticated measures of body composition, such as through the double-labelled water method. Until such validation, the method remains untested and conclusions drawn using the condition indices derived must be considered with caution.

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