# Investigating the links between behavioural types and mate choice in a polygynous pinniped

SAMUEL IAN HARDMAN

**How to cite:**

HARDMAN, SAMUEL IAN (2014) Investigating the links between behavioural types and mate choice in a polygynous pinniped. Masters thesis, Durham University.

# Investigating the links between behavioural types and mate choice in a polygynous pinniped

By

Samuel Ian Hardman

School of Biological and Biomedical Sciences

Durham University

UK

2013

This thesis is submitted in candidature for the degree of

Master of Science by research (MRes)

# Abstract

Consistent individual differences in behaviour (CIDs) or behavioural types have now been shown in a broad range of taxa, from Cnidarians to birds and mammals. An individual's behavioural type can influence may aspects of its life history including foraging strategies and mate choice decisions. Grey seals (*Halichoerus grypus*) have been shown to exhibit behavioural types ranging from proactive to reactive but the implications of this on sexual behaviour and mate choice decisions have not yet been investigated. This study tests the prediction that females with a more reactive personality type should be more choosy in their mate choice decisions.

Behavioural observations were made at two distinct sites within Donna Nook breeding colony on the Lincolnshire coast, UK. The RAF site was characterised by flat topography and very low human presence while the public site was more topographically varied and had a high level of human presence. The behavioural types of females were determined by examining the rate at which they performed pup-checking behaviour in both disturbed and undisturbed situations. Females were considered to have a more proactive behavioural type if they maintained a relatively constant rate of pup-checking across situations and a more reactive behavioural type if their rate of pup-checking changed across situations. Owing to their tendency to be found on the edges of the colony where they are subject to increased harassment by transient males it was predicted that females with a more reactive behavioural type would show frequent and intense aggression towards males. In contrast, due their tendency to occupy the best pupping sites at the centre of the colony which are occupied by more dominant males it was predicted that females with a more proactive behavioural type would show fewer and less intense aggressive interactions towards males. This pattern would indicate female mate choice by showing that females respond more aggressively to low ranking transient males than they do to more dominant males suggesting a preference for the latter. Detailed accounts of all aggressive interactions involving focal females were recorded *ad-lib* in the field.

In agreement with previous studies, evidence of CIDs in the pup-check rates of females in undisturbed situations was identified at this colony. However, in contrast with past research no evidence of CIDs in disturbed situations was found. A significant difference was found in the mean pup-check rates of females at the two study sites with females at the less disturbed RAF site pup-checking at a significantly greater rate. The data also showed significant differences in rates and intensities of aggression between the two study sites with females at the RAF site showing a higher rate but lower intensity of aggression than those at the public site. However, no evidence was found to support the hypothesis that reactive females show higher level of aggression towards males than do proactive females. Thus, no evidence of female mate choice in grey seals was found in this study. Despite this, potentially interesting effects of topography and/or anthropogenic presence on grey seal behaviour were found and these may prove to be interesting avenues for future research.

# Acknowledgements

This thesis would not have been possible without the help and support of a large number of people to whom I am very grateful. Firstly I would like to thank Sean Twiss for being an all round fantastic supervisor and endless source of knowledge and advice, it has been very much appreciated. I would also like to thank Amy Bishop for reviewing my work at various stages, for the many useful discussions and for putting up with my constant requests for help with R! I am extremely grateful to Isabel Richardson for drawing the image which graces the cover of this thesis, it is perfect, thank you. I would to thank Brenda Mallett for the financial assistance without which this project could never have happened. To Charlotte Cairns and James Stewart for encouraging me to apply to Durham University in the first place I am also very grateful. Finally I would like to thank all the people who have encouraged and helped me in numerous other ways over the course of this project, in no particular order they are Alke Voskamp, Bethan Hindle, Naiara O'Mahony, Nathalie Lombaert, Charlotte Walsh, Tom Mason, Hagen O'Neill, Emily Perrin, Georgina Palmer, Noel Helliwell, Olly Kinsey, Jamie Alison, Mike Harrap, Christine Howard, Mike Forty, David Baker, Niall Gauld, Steve Hancock and Andy Lloyd.

# 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.

# Contents

## Chapter one: Introduction

## Chapter two: Fieldwork methods

## Chapter three: Analytical methods

## Chapter four: Results

## Chapter five: Discussion

## List of Tables

# List of figures

# Chapter one: Introduction

## ***1.0. Aims of this study***

This thesis builds upon past research conducted at North Rona, Scotland, which has shown that grey seals (*Halichoerus grypus*) exhibit consistent individual differences (CIDs) in behaviour indicative of proactive and reactive behavioural types (Twiss and Franklin, 2010, Twiss *et al.*, 2011, Twiss *et al.*, 2012). Recent research has shown that there may be a link between these behavioural types and female choosiness of mating partners in this species (Twiss *et al.* 2012). This study examines the prevalence of CIDs and their potential link to female mate choice in grey seals at Donna Nook grey seal colony on the east coast of England.

Data from Twiss *et al.* (2012) suggests that female grey seals with a more proactive behavioural type spend more time in aggression with other adult females than they do with males, whilst females with a more reactive behavioural type show more aggression towards males than they do females. This stark contrast may simply be a product of the location of proactive and reactive females on the colony. High levels of aggression shown towards males by reactive females could be a result of their tendency to be found in low density areas towards the edges of the colony where they are subject to transient male incursions and harassment (Boness *et al.* 1995, Lidgard *et al.* 2001, Lidgard 2003). Alternatively it could indicate that more reactive females show a high degree of choosiness in their potential mating partners. In contrast, proactive females are typically more aggressive than reactive females and this may enable them to secure preferred pupping sites in high density areas of the colony which tend to be occupied by more dominant males (Pomeroy *et al.* 1994, Twiss *et al*. 2000). In this case since proactive females have access to dominant males by default they have little need to be choosy about their mating partners. This potentially explains why proactive females show lower levels of aggression towards males. The high levels of aggression shown towards other females may be explained by their close proximity on the colony combined with a proactive 'strategy' of competing for access to the best pupping sites (Pomeroy *et al.* 2000). These explanations are not mutually exclusive and may all contribute in some way to the observed patterns of aggression.

To date, all studies of personality in female grey seals have been based on data collected from just one colony (North Rona) (Twiss *et al.* 2011, 2012). There is therefore a need to examine personality in this species at another colony in order to establish if personality is a general feature of grey seals or just a characteristic of seals at the North Rona colony. In addition, previous studies of personality in this species have used experimental techniques which may not always be possible at other locations (Twiss *et al.* 2011, 2012), for example at the publicly accessible area of the Donna Nook colony experimentation is not feasible. The development of a purely observational method of examining personality could therefore prove extremely useful for further studies of personality in grey seals and could possibly be extended for use in other species. The aims of this research are therefore: -

- To use methods based on those described by Twiss *et al.* (2012) to look for consistent individual differences in behaviour in female grey seals at a different colony to that previously studied and to test the effectiveness of an entirely 'hands-off' approach to the study of animal personality.
- To determine if there are individually consistent differences in levels of aggression shown by female grey seals to other female and male conspecifics.
- To determine whether differences in female-male aggression can be explained solely by male status or familiarity between males and females or, whether female aggression towards males is also dependent on their behavioural type and so indicates differences in choosiness of mating partners.

## ***1.1. The biology of female mate choice***

Mate choice refers to the process whereby individuals of one sex choose mates of the opposite sex on the basis of desirable characteristics such as dominance status (Horne and Ylönen, 1996), ornamentation (Darwin 1871, Andersson, 1982), possession of resources (Jennions and Petrie, 1997), provision with nuptial gifts (Vahed, 1998), unrelatedness (Hoffman *et al.*, 2007), maturity, fertility, colouration and vocal or olfactory displays (Clutton-Brock and McAuliffe, 2009). In sexually reproducing species it is usually the males that compete for access to females whilst females exercise mate choice. This process can lead to significant variation in reproductive success between the sexes (Trivers, 1972). In most species the majority of females are able to successfully reproduce each breeding season, however, in any one population there may be just a few dominant males accounting for almost all male reproductive success.

Trivers (1972) suggested that the differential investment of resources into gametes and parental care leads to sexual competition whereby the sex that invested the least would compete among themselves to mate with those that invested most. In the vast majority of species it is the females that invest the most resources into each reproductive effort (Andersson, 1994). Females produce relatively few large gametes whilst males produce large numbers of small gametes. In addition, females often invest heavily in other types of parental care such as child-bearing and post-partum care (Trivers, 1972, Kokko and Jennions, 2008). Since males typically invest few resources into each of their offspring they are able to increase their reproductive success by mating multiple times with many different females. Females however, can only increase their reproductive success by converting resources into gametes and offspring at a faster rate (Trivers, 1972). Furthermore, for many species the high cost of reproduction restricts females to reproducing just once per year. Consequently males typically have a much greater potential reproductive rate than females do (Bateman, 1948). As male investment in reproduction is low the potential pay-off to males for multiple mating is very high, whilst the cost of mating with sub-optimal females is low. In contrast, since females invest heavily in reproduction, mating with sub-optimal males potentially carries a high fitness cost. Females are therefore expected to be far more discriminating in their choice of mates than males are as, for them, it is vital that each reproductive effort is as close to optimal as possible (Darwin, 1871, Fisher, 1930, Bateman, 1948, Trivers, 1972, Janetos, 1980, Hoffman *et al.*, 2007). Reproduction may impose non-trivial costs on males such as the costs of producing sperm which, when sperm competition is a factor, may be produced in large quantities (Dewsbury, 1982, Andersson and Simmons, 2006). An increased energy expenditure in the production of sexually selected characters or displays, or imposed by competition with other males may also be substantial (Maynard Smith *et al.*, 1988, Møller, 1989, Kotiaho, 2001). Nevertheless, in most cases it is the females that invest the most into each reproductive attempt and yet, with few exceptions, they cannot reproduce as rapidly as males. As a result females are a scarce resource for which males must compete (Trivers, 1972). This is the basis of sexual competition and mate choice.

In order to understand sexual selection and the evolution of mating patterns it is necessary to determine how individuals of each sex maximise their reproductive success (LeBoeuf, 1974, Emlen and Oring, 1977, Reiter *et al.*, 1981). Amongst pinnipeds mating patterns are generally polygynous but individuals may adopt a range of strategies from extreme polygyny to serial monogamy, and there are even examples of mate fidelity (Reiter *et al.*, 1981, Riedman, 1989, Amos *et al.*, 1995, Cappozzo, 2001, Lidgard *et al.*, 2004). The grey seal, *Halichoerus grypus*, is a sexually dimorphic pinniped with a mating pattern that has been described as terrestrial polygyny (Boness and James, 1979, Twiss, 1991). Each breeding season females come ashore to give birth to a single pup. Towards the end of lactation they come into oestrus and males compete aggressively to maintain position amongst groups of females that may change over time (Twiss, 1991, Twiss *et al.*, 1998, Lidgard *et al.* 2005). Mating success favours those that are able to maintain position within the colony, however, the extent to which male grey seals are able to do this varies substantially between colonies due to differing population densities and landscape topography (Anderson and Harwood, 1985, Twiss, 1991, Amos *et al.*, 1993, Twiss and Thomas, 1998, Pomeroy *et al.* 2001, Twiss *et al.* 2006). For example, Anderson and Harwood (1985) found that there was a skew in the sex ratio of grey seal breeding colonies from 2:1 female to male on Sable Island, Canada, where the wide sandy beach facilitates easy access for males, to 7:1 on the cliff bound island of North Rona, Scotland, where access is only possible at a few locations via rocky gullies. The mating pattern of grey seals has therefore been described by Riedman (1989) as varying from extreme to moderate polygyny with pronounced sexual dimorphism. However, this description assumes that the apparent variation in sex ratios translates into variation in reproductive success but this may not be the case. On grey seal breeding colonies subordinate males are excluded by more dominant males who compete with each other for access to females, there is therefore significant variation in the number of successful copulations achieved by individual males (Twiss, 1991). Amos *et al.* (1993), questioned whether male mating success necessarily translates into reproductive success and suggested two reasons why it may not. First, females have been observed to mate with more than one male (Twiss, 1991), and second, when females leave the colony the dominant males can no longer exert the same control over access, this may give females the opportunity to mate with peripheral males outside of the colony. Paternity testing by genetic analyses of pups on North Rona revealed that whilst dominant males did account for a disproportionately high number of paternities, it was still fewer than what was predicted given their mating opportunities (Amos *et al.* 1993). This suggests that females may have some degree of control over which male fathers their pup and it is not always the most behaviourally dominant males that are successful.

Although the mating pattern of grey seals has been extensively studied, research has often been focussed on male reproductive success and strategy (Twiss, 1991, Amos *et al.*, 1993, Twiss *et al.*, 1998, Lidgard *et al.*, 2004, Twiss *et al.*, 2006, Boness and James, 2009) with little attention given to the possibility of female choice (*sensu* Clutton-Brock and McAuliffe 2009). This oversight is surprising given that male grey seals invest nothing in their offspring other than their genes; selection should therefore favour females that choose mates on the basis of desirable and heritable characteristics such as size and dominance (Cox and LeBoeuf, 1977) so long as the cost of choice is not greater than the benefits. This view is supported by a growing number of studies showing that female mammals often show consistent preferences for particular categories of males that are likely to provide their offspring with significant fitness benefits (Moss, 1983, Jennions and Petrie, 2000, Roberts and Gosling, 2003, Hoffman *et al.*, 2007, Clutton-Brock and McAuliffe, 2009). However, for female grey seals active mate choice may impose costs that outweigh the benefits. In this species females come ashore to give birth to a single pup before mating and returning to the sea. When their pups are born they are small, vulnerable and require a regular supply of maternal milk (Reidman, 1989). It could therefore be argued that for a female to go and find a mate would leave her pup at high risk of harm since long term mother-pup separation is known to lead to starvation and death (Anderson *et al.* 1979). This is not true for all pinnipeds however, for example, some studies suggest that otariids show female mate choice (Goldsworthy *et al.* 1999, Insley, 2000, Hoffman *et al.* 2007), and yet they typically enter oestrus immediately or within a very few days post-partum (Gentry, 1998). This means that if they are to express active mate choice they must leave their pups unattended at a very early stage in their development to go and find a mate. However, otariids are “income breeders” which means that females need to regularly leave their pup to find food if they are to provide a constant supply of milk (Gentry, 1998, Trillmich *et al.* 2006). Otariid pups may therefore be relatively well adapted to coping during long periods of mother-pup separation and this in turn may provide opportunities for active female mate choice. In contrast, phocids, such as grey seals, are “capital breeders”, they rely on stored body fat to provide milk for their pup and do not leave their pupping site to find food until their pup is weaned (Trillmich *et al.* 2006). As a result, phocid pups may not be so well adapted to coping with long periods of mother-pup separation and this may reduce opportunities for active female mate choice in phocid species. However, phocid females are thought to enter oestrus around 16 days after giving birth (Pomeroy, 1999). This is much later than in otariid species and by this point their pups are generally well developed and approaching full independence (Hoffman *et al.* 2003). The risk to the pup of being left unattended at this stage in their development is therefore likely to be low and this leaves open the possibility of female choice.

Another way in which female mate choice may be costly is through increased male harassment as females actively move through the colony in the search for a mate. Whilst there is little evidence for this it has been suggested that grey seals show some degree of inter-annual mate fidelity (Amos *et al.* 1995);  this could reduce the level of male harassment towards females, as females may preferentially select known males, reducing the need to move around the colony searching for potential mates. In contrast, Twiss *et al.* (2006) observed females leaving their pup to mate with males outside of their local male's home range. This strongly suggests that females are able to express some degree of mate choice, and that the costs of leaving their pups are outweighed by the benefits of mate choice.

There is also evidence of behavioural mechanisms that females may use to express mate choice. Anderson *et al.* (1975) found that grey seal cows with pups exhibited a strong aggressive response when approached by bulls. They suggested that this response is a behavioural mechanism that ensures that females only mate with those males that are able to overcome female aggression and are therefore likely to be the fittest. This view has since been supported by Boness *et al.* (1982) who, in a study of grey seals at Sable Island, Canada, found that females with pups and in oestrus were significantly more likely to show aggression towards an approaching male than they were towards approaching females. Furthermore, Boness *et al.* (1982) also found that females were less aggressive towards tenured males, defined as males which maintained their position on the colony for two or more consecutive days, than they were towards transient males, defined as those present on the colony for less than two days. However, in this case it is difficult to disentangle female choice from familiarity since the preferred males were also those that had been on the colony for several days. Cox and LeBoeuf (1977), studying northern elephant seals, *Mirounga angustirostris*, found that females respond to the copulatory attempts of males with loud protestations and vigorous escape movements. This attracts the attentions of nearby males of varying social rank who compete with each other and aggressively try to prevent other males from mating. By inciting competition in this way females may effectively prevent low ranking males from successfully mating and ensure that they only mate with the most dominant, and presumably fittest, males. Boness *et al.* (1982) propose that since aggression by grey seals occurs under similar conditions to those observed for northern elephant seals, then grey seals may also incite competition from tenured males and so prevent successful copulation attempts by low ranking males. Although these findings are consistent with the hypothesis that incitation of male aggression serves as a form of female mate choice they are difficult to test. Male aggression may prevent low ranking males from mating, however, this does not mean that females are making an active choice. In contrast, it may be that mate choice is constrained by male aggression as females have fewer mates to choose from, furthermore, it is difficult to exclude the possibility that female matings with dominant males do not occur as a result of male coercion rather than female choice (Clutton-Brock and McAuliffe, 2009).

Females may choose males either for direct benefits such as resources or territory, or for indirect benefits such as good genes which enhance offspring fitness (Hamilton and Zuk, 1982, Vahed, 1998, Amos *et al.* 2001, Clutton-Brock and McAuliffe, 2009). Since genes cannot be assessed directly, females choosing for indirect benefits should choose males on the basis of characteristics which are themselves the product of good genes such as size or dominance (Clutton-Brock and McAuliffe, 2009). In their study of northern elephant seals Cox and LeBoeuf (1977) put forward several possible reasons for why this should be. Firstly, in order to achieve high social rank male elephant seals must live to be at least 10 years of age, since male mortality in this species is high very few individuals do this. If longevity has a genetic component then males that do survive to 10 years old have demonstrated a high level of fitness that may be passed on to future offspring. In this case females are choosing genes ‘for’ longevity, an important aspect of fitness. Another possibility is that by out-competing other males in the colony the individuals with the highest social rank are demonstrating that they are physiologically fit, this trait may also have a genetic component and so be inherited by the next generation. Finally, females may prefer dominant males because they are more sexually experienced, possibly because this increases the possibility of reproductive success (Cox and LeBoeuf, 1977). It is not yet clear if female grey seals show a preference for mating with dominant males as female northern elephant seals appear to. While it has been suggested that grey seal females show a preference for tenured males (Boness *et al.* 1982), there is also evidence that the reproductive success of tenured males is less than would be expected if this were the case (Amos *et al.* 1993). In principle female grey seals could gain indirect benefits from choosing to mate with more dominant males, however, with the evidence currently available this possibility cannot be confirmed or rejected.

Females may also choose males on the basis of heterozygosity which, in the Antarctic fur seal, *Arctocephalus gazella*, has been found to be a predictor of male reproductive quality for traits such as longevity and ability to hold territories (Hoffman *et al.*, 2007). In this species females may therefore increase the fitness of their male offspring by choosing to mate with males whose genotypes show substantial heterozygosity and are dissimilar to their own (Clutton-Brock and McAuliffe, 2009). Breeding with close relatives is known to reduce the fitness of offspring produced through inbreeding depression (Maynard Smith, 1956); females are therefore also expected to choose to mate with unrelated males so long as they have a means of determining relatedness.

Since male pinnipeds provide their mates with no material contribution at all, female choice, if it exists, must be for indirect benefits (Clutton-Brock and McAuliffe, 2009). Although female choice in grey seals has been proposed before, there are as yet no studies investigating what male traits females may be choosing for. However, Hoffman *et al.* (2007) studying another polygynous pinniped, the Antarctic fur seal, found that a substantial number of females did not conceive to their nearest male, with some travelling to males up to 35m away before returning to their pupping location. Furthermore, they found that as females moved further away the average heterozygosity of the available males increased whilst their relatedness decreased. This suggests that females are actively choosing males on the basis of their being heterozygous and unrelated. Hoffman *et al.* (2007) suggest that visual cues such as size, condition or dominant behaviours may help females choose mates, alternatively they may use olfactory cues from the MHC (Clutton-Brock and McAuliffe, 2009). Although Hoffman *et al.* (2007) appears to provide evidence of female mate choice their study has been strongly criticised for failing to consider previously published data which appears to undermine their conclusions. Kotiaho *et al.* (2008a) found that in Antarctic fur seals both males and females are relatively static but that occasionally individuals of either sex may move 20-30 metres from where they were first observed. This contradicts Hoffman *et al.* (2007) who suggested that males remained static whilst females moved to actively find mates. Furthermore, 75% of females mated within 6 metres of the location at which they were first observed and only 8% travelled further than 10 metres (Kotiaho *et al.* 2008a). If moving a greater distance means that males become more distantly related, and therefore more desirable, as Hoffman *et al.* (2007) suggest, then it appears that most females are not mating with preferred males. The conclusions drawn by Hoffman *et al.* (2007) should therefore be taken with extreme caution.

Studies of female choice have often focused on male qualities such as size (Reiter *et al.*, 1981), dominance (Horne and Ylönen, 1996), or ornamentation (Andersson, 1982, Hamilton and Zuk, 1982). In these cases all females might be expected to choose the same ‘best’ male in order to produce offspring which also possess desirable characteristics (Amos *et al.*, 2001). However, directional female choice for the same male characters is expected to erode genetic diversity leading to inbreeding depression and reduced offspring fitness (Fisher, 1930, Kotiaho *et al.*, 2008b). Females might therefore be expected to choose mates that are genetically diverse in order to produce genetically diverse offspring and reduce the risk of inbreeding (Amos *et al.*, 2001). In a genetic study of grey seals from the colony at North Rona, Amos *et al.* (2001) found that pups who were maternal half-siblings were significantly more genetically diverse than would be expected from random mating. This result implies some form of female choice for genetically diverse mates. Amos *et al.* (2001) consider behavioural mechanisms of female choice unlikely and instead suggest a form of post-copulatory female choice is operating. Twiss (1991) found that females mate on average 2.9 times each breeding season whilst Amos *et al.* (2001) consider the true figure to be nearer to six involving more than one partner. This allows for the possibility of sperm competition and/or cryptic female choice. Although there is currently no direct evidence for this, Amos *et al.* (2001) suggest that anti-sperm antibodies within the cervical mucus of female grey seals may develop in response to exposure to sperm. If correct, immuno-intolerance could develop towards the sperm of each successive partner, and possibly also their close relatives, causing them to be selected against in future encounters. Post-copulatory choice was also suggested as a possible mechanism of female choice by Twiss *et al.* (2006) who conducted a large scale spatio-temporal analysis of paternities in grey seals on North Rona. They found that while over 80% of paternities could be assigned to known males within each female’s local area, a minority of 10-20% did not have their pups sired by local males. Twiss *et al.* (2006) observed some females leave their local male's home ranges temporarily, enter another males home range, mate, then return to their pups. This suggests that females may be actively choosing their mates or choosing to mate multiple times with several different males. In the latter case post-copulatory choice may be operating but this requires further research.

## ***1.2. An introduction to behavioural types or 'personalities'.***

Darwin (1859) recognised that individual differences are of fundamental importance to biology as they provide the raw material on which natural selection acts (Thornton and Lukas, 2012). Following this insight countless studies have investigated individual physical (e.g. colour) and psychological (e.g. cognitive performance) differences in a wide range of species from birds and mammals (Hill, 1990, Luhrs *et al.* 2012, Thornton and Lukas, 2012) to insects (Cook *et al.* 2012). In contrast, individual differences in behaviour, although intensely studied in humans (Caprara *et al.* 2000), received little attention in non-human animals, possibly due to ethologists' focus on species level adaptations and 'instinct' (Tinbergen, 1951, Thornton and Lukas, 2012). Although some animals were acknowledged to have distinct personalities (Darwin, 1871), scientific studies of animal behaviour traditionally viewed individual behavioural differences as statistical noise around an adaptive population mean and they were consequently ignored or overlooked (Réale *et al.* 2007, Smith and Blumstein, 2008, Thornton and Lukas, 2012). What studies there were tended to examine differences between different classes of organisms (e.g. age or sex) and did not examine behavioural differences of individuals within classes (Loughry and Lazari, 1994). In recent years however, this situation has changed. Long-term studies of wild and captive individually identifiable animals have allowed researchers to examine individual behavioural responses to changes in the physical and social environment across time, contexts and situations (Dingemanse *et al.* 2004, van Oers *et al.* 2008, Minderman *et al.* 2009, Briffa and Greenaway 2011, Betini *et al.* 2012, Twiss *et al.* 2012, Blumstein *et al.* 2013). These studies have revealed that many animals show consistent individual differences (CIDs) in behaviour indicative of behavioural types or ‘personality’.

Within the behavioural and evolutionary ecology literature numerous different terms are used to refer to personality including “coping styles”, “temperament” and “behavioural types” (Koolhaas *et al.* 1999, Réale *et al.* 2007, Twiss *et al.* 2012). What all of these terms refer to are consistent, coherent and repeatable differences in patterns of behaviour that distinguish individuals from other members of their species and their age and sex class (Lowe and Bradshaw, 2001, Twiss *et al.* 2011). For example, some individuals may be consistently more aggressive (Twiss *et al.* 2012) or more bold (Williams *et al.*, 2012) than others. Although this definition does imply limited behavioural plasticity individuals may still modulate their behavioural responses to some degree. To be considered indicative of personality only the rank order of differences between individuals need be maintained (Sih *et al.* 2004, Sih *et al.* 2010).

Personalities have now been documented in a broad range of taxa including birds (Dingemanse *et al.* 2004, Carere *et al.* 2005, van Oers *et al.* 2008, Minderman *et al.* 2009, Betini *et al.* 2012, Williams *et al.*, 2012), mammals (Lowe and Bradshaw, 2001, Twiss *et al.* 2012, Blumstein *et al.* 2013), insects (Schuett *et al.* 2011, Tremmel and Müller 2012) and even cnidarians (Briffa and Greenaway 2011). These and similar studies often interpret personality measures as bimodal variables with individuals falling along a smooth continuum between two extremes (e.g. shy-bold: Williams *et al.*, 2012; fast-slow: van Oers *et al.* 2008, Dingemanse *et al.* 2004, proactive-reactive: Twiss *et al.* 2012). Other forms of individual differences in behaviour such as discrete alternative mating strategies (e.g. Hutchings *et al.* 1988) are not generally considered to be elements of personality, possibly because such individuals fall into different *classes* (e.g. parr and anadromous salmon). Many of the different terms used to describe personality are synonymous for example, proactive, fast and bold all have very similar definitions as do reactive, slow and shy. Twiss *et al.* (2012) placed grey seals on a continuum from proactive at one end to reactive at the other. Proactive individuals tend to be aggressive, show bold exploratory behaviour, are relatively insensitive to external stimuli and readily form persistent routines. In contrast, reactive individuals show less aggression, pay careful attention to external stimuli and adapt cautiously to changing environments (Koolhaas *et al.*, 1999, Twiss *et al.* 2012).

Recent theoretical (Smith and Blumstein, 2008) and empirical (Dingemanse *et al.* 2004) studies have shown that CIDs can impact significantly on ecological and behavioural processes. For example, whereas behavioural ecologists have traditionally treated animal behaviours as infinitely plastic, with individuals exhibiting optimum behaviours across contexts, CID's by definition imply limited behavioural plasticity (Sih *et al.*, 2004). Depending on the environmental conditions, animals with different behavioural types can experience vastly different fitness outcomes. Stable environments generally lead to increased fitness for individuals with a more proactive, bold or fast personality type, whilst in fluctuating environmental conditions individuals with a more reactive, shy or slow personality type fair far better (Dingemanse *et al.* 2004, Smith and Blumstein, 2008, Twiss *et al.* 2012) . Personality types at any point on the proactive-reactive continuum can lead to high levels of fitness if the environmental conditions are favourable, personality types can therefore be considered to represent alternative, but equivalent, adaptive strategies (Sih *et al.* 2004, Twiss *et al.* 2012).

### ***1.3. Links between behavioural types and mate choice***

One area where personality may have a significant influence is on processes of sexual selection and mate choice. A key prediction of mate choice theory is that females should choose males that in some way enhance their fitness, either directly (e.g. nuptial gifts; Vahed, 1998) or indirectly ('good genes' that enhance offspring fitness; Clutton-Brock and McAuliffe, 2009). A male's personality type is an indication of his ability to cope with a range of different environmental conditions or a subset of environmental conditions, for example, boldness may influence foraging, anti-predator, social and dispersal behaviour (Koolhaas *et al.* 1999, Sih *et al.* 2010). Since personality types are known to be at least partially heritable (Dingemanse *et al.* 2002, Drent *et al.* 2003, van Oers *et al.* 2005), female choice of males based on male personality could indirectly affect the fitness of her offspring (Dingemanse *et al.* 2004). In species in which males provide parental care females may also gain direct benefits, such as increased provisioning for their young, by choosing males according to their personality type (Dingemanse *et al.* 2004). Bearing these possibilities in mind it might be expected that females would factor personality into their mate choice decisions. Several recently published empirical studies appear to support this assumption (Dingemanse *et al.* 2004, van Oers *et al.* 2008, Schuett *et al.* 2010, Williams *et al.*, 2012).

If personality type is an indication of a male's ability to cope with specific environmental pressures then it might be expected that females would have different mate preferences depending on the environmental conditions at the time. In great tits, *Parus major*, the fitness of females is a product of both the personality of their mate and the environmental conditions. In years when food is abundant males with a bold or fast personality type tend to do very well whereas, in years when food is scarce shy or slow males fair better (Dingemanse *et al.* 2004). Dingemanse *et al.* (2004) found that female preference for males with different personality types fluctuated from year to year depending on the abundance of food, with slow individuals favoured in years when food was scarce and fast individuals favoured when food was abundant. This potentially provides a solution to the 'lek paradox' in which female preference for the same male traits erodes genetic diversity until there is no difference between males and so reason for females to be choosy.

Personality may also lead to assortative mating in some species, with females choosing males on the basis that their combined personality types lead to optimal fitness outcomes. Van Oers *et al.* (2008) found that mate preferences were influenced by personality type in great tits. Their study showed that when a female was paired with a male of a similar and extreme personality type (i.e. both female and male at the extreme fast or slow end of the continuum), her nest was significantly more likely to contain chicks sired by another male. There is as yet no clear explanation for why this should be, however, one possibility is that the females are aiming to maximise the genetic variability of their broods though extra-pair copulations. The selective pressures acting on the offspring are expected to vary in space and time with different fitness implications for birds with different personality types (Dingemanse *et al.* 2004, van Oers *et al.* 2008). In this case a genetically variable brood provides the female with some insurance against the risk of not contributing any successful offspring to the next breeding season. As personality is known to be heritable in this species (Dingemanse *et al.* 2002, Drent *et al.* 2003), pairs in which both sexes have an intermediate personality type are expected to produce the most genetically variable broods, whilst pairs in which both sexes have the same extreme personality type produce broods with little genetic variability. In pairs with extreme personalities the female may be able to increase the genetic variability of her brood by mating outside of her social pair with males with a different personality type to her own. Although this result was not supported by van Oers *et al.* (2008), the authors do state that their sample size was small with low power meaning that this hypothesis cannot yet be ruled out.

Sexual selection on personality types has also been shown in other species including Gouldian finches, *Erythrura gouldiae*, where females seem to show a preference for males with more aggressive personalities, possibly because they are able to compete more successfully for access to the best foraging locations (Williams *et al.*, 2012). Schuett *et al.* (2010) suggest that personality traits could signal male quality to females. They cite a study of collared flycatchers, *Ficedula albicollis*, which found that males that were bolder and took more risks were able to bond with females more quickly than males that were cautious and avoided risks (Garamszegi *et al.*, 2008). This may indicate that risk taking males are of superior quality or that they are more likely to take risks and commit to raising offspring due to low prospects for future reproduction (Schuett *et al.*, 2010). Although more risk averse males may not be preferred by females in this species, it is possible that by living longer they are able to achieve the same level of reproductive success as bolder individuals on average over their lifetimes. This would explain how different personality can be maintained within a single species.

Although studies such as those cited above do indicate that mate choices may be based on personality they have tended to focus on captive, or semi-wild, birds. This imposes severe limitations on how broadly the results of these studies can be interpreted. For example, it is not yet known if personality is a significant factor in mate choice decisions across the animal kingdom or whether it only applies to a few species of easily reared passerine birds. It is also not known how or if these results apply to animals in the wild since all of the research in this area to date has involved either a small or large degree of experimental manipulation. Finally, past studies linking personality and mate choice have typically focussed on species which form extended pair bonds and in which both the male and female provide parental care. In such cases it is clear to see how the personality type of both sexes could impact upon their success at rearing offspring. It is less clear to see how personality may affect mate choice decisions in polygynous species in which the absence of pair bonds and male parental care means only indirect benefits are at stake.

These gaps in our current knowledge expose a clear need for further research looking at 1) animals other than birds, 2) wild animals in their natural environment and 3) a variety of animals with a broad range of different mating patterns. The grey seal is an ideal study animal in this regard since there is previous evidence of personality in this species (Twiss *et al.* 2011, Twiss *et al.* 2012), it has a very different mating pattern to most birds, it is easily observable in the wild and males provide no parental care.

### ***1.4. Measuring 'personality'***

Just as in humans, many animals show individual differences in behavioural tendencies. When behaviours within individuals remain relatively consistent over time whilst being different from other animals of the same species, sex and age they are considered to be aspects of personality (Koolhaas *et al.* 1999, Réale *et al.* 2007). Since different species differ radically in their range of behaviours there can be no one single test of personality that can be applied to all species. Instead tests have be species specific in their design but still align with the generally accepted personality axes (e.g. fast-slow, bold-shy or proactive-reactive). This means that personality studies may not be directly comparable across species, although with careful study design this should be possible to some degree.

Studies of personality typically focus on one or a few elements of an animal's behavioural repertoire in which there is recognised to be individual variation within the species. For example, in great tits (*Parus major*) exploratory behaviour has been used as a measure of personality. Dingemanse *et al.* (2002) introduced wild-caught great tits into a sealed room containing five artificial trees, they recorded the total number of flights and hops the birds made within 2 minutes and used this as a measure of the birds exploratory behaviour. When some of the same birds were re-caught from the wild up to six months later they were found to show the same level of exploratory behaviour as they had previously. This study showed that great tits are individually consistent in their willingness to explore a novel environment and this has been taken as a measure of personality.

Briffa and Greenaway (2011) were able to identify personalities in a sea anemone (*Actinia equina*) by examining the repeatability of a startle response in individuals. This was induced by discharging a syringe of seawater into the anemones' oral disc causing them to retract their tentacles. The duration of this response was recorded three times over 14 days and shown to differ between individuals but remain consistent within individuals. This study shows that even relatively simple animals such as anemones have personalities.

Twiss *et al.* (2011, 2012) examined the personality types of wild female grey seals at a breeding colony in North Rona, Scotland, using the rate at which females turned to check on their pup as a behavioural metric. Pup check rates were examined in two situations, undisturbed and disturbed, where disturbance was induced using a remote controlled vehicle to deliver a novel auditory stimulus (a pre-recorded wolf call) within 2 metres of the focal seal. By comparing the pup-check rates within and across situations, Twiss *et al.* (2011, 2012) were able to place individuals along a smooth continuum of personality types, from proactive to reactive. Those classed as more proactive showed consistency in pup-check rates both within and across situations, their pup-check rates did not change after disturbance. In contrast, those classed as more reactive showed consistency in pup-check rates within, but not across, situations, they responded to disturbance by increasing their pup-check rate.

These are just some examples from an expansive literature showing that personality is common and widespread in the animal kingdom. Studies investigating personality can potentially use any behaviour to measure it, so long as there are consistent individual differences in that behaviour between individuals within the same species and sex and age class. However, if studies of personality in different species are to be comparable it is important to determine how different measures of personality relate to one of main recognised personality axes (fast-slow, shy-bold, proactive-reactive).

### ***1.5. The advantages and disadvantages of experimental and observational personality studies***

The ultimate aim of almost every study of animal behaviour is reveal how animals behave and why they do so in their *natural* environments. There are two ways in which such studies can proceed, either by behavioural observation in which the researcher does not manipulate or intervene in events, or by experiment in which events are deliberately controlled or manipulated in order to test a specific hypothesis (Stamp Dawkins, 2007). The major benefit of experimental studies is that by carefully controlling different variables correlation can be convincingly separated from causation. Experiment is therefore the “gold standard” of the scientific method and the most effective means available of disentangling cause and effect (Stamp Dawkins, 2007). There are however many instances where experimental studies may not be possible or even desirable. For example, some animals may not respond well to human intervention either in the field or in the laboratory. There may also be practical difficulties with the experimental approach such as when working with animals in extreme or inaccessible environments. Ethical considerations must also be taken into account, for most proposed research it is necessary to pass an internal ethical review board and to meet the guidelines of organisations such as the Association for the Study of Animal behaviour (Rollin & Kessel, 1998) if the work is to be authorised and published. The ethical treatment of study animals is also important from a scientific perspective, as any adverse treatment of study animals could alter normal behaviour patterns and so invalidate any results emerging from the study. Although experimental techniques take pride of place in animal behaviour research, purely observational studies still have an important role to play and may in many cases prove advantageous over the experimental method. One major benefit of observation over experimentation is that it allows researchers to see how animals really behave in their natural environments without human interference. Whilst well designed experimental studies may try to minimise their impact on the animals' natural behaviour there is always likely to be some impact caused by disturbance and artificial conditions. When well designed, and with clearly defined research questions, observational studies can be just as effective as their experimental counterparts (Tinbergen, 1963). However, in studies of animal personality, and animal behaviour in general, it is the experimental technique that is most often used. Animals used in these studies may be either kept in laboratory conditions for their whole lives (Colléter and Brown, 2011, Schuett *et al.* 2011, Williams *et al.* 2012), taken from the wild for the duration of the experiment and then released (Dingemanse *et al.* 2002, van Oers *et al.* 2008, Minderman *et al.* 2009), or in some cases experimental manipulation may take place on wild animals in their natural habitat (Garamszegi *et al.*, 2008, Briffa and Greenaway, 2011, Twiss *et al.* 2011, Betini *et al.* 2012, Twiss *et al.* 2012).

Despite the surge in interest in animal personality over the past decade there have been very few studies looking at animal personalities in the wild and even fewer that did not use experimental manipulation (although see Réale *et al.* 2000). One possible reason for this is that experimental studies are able to control for external factors affecting animal behaviour much more easily than observational studies can. Laboratory studies in particular can be conducted in precisely controlled environments where factors such as temperature, weather and noise are standardised for all animals involved (Colléter and Brown, 2011, Schuett *et al.* 2011, Williams *et al.* 2012). This may be particularly important when looking at fine-scale behavioural differences between individuals as external factors could alter individual behaviours in a way that masks personality (Killen *et al.* 2013). However, although conducting observational studies of personality in wild animals may be difficult, such an approach should not be ignored. Controlled experimental studies have provided compelling evidence that animals do have personalities. As a result, studies of animal personality are beginning to shift their attention away from establishing that personality in animals exists and towards learning what the functional implications of personalities are to individuals in the wild (Dingemanse *et al.* 2004, Smith and Blumstein, 2008, van Oers *et al.* 2008, Minderman *et al.* 2009, Betini and Norris, 2012, Twiss *et al.* 2012, Wolf and Weissing, 2012).

There are currently no studies of animal personality in which there has been no human intervention. However, there are several studies of personality in wild animals in which the experimental component was kept to a minimum and these have yielded interesting results. For example, personality has been linked to individual fitness (Dingemanse *et al.* 2004, Betini and Norris, 2012, Twiss *et al.* 2012), environmental sensitivity (Minderman *et al.* 2009) and mate choice decisions (Dingemanse *et al.* 2004, van Oers *et al.* 2008). Although their study involved some experimentation Twiss *et al.* (2012) were able to identify repeatability in undisturbed pup-check rates in grey seals based solely on observation. In addition, there are numerous examples of observational studies of animal behaviour that have produced interesting and useful results despite a lack of experimentation (e.g. Twiss *et al.* 2000, Cäsar *et al.* 2012, Bishop *et al.* 2013). This suggests that observational studies that are carefully designed using prior knowledge of the study species and site, and building on the results of previous experimental studies can provide valuable information into the prevalence of personality in wild animals and its' functional implications.

## ***1.6. The biology of grey seals: Their classification, range, diet and reproductive system***

The grey seal, originally described by Fabricius in 1791, is a pinniped belonging to the family Phocidae, members of which can be distinguished by their short forelimbs, short vibrissae, lack of external ear flaps and a pelvis that does not allow for quadrupedal locomotion (Beaumont and Goold, 2007). It is the largest member of the sub-family Phocinae, the northern seals, and is most closely related to members of the genus *Pusa* including *P. caspica,* the Caspian seal, *P. hispida,* the ringed seal and *P. sibirica,* the Baikal seal (Higdon *et al.* 2007).

Grey seals inhabit an extensive northern range stretching from the east coast of Canada to Iceland, the United Kingdom, Scandinavia and the Baltic, there is also a small population in France (Reidman, 1989) (Fig. 1). The global grey seal population is estimated at around 290,000 – 300,000 individuals split between three distinct populations termed the North-west Atlantic, North-east Atlantic and Baltic stocks (Hammond *et al.* 2005, Beaumont and Goold, 2007). Approximately 38% of this population is found in the UK (SCOS, 2012). Although widely dispersed, grey seals tend to prefer coastal waters where they forage on a wide range of food items including cod, herring, mackerel, squid, sand eels and dragonets (Reidman *et al.*1989, Defra, 2005). Like all pinnipeds, grey seals are semi-aquatic, periodically hauling out onto ice, rocks and sandy beaches to rest, moult, breed and give birth.

Although there are reports of aquatic matings (e.g. Watkins, 1990), grey seals typically mate on land with the exact timing of the breeding season varying according to the geographical location (Reidman, 1989, Worthington Wilmer *et al.* 1999). At Sable Island, Canada, the breeding season runs from December to mid-February (Boness and James 1979), at North Rona, Scotland, from September to November (Twiss *et al.* 2012) and at Donna Nook, England, from late October to mid-December (pers. obs.). During this period of 6-7 weeks there is a constant turnover of females which arrive, give birth to a single pup, mate and return to the sea. Individual females usually stay on the colony for 18-20 days (Pomeroy *et al.* 1999). Males compete with each other during the breeding season for the best positions within the colony where they are able to access, and potentially mate with, as many females as possible. Although not as skewed as in some other pinniped species such as northern elephant seals (LeBoeuf, 1974), the mating system of grey seals does lead to skewed male reproductive success where a few dominant males sire almost all of the next years offspring (Twiss *et al.* 2006). Neither male nor female eat during the breeding season, instead they rely entirely on their fat stores for energy and, for females, for milk to feed their pup (Reidman, 1989).

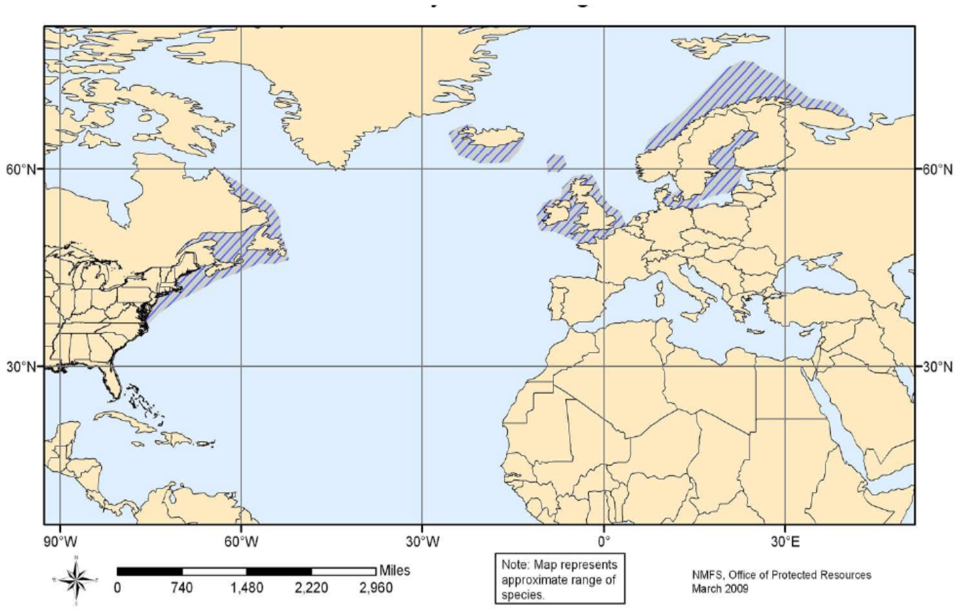

**Figure 1.** Map showing the geographic range of the grey seal (shaded area). *Image from the NOAA* (2013).

## *1.7. The grey seal as a model organism for studying personality and mate choice in the wild*

A large proportion of studies in the behavioural ecology literature are conducted using species which can be easily reared, handled and manipulated in laboratory conditions. Commonly used species include fruit flies, *Drosophila melanogaster* (Friberg *et al.* 2003), great tits, (van Oers *et al.* 2008), Gouldian finches, (Williams *et al.* 2012) and mice, *Mus musculus* (Roberts and Gosling, 2003). Studies of larger animals, with the exception of some primates, are much less common and studies of marine mammal behaviour are very infrequent. One of the main reasons for this may be that large animals are difficult to rear, handle, and house. Studies of marine mammals face the the added difficulty of their aquatic environment which can make long term observation of individuals challenging (Mann, 1999).

Grey seals provide an ideal solution to this problem. They are a large species of marine mammal which during the annual breeding season spends several weeks hauled out on land, often in relatively easily accessible locations (Bishop *et al.* 2013). They also have individually unique pelage patterns meaning that individuals can be reliably re-identified using a range of different photo-identification techniques (Hiby *et al.* 1990, Karlsson *et al.* 2005, Beaumont and Goold, 2007) which removes the need to handle the animals to apply tags or brands (Twiss *et al.* 2011). Once hauled out, individuals do not tend to move far (pers. obs.) meaning that 'known' individuals can be observed for several days or even weeks at a time (Twiss *et al.* 2011). In addition, there is evidence of inter-annual site fidelity in this species making it possible to re-observe the same animals over multiple breeding seasons (Pomeroy *et al.* 2000). These characteristics mean that grey seals are relatively easy to study in the wild and make them an ideal study species for investigating behavioural questions in a large, wild, marine mammal.

# Chapter two: Fieldwork Methods

## 2.0. The study sites

All fieldwork for this study took place at Donna Nook grey seal colony on the north Lincolnshire coast, England (53°28'35.56"N, 0°8'50.95"E) (Figs. 2 & 3). This site is managed by the Lincolnshire Wildlife Trust and spans a Royal Air Force (RAF) training range. Donna Nook is the largest grey seal colony on the British mainland with an estimated population of 5,300 adults (Lidstone-Scott, pers. Comm.). It is also rapidly expanding with pup production doubling from 600 in 2000, to 1400 in 2011 (SCOS, 2012). The breeding season at Donna Nook lasts for 6-8 weeks with individual females coming ashore for 18-20 days. There is therefore a constant turnover of females with the colony reaching its peak density during the last week of November (Fig. 8).

The topography of Donna Nook is mixed, to the north the reserve is topographically varied consisting of low dunes, mud-flats and muddy pools formed by the deposition of material from the nearby river Humber. Here the dunes have trapped areas of salt marsh behind them allowing some vegetation such as samphire (*Salicornia europaea*) and sea buckthorn (*Hippophae rhamnoides*) to develop. Seaward, and to the south, the topography becomes much more uniform consisting almost entirely of mud flats and sand stretching approximately one mile from the dunes to the sea (Bishop *et al.* 2013).

The colony at Donna Nook can be divided into two parts with an outer colony forming along the shoreline and an inner colony forming around one mile further inland by the dunes (Bishop *et al.* 2013). The inner colony itself can be further divided into northern and southern aggregations. To the north the site is open to the public (hereafter referred to as the public site) and the seals here experience high levels of anthropogenic presence caused by up to 70,000 visitors per year (SCOS, 2012). In an attempt to reduce contact between humans and the seals, a fence has been erected at the public site which prevents people from walking out onto the colony and stops the seals from moving further inland than the edge of the dunes. To the south the site (hereafter the RAF site) is used as an RAF training range and is closed to the public year round. This greatly reduces the number of humans present at this site, however, both sites are subject to high noise levels caused by low flying aircraft. Grey seals typically haul out on remote uninhabited islands or undisturbed coasts, Donna Nook is therefore quite unusual as the seals there appear to have become habituated to high levels of human presence (SCOS, 2012).

**Figure 2.** The location of the study sites within the UK (Image: Google Inc. 2013).

**Figure 3.** An aerial photograph of the two study sites (Image: Google Inc. 2013).

**Figure 4.** A photo of the RAF study site. The topography here is flat and fairly uniform (image: Hardman, S. 2012).

**Figure 5.** The public site. The topography here is varied, consisting of dunes, muddy pools and vegetation (Image: Bishop, A. 2012).

### ***2.1. Dates, times and locations of study***

All field work for this study took place between November 4th and December 14th 2012. Observations were made every day during this period (with the exceptions of the 21st, and 25th November due to bad weather) for approximately eight hours a day, from 8am to 4pm. Observations were made at both the public and RAF sites on alternating days. Observations at the public site were made outside from the fence-line whilst at the RAF site a wooden hide placed on the dune was used. At both sites the distance from the observation point to the seals was minimal, in most cases the closest seal was within five metres. To simplify analyses, the dates recorded during field observations were converted to day of season (DOS), a continuous scale starting with day one on the 26th October and ending with DOS 49 on December 14th. The 26th October was chosen as a starting point as it allowed data from this study to be easily compared with another study which started on this date at the same location.

### ***2.2. Identifying individual females by photo ID***

Grey seals have individually unique pelage patterns which allows for easy and reliable identification of individuals without the need for tags, brands or other artificial markings (Twiss *et al.* 2012). Every female seal observed for this study was assigned a unique ID number (public site $n$ = 65, RAF site $n$ = 68) and was photographed multiple times from both the left and right flanks so as to record pelage patterns in as much detail as possible. Weather-resistant survey books (Chartwell survey book 2026) were also used to record unique individual markings by sketching in the field. All photographs were matched to the seal's ID number and added to a digital photo catalogue from which individuals observed in future encounters could be reliably re-identified by their pelage patterns. Photographs were taken using a Canon 500D dSLR fitted with a Tamron 70-300mm zoom lens. IDs for male seals observed on the study sites were obtained from Bishop and Stewart (pers. comm.) and were also used in the analyses.

### ***2.3. Mapping the locations of individually identified females***

A key prediction of this study was that proactive females should occupy high density areas of the colony whilst reactive females should occupy low density areas of the colony. In order to test these hypotheses the positions of all females, males and pups observed on the study sites (together with IDs when known) were recorded hourly each day.

At the RAF site a scaled aerial photograph of the site taken from Google maps was used, onto which the hourly positions of all females, males and pups within the study site were recorded (Fig 6.). Up to three maps were used each day with one map being used to record up to three hours of observations. The position of aerial training markers along the horizon of the study site were used as reference points to improve the accuracy with which the position of individual seals could be recorded. A Nikon 550 laser range-finder was used to accurately judge the distance of individual seals from the position of the hide.

Due to difficulties obtaining sufficient data from the initial observation position at the public site it was necessary to move to a new location for which aerial maps were not available. An alternative approach was therefore used. Proximity maps consisting of five concentric circles were used to record the *relative* position of female grey seals to each other (Fig 7.). The distance between each concentric circle represented one seal body length (approx 2 metres). One proximity map was produced for every animal observed on the study site each hour with the focal seal at the centre and the position of all other seals (including males and pups) up to five body lengths (10 metres) away recorded around her. When known, individual IDs were also recorded onto the maps. The distance of ten metres was chosen as Twiss *et al.* (2000) determined that this would include sufficient and relevant information about the density of other seals around focal females. Although proximity maps did not provide information on the geographic location of individuals they did provide detailed information about the concentration of individuals around each focal seal and so provided a measure of the relative density of the colony at which each seal was observed.

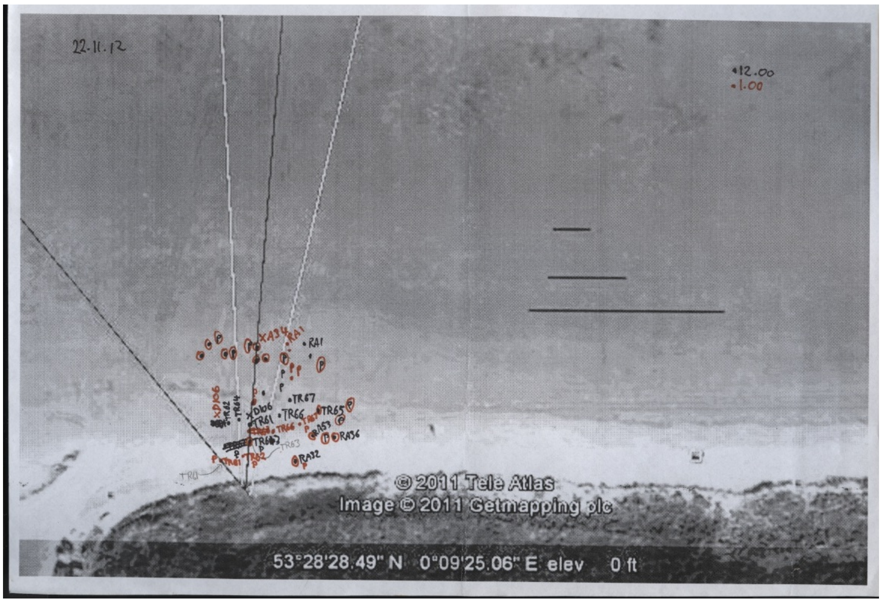

**Figure 6.** An example of the aerial maps used at the RAF site. The vertical lines on the map point to aircraft targets on the beach which were used as an aid to marking the location of seals accurately on the map. The point at which the lines converge was the location of the hide. The horizontal scale bars to the right represent 10, 20 and 50 metres respectively.

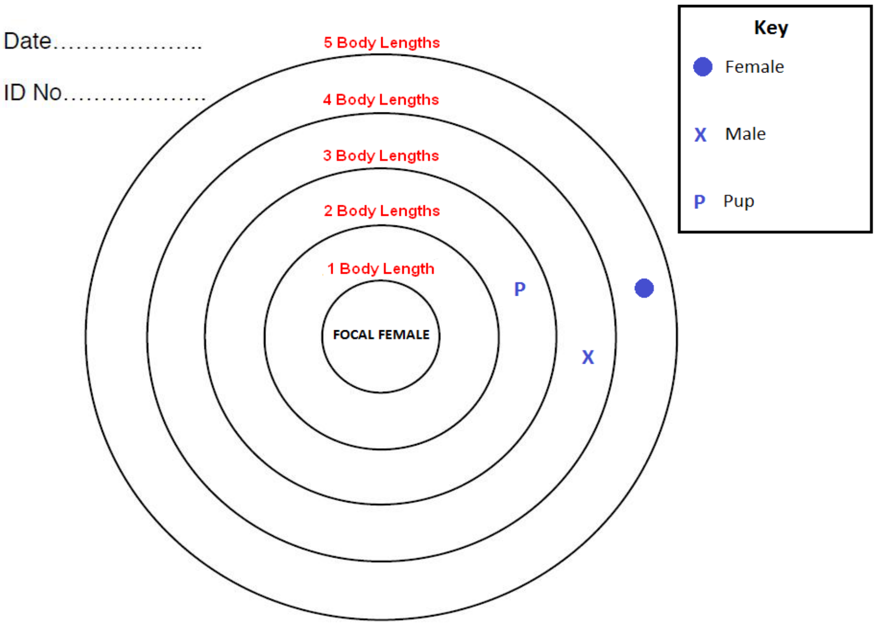

**Figure 7.** An example of the proximity maps used at the public site. One map was used per seal per hour. The focal seal was placed at the centre of the map and the position of other seals within ten metres marked on the map around her. The distance between circles represents one seal body length up to a distance of five body lengths (approximately 10 metres). Dots were used to mark females, X's for males and P's for pups.

### ***2.4. Recording individual aggressive behaviours by ad-lib observation***

Both female-female and female-male aggressive interactions (defined as consisting of one or more behaviours explained below) were recorded *ad-lib* during observation periods in a weather-resistant field notebook (Chartwell survey book 2426). The IDs of the interactants, the time, date and study site were recorded. In addition, an ethogram (based on that used by Culloch, 2012) was used in the field to record the general occurrence of specific aggressive behaviours defined as follows:

***Open mouth threat (OMT)*** – This is the most common aggressive behaviour used on the colony and consists of an open mouth gape, revealing teeth, directed at another individual.

***Flippering (FL)*** – another common aggressive behaviour in which individuals scratch each other using the claws on their flippers. This behaviour can vary in intensity from mild to strong aggression.

***Lunge (LU)*** – Quickly stretching out the neck with teeth bared so as to lunge at another individual.

***Bite (B)*** – Rarely used by females except in some very aggressive encounters.

***Chase (CH)*** – On some occasions the 'winner' of an aggressive encounter will chase the 'loser'.

***Growl (GR)*** – A clearly audible growling noise directed towards another individual or individuals.

The intensity of each aggressive interaction was also recorded qualitatively using a five point scale where 1 = very mild aggression and 5 = very strong aggression. This meant that it was not only possible to examine differences in rates of aggression between individuals, but also differences in intensity. Each point of the scale was defined as follows:

***1*** – No contact or significant movement, only open mouth threats or clearly directed growls.

***2*** – No contact, or very brief insubstantial contact, individuals may move to confront one another with open mouth threats and growls.

***3*** – Some contact that persists for less than 5 seconds, open mouth threats, growls, lunges and flippering.

***4*** – Contact for more than five seconds, open mouth threats, growls, lunges and flippering.

***5*** – Prolonged and very aggressive contact including open mouth threats, growls, lunges, flippering and biting.

## *2.5. Recording the stage of pups belonging to individually identified females*

Grey seals give birth to altricial and relatively helpless young which develop rapidly, passing through several developmental stages before all parental care stops after around 18 days. It has been suggested that pup stage is linked to female aggression as females are more aggressive when their pups are young and vulnerable than when they are older and more robust (Boness *et al.* 1982). When possible pup stage was therefore recorded for all females for which IDs were known and which were observed with their pup in the field. Depending on their age grey seal pups can be categorised into one of five developmental stages which, based on Kovacs and Lavigne (1986), can be defined as:

***Stage one:*** Newborns, pale yellowish tint to the fur, skin in loose folds and awkward locomotion.

***Stage two:*** Well defined neck, trunk of body a cylindrical shape, white fur.

***Stage three:*** Neck and trunk of body combine to form a fusiform shape, fur greyish white.

***Stage four:*** Lanugo (white neonatal fur) being shed from anywhere on the body except the face. Body shape now large and round with no loose skin.

***Stage five:*** Lanugo now completely shed revealing the underlying juvenile pelage underneath. A few isolated tufts of white fur <5cm in diameter may still remain.

### *2.6. Investigating CIDs in female grey seals in disturbed and undisturbed states*

Consistent individual differences in the behaviour of individual female grey seals were measured by examining the consistency of their pup-check rates across time and situations. Pup-checks were defined as situations in which the female turns her head to make a clear and directed look at her pup and can be considered a measure of maternal attentiveness (Twiss *et al.* 2012). Individual consistency in pup-check rates were assessed in two situations; disturbed and undisturbed. These were defined as follows:

> **Disturbed:** the period immediately following an aggressive interaction with an adult conspecific or conspecifics.
>
> **Undisturbed:** Periods in which individuals had not received any form of external disturbance (e.g. aggression from other seals or human disturbance such as noise) for at least ten minutes. This time period was chosen as it was observed that females generally returned to their pre-disturbance pup-checking rates after around ten minutes.

### *2.7. Using video focals to record individual pup-check rates for use as a measure of CIDs*

Data on pup-check rates were collected by *ad-lib* focal recording (Altmann, 1974) of individually identified female grey seals using a Panasonic HC-V520 digital video camera mounted on either a tripod (public site) or a clamp mount (RAF site). Disturbed video focals were recorded for up to 20 minutes starting from the moment the aggressive interaction ended. This time period was chosen as, without additional disturbance, almost all females returned to an undisturbed state in ten minutes or less. Following the methods used by Twiss *et al.* (2012) undisturbed video focals were recorded for a minimum of 20 minutes and up to half an hour. If the focal animal(s) were disturbed during this period the recording was stopped and only footage from before the disturbance used. Undisturbed video focals shorter than 20 minutes were discarded.

# Chapter three: Analytical methods

## *3.0. Examining colony density*

### *3.0.1. Converting paper maps into a GIS database*

The paper maps used at the RAF site were scanned as high resolution JPEG images, loaded into ARCinfo version 10.0 and georectified by matching landscape features visible on the maps with their coordinates (OSGB) obtained from Google earth (Version 7.1.1.1888, Google inc. 2013). ARCmap version 10.1 was then used to overlay points showing the hourly positions of all mapped seals along with their IDs when known. These data were converted into a spatial database which provided hourly records of the location of females together with information on the colony density within ten metres of focal females and the presence, absence and the duration of stay of all identified individuals on the colony.

### *3.0.2. Converting paper proximity maps into an excel database*

Data from the proximity maps were entered into a Microsoft Excel spreadsheet. This database was used to extract information on colony density within ten metres of focal females and the presence, absence and duration of stay of females on the colony.

### *3.0.3. Calculating colony density around focal females*

To calculate the colony density around observed females each day a count of the number of other seals falling within a ten metre radius of their position was extracted from either a GIS database (RAF site) or from an excel spreadsheet (public site) for each female at hourly points each day. The hourly figures were summed for each day then divided by the number of hours of observation to provide a mean hourly colony density figure for each female per day. Only individuals with at least three hours of continuous observation on the day in question were included. This figure provides a measure of the density of other seals around focal females and can be used as a rough proxy for colony density. High values suggest that the female was located in a high density area of the colony, whilst low values suggest a low density area.

### ***3.1. Examining how the density of seals in close proximity to focal females changed over time***

Increased colony density was expected to lead to higher rates of aggression through increased female-female and female-male contact. To investigate this possibility it was necessary to establish if colony density did change significantly over the breeding season. To accomplish this the relationship between the local colony density around individual females and the day of season was examined using a Generalised Additive Model (GAM). This method was chosen as the relationship between these two variables was not linear. GAMs are non-parametric extensions of Generalised Linear Models which allowed the data themselves to suggest the pattern of colony density over time, rather than requiring the parametric form of the data to be specified prior to modelling (Boveng *et al.* 2003, Bishop, 2011). Analysis was conducted using the package *mgcv* (Wood, 2011) and the function *gam.* The model was run with a Gaussian error distribution with a mean determined by an identity link function.

### ***3.2. Examining pup-check rates of individual females***

#### *3.2.1. Extracting pup-check rates from video focals*

Pup-check data were extracted from 190 videos totalling 87 hours of video footage (RAF site = 46hrs, public site = 41hrs). 65 videos were of females recorded after a disturbance (RAF site = 33, public site = 32) and 125 were of females in undisturbed states (RAF site = 67, public site = 58). Videos were played back in the laboratory at real-time speed using VLC player (version 2.07) and data extracted using a custom Microsoft excel VBA data recording program. Frequencies of pup-checking were standardised to rates per minute.

#### *3.2.2. Comparing pup-check rates between the RAF and public sites*

Differences in both undisturbed and disturbed pup-check rates between the two study sites were examined statistically using GLMM models with female ID set as a random effect to account for pseudo-replication. In both cases the data were log transformed to comply with the requirement for normality and the models run with a Gaussian error distribution with a mean determined by an identity link function. All valid records (using the criteria described above) were included in these analyses. Analyses were conducted using the package *lme4* (Bates *et al.* 2013) and the function *lmer.* A *P*-value was calculated using the package languageR (Baayen 2011) and the function *pvals.fnc.*

## ***3.3. Examining individual daily rates and intensities of aggression***

### *3.3.1. Preparing aggression data for analyses*

Due to slight differences in the duration of observations each day the number of daily aggressive interactions for each individual observed was standardised to 8 hour observation periods. These were then converted into mean rates$^{-hr}$ of aggressive interactions per day for all observed females. Some females were observed to show very high levels of aggression for very short periods of time, such as when first arriving on, or leaving, the study site. Extrapolating aggression rates from these short periods of observation to a mean daily rate$^{-hr}$ could therefore have led to inaccurate and skewed results. To minimise the risk of greatly over or under estimating an individual's mean daily rate$^{-hr}$ of aggressive interactions, these were only calculated for females that had been mapped for at least three consecutive hours on the study site on the day in question.

### *3.3.2. Comparing rates and intensities of aggression between the RAF and public sites*

To examine differences in the daily rates of aggression between females at the RAF and public sites a GLMM model was used with female ID set as a random effect to account for pseudo-replication. The data were log transformed to comply with the requirement for normality and the model run with a Gaussian error distribution with a mean determined by an identity link function. Analysis was conducted using the package *lme4* (Bates *et al.* 2013) and the function *lmer.* A *P*-value was calculated using the package languageR (Baayen 2011) and the function *pvals.fnc.* The same procedure was followed to examine the difference in daily aggression intensity scores between females at the RAF and public sites with the score per interaction replacing the number of interactions each day in the model.

## ***3.4. Investigating the possibility of using pup-check rates as a behavioural metric***

### *3.4.1. Using ICC tests to examine consistent individual differences in pup-checking behaviour*

Individual consistency in pup-check rates were analysed using a one-way intra-class correlation (ICC) model. This model examines the similarity of repeated measures within groups (or classes). In this case the IDs of individual females were the groups and their pup-check rates per minute were the measures tested for repeatability. The repeatability of pup-check rates in disturbed and undisturbed situations were tested separately. Only female IDs with at least two disturbed or two undisturbed measures of their pup-check rate were included in these analyses. All analyses were conducted using the R package *ICC* (Wolak *et al.* 2012) and the function *ICCest*. This function does not provide *P*-values so these were calculated *post-hoc* using an ANOVA test to test the null hypothesis that individual repeatability of pup-check rates was not greater than 0. All results giving a significant *P*-value (≤0.05) are considered to be repeatable.

### 3.4.2. Proactive and reactive behavioural types

Twiss *et al.* (2012) found that within individuals pup-check rates were highly repeatable, both before and after disturbance. Based on this they were able to classify females as having either ‘proactive’ or ‘reactive’ behavioural types based on the difference in their pup-check rates between disturbed and undisturbed states where disturbance was induced using a pre-recorded wolf call as a standardised stimulus. Females that significantly increased their pup-check rate from undisturbed and disturbed situations were classed as reactive whilst those that did not change their pup-check rates were classed as proactive. In the present study it was not possible to use a standardised stimulus to induce disturbance and so natural disturbances (fights) were used instead. This meant that it was not possible to use the same measures of proactivity and reactivity as Twiss *et al.* (2012) used, as using natural disturbances led to high levels of individual variation in disturbed pup-check rates which were not repeatable when tested (see section 4.4.).

An alternative measure of proactivity-reactivity was therefore used. Based on the assumption that over multiple observations a females maximum and minimum pup-check rates would be observed, all females for which pup-check rates had been recorded in at least two undisturbed *and* two disturbed situations were assigned a score equal to the difference between their minimum observed undisturbed pup-check rate and their maximum disturbed pup-check rate. This equates to the greatest observed difference in pup-check rates for each individual. This figure was then converted into a score from zero to one (hereafter termed the 'proactive-reactive score') where a score of zero was given to the individual with the least observed difference in pup-check rates and a score of one to the individual with the greatest. On this scale females with low scores are considered proactive (their pup-check rates do not change across situations) whilst those with high scores are considered reactive (they react to disturbance by increasing their pup-check rate). It is possible that the assumption that over multiple tests each seal's minimum and maximum pup-check rates were observed was not met in every case. However, given the long periods of observation and multiple measures of pup-check rates recorded for all individuals included in the analyses, it is likely that the data presented here are a reasonable approximation of this assumption.

### ***3.5. Examining which factors best explain female-male and female-female aggression using Generalised Linear Mixed Models (GLMMs)***

GLMMs are similar to the more commonly used Generalised Linear Models (GLMs) except that they allow for both random and fixed effects to be incorporated into the models. Unlike GLMs, GLMMs do not require that all samples in the model are independent of each other, they are therefore particularly useful in cases where pseudo-replication is a problem. For example, where there are multiple measures taken from the same sample, site or individual (Bolker *et al.* 2009).

To examine factors potentially contributing to individual variation in the number of daily aggressive interactions shown by females, GLMMs were used with female ID set as the random effect to account for unequal sampling and pseudo-replication. All analyses were conducted using the package *lme4* (Bates *et al.* 2013) and the function *lmer.* Separate models were created to examine aggressive interactions shown towards males, towards other females, and towards males and females combined (a general explanation for female aggression). In each case a global model was first created including colony density, proactive-reactive score and pup-stage set as fixed effects. These variables were chosen as fixed effects as both pup stage and colony density have been linked to increased rates of aggression in previous studies (Boness *et al.* 1982, Pomeroy *et al.* 2000), while including proactive-reactive scores allowed the hypothesis that aggression is linked to personality to be tested. Where necessary the data for the number of daily aggressive interactions were either log or square root transformed to meet normality requirements. In one case (aggression towards males and females combined) it was necessary to square root transform the data twice. All GLMM models were run with a Gaussian error and an identity link function.

AIC scores were generated from each global model for all possible combinations of fixed predictor variables using the package *MuMIn* (Barton, 2013) and the function *dredge.* ΔAIC scores were also calculated as the difference between the lowest AIC score and the AIC score of every other possible model. The top three models for each case were selected based on Richards (2008) rules for AIC selection. These rules state that all models with a ΔAIC score of six or less are retained and of these the more complex models which do not have a ΔAIC score lower than that of the simpler models in which they are nested should be removed. Of the remaining models the most parsimonious (the model which includes the fewest fixed effects) is considered the best model. When models are equally parsimonious and have a less than two point difference in ΔAIC scores, they are both considered equally valid. For all the best fitting models in this study Markov Chain Monte Carlo (MCMC) estimated *P*-values were calculated *post-hoc* using the package *languageR* (Baayen, 2011) and the function *pvals.fnc*. For information models with ΔAIC scores above six are included in the results but were not considered when drawing conclusions (section 4.5).

Initially the proactive-reactive score of individuals were included in the models however, this had the effect of greatly reducing sample sizes and thereby reducing confidence in the results. To solve this problem each model was repeated following the procedure described above but with proactive-reactive scores excluded. All selected models are presented in the results chapter (4.5).

### ***3.6. Analytical software used***

All numerical data for this study was entered into Microsoft Excel 2007 and saved as csv files prior to analysis. All statistical analyses were conducted using R studio version 0.97.336 (R Studio, 2012). Map data was entered into a GIS database prior to analysis using ARCinfo version 10.0 and ARCmap version 10.1.

# Chapter four: Results

## *4.0. Data describing the study sites*

### *4.0.1. The number of females observed and the number of individual records of pup-check rates*

133 females were individually identified in the field from their pelage patterns and assigned ID numbers (RAF site = 68, public site = 65). From these 1396 aggressive interactions were recorded (RAF site = 774, public site = 622). Multiple records of pup-check rates were obtained for 33 females when undisturbed (number of tests = 140), and for 24 females when disturbed (number of tests = 76). Of these 22 females had multiple records for both undisturbed and disturbed pup-check rates (*n* = 152) allowing proactive-reactive scores to be calculated. All females with ID numbers were recorded onto maps in the field.

### ***4.1. Changes in the density of the colony within a ten metre radius of focal females over the breeding season***

A significant non-linear relationship was found between colony density and the day of season (unique IDs = 77, *n* = 267, *P* = <0.001, F = 18.31, $R^2_{adj}$ = 0.353). Colony density around focal females peaked around the 25th November (DOS 30) before declining rapidly as females left the beach (Fig. 10).

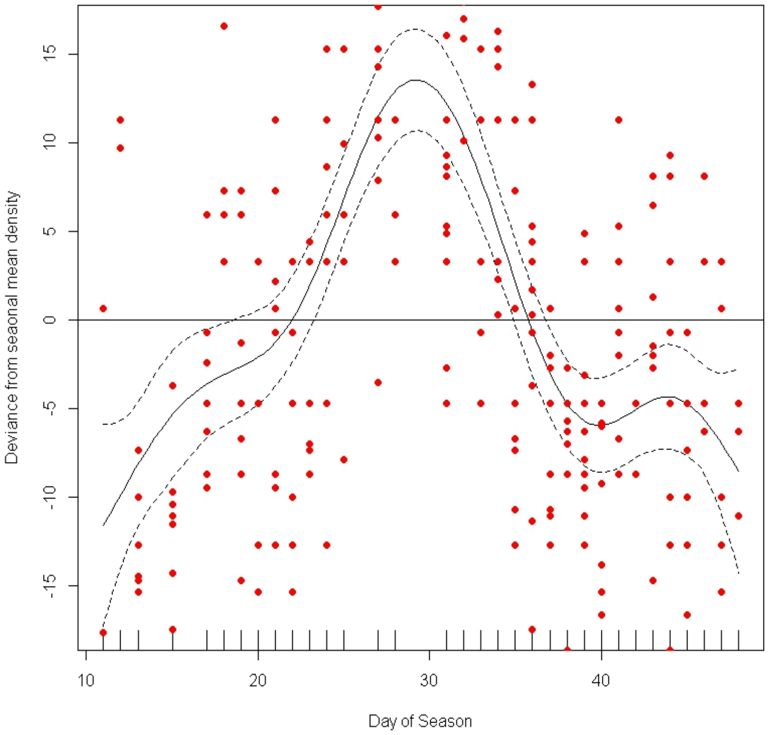

**Figure 8.** A plot of a Generalised Additive Model (GAM) showing the local colony density around focal females plotted against the day of season. The local colony density around individuals is calculated as the mean number of seals per hour per day within a 10m radius of each focal seal. For each day on this plot the points represent the local colony density per hour around individually identified females. The zero on the *y* axis (shown by a horizontal line) marks the mean local colony density around all females averaged across the whole season. Any point above this line represents higher than average local colony density whilst those below it represent below average local colony density. The solid curved line represents the effect and the dashed lines the 95% confidence intervals. This plot shows a significant effect of the day of season on colony density (unique IDs = 77, *n* = 267, *P* = <0.001, F = 18.31, $R^2_{adj}$ = 0.353).

## ***4.2. Visitor numbers at the RAF and public sites***

Donna Nook received an estimated 51,970 visitors during the 2012-2013 season. Of these 20,830 (40.08%) visited on weekdays (Monday – Friday) (Fig. 8), and 31,140 (59.92%) visited on weekends (Fig. 9) (Lidstone-Scott, unpublished data).

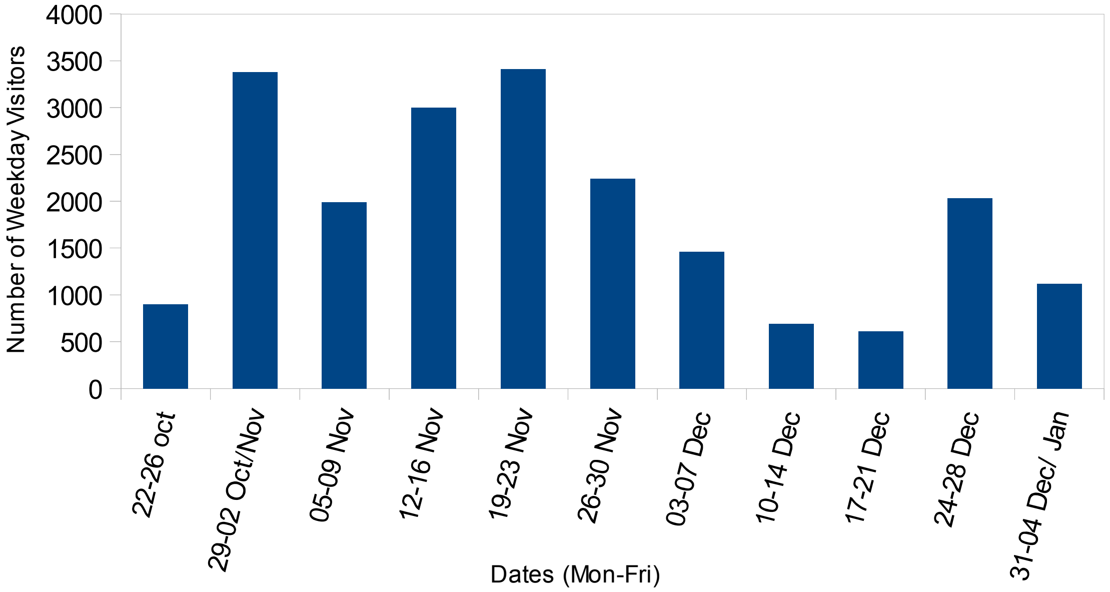

**Figure 9.** The total number of visitors to Donna Nook each week, from the 22nd October 2012 to the 4th January 2013. Figures shown are sum totals from Monday to Friday each week.

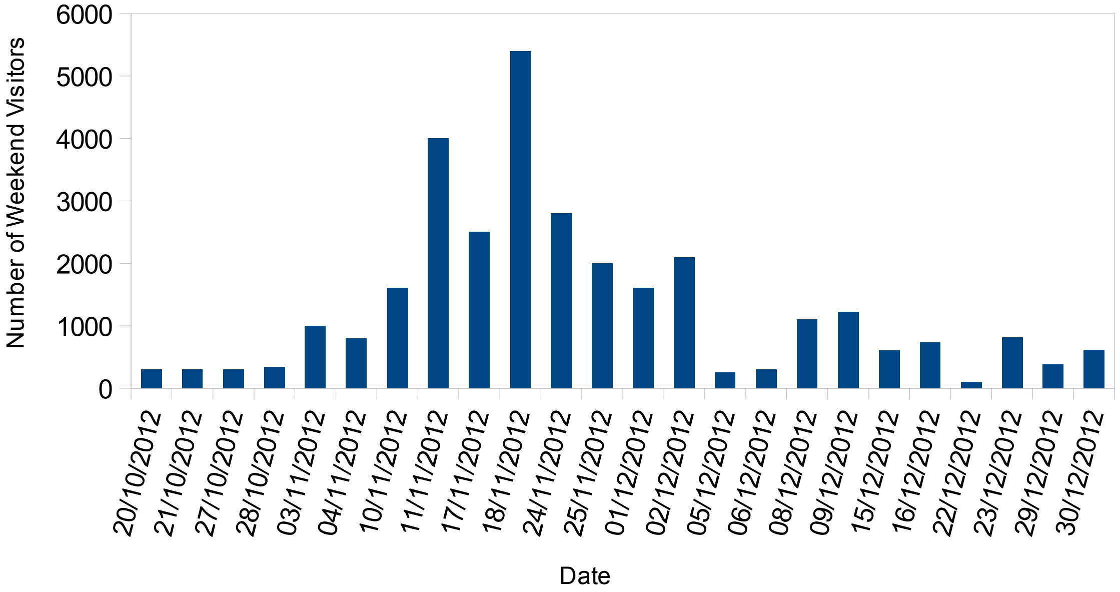

**Figure 10.** The number of visitors to Donna Nook on weekends, from the 20th October 2012 to 30th December 2012. Numbers for both Saturday and Sunday each week are shown.

## ***4.3. A comparison of the RAF and public sites***

### *4.3.1. Comparison of colony density at the RAF and public sites*

The mean number of females observed within a ten metre radius of focal seals at hourly points each day was slightly higher at the public site (2.32), than at the RAF site (2.23), however this difference was not significant (Wilcoxon test, W = 10145, *P* = 0.29). The mean number of males observed within ten metres of focal females at hourly points each day was slightly higher at the RAF site (0.299), than at the public site (0.294), but this result was also not significant (Wilcoxon test, W = 11761, *P* = 0.21).

### *4.3.2. A comparison of mean pup-check rates per minute$^{-1}$ at the RAF and public sites*

Undisturbed pup-check rates per minute$^{-1}$ were found to be higher at the RAF site than at the public site. However, this result was not significant (GLMM *P* = 0.08, *n* = 140) (Fig. 11, table 1). No significant difference was found in disturbed pup-check rates between the two sites.

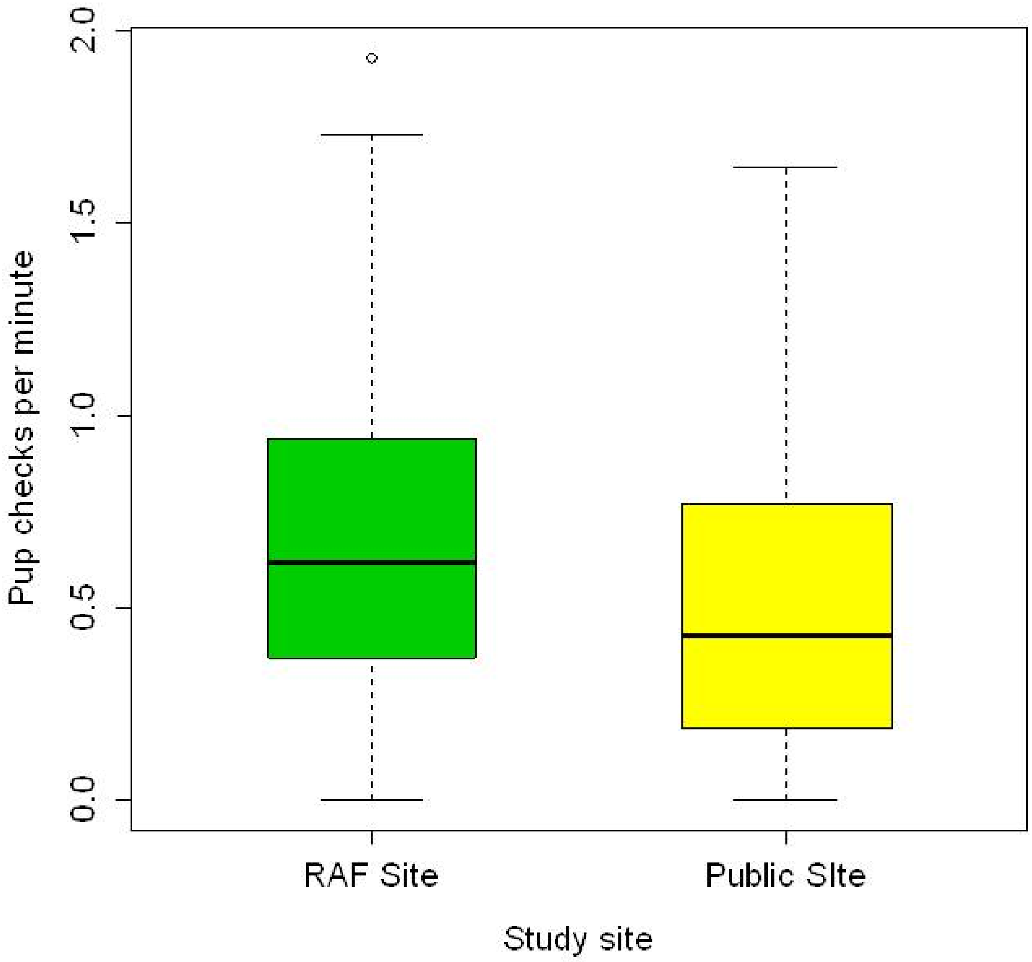

**Figure 11.** The rate of pup-checking by undisturbed female grey seals at the RAF (*n* =85) and public sites (*n* = 55). Boxes show the median (RAF site = 0.62, public site = 0.43), upper and lower quartiles (top and bottom of the boxes), and minimum and maximum values (shown by the dashed lines). The outlier at the RAF site represents female ID 3042 for which the undisturbed pup-check rate was recorded twice, at 1.73 and 1.93 checks per minute respectively.

**Table 1.** The results of a GLMM model used to predict the effect of the study site on the pup-check rate per minute of undisturbed females. These data were normalised by log-transformation. The model is fit with a Gaussian (identity-link) distribution and has female ID set as a random effect. This method was used as it accounts for pseudo-replication caused by included repeated measures from the same individuals in the analysis. This result was not significant although it was marginal.

| **df** | **Number of obs.** | **Number of individuals** | **AICc** | **ΔAIC** | **Intercept estimate (*P*-value)** | **Effect of site estimate (*P*-value)** |
|---|---|---|---|---|---|---|
| 4 | 140 | 48 | 146.5 | 0 | 0.4951<br>(0.0000) | 1.702<br>(0.0853) |

### *4.3.3. A comparison of the number of daily aggressive interactions by females at the RAF and public sites*

The mean number of aggressive interactions individual females were involved in each day was found to be higher at the RAF than at the public site. This result was significant (GLMM, $P = 0.0038$, number of daily records = 361 (RAF site = 158, public site = 203)) (Fig. 12, Table 2).

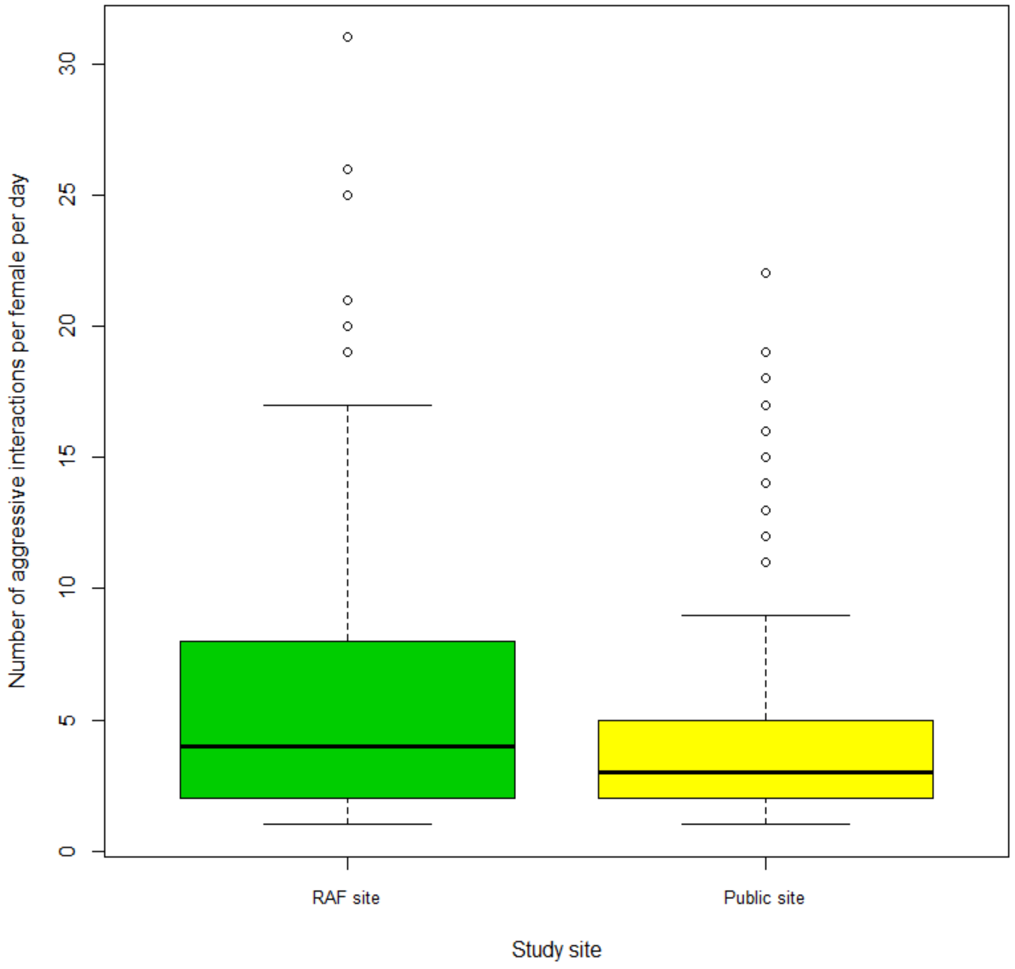

**Figure 12.** The number of daily aggressive interactions of individual females at the RAF and public sites (RAF site median = 4, public site median = 3). Boxes show the median (RAF site = 4, public site = 3), upper and lower quartiles (top and bottom of the boxes), and minimum and maximum values (shown by the dashed lines).

**Table 2.** The results of a GLMM model used to predict the effect of the study site on the number of aggressive interactions females were involved in each day. These data were normalised by log-transformation. The model is fit with a Gaussian (identity-link) distribution and has female ID set as a random effect. This method was used as it accounts for pseudo-replication caused by included repeated measures from the same individuals in the analysis. This result shows that females at the RAF site are involved in significantly more aggressive interactions each day than females at the public site..

| **df** | **Number of obs.** | **Number of individuals** | **AICc** | **ΔAIC** | **Intercept estimate (*P*-value)** | **Effect of site estimate (*P*-value)** |
|---|---|---|---|---|---|---|
| 4 | 361 | 99 | 204.3 | 0 | 4.209<br>(0.0000) | 1.754<br>(0.0038) |

### *4.3.4. A comparison of the mean daily aggression intensity scores of females at the RAF and public sites*

In contrast to the number of daily aggressive interactions, the mean daily aggression intensity scores of individual females were found to be higher at the public than at the RAF site (GLMM, *P* = 0.0141, *n* = 297 (RAF site = 129, public site = 168)) (Fig. 13, table 3).

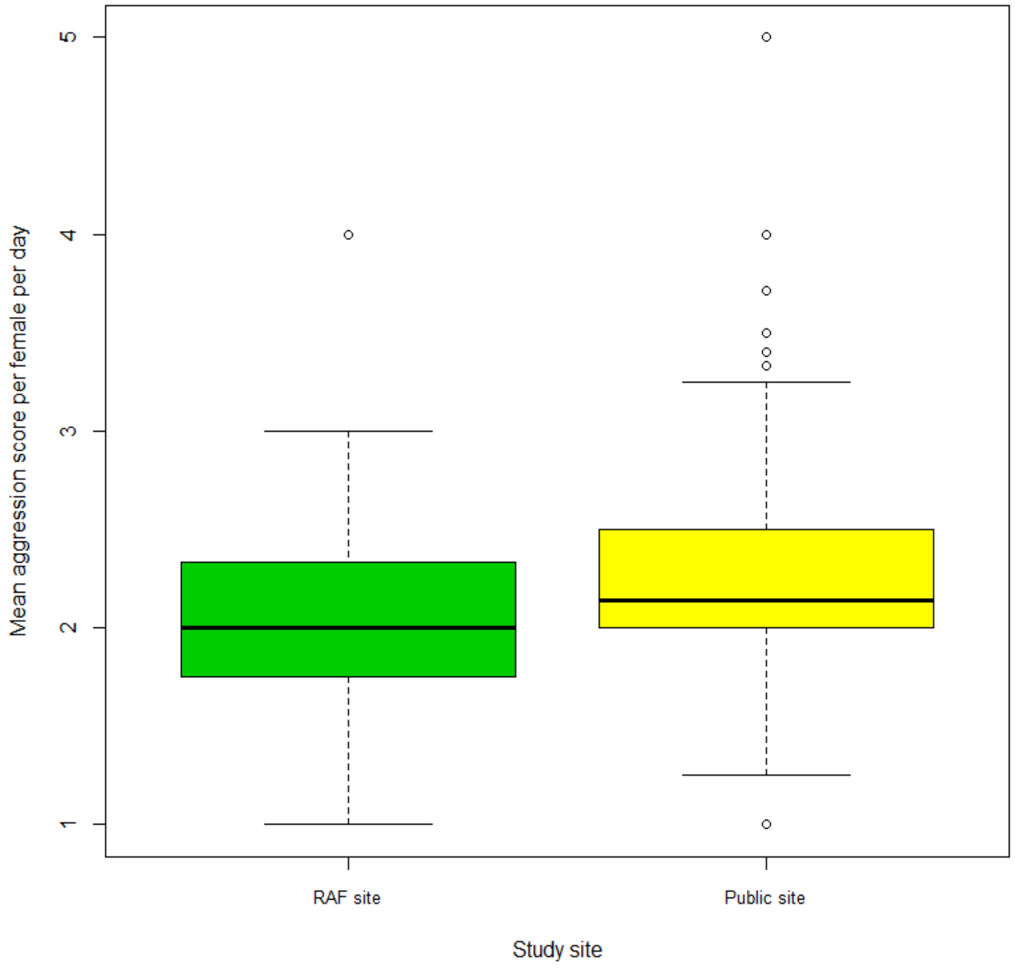

**Figure 13.** The mean daily aggression intensity scores of females at the RAF and public sites (RAF site median = 2. public site median = 2.02). Boxes show the median (RAF site = 2, public site = 2.02), upper and lower quartiles (top and bottom of the boxes), and minimum and maximum values (shown by the dashed lines).

**Table 3.** The results of a GLMM model used to predict the effect of the study site on the aggression intensity scores of females. These data were normalised by log-transformation. The model is fit with a Gaussian (identity-link) distribution and has female ID set as a random effect. This method was used as it accounts for pseudo-replication caused by included repeated measures from the same individuals in the analysis. This result shows that females at the public site had higher scores on average than females at the RAF site.

| df | Number of obs. | Number of individuals | AICc | ΔAIC | Intercept estimate (*P*-value) | Effect of site estimate (*P*-value) |
|---|---|---|---|---|---|---|
| 4 | 297 | 94 | 595.6 | 0 | 2.226<br>(0.0000) | -0.192<br>(0.0114) |

### ***4.4. Results of intra-class correlation (ICC) tests used to examine individual repeatability of pup-check rates***

Intra-class correlation (ICC) tests showed that individuals had a significant degree of repeatability in their pup-check rates when undisturbed at the RAF site (unique IDs = 18, *n* = 85, *P* = 0.00006), the public site (unique IDs = 15, *n* = 55, *P* = 0.01580), and when females from both sites were combined (unique IDs = 33, *n* = 140, *P* = <0.00001) (table 4, Fig. 14). However, although the frequency of disturbed pup-check rates increased significantly after disturbance (Wilcoxon test, W = 9878, *P* = <0.001), individuals were not consistent in their pup-check rates when disturbed at either the RAF site (unique IDs =12, *n* = 42, *P* = >0.05), the public site (unique IDs = 12, *n* = 34, *P* = >0.05), or when study sites were combined (unique IDs = 24, *n* = 76, *P* = >0.05) (Table 4).

**Table 4.** Intra-class correlation (ICC) scores for the pup-check rates (per minute) of females from the RAF site, public site and both sites combined, in both undisturbed and disturbed states. Only individuals where pup-check rates were known from at least two undisturbed and two disturbed video focal were included. The scores show how closely correlated (how repeatable) pup-check rates were within individuals and how different they were between individuals. ICC scores are from 0 to 1 where one is perfect correlation (i.e. pup checks rates are identical across tests) and 0 is no correlation. P-values are also given where significance (≤0.05) indicates a strong correlation.

| Test | ICC score | Lower CI | Upper CI | N | Mean number of measurements per group (k) | Within individual or group variance | Among individual or group variance | *P*-value |
|---|---|---|---|---|---|---|---|---|
| **Undisturbed females from both sites** | 0.390066 | 0.2084969 | 0.5856302 | 33 | 3.754 | 0.1140009 | 0.07290603 | <0.00001 *** |
| **Undisturbed females from the RAF site only** | 0.4068411 | 0.1797167 | 0.6631104 | 18 | 4.159443 | 0.1085352 | 0.07444311 | 0.00006 *** |
| **Undisturbed females from the public site only** | 0.3144253 | 0.02519976 | 0.6394942 | 15 | 3.198251 | 0.1233247 | 0.05656042 | 0.01580 * |
| **Females from both sites recorded after a disturbance** | 0.06116239 | -0.1430677 | 0.3342702 | 25 | 3.023026 | 0.6645446 | 0.04329304 | 0.31000 |
| **Females from the RAF site recorded after a disturbance** | -0.1109742 | -0.2904556 | 0.2421227 | 13 | 3.198413 | 0.6643883 | -0.06636514 | 0.75600 |
| **Females from the public site recorded after a disturbance** | 0.2333056 | -0.1204477 | 0.637474 | 12 | 2.796791 | 0.6647505 | 0.202284 | 0.11 |

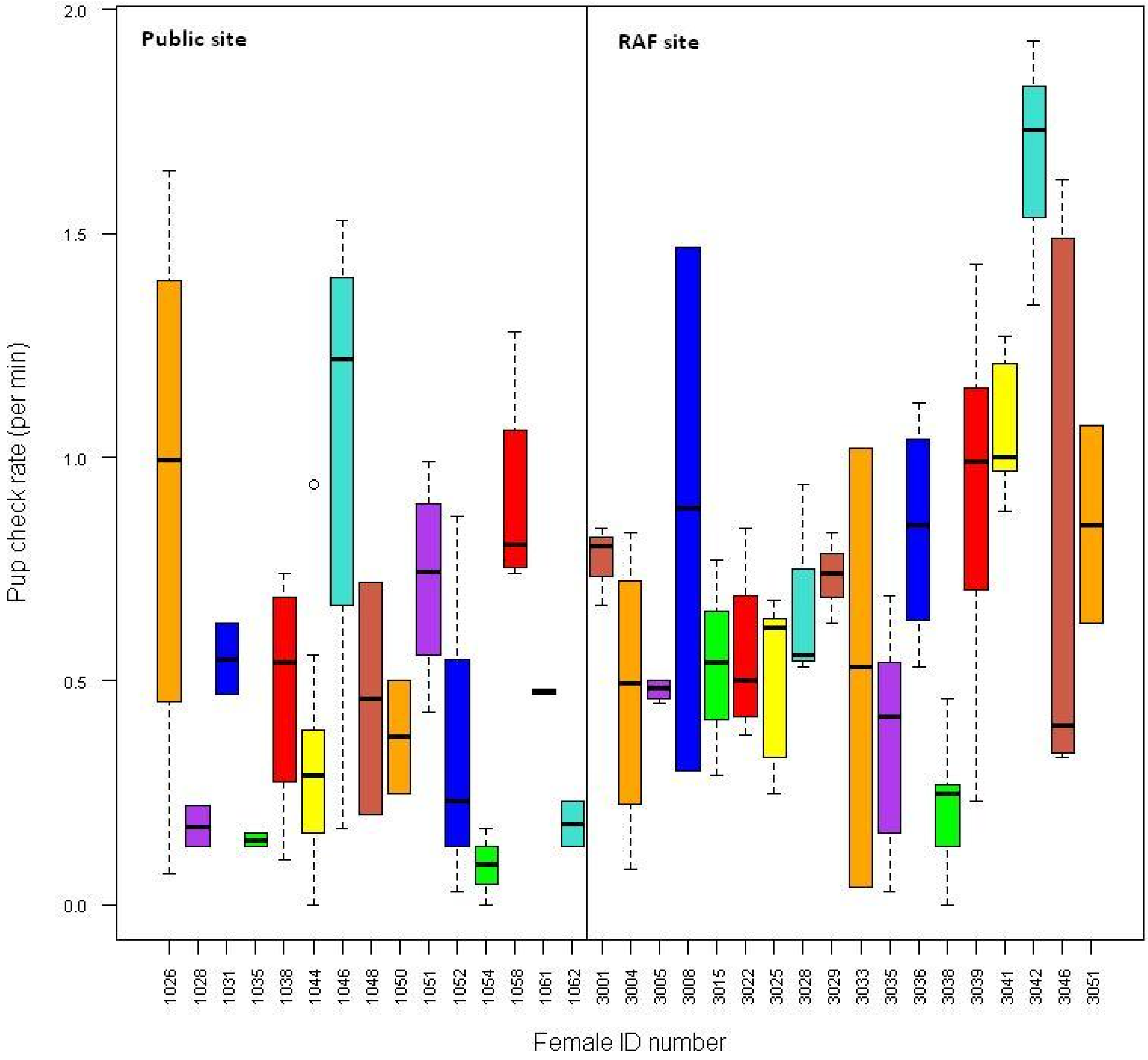

**Figure 14.** Box plots showing the variability in undisturbed pup-check rates (per minute) within individual females from the public and RAF sites. ICC tests revealed that both the public site (ICC test, unique IDs = 15, *n* = 55, *P* = 0.0158) and RAF site (ICC test, unique IDs = 18, *n* = 85, *P* = 0.00006) showed significant repeatability in pup-checking rates in undisturbed situations. When the data from both sites were combined this result remained (ICC test, unique IDs = 33, *n* = 140, *P* = 0.00001).

## ***4.5. Results of Generalised Linear Mixed Models (GLMMs) used to examine factors explaining female aggression***

Tables 2-7 show the results of GLMM models examining potential factors explaining individual daily rates of aggressive interactions of females towards males, other females and males and females combined. Due to a substantial reduction in sample size when a measure of personality (proactive-reactive score) was included, two sets of models are presented in each case, one including proactive-reactive scores as fixed effects and one excluding them. The models presented in each table are those that best predict female aggression according to their AIC scores (Richards, 2008). For each predictor variable *P*-values are provided where $P \leq 0.05$ indicates a significant effect on female aggression.

### *4.5.1. Results of GLMMs examining female-male aggression*

When proactive-reactive score was included as a fixed effect in the global model the best fitting model after model selection was the null model (model 1, table 5). The second best fitting model (model 2, table 5) retained proactive-reactive scores as a fixed effect however, this was not significant ($P = 0.9$). The third best fitting model (model 3, table 5) retained pup stage as a fixed effect but no pup stage had a significant effect.

When proactive-reactive scores were removed from the global model the best fitting model after model selection was again the null model (model 4, table 6). The next best fitting model (model 5, table 6) retained pup stage as a fixed effect, however, the ΔAIC score of 6.99 means that this model should be rejected under Richards (2008) rules for model selection. Model 6 (table 6) retained colony density as a fixed effect however, it had a ΔAIC score of 10.17 meaning that it too should be rejected.

**Table 5.** The top three GLMM models predicting the number of daily aggressive interactions of individual females towards males with proactive-reactive score, colony density and pup stage included as fixed effects. Models are fit with a Gaussian (identity-link) distribution and have female ID set as a random effect. The data for the number of daily aggressive interactions were log transformed to comply with the requirement for normality. AICc = the corrected AIC score after model selection, ΔAIC = the difference between the AICc score of the model in question and the best fitting model.

| Model | df | No. of obs. | No. of individuals | AICc | ΔAIC | Intercept estimate (*P*-value) | Local colony density estimate (*P*-value) | Pup stage 2 estimate (*P*-value) | Pup stage 3 estimate (*P*-value) | Pup stage 4 estimate (*P*-value) | Proactive-reactive score estimate (*P*-value) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **M1:** Null model | 7 | 32 | 15 | 65 | 0.00 | 1.559 (0.0000) | - | - | - | - | - |
| **M2:** Proactive-reactive score only | 4 | 32 | 15 | 66.9 | 1.83 | 1.5738 (0.0000) | - | - | - | - | -0.0541 (0.929) |
| **M3:** Pup stage only | 6 | 32 | 15 | 70.2 | 5.19 | 0.8267 (0.1584) | - | 0.6054 (0.3293) | 0.9369 (0.1230) | 0.5762 (0.3461) | - |

**Table 6.** The top three GLMM models examining factors predicting the number of daily aggressive interactions of individual females towards males with colony density and pup stage set as fixed effects. Proactive-reactive score is excluded from these models. Models are fit with a Gaussian (identity-link) distribution and have female ID set as a random effect. The data for the number of daily aggressive interactions were square root transformed twice to comply with the requirement for normality. AICc = the corrected AIC score after model selection, ΔAIC = the difference between the AICc score of the model in question and the best fitting model.

| **Model** | **df** | **No. of obs.** | **No. of individuals** | **AICc** | **ΔAIC** | **Intercept estimate (*P*-value)** | **Local colony density estimate (*P*-value)** | **Pup stage 2 estimate (*P*-value)** | **Pup stage 3 estimate (*P*-value)** | **Pup stage 4 estimate (*P*-value)** |
|---|---|---|---|---|---|---|---|---|---|---|
| **M4:** Null model | 3 | 70 | 34 | 145.8 | 0.00 | 1.6778<br>(0.0000) | - | - | - | - |
| **M5:** Pup stage only | 6 | 70 | 34 | 152.8 | 6.99 | 1.9185<br>(0.0000) | - | -0.3076<br>(0.2449) | -0.1738<br>(0.4910) | -0.4823<br>(0.0890) |
| **M6:** Colony density only | 4 | 70 | 34 | 156 | 10.17 | 1.7281<br>(0.0000) | -0.0026<br>(0.716) | - | - | - |

### *4.5.2. Results of GLMMs examining female-female aggression*

When proactive-reactive reactive score was included the global model as a fixed effect it was retained after model selection in both models 7 and 8 (table 7). The best fitting model was model 7 which retained both pup stage and proactive-reactive score, of these, females having a pup at stage four were found to show significantly increased rates of female-female aggression (*P* = 0.01). The next best fitting model (model 8, table 7) retained pup-stage, proactive-reactive score and an interaction between pup stage and proactive-reactive score. However none of these results were significant.

When the proactive-reactive score was excluded from the global model, pup stage was again retained in the best fitting model (model 9, table 8). This result also showed that females significantly increased their rates of female-female aggression when they had a pup at stage three (*P* = 0.0311), or stage four (*P* = 0.0002), whilst the significance of having a pup at stage two was marginal (*P* = 0.0544). The next best fitting model was the null model (model 10, table 8) whilst the third best fitting model (model 11, table 10) retained both pup stage colony density. Model 11 also showed that females significantly increased their rate of female-female aggression if they had a pup at stage 2 (*P* = 0.04), stage 3 (*P* = 0.02), or stage 4 (*P* = 0.0001). However, the ΔAIC score of model 11 was 8.97 suggesting that it is not a good fit for the data and should therefore be rejected (Richards, 2008).

**Table 7.** The top two GLMM models predicting the number of daily aggressive interactions of individual females towards other females with proactive-reactive score, colony density and pup stage included as fixed effects. Models are fit with a Gaussian (identity-link) distribution and have female ID set as a random effect. The data for the number of daily aggressive interactions were log transformed to comply with the requirement for normality. AICc = the corrected AIC score after model selection, ΔAIC = the difference between the AICc score of the model in question and the best fitting model.

| Model | df | No. of obs. | No. of individuals | AICc | ΔAIC | Intercept estimate (*P*-value) | Pup stage 2 estimate (*P*-value) | Pup stage 3 estimate (*P*-value) | Pup stage 4 estimate (*P*-value) | Proactive-reactive score estimate (*P*-value) | Pup stage 2 X proactive-reactive score | Pup stage 3 X proactive-reactive score | Pup stage 4 X proactive-reactive score |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **M7: Pup stage + proactive-reactive score only** | 7 | 77 | 20 | 204.3 | 0 | 1.639 (0.000) | -0.1446 (0.6404) | -0.1488 (0.5684) | -0.7795 (0.0135) | 0.3435 (0.1004) | - | - | - |
| **M8: Pup stage + proactive-reactive score + pup stage* proactive / reactive score** | 10 | 77 | 20 | 207.9 | 3.58 | 1.33692 (0.0005) | 0.0823 (0.8663) | 0.2191 (0.6086) | -0.0746 (0.8986) | 0.7922 (0.0738) | -0.3108 (0.6128) | -0.5799 (0.2968) | -0.9685 (0.1561) |

**Table 8.** The top three GLMM models predicting the number of daily aggressive interactions of individual females towards other females with colony density and pup stage set as fixed effects. Proactive-reactive score is excluded from these models. Models are fit with a Gaussian (identity-link) distribution and have female ID set as a random effect. The data for the number of daily aggressive interactions were log transformed to comply with the requirement for normality. AICc = the corrected AIC score after model selection, ΔAIC = the difference between the AICc score of the model in question and the best fitting model.

| Model | df | No. of obs. | No. of individuals | AICc | ΔAIC | Intercept estimate (*P*-value) | Pup stage 2 estimate (*P*-value) | Pup stage 3 estimate (*P*-value) | Pup stage 4 estimate (*P*-value) | Local colony density (*P*-value) |
|---|---|---|---|---|---|---|---|---|---|---|
| **M9: Pup stage only** | 6 | 143 | 52 | 356.6 | 0 | 1.9634 (0.0000) | -0.3899 (0.0544) | -0.3836 (0.0311) | -0.8469 (0.0002) | - |
| **M10: Null model** | 3 | 143 | 52 | 359.6 | 2.99 | 1.611 (0.0000) | - | - | - | - |
| **M11: Pup stage and colony density** | 7 | 143 | 52 | 365.6 | 8.97 | 2.1246 (0.0000) | -0.4037 (0.0460) | -0.3896 (0.0280) | -0.8717 (0.0001) | --0.0077 (0.1983) |

### *4.5.3. Results of GLMMs examining all aggressive interactions (Female-male and female-female) combined*

When proactive-reactive score was included as a fixed effect in the global model, the best fitting model after model selection was the null model (model 12, table 9). This suggests that no fixed effects adequately predicted female-male aggression. The next best fitting model (model 13, table 9) retained the proactive-reactive score as a fixed effect, however, this was not significant ($P = 0.11$). Model 14 (table 9) retained colony density as a fixed effect but this was also not significant ($P = 0.36$) and had an ΔAIC score of 11.2 which, under Richards (2008) rules for AIC selection, means it should be rejected.

When proactive-reactive score was excluded from the global model, the null model was once again the best fitting model after model selection (model 15, table 10). The next best fitting model (model 16, table 10) retained pup stage as a fixed effect and this was significant for females which had a pup at stage four ($P = 0.01$), suggesting they show increased aggression towards males. This model also showed marginal results for pup stages two ($P = 0.07$) and three ($P = 0.07$). However, the ΔAIC score for model 16 was 9.58 meaning that it should be rejected under Richards (2008) rules for model selection. Model 17 retained local colony density as a fixed effect but this result was not significant ($P = 0.1$) and the ΔAIC score of 31.6 means this model should also be rejected (Richards, 2008).

**Table 9.** The top three GLMM models predicting the number of daily aggressive interactions of individual females towards both males and females, with proactive-reactive score, colony density and pup stage included as fixed effects. Models are fit with a Gaussian (identity-link) distribution and have female ID set as a random effect. The data for the number of daily aggressive interactions were log transformed to comply with the requirement for normality. AICc = the corrected AIC score after model selection, ΔAIC = the difference between the AICc score of the model in question and the best fitting model.

| **Model** | **df** | **No. of obs.** | **No. of individuals** | **AICc** | **ΔAIC** | **Intercept estimate (*P*-value)** | **Local colony density estimate (*P*-value)** | **Pup stage 2 estimate (*P*-value)** | **Pup stage 3 estimate (*P*-value)** | **Pup stage 4 estimate (*P*-value)** | **Proactive-reactive score estimate (*P*-value)** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **M12: Null model** | 3 | 70 | 20 | 37.8 | 0 | 1.672 (0.0000) | - | - | - | - | **-** |
| **M13: Proactive -reactive score only** | 4 | 70 | 20 | 40.2 | 2.4 | 1.5805 (0.0000) | - | - | - | - | 0.2415 (0.1135) |
| **M14: Colony density only** | 4 | 70 | 20 | 49.1 | 11.29 | 1.7320 (0.0000) | -0.0026 (0.3661) | - | - | - | - |

**Table 10.** The top three GLMM models predicting the number of daily aggressive interactions of individual females towards both males and females, with colony density and pup stage set as fixed effects. Proactive-reactive score is excluded from these models. Models are fit with a Gaussian (identity-link) distribution and have female ID set as a random effect. The data for the number of daily aggressive interactions were log transformed to comply with the requirement for normality. AICc = the corrected AIC score after model selection, ΔAIC = the difference between the AICc score of the model in question and the best fitting model.

| **Model** | **df** | **No. of obs.** | **No. of individuals** | **AICc** | **ΔAIC** | **Intercept estimate (*P*-value)** | **Local colony density estimate (*P*-value)** | **Pup stage 2 estimate (*P*-value)** | **Pup stage 3 estimate (*P*-value)** | **Pup stage 4 estimate (*P*-value)** |
|---|---|---|---|---|---|---|---|---|---|---|
| **M15: Null model** | 3 | 119 | 48 | 78 | 0 | 1.669<br>(0.0000) | - | - | - | - |
| **M16: Pup stage only** | 6 | 119 | 48 | 87.6 | 9.58 | 1.7960<br>(0.0000) | - | -0.1516<br>(0.0709) | -0.1394<br>(0.0762) | -0.2567<br>(0.0111) |
| **M17: Colony density only** | 4 | 119 | 48 | 130.6 | 31.69 | 1.7410<br>(0.0000) | -0.0034<br>(0.194) | - | - | - |

# Chapter five: Discussion

## *5.0. Evidence of behavioural types, or 'personality', in female grey seals at Donna Nook*

The results of this study show that, when undisturbed, female grey seals at Donna Nook do show consistent individual differences in their pup-check rates indicative of behavioural types, or personalities (table 12). However, in situations immediately following a disturbance females did not show consistency in their pup-check rates. These results differ from those of Twiss *et al.* (2012) which found that females at North Rona show consistency in their pup-checking rates in both undisturbed and disturbed situations. The difference in the results between the present study and that at North Rona may be explained by differences in the methods used. As a means of inducing disturbance Twiss *et al.* (2011, 2012) used a remote controlled vehicle to deliver a disturbance inducing stimulus (a pre-recorded wolf call) to within 2 metres of each focal female. This technique meant that disturbances at the North Rona colony were highly standardised, possibly leading to more consistent rates of pup-checking. Due to the high number of visitors It was not possible to use this, or similar, techniques at Donna Nook. Instead, a fully 'hands-off' approach was used where disturbance was defined as the period immediately following an aggressive interaction with a conspecific. Whilst the data do show that females increased their pup-check rates after these disturbances, the level of aggression involved in each disturbance was not standardised. In some cases interactions between individuals were prolonged and highly aggressive, whilst in others interactions were brief and the level of aggression was mild. Females might be expected to respond to intense aggression by substantially increasing their pup-check rate, while mild aggressive encounters may not initiate much response at all. The intensity of aggressive interactions might also depend on what factors caused the aggression and how much of a threat to her pup the female perceives there to be. This could depend on a number of different factors, for example, a female may perceive a greater threat when she cannot easily back away due to landscape features or other seals blocking her way. Alternatively, the position of the opponent relative to the female's pup could also alter her perception of the danger and in different situations this could potentially lead to very different pup-check rates. In this study the intensity of aggression and the perceived threat level were not factored into the analyses of repeatability and this may explain why such wide variation in disturbed pup-check rates were observed. An obvious solution to this problem would be to only examine repeatability in cases where the conditions and disturbance level of the interactions was judged to be approximately equal (i.e. equal aggression intensity scores), however, this was not possible in this study due to limitations of sample size.

An alternative explanation for the observed differences in the patterns of individual consistency in pup-checking rates between female grey seals at North Rona and at Donna Nook is that differences in environmental, social and anthropogenic factors are responsible. North Rona is a small, isolated, cliff-bound island off the north coast of Scotland (Anderson and Harwood, 1985). It is uninhabited and receives very little human disturbance. In contrast, Donna Nook is situated on the east coast of the English mainland, within easy reach of cities such as Hull and Lincoln. At peak season it receives several thousand visitors each day (figures 8 and 9; Lidstone-Scott, unpublished data), and is also exposed to high levels of noise caused by military aircraft using the site as a training range. Killen *et al.* (2013) suggest that factors such as high levels of noise pollution could increase an animal's perceived threat level and so cause a reduction in activity (increased shyness). By altering animal behaviours in this way noise and/or other disturbances could effectively mask natural behaviour patterns making them difficult or impossible to detect by observation alone. At Donna Nook visitor numbers fluctuated substantially across the breeding season, from a few hundred, or less, on some days to a peak of 5400 on the 18th November (figures 8 and 9; Lidstone-Scott, unpublished data). In addition, aircraft from the nearby RAF base were never active on weekends but were often very active on weekdays. It may be that these external factors created a constant and prolonged background of disturbance which combined with disturbances from aggressive interactions caused females to increase their pup-check rates even further. In this case disturbed pup-check rates would fluctuate according to the number of visitors and/or noise levels from low flying aircraft and this could have made natural repeatability in pup-check rates hard to detect. Another possibility for the observed differences between this study and that at Donna Nook is that high levels of continuous anthropogenic presence at Donna Nook may have led to an increased baseline (undisturbed) pup-check rate. This in turn could make individual differences in disturbed pup-check rates hard to detect as individuals with elevated pup-check rates due to continuous disturbance may be constrained in their ability to increase them further (Killen *et al.* 2013). In support of this hypothesis, the mean undisturbed pup-check rate at Donna Nook was 0.61 pup-checks per minute while at North Rona it has been recorded at 0.42 pup-checks per minute (Twiss *et al.* 2011), this represents an difference of just over 31%. If this difference is the reason why consistency in disturbed pup-check rates could not be found at Donna Nook it suggests that while pup-check rates are an appropriate metric of behavioural types at quiet or isolated sites, they are not suitable for sites which are exposed to a high level of human activity. Instead, an alternative metric such as time spent alert could potentially be used at these sites. Future studies should therefore examine the possibility of alternative behavioural measurements which could be used to identify behavioural types at locations where the pup-check rates of females may not be a suitable metric.

Human disturbance has been shown to influence the behaviour of a range of pinniped species. For example, Hoover-Miller *et al.* (2013) found that in common seals, *Phoca vitulina*, disturbance can disrupt resting and nursing behaviour. In a separate study, Engelhard *et al.* (2002) found that female southern elephant seals, *Mirounga leonina*, in mother-pup pairs increased their alertness (defined as the number of times per hour that individuals raised their head) three-fold after disturbance by humans. This definition of alertness could be considered a measure of maternal attentiveness and is similar to the pup-check rate used as a measure of attentiveness in this study. However, given that rates of pup-checking in this study were found to be lower at the public site than at the RAF site (Fig. 11) it would appear that anthropogenic disturbance does not affect grey seals in quite the same way, or that the seals at Donna Nook have become fully habituated to a high anthropogenic presence. The impact on pinnipeds of noise from aircraft has also been studied, however, no clear impact on behaviour has been found. Perry *et al.* (2002) found that sonic booms caused by Concorde flyovers caused common seals to become more vigilant, however there was no clear effect on grey seal behaviour. Similarly, Manci *et al.* (1988), examined the impact of sonic booms caused by aircraft on the behaviour of common seals and northern elephant seals on the California channel islands and found no significant effect. These studies suggest that noise from aircraft is unlikely to have an influence on pup-check rates at Donna Nook. However, there is good evidence supporting the idea that direct human disturbance caused by visitors to the site could influence pup-check rates (Engelhard *et al.* 2002, Hoover-Miller *et al.* 2013). This may explain why no consistency in disturbed pup-check rates was found at Donna Nook whilst a high degree of consistency was found at North Rona. However, if this is the case it remains to be explained why undisturbed pup-check rates were consistent and were not affected by disturbance in the same way. It could be that high levels of disturbance causes seals to become more responsive to direct stimuli. In this case undisturbed pup-check rates may remain consistent within individuals as they are a passive behaviour and not a response to any direct stimulus. In contrast, pup-checking in disturbed situations appears to increase as a direct response to aggression and this may be compounded by the addition of disturbance from human visitors (Engelhard *et al.* 2002, Hoover-Miller *et al.* 2013).

If human disturbance does explain the high individual variance in rates of pup-checking immediately following aggressive disturbances it remains to be explained why high variance was also found at the RAF site, where there was very little human activity. It may be that females that are more sensitive to disturbance actively choose the quieter RAF site but respond more strongly to occasional disturbances such as noise made by researchers (Engelhard *et al.* 2002). Kucey (2005), found that in Steller sea lions, *Eumetopias jubatus*, disturbances resulted in fewer animals using haul-outs during the post-disturbance period. This suggests that in Steller sea lions individuals do actively choose sites with little disturbance and will change site if necessary. However, this study looked at choice of haul-out sites and not pupping sites and so cannot be compared directly to grey seals at Donna Nook. Even if grey seals do avoid disturbance at haul-out sites as Steller sea lions appear to, they may have fixed pupping sites which do not change regardless of disturbance levels. In support of this possibility Pomeroy *et al.* (2000) found that grey seal mothers at the Isle of May colony typically returned and gave birth within 25 metres of their previous years pupping site. A similar result was found by Pomeroy *et al.* (1994) on North Rona where females typically return each year to within 55 metres of their previous years pupping site. While no detailed figures are available for how site faithful females are at Donna Nook, previously identified females have been re-sighted in approximately the same location for two consecutive years (pers. obs.), while previously identified males have been re-sighted for at least three consecutive years (Bishop, pers. comm.). In addition to a tendency to be site faithful, once females have given birth they must defend and provide milk for their pups which are relatively helpless cannot move far, especially in the first few days after birth. This means that females are generally constrained to a relatively small area for the duration of the maternal care period and so cannot easily avoid disturbance once they have given birth (Anderson *et al.* 1979). At the public site females were observed very close to fence-line, allowing visitors to get within touching distance (pers. obs.). Despite this they did not move away from the fence or show any behavioural signs of stress or disturbance. Even after their pups are born females are able to move short distances and often do so to avoid conflict with other seals (pers. obs.). That females remained close to the fence for prolonged periods of time suggests that they may not be negatively impacted by human visitors. However, as females are known to return to approximately the same place each year to give birth (Pomeroy *et al.* 2000), it is also possible that the instinct driving them to return to the same pupping site each year overrides the pressure to move away from disturbances. It should be considered however, that even if females are constrained to return to within 25 metres of their previous pupping site this would still allow them to move well away from the fence if the disturbance there was a significant problem. There may therefore be benefits to being near to the fence such as reduced exposure to harassment by transient males or a lower risk of disturbance from high tides which in previous years have flooded the site causing high levels of pup mortality (Bishop, pers. comm.). These possibilities have not yet been investigated.

In this study an entirely 'hands-off' approach to measuring personality was trialled. This is very unusual for studies of this type which typically use highly controlled experimental designs (Dingemanse *et al.* 2002, van Oers *et al.* 2008, Minderman *et al.* 2009, Colléter and Brown, 2011, Schuett *et al.* 2011, Williams *et al.* 2012). The main advantage of experimental studies is that they largely remove the influence of external factors which can make fine-scale differences between individuals hard to detect. However, it could be argued that by controlling the external environment these studies do not truly reflect natural conditions and so may not accurately describe prevalence or functional importance of personality in the wild (Stamp Dawkins, 2007). That undisturbed pup-check rates were not shown to be repeatable in this study could point to a weakness with purely observational study techniques or it could be due to differences between the study sites at North Rona and Donna Nook. For example, Culloch (pers. comm.) suggested that pup-check rates may be a good measure of personality at North Rona because it is a relatively unstressed location, while at sites such as Donna Nook it may not be appropriate as individual differences could be masked by continuous external disturbances and stress which cause females to have constantly elevated pup-check rates with little variation. One way of testing which of these hypotheses best explains the results would be to repeat this study but using the techniques employed by Twiss *et al.* (2011, 2012) to reduce the impact of confounding variables. This would effectively confirm or rule out the hypothesis that it is the absence of a standardised method of inducing disturbance that has caused the difference between this study and that of Twiss *et al.* (2011, 2012).

Although this study did not find individual consistency in pup-check rates in disturbed situations it was able to show a high degree of repeatability in undisturbed pup-check rates and this meets most definitions of personality (Koolhaas *et al.* 1999, Réale *et al.* 2007). These results therefore support the conclusions of Twiss *et al.* (2011, 2012) that grey seals do have personalities and this can now be confirmed at two distinct sites, North Rona and Donna Nook.

### ***5.1. Differences in mean daily rates of aggression between the RAF and public sites***

The results of this study show that females at the RAF site were involved in significantly more aggressive interactions each day than females art the public site. There are several possible explanations for this. Boness *et al.* (1982) found that females are intolerant of other seals that come within two body lengths of them. This suggests that colony density could be a major factor driving aggressive interactions, where high density leads to increased conflict. This view is supported by Stephenson *et al.* (2007) who used the number of pups at stages one to three occurring within 2x2m grid squares as a proxy for colony density on the Isle of May, Scotland. They found that the presence of pups in neighbouring grid squares of focal females was a significant predictor of aggressive interactions observed throughout the breeding season ($P$ = <0.001). This result shows that, on the Isle of May, increased colony density does lead to higher rates of aggression. At Donna Nook males were found to be closer to their nearest neighbouring male at the RAF site (17.86m) than they were at the public site (19.25m) (Bishop, unpublished data). This suggests that colony density was higher at the RAF than at the public site and, although not directly applicable to females, could explain the difference in aggression between the two sites. However, data from this study shows that the mean number of males observed within ten metres of focal females at hourly intervals was only slightly higher at the RAF site (0.299) than at the public site (0.294) and this was not significant ($P$ = 0.21). A similar result was found for females which were observed in higher densities at the public site (2.32), than at the RAF site (2.23), but this difference was also not significant ($P$ = 0.29). Although these data appear to contradict those of Bishop (unpublished data), they do not necessarily do so since they are not directly comparable. However, the results from this study do suggest that mean colony densities may not actually differ between sites, in which case an alternative explanation for the difference in rates of aggression is needed.

At Donna Nook the topography differs considerably between the two study sites and this may partially explain the observed difference in daily rates of aggression. The RAF site is an open, flat and sandy beach with little variation or landscape features. In contrast, the public site is more topographically varied, consisting of dunes, muddy pools and vegetation (Figs. 4 and 5).

It may be that although colony density is the same *on average* between the two sites they could differ substantially at different times due to environmental and topographical influences. Due to its more open and flat topography the RAF site appears to be more susceptible to high tides which force all seals on the beach to move inland towards the dunes. This effectively increases colony density for the duration of the tide and may therefore temporarily increase the rate of aggression. At the public site the high tides seem to be buffered by the dunes and vegetation which, except in the case of storm surges, reduces their effect. This makes the environment at the public site much more stable than that at the RAF site. It is possible that by causing a temporary increase in colony density, high tides could lead to periods of intense aggression at the RAF site which pushes up the mean daily rate of aggressive interactions. Another possibility is that aggression may depend to some degree on neighbour familiarity with seals that have been associated with each other for long periods of time showing reduced aggression towards each other. Given that female grey seals generally return to the same pupping site each year this may allow for familiarity to build up over several years. By forcing females to move, high tides could lead to increased aggression as females which are unfamiliar with each other are forced into close contact.

The variation in topography may also lead to differences in the social structure of females at the two sites which could affect rates of aggression. The dunes and pools at the public site appear to limit the areas on which females choose to gather once on land. This leads to a clumped distribution of females at the public site with individuals gathering between the dunes and around the pools (Bishop, pers. comm.). Due to its open topography the RAF site is quite different, with individuals being relatively evenly dispersed. Pomeroy *et al.* (2005) suggest that the physical landscape affects the probability of non-random associations between individuals in colonially breeding animals such as grey seals. At sites such as Sable Island, Canada, where the landscape is very open and flat there may be little opportunity for long-term associations between females to form (Boness and James, 1979, Pomeroy *et al.* 2005). In contrast, on sites such as North Rona and the Isle of May where access is restricted or breeding space is limited, individuals may be constrained to certain areas and so form clumped distributions in close association with other females (Pomeroy *et al.* 2005). In a number of species it has been suggested that this provides the necessary conditions for the emergence of sociality, which may in turn lead to reciprocity in behaviour, reinforced bonds and conflict reduction (Michod, 2000, Utne-Palm and Hart, 2000, Zuri and Rado, 2000, Pomeroy *et al.*

2005). Pomeroy *et al.* (2005) found evidence of this on North Rona where 91% of grey seal mothers showed intra-annual association with at least one other female in their study. Given that grey seals are known to show inter-annual breeding site fidelity (Pomeroy *et al.* 2000), it is also possible that individuals could form associations lasting several years. Evidence of this was found at North Rona where some females showed inter-annual associations, even when they changed pupping locations. Association between females potentially leads to fitness benefits, either in reduced costs to the mother, or an improved performance in raising offspring. One way in which fitness may be increased is by reduced aggression between familiar associates, which leads to reduced energetic costs and the potential for greater maternal investment (Pomeroy *et al.* 2005). If the topography at the the public site favours the formation of long-term associations between individuals, whilst the RAF site does not, this may explain the difference in rates of aggression between the two sites. There have so far been no studies investigating this intriguing possibility however, it may prove a fruitful area for future research.

### ***5.2. Differences in mean daily aggression intensity scores between the RAF and public sites***

In contrast to the observed differences in daily rates of aggression at the RAF and public sites, it was found that daily aggression scores were significantly higher at the public site than at the RAF site ($P = 0.0141$). This suggests that although they get into fewer aggressive interactions, when females at the public site do fight they are considerably more aggressive. This result could be explained by environmental differences between the two sites. Due to its topography the public site may be more environmentally stable than the RAF site as the dunes and vegetation reduce the impact of cyclical high tides. In this case the public site might be favoured by seals with a more proactive personality type which are not able to readily adapt to changing conditions. Since the RAF site does seem to be more severely affected by high tides it may be occupied by more reactive individuals which show greater behavioural flexibility and are therefore better able to cope with a changing environment (Twiss *et al.* 2012). If correct, this would explain why higher aggression intensity scores were observed at the public site, since more proactive individuals typically show higher levels of aggression than more reactive individuals (Koolhaas *et al.* 1999, Koolhaas *et al.* 2010, Twiss *et al.* 2012). However, as discussed previously, although the public site may not be strongly impacted by high tides, it is affected by human disturbance whilst the RAF site is not. Since human disturbance has been shown to negatively affect a range of different species (Manci *et al.* 1998, Engelhard *et al.* 2002, Kucey, 2005, Hoover-Miller *et al.* 2013), it is likely to have a negative impact on grey seals as well. It therefore seems unlikely that proactive females would actively avoid a quiet beach with occasional high tides, and move to one with constant human disturbance. An alternative explanation for the higher aggression intensity scores at the public site could be that since this site is not so strongly influenced by disruptive high tides there may be a high level of familiarity and social stability between neighbouring seals there. This could potentially reduce the rate of low-level aggression while only more serious disputes lead to full contact and more intense aggressive interactions. This possibility is supported by the results of this study which found a lower rate but higher intensity of aggression at the public site (figures 12 and 13). Despite this, the observed difference in the intensity of aggression at the two sites is difficult to explain with currently available data. It could simply be due to very local differences in the observation areas which may have disappeared if a broader area was studied. Alternatively, it could be due to random sampling biases and limited sample size. Future work should therefore focus on recording behaviour across a much wider area at both sites and increasing sample sizes to minimise biases. It is possible that differences in social stability between the RAF and public sites explain the observed differences in the intensity of aggression between them. To address this future studies should attempt to quantify the social environment at both study sites and factor this in to their analyses. If new techniques can be developed to more reliably identify the personality types of individuals at Donna Nook this would also be beneficial as it would allow the hypothesis that different personality types have different site preferences to be confirmed or ruled out.

### ***5.3. Differences in pup-check rates between the RAF and public sites***

Pup-check rates after disturbances were found to be significantly higher at the RAF than at the public site ($P = <0.001$). This difference may be simply explained as a side effect of the higher rates of aggressive interactions at this site which could maintain higher rates of pup-checking if females do not return to a fully undisturbed state between interactions. Alternatively, it may be that because the RAF site is not affected by human visitors it is favoured by seals which are less tolerant of disturbance, while seals that are not so affected by disturbance could choose to settle on either site. In this case the higher pup-check rates observed at the RAF site could simply be due an intrinsic higher level of alertness or sensitivity to disturbance expressed by females at this site and this would suggest that the site is favoured by more reactive females. If this is the case then it can be predicted that if females were moved from the RAF to the public site their pup-check rates would be even higher. However, this possibility cannot be tested at Donna Nook for ethical and practical reasons and due to restrictions on handling animals at this site. A third possible explanation for the observed result is that because disturbance at the public site is fairly constant (at least during daylight hours) the seals there have become habituated to it and so have a higher tolerance threshold to disturbances than seals at the RAF site which are not exposed to constant disturbance. Because the RAF site is generally quiet, with only occasional disturbances from aircraft and none from human visitors, the seals there may not be habituated to disturbance and so respond much more strongly to disturbance caused by aggression from con-specifics. Given the evidence that grey seals are highly site faithful (Pomeroy *et al.* 2000), it is possible that habituation could be built up over a number of years as seals return to the same site annually. This could be tested by examining the mean pup-check rates of previously identified females over successive years to see if their pup-check rates decline as the females get older. However, while this is possible in principle, ageing seals accurately is difficult without either a long-term photo-id dataset, or by invasive procedures such as removing teeth (Mansfield, 1991). It would also be very difficult to effectively separate the effects of habituation to disturbance from the effects of age alone, pup-rearing experience, environmental factors and even the behaviour of the pup itself. The habituation hypothesis could also be tested by moving animals to different sites and examining their response, however, this is not possible at Donna Nook for reasons discussed above.

## ***5.4. Links between behavioural type and mate choice***

Generalised linear mixed models were used to examine if there was a significant link between the rate of female aggression towards males and their behavioural type, or, whether aggression is better explained by other factors or a combination of factors. The same models were also used to examine female-female aggression and all aggressive interactions combined.

### *5.4.1. Factors explaining female-male aggression*

Analyses of female-male aggressive interactions using GLMM models revealed that the null model was the best fitting model, both when a measure of behavioural type was included (table 5) and when it was excluded as a fixed effect (table 6). This result means that in this study the behavioural type of females could not predict their rate of aggression towards males. This suggests that there is no link between behavioural type and mate choice in female grey seals at Donna Nook. These results are surprising given that behavioural type has been linked to mate choice in a range of other species (Dingemanse *et al.*2004, van Oers *et al*. 2008, Schuett *et al.* 2010, Williams *et al.* 2012) and preliminary evidence has also suggested that there is a link between mate choice and behavioural type in grey seals (Twiss *et al.* 2012). It may be that the small sample size of only 15 individuals meant that an effect of behavioural type on mate choice was not statistically detectable due to low power (table 5). Future studies at Donna Nook should therefore concentrate on recording additional female-male aggressive interactions and identifying female behavioural types to increase the sample size. As females are known to be relatively site faithful (Pomeroy *et al.* 2000) it may be possible to observe the same individuals over several years which would be beneficial as these individuals would have known behavioural types. This would also allow the behaviour of known females to be compared against a range of different males since the rate of male turnover is known to be higher than that of females (Twiss, pers. comm.).

Another potential explanation for the results presented here could be that the areas of the beach from which behavioural data were collected only contained females with very similar behavioural types that were not representative of the colony as a whole. If the range of behavioural types observed was too narrow then a real effect of behavioural type on rates of female-male aggression could have been missed. This is a possibility since at both the RAF and public sites behavioural observations were only made on seals occupying the relatively sheltered areas of the beach from the edge of the dunes up to no more than 100m out towards the sea. Since these areas provide some shelter from harassment by transient males they may be predominantly occupied by more aggressive proactive females which are able to out-compete the less aggressive reactive females in competition for the best pupping locations. This could have potentially caused a bias in this study whereby behavioural observations may inadvertently have been mostly focussed on more proactive over reactive females and this may have obscured a real link between behavioural type and mate choice. However, this explanation seems unlikely as the range of observed pup-checking rates, and the range of differences between disturbed and undisturbed pup-check rates within individuals (figure 14) are comparable to those found by Twiss *et al.* (2012). This suggests that there was no bias favouring observation of more proactive over more reactive females. To confirm these results future studies should aim to collect behavioural observations over a more extensive area including females both close to the dunes and further out towards the sea. More generally, future studies could benefit from mapping the positions of seals relative to their behavioural types to confirm or reject the possibility that different behavioural types have different habitat preferences.

### *5.4.2. Factors explaining female-female aggression*

When a GLMM model was constructed which included a measure of behavioural type as a fixed effect, female-female aggression was found to be best explained by an interaction between the stage of a females pup and her behavioural type (table 7). When the model was re-run excluding behavioural type the effect of pup-stage on aggression was retained (table 8). In agreement with previous studies these results suggests that females with a more proactive behavioural type tend to be more aggressive than those with a more reactive behavioural type (Koolhaas *et al.*, 1999, Twiss *et al.* 2012). Surprisingly, the effect of pup stage on aggression differed from expectation as females appear to show increased aggression towards other females when their pup is relatively robust at stages three and four, but not at stage two when it is more vulnerable (Reidman, 1989; tables 7 and 8). This result is in contrast to previous research which found that female aggression in grey seals (Boness *et al.* 1982) and northern elephant seals (Christenson and Le Boeuf, 1978) was greatest when pups were newborn and declined as they matured. While pup defence is likely to explain some of the female-female aggressive interactions observed at Donna Nook, it cannot explain why females should become more aggressive as their pups grow older. An alternative explanation for this result is that females with later stage pups are entering oestrus and this causes heightened aggression due to increased male harassment or increased sensitivity to harassment (Boness *et al.* 1982). Grey seal females begin to accept mates as they come into oestrus (Reidman, 1989) and Bishop (pers. comm.) found that females at Donna Nook typically mated when their pups were between stages three and four. This timing matches well with the observed increase in aggression (tables 7 and 8). Boness *et al.* (1982) also found that females entering oestrus were more aggressive, however, in contrast to the current study this effect was only shown to apply to aggression towards males and not to females. Why females at Donna Nook showed the opposite trend is difficult to explain with the available data. It may be that females show a general increase in sensitivity to disturbance, and therefore increased aggression, as they approach oestrus but that this effect was not detected for female-male aggression due to limited sample size. It could also be that females are more active during this time, possibly because their pups are becoming more active, and this may lead to increased contact and conflict with other seals on the beach. These possibilities could be investigated in future studies. The dataset for female-female aggression is slightly larger than that for female-male aggression and this may have made an increase in aggression statistically detectable in this case. To confirm or reject this hypothesis future studies should concentrate on collecting data on female-male aggressive interactions as this would increase the sample size and so make it possible to confirm or reject the possibility that females are generally aggressive during oestrus. Alternatively, data may already exist from long running studies on grey seal behaviour such as those on North Rona (e.g. Anderson *et al.* 1975), the Isle of May (e.g. Pomeroy *et al.* 2000) and Sable Island (e.g. Boness and James 2009). However, including data from these studies would not allow the possibility that this behaviour is unique to Donna Nook to be investigated. Since a behaviour in male grey seals that is unique to Donna Nook and some other nearby sites has recently been identified (Bishop *et al.* 2013) this possibility should not be ruled out.

### *5.4.3. Factors explaining female aggression in general*

The GLMM models used to examine potential explanations for female aggression towards both males and females combined revealed that the best fitting model was the null model, both when a measure of behavioural type was included and when it was excluded (tables 9 and 10). This result suggests that at the scale of this study aggression at Donna Nook cannot be adequately predicted by either colony density, the stage of a females pup or her behavioural type. Given that the sample size for these models was reasonable (20 individuals and 70 observations when behavioural type was included, 48 individuals and 119 observations when excluded) it is unlikely that problems with sample size or biases have affected these results. Previous attempts to identify the functions of female aggression have not been able to draw any definitive conclusions (Christenson and Le Boeuf, 1978, Boness *et al.* 1982). It may be that one function of female aggression is pup defence but the level of aggression does not change with pup stage as might be expected. It could also be that female aggression is linked to mate choice but not also to behavioural type as was predicted in this study. Future research may be able to test some alternative hypotheses using different methods to those used here, however, it is likely that some degree of experimental manipulation will be necessary if the functions of female aggression are to be revealed.

## ***5.5. Summary of results***

Donna Nook is home to a large and rapidly expanding population of grey seals which has more than doubled in size since 2000. With an estimated population of 5,300 adults it is the largest grey seal colony on the British mainland and is therefore of national importance (Lidstone-Scott, pers. Comm.). During this study detailed behavioural observations were made on two sites at Donna Nook during the breeding season in November and December 2012. The RAF site is flat and sandy and is closed to visitors while the public site is more topographically varied consisting of low dunes, mud flats and muddy pools. Females within the study areas on each site were individually identified by their unique pelage patterns and each was assigned an ID number and photographs kept in a photo catalogue. In total 68 females were identified from the RAF site and 65 from the public site.

Based on the methods developed by Twiss *et al.* (2012) the behavioural types of focal female grey seals were estimated by comparing the pup-check rates of focal females in disturbed and undisturbed situations. The behavioural type of females whose pup-check rate remained relatively constant across situations was considered to be more proactive while females whose pup-check rate changed across situations were considered to be more reactive. Twiss *et al.* (2012) was able to identify proactive and reactive behavioural types in grey seals on the island of North Rona. In their study a wolf call played from a remote controlled vehicle was used to induce disturbance in focal seals (Twiss *et al.* 2012). Due to the large number of visiting public a similar experimental approach to inducing disturbance was not possible at Donna Nook. Instead, the pup-check rates of females were recorded during both quiet undisturbed periods and also immediately after aggressive interactions. This allowed for the the pup-check rates of females in undisturbed and disturbed situations to be compared. A key aim of this study was to look for consistent individual differences in behaviour in grey seals at a different colony to that studied by Twiss *et al.* (2012) and to test the effectiveness of an entirely 'hands-off' approach to studies of behavioural types with no experimental manipulation. The results of this study show that in undisturbed situations grey seals at Donna Nook do show consistent individual differences in their pup-check rates indicative of behavioural types in seals at this site. However, although females did significantly increase their pup-check rates after disturbances, they did not show consistency in their pup-check rates in disturbed situations (section 4.4.). It is possible that significant repeatability of pup-check rates was not found in disturbed situations due the inability of this study to control the duration and intensity of the aggressive interactions that occurred immediately prior to disturbed pup-check rates being recorded. Alternatively, it may be that the sample size taken from observations of just 12 individuals from each study site was not large enough to reveal any significant patterns. Despite this, female grey seals were shown to exhibit significant repeatability in their pup-check rates in undisturbed situations. This result partially confirms that of Twiss *et al.* (2012) and suggests that a 'hands-off' study can work in certain situations but may not be suitable for situations which are very variable or unpredictable.

A second key aim of this study was to determine if there are individually consistent differences in levels of aggression shown by females towards female and male conspecifics and to determine whether these differences could be related to behavioural type. The aggressive interactions of all females with IDs were recorded in the field together with a record of which aggressive behaviours were used in each interaction and a measure of how aggressive the interaction was based on a score from 1 (mild aggression) to 5 (strong aggression). This provided data on the both the intensity and frequency of aggressive interactions involving focal females and conspecifics. Statistical models were used to reveal which factors best explained female aggression towards conspecifics. These revealed that female-female aggression was best explained by both behavioural type and pup-stage, with females with a more proactive behavioural type which also have a pup at stage four significantly more likely than other females to exhibit female-female aggression (section 4.5.2.). This result suggests that the behavioural type of females may influence their level of aggression towards other females but only at certain times such as when they have a late stage pup. In contrast to female-female aggression, no evidence was found to support the hypothesis that behavioural type could predict the level of female aggression towards males and no model outperformed the null model (section 4.5.1.). It is possible that this negative result is due to the small sample size of observations from only 15 individuals. Alternatively, it may be that the study sites that were observed covered too small an area of the beach and did not contain individuals with sufficient variation in female behavioural types to detect differences in levels of aggression towards males. Further studies which could increase the sample size of the present study may therefore be beneficial. At present however the hypothesis that more reactive females exhibit greater choosiness of mating partners than do more proactive females must be rejected.

The data collected during this study allowed for the behaviour of grey seals to be compared between two sites with differing topography and levels of human presence. A comparison of pup-check rates between the two sites revealed a higher average rate of pup-checking at the RAF site than at the public site although this was not significant (section 4.3.2.). When the frequency of aggressive interactions were compared between the two sites females at the RAF site were found to be involved in significantly more aggressive interactions than females at the public site (section 4.3.3). In contrast, when the intensity of aggressive interactions was compared between the two sites females at the public site were found to be significantly more aggressive than females at the RAF site (section 4.3.4.). Thus, females at the public site were involved in fewer aggressive interactions than females at the RAF site but when they did fight the intensity of aggression was higher on average. Females at the RAF site fought more often than females at the public site but when they did so the intensity of aggression was lower on average. These results suggest that topography, human presence or both can potentially affect the behaviour of grey seals in significant ways and may provide interesting avenues for future research.

## ***5.6. Concluding remarks***

This study represents the first attempt to identify personality in wild grey seals using an entirely hands off approach. It is also the first study to look for personality in grey seals at Donna Nook. The results show that in undisturbed situations female grey seals do show consistent individual differences in their pup-check rates and this matches most definitions of personality (Koolhaas *et al.* 1999, Réale *et al.* 2007). This study can therefore confirm the results of Twiss *et al.* (2011,

2012) who identified personality in grey seals on North Rona, and extend them to a new and very different study site. This result suggests that CIDs in vigilance type behaviours such as pup-checking are not limited to just one colony but may be a general feature of the species as a whole. This possibility could be further tested by looking for CIDs in pup-check rates in females from very distant populations such as that on Sable Island, Canada. In contrast to Twiss *et al.* (2012) this study this study did not find evidence of consistent individual differences in pup-check rates in the period immediately following a disturbance, however, this is potentially explained by environmental factors such as high visitor numbers and aircraft disturbance which are present at Donna Nook but not present at North Rona. This result may also point to limitations with an entirely hands-off approach to personality studies. Future studies of personality at Donna Nook may benefit from choosing an alternative behaviour to pup-check rates from which to look for consistent individual differences as this may allow for individual consistency to be identified in both disturbed and undisturbed situations. Alternatively, experimental manipulations could be used to provide a more controlled and standardised means of looking for evidence of CIDs in pup-check rates.

Interestingly the results of this study identified statistically significant differences in both the rate and the intensity of aggression at the RAF and public sites. Furthermore, while the females at the RAF site were found to be involved in significantly more aggressive interactions, the intensity of aggression was found to be significantly higher at the public site. These results may be explained by numerous factors including differences in colony density, topography or the differential influence of tides at the two sites. This study could not identify the functions of female aggression however, examining the reasons for the differences in rates and intensity of aggression between the two study sites could potentially provide a good starting point for future research in this area.

A significant difference was found in disturbed pup-check rates between the two study sites with the rate at the RAF site being higher than at the public site. A simple explanation for this is that the higher pup-check rate at the RAF site is a side effect of the higher rate of aggression there. However, other possibilities cannot yet be ruled out. For example, because there are no visitors at the RAF site it may be favoured by seals that are intolerant of disturbance. This could mean that they are much more sensitive to disturbances when they do occur and so show an elevated pup-check rate on average. Alternatively, it could be that seals at the public site habituate to disturbances due to the high anthropogenic presence there and so are not so sensitive to disturbance. Determining if either of these hypotheses is correct is difficult, however, one potential avenue for future research could be to compare pup-check rates in grey seals at a range of sites around the world to examine if there is any correlation between mean disturbed pup-check rates and level of background or continuous disturbance.

The main aim of this study was to look for a link between the behavioural type of females and their level of aggression towards males. Data from Twiss *et al.* (2012) suggested that proactive and reactive females may show differences in their levels of aggression towards males and this appeared to be linked to how easily they could access dominant, and therefore more desirable, males as mating partners. The hypothesis therefore was that reactive females would show increased aggression towards males due to their position on the edge of colony where they are subject to transient male harassment. In contrast proactive females were predicted to show lower aggression towards males as they tend to be found in high density areas of the colony where they have easy access to the most dominant males. The results of this study do not support this hypothesis. The GLMM models used to examine the causes of female aggression show that a female's behavioural type does not predict her level of aggression towards males. This result does not mean that females do not express mate choice but it does suggest that mate choice is not linked to behavioural type in any significant way. It may be that a significant result would have been found had a larger sample size been available, however this was not possible during this study as females at Donna Nook only come ashore for a short period each year and they were observed for the entirety of this time. It is also possible that the relatively small study areas observed for this study contained only males of equal, and fairly dominant, rank. In this case females would have little need to express choice since all males would be equally desirable. This possibility is plausible since this study focussed on females that were close to the dunes and far from the sea while more transient males were often observed at the edges of the colony and further out towards the shoreline. Future studies should therefore be designed so as to include a range of males with different levels of dominance so that females have the potential to choose between them.

One intriguing possibility is that rather than selecting the best male to mate with, females may exercise cryptic mate choice or mate with several males to induce sperm competition (Amos *et al.* 2001). At Donna Nook females were observed to mate with multiple males (pers. obs.). This has also been observed on North Rona where female grey seals often mate with several of the most dominant males (Twiss *et al.* 2006). This suggests that on North

Rona females may choose a selection of the best males to mate with and then allow them to compete via sperm competition. Although this mechanism of choice has never been examined in pinnipeds before, it has been shown in other mammals. For example in the promiscuous rodent the common yellow-toothed cavy, *Galea musteloides*, females that mated with multiple males were found to have more surviving offspring than those that mated with only one (Keil and Sascher, 2010). A similar result has been found in bank voles, *Clethrionomys glareolus*, where the offspring of females that mated with multiple males had significantly higher reproductive success than did the offspring of females that mated only once (Klemme *et al.* 2008). These studies suggest that in these two species there is a fitness advantage to multiple matings potentially mediated through some form of post-copulatory choice. There are good theoretical reasons for proposing that female grey seals should express some degree of mate choice (Cox and LeBoeuf, 1977, Clutton-Brock and McAuliffe 2009), yet despite evidence of mate choice in other pinnipeds (Goldsworthy *et al.* 1999, Insley, 2000, Hoffman *et al.* 2007) the evidence of mate choice in grey seals remains circumstantial and inconclusive (Anderson *et al.* 1975, Boness *et al.* 1982, Twiss *et al.* 2006). Future studies of mate choice in this species may therefore benefit from examining potential physiological, rather than just behavioural, mechanisms of mate choice.

When GLMM models were used to examine the causes of female-female aggression it was found that both the stage of the females pup and her behavioural type may have some effect (tables 7 and 8). Interestingly, females were found to be most aggressive when their pups were at stages three and four and least aggressive when their pups were younger and presumably more vulnerable. It may be that the increased aggression is actually caused by oestrus which tends to occur when females pups are at stages three or four (Bishop, pers. comm.). During oestrus females may be both more sensitive to the approaches of unwanted males and be actively searching for a desirable mate, this could bring them into increased contact with other seals and so increase their rate of involvement in aggressive interactions. However, the effect of pup stage and behavioural type were not found for either female-male aggressive interactions or female-female and female-male interactions combined. This result must therefore be treated with caution and regarded as a speculative relationship due to small sample size, however, it does suggest that females with a more proactive behavioural type and a late stage pup are likely to be more aggressive.

The results of this study cannot confirm the hypothesis that the behavioural type of female grey seals is linked to their choosiness of mating partners. However it can confirm that grey seals do show consistent individual differences in behaviour indicative of behavioural types or personalities. Furthermore, significant differences were found in the rate of pup-checking by females and in their rates and intensities of aggressive interactions between the RAF and public sites. These results suggest that topography and/or disturbance or a combination of these factors can have significant effects on seal behaviour. These results have potentially important implications for conservation. For example, if disturbance leads to an increased pup-checking rate this may reduce the amount of time or energy that a female can invest in her pup and so have a direct effect on fitness (Engelhard *et al.* 2002, Hoover-Miller *et al.* 2013). Future studies may therefore wish to follow up on these results to provide a more detailed analysis of the effects of topography and disturbance on the behaviours of grey seals and how this affects their reproductive success.

# References

Altmann, J. (1974). Observational study of behaviour: sampling methods. *Behaviour*, 49, 227-265.

Amos, B., Twiss, S., Pomeroy, P. & Anderson, S. (1995). Evidence for mate fidelity in the gray seal. *Science*, 268, 1897.

Amos, W., Twiss, S., Pomeroy, P. P. & Anderson, S. S. (1993). Male mating success and paternity in the grey seal, *Halichoerus grypus*: a study using DNA fingerprinting. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 252, 199-207.

Amos, W., Worthington Wilmer, J. & Kokko, H. (2001). Do female grey seals select genetically diverse mates? *Animal Behaviour*, 62, 157-164.

Anderson, S. S. & Harwood, J. (1985). Time budgets and topography: how energy reserves and terrain determine the breeding behaviour of grey seals. *Animal Behaviour*, 33, 1343-1348.

Anderson, S. S., Baker, J. R., Prime, J. H., & Baird, A. (1979). Mortality in grey seal pups: incidence and causes. *Journal of Zoology*, 189 (3), 407-417.

Anderson, S. S., Burton, R. W. & Summers, C. F. (1975). Behaviour of Grey seals (*Halichoerus grypus*) during a breeding season at North Rona. *Journal of Zoology*, 177, 179-195.

Andersson, M (1994). *Sexual Selection*. Princeton: Princeton University Press.

Andersson, M. (1982). Female choice selects for extreme tail length in a widowbird. *Nature*, 299, 818-820.

Andersson, M. & Simmons, L. W. (2006). Sexual selection and mate choice. *Trends in Ecology & Evolution*, 21, 296-302.

Baayen, R. H. (2011). languageR: Data sets and functions with "Analyzing Linguistic Data: A practical introduction to statistics".. R packageversion 1.4. http://CRAN.Rproject.org/package=languageR

Barton, K. (2013). MuMIn: Multi-model inference. R package version 1.9.0. http://CRAN.R-project.org/package=MuMIn

Bateman, A. J. (1948). Intra-sexual selection in Drosophila. *Heredity*, 2, 349.

Bates, D., Maechler, M. & Bolker, B. (2013). lme4: Linear mixed-effects models using S4 classes. R package version 0.999999-2. http://CRAN.R-project.org/package=lme4

Beaumont, E. S. & Goold, J. C. (2007). Cheap and accessible method to aid individual photo-identification of grey seals, *Halichoerus grypus*. *Journal of the Marine Biological Association of the United Kingdom*. 87 (5), 1337-1343.

Betini, G. S., & Norris, D. R. (2012). The relationship between personality and plasticity in tree swallow aggression and the consequences for reproductive success. *Animal Behaviour*, 83 (1), 137-143.

Bishop, A. (2011). Selection of haul-out substrate by harbor seals *(Phoca vitulina*) associated with tidewater glaciers in Kenai Fjords national park, Alaska (Masters thesis, Duke University).

Bishop, A., Lidstone-Scott, R., Pomeroy, P. & Twiss, S. (2013). Body Slap: An Innovative Aggressive Display by Breeding Male Grey Seals (*Halichoerus grypus*). *Marine Mammal Science*. doi: 10.1111/mms.12059

Blumstein, D. T., Petelle, M. B. & Wey, T. W. (2013). Defensive and social aggression: repeatable but independent. *Behavioural Ecology*, 24 (2), 457 – 461.

Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H., & White, J. S. S. (2009). Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in ecology & evolution*, 24 (3), 127-135.

Boness, D. J. & James, H. (1979). Reproductive behaviour of the grey seal (*Halichoerus grypus*) on Sable Island, Nova Scotia. *Journal of Zoology*, 188, 477-500.

Boness, D. J., Anderson, S. S. & Cox, C. R. (1982). Functions of female aggression during the pupping and mating season of grey seals, *Halichoerus grypus* (Fabricius). *Canadian Journal of Zoology*, 60, 2270-2278.

Boness, D. J., Bowen, W. D., & Iverson, S. J. (1995). Does male harassment of females contribute to reproductive synchrony in the grey seal by affecting maternal performance?. *Behavioral Ecology and Sociobiology*, 36 (1), 1-10.

Boveng, P. L., Bengston, J. L., Withrow, D. E., Cesarone, J. C., Simpkins, M. A., Frost, K. J., & Burns, J. J. (2003). The abundance of harbor seals in the Gulf of Alaska. *Marine Mammal Science*, 19 (1), 111-127.

Briffa, M. & Greenaway, J. (2011). High in situ repeatability of behaviour indicates animal personality in the beadlet anemone *Actinia equina* (Cnidaria). *PLoS ONE*, 6, e21963.

Capozzo, H. L. (2001). New perspectives on the behavioural ecology of pinnipeds. Marine mammals: biology and conservation. New York: Broché.

Caprara, G. V. and Cervone, D. (2000). Personality: Determinants, dynamics, and potentials. Cambridge University Press.

Carere, C., Drent, P. J., Privitera, L., Koolhaas, J. M. & Groothius, T. G. G. (2005). Personalities in great tits, *Parus major*: stability and consistency. *Animal Behaviour*. 70, 795–805.

Cäsar, C., Byrne, R., Young, R. J. & Zuberbühler, K. (2012). The alarm call system of wild black-fronted titi monkeys, *Callicebus nigrifrons*. Behavioral *Ecology and Sociobiology*. 66 (5), 653-667.

Christenson, T. E., & Le Boeuf, B. J. (1978). Aggression in the female northern elephant seal, *Mirounga angustirostris*. *Behaviour*, 158-172.

Clutton-Brock, T. & McAuliffe, K. (2009). Female mate choice in mammals. *The Quarterly review of biology*, 84, 3-27.

Colléter, M. & Brown, C. (2011). Personality traits predict hierarchy rank in male rainbowfish social groups. *Animal Behaviour*. 81 (6), 1231–1237.

Cook, L. M., Grant, B. S., Saccheri, I. J. and Mallet, J. (2012). Selective bird predation on the peppered moth: the last experiment of Michael Majerus. *Biology Letters*. 8, 609–612.

Cox, C. R. & LeBeouf, B. J. (1977). Female Incitation of Male Competition: A Mechanism in Sexual Selection. *The American Naturalist*, 111, 317-335.

Culloch, R. M. (2012). The application of modern statistical approaches to identify consistent individual differences in the behaviour of wild postpartum female grey seals (*Halichoerus grypus*). PhD Thesis, Durham University.

Darwin, C. (1859). *On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life*. London: John Murray.

Darwin, C. (1871). *The Descent of Man, and Selection in Relation to Sex*. London: John Murray.

DEFRA. (2005). Grey Seal diet Composition and Fish Consumption in the North Sea (Defra project code: MF0319).

Dewsbury, D. A. (1982). Ejaculate cost and male choice. American Naturalist, 601-610.

Dingemanse, N. J., Both, C., Drent, P. J. & Tinbergen, J. M. (2004). Fitness consequences of avian personalities in a fluctuating environment. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, 271, 847-852.

Dingemanse, N. J., Both, C., Drent, P.J., van Oers, K. & van Noordwijk, A.J. (2002). Repeatability and heritability of exploratory behavior in wild great tits. *Animal Behaviour*, 64, 929–938.

Drent, P. J., van Oers, K. & van Noordwijk, A.J. (2003). Realized heritability of personalities in the great tit (*Parus major*). *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 270, 45–51.

Emlen, S. T., & Oring, L. W. (1977). Ecology, sexual selection, and the evolution of mating systems. *Science*, 197 (4300), 215-223.

Engelhard, G. H., Baarspul, A. N., Broekman, M., Creuwels, J. C., & Reijnders, P. J. (2002). Human disturbance, nursing behaviour, and lactational pup growth in a declining southern elephant seal *(Mirounga leonina)* population. *Canadian Journal of Zoology*, 80 (11), 1876-1886.

Fisher, R. A. (1930). The genetical theory of natural selection, Oxford, Clarendon Press.

Friberg, U., & Arnqvist, G. (2003). Fitness effects of female mate choice: preferred males are detrimental for *Drosophila melanogaster* females. *Journal of evolutionary biology*, 16 (5), 797-811.

Garamszegi, L. Z., Eens, M. & Török, J. (2008). Birds reveal their personality when singing. *PLoS ONE*, 3, e2647.

Gentry, R. L. (1998). *Behavior and ecology of the northern fur seal*. Princeton University Press.

Goldsworthy, S. D., Boness, D. J., & Fleischer, R. C. (1999). Mate choice among sympatric fur seals: female preference for conphenotypic males. *Behavioral Ecology and Sociobiology*, 45 (3-4), 253-267.

Google inc. (2009). Google Earth (Version 5.1.3533.1731) [Software]. Available from http://www.google.co.uk/intl/en_uk/earth/index.html

Hamilton, W. D. & Zuk, M. (1982). Heritable true fitness and bright birds: a role for parasites? *Science*, 218, 384-387.

Hammond, P.S., Northridge, S.P., Thompson, D., Gordon, J.C.D., Hall, A.J., Duck, C. D., Aarts, G., Cunningham, L., Embling, C.B. & Matthiopoulos, J. (2006). Background information on marine mammals for Strategic Environmental Assessment 7. Report to the Department of Trade and Industry. Available at www.gov.uk/government/uploads/system/uploads/attachment_data/file/197041/SEA7_Mammals_SMRU.pdf

Hiby, L. & Lovell, P. (1990). Computer aided matching of natural markings: a prototype system for grey seals. Report of the International Whaling Commission Special Issue 12, 57–61.

Higdon, J. W., Bininda-Emonds, O. R., Beck, R. M., & Ferguson, S. H. (2007). Phylogeny and divergence of the pinnipeds (Carnivora: Mammalia) assessed using a multigene dataset. *BMC Evolutionary Biology*, 7 (1), 216.

Hill, G. E. (1990). Female house finches prefer colourful males: sexual selection for a condition dependent trait. *Animal Behaviour*. 40 (3), 563–572.

Hoffman, J. I., Boyd, I. L., & Amos, W. (2003). Male reproductive strategy and the importance of maternal status in the Antarctic fur seal *Arctocephalus gazella*. *Evolution*, 57 (8), 1917-1930.

Hoffman, J., Forcada, J., Trathan, P. & Amos, W. (2007). Female fur seals show active choice for males that are heterozygous and unrelated. *Nature*, 445, 912-914.

Hoover-Miller, A., Bishop, A., Prewitt, J., Conlon, S., Jezierski, C., & Armato, P. (2013). Efficacy of voluntary mitigation in reducing harbor seal disturbance. *The Journal of Wildlife Management*, 77 (4), 689-700.

Horne, T. J. & Ylönen, H. (1996). Female bank voles (*Clethrionomys glareolus*) prefer dominant males; but what if there is no choice? *Behavioral Ecology and Sociobiology*, 38, 401-405.

Hutchings, J. A., & Myers, R. A. (1988). Mating success of alternative maturation phenotypes in male Atlantic salmon, *Salmo salar. Oecologia*, 75 (2), 169-174.

Insley, S. J. (2000). Long-term vocal recognition in the northern fur seal. *Nature*, 406 (6794), 404.

Janetos, A. C. (1980). Strategies of female mate choice: a theoretical analysis. *Behavioral Ecology and Sociobiology*, 7, 107-112.

Jennions, M. D. & Petrie, M. (1997). Variation in mate choice and mating preferences: a review of causes and consequences. *Biological Reviews*, 72, 283-327.

Jennions, M. D. & Petrie, M. (2000). Why do females mate multiply? A review of the genetic benefits. *Biological Reviews*, 75, 21-64.

Karlsson, O., Hiby, L., Lundberg, T., Jüssi, M., Jüssi, I., & Helander, B. (2005). Photo identification, site fidelity, and movement of female gray seals (*Halichoerus grypus*) between haul-outs in the Baltic Sea. *AMBIO: A Journal of the Human Environment*, 34 (8), 628-634.

Keil, A., & Sachser, N. (1998). Reproductive benefits from female promiscuous mating in a small mammal. *Ethology*, 104 (11), 897-903.

Killen, S. S., Marras, S., Metcalfe, N. B., McKenzie, D. J., & Domenici, P. (2013). Environmental stressors alter relationships between physiology and behaviour. *Trends in Ecology & Evolution*, 28 (11),  651–658.

Klemme, I., Ylönen, H., & Eccard, J. A. (2008). Long-term fitness benefits of polyandry in a small mammal, the bank vole *Clethrionomys glareolus*. *Proceedings of the Royal Society B: Biological Sciences*, 275 (1638), 1095-1100.

Kokko, H. & Jennions, M. D. (2008). Parental investment, sexual selection and sex ratios. *Journal of evolutionary biology*, 21, 919-948.

Koolhaas, J. M., De Boer, S. F., Coppens, C. M., & Buwalda, B. (2010). Neuroendocrinology of coping styles: towards understanding the biology of individual variation. *Frontiers in neuroendocrinology*, 31 (3), 307-321.

Koolhaas, J., Korte, S., De Boer, S., Van Der Vegt, B., Van Reenen, C., Hopster, H., De Jong, I., Ruis, M. & Blokhuis, H. (1999). Coping styles in animals: current status in behavior and stress-physiology. *Neuroscience and biobehavioral reviews*, 23, 925-936.

Kotiaho, J. S. (2001). Costs of sexual traits: a mismatch between theoretical considerations and empirical evidence. *Biological Reviews*, 76, 365-376.

Kotiaho, J. S., Lebas, N. R., Puurtinen, M. & Tomkins, J. L. (2008a). On female choice, heterozygosity and the lek paradox. *Animal Behaviour,* 75, 1.

Kotiaho, J. S., Lebas, N. R., Puurtinen, M. & Tomkins, J. L. (2008b). On the resolution of the lek paradox. *Trends in Ecology and Evolution*, 23, 1-3.

Kovacs, K. M., & Lavigne, D. M. (1986). Growth of grey seal (*Halichoerus grypus*) neonates: differential maternal investment in the sexes. *Canadian Journal of Zoology*, 64 (9), 1937-1943.

Kucey, L. (2005). Human disturbance and the hauling out behaviour of Steller sea lions *(Eumetopias jubatus)* (Masters thesis, University of British Columbia).

Le Boeuf, B. J. (1974). Male-male competition and reproductive success in elephant seals. *American Zoologist*, 14 (1), 163-176.

Lidgard, D. C. (2003). Intraspecific variation in male mating behaviour in the grey seal: linking phenotype, behaviour and success. PhD Thesis, Université de La Rochelle.

Lidgard, D. C., Boness, D. J., & Bowen, W. D. (2001). A novel mobile approach to investigating mating tactics in male grey seals *(Halichoerus grypus). Journal of Zoology*, 255 (3), 313-320.

Lidgard, D. C., Boness, D. J., Bowen, W. D. & McMillan, J. I. (2005). State-dependent male mating tactics in the grey seal: the importance of body size. *Behavioral Ecology*, 16, 541-549.

Lidgard, D. C., Boness, D. J., Bowen, W. D., McMillan, J. I. & Fleischer, R. C. (2004). The rate of fertilization in male mating tactics of the polygynous grey seal. *Molecular Ecology*, 13, 3543-3548.

Loughry, W. J. & Lazari, A. (1994). The ontogeny of individuality in black-tailed prairie dogs, *Cynomys ludovicianus. Canadian Journal of Zoology*, 72, 1280-1286.

Lowe, S. E. & Bradshaw, J. W. S. (2001). Ontogeny of individuality in the domestic cat in the home environment. *Animal Behaviour*, 61, 231-237.

Luhrs, M.L., Dammhahn, M. & Kappeler, P. (2012). Strength in numbers: males in a carnivore grow bigger when they associate and hunt cooperatively, *Behavioral Ecology,* 24 (1) 21-28.

Manci, K. M., Gladwin, D. N., & Villella, R. (1988). Effects of aircraft noise and sonic booms on domestic animals and wildlife: bibliographic abstracts (No. NERC-88/32). National Ecology Research Center Fort Collins Co.

Mann, J. (2006). Behavioral sampling methods for cetaceans: a review and critique. *Marine Mammal Science*, 15 (1), 102–122.

Mansfield, A. W. (1991). Accuracy of age determination in the grey seal *Halichoerus grypus* of eastern Canada. *Marine mammal science*, 7 (1), 44-49.

Maynard Smith, J. (1956). Fertility, mating behaviour and sexual selection in *Drosophila subobscura*. *Journal of genetics*, 54 (2), 261-279.

Maynard Smith, J., Harper, D. G. C. & Brookfield, J. F. Y. (1988). The Evolution of Aggression: Can Selection Generate Variability? [and Discussion]. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, 319, 557-570.

Michod, R. E. (2000). *Darwinian dynamics: evolutionary transitions in fitness and individuality*. Princeton University Press.

Minderman, J., Reid, J. M., Evans, P. G. H., & Whittingham, M. J. (2009). Personality traits In wild starlings: exploration behavior and environmental sensitivity. *Behavioural Ecology*, 20 (4) 830–837.

Møller, A. P. (1989). Viability costs of male tail ornaments in a swallow. *Nature*, 339, 132-135.

Moss, C. J. (1983). Oestrous behaviour and female choice in the African elephant. *Behaviour*, 86 (3), 167-196.

NOAA. (2013). *Gray Seal (Halichoerus grypus).* Available: http://www.nmfs.noaa.gov/pr/species/mammals/pinnipeds/grayseal.htm. Last accessed 14/12/2013.

Perry, E. A., Boness, D. J., & Insley, S. J. (2002). Effects of sonic booms on breeding gray seals and harbor seals on Sable Island, Canada. *The Journal of the Acoustical Society of America*, 111, 599.

Pomeroy, P. P. (1999). Breeding behaviour and oestrus in free-ranging grey seals. *European Research on Cetaceans*, 12, 411-413.

Pomeroy, P. P., Anderson, S. S., Twiss, S. D., & McConnell, B. J. (1994). Dispersion and site fidelity of breeding female grey seals (*Halichoerus grypus*) on North Rona, Scotland. *Journal of Zoology*, 233 (3), 429-447.

Pomeroy, P. P., Redman, P. R., Ruddell, S. J. S., Duck, C. D., & Twiss, S. D. (2005). Breeding site choice fails to explain interannual associations of female grey seals. *Behavioral Ecology and Sociobiology*, 57 (6), 546-556.

Pomeroy, P. P., Twiss, S. D., & Duck, C. D. (2000). Expansion of a grey seal (*Halichoerus grypus*) breeding colony: changes in pupping site use at the Isle of May, Scotland. *Journal of Zoology*, 250 (1), 1-12.

Pomeroy, P., Wilmer, J. W., Amos, W. & Twiss, S. (2001). Reproductive performance links to fine-scale spatial patterns of female grey seal relatedness. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 268, 711-717.

R Studio. (2012). RStudio: Integrated development environment for R (Version 0.97.336) [Computer software]. Boston, MA. Retrieved May 20, 2013. Available from http://www.rstudio.org/

Réale, D., Gallant, B. Y., LeBlanc, M. & Festa-Bianchet, M. (2000). Consistency of temperament in bighorn ewes and correlates with behaviour and life history. Animal *Behaviour.* 60, 589–597.

Réale, D., Reader, S. M., Sol, D., McDougall, P. T. & Dingemanse, N. J. (2007). Integrating animal temperament within ecology and evolution. *Biological Reviews*, 82, 291-318.

Reiter, J., Panken, K. J. & LeBeouf, B. J. (1981). Female competition and reproductive success in northern elephant seals. *Animal Behaviour*, 29, 670-687.

Richards, S. A. (2008). Dealing with overdispersed count data in applied ecology. *Journal of Applied Ecology,* 45, 218-227.

Riedman, M. (1989). The pinnipeds: seals, sea lions, and walruses, University of California Press.

Roberts, S. C. & Gosling, L. M. (2003). Genetic similarity and quality interact in mate choice decisions by female mice. *Nature genetics*, 35, 103-106.

Rollin, B. E., & Kessel, M. L. (1998). Guidelines for the treatment of animals in behavioural research and teaching. *Animal Behaviour*, 55, 251-257.

Schuett, W., Dall, S. R. X., Baeumer, J., Kloesener, M. H., Nakagawa, S. Beinlich, F. & Eggers, T (2011). "Personality" variation in a clonal insect: The pea aphid *Acyrthosiphon pisum*. *Developmental Psychobiology*, 53 (6), 631–640.

Schuett, W., Tregenza, T. & Dall, S. R. X. (2010). Sexual selection and animal personality *Biological Reviews*, 85, 217-246.

SCOS Advice, (2012). Scientific advice on matters related to the management of seal populations: 2012. Available at http://www.smru.standrews.ac.uk/documents/1199.pdf

Sih, A., Bell, A. & Johnson, J. C. (2004). Behavioral syndromes: an ecological and evolutionary overview. *Trends in Ecology & Evolution*, 19, 372-378.

Sih, A., Bell, A. & Johnson, J. C. (2010). Behavioural Syndromes. In: Westneat, D. F. & Fox, C.W. *Evolutionary Behavioural Ecology*. New York: Oxford University Press. 516-530.

Smith, B. R. & Blumstein, D. T. (2008). Fitness consequences of personality: a meta-analysis. *Behavioral Ecology*, 19, 448-455.

Stamp Dawkins, M. (2007). Observing Animal Behaviour. New York: Oxford University Press.

Stephenson, C. M., Matthiopoulos, J., & Harwood, J. (2007). Influence of the physical environment and conspecific aggression on the spatial arrangement of breeding grey seals. *Ecological Informatics*, 2 (4), 308-317.

Thornton, A. & Lukas, D. (2012). Individual variation in cognitive performance: developmental and evolutionary perspectives. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, 367, 2773-2783.

Tinbergen, N. (1951). The study of instinct. Oxford, UK: Oxford University Press.

Tinbergen, N. (1963). On aims and methods of ethology. *Zeitschrift für Tierpsychologie*, 20 (4), 410-433.

Tremmel, M. & Müller, C. (2013). Insect personality depends on environmental conditions *Behavioural Ecology*. 24 (2), 386-392.

Trillmich, F., & Weissing, F. J. (2006). Lactation patterns of pinnipeds are not explained by optimization of maternal energy delivery rates. *Behavioral Ecology and Sociobiology*, 60 (2), 137-149.

Trivers, R. L. (1972) Parental investment and sexual selection. In B. Campbell (Ed.) Sexual selection and the descent of man, 1871-1971 (pp 136–179). Chicago, Aldine.

Twiss, S. D. (1991). Behavioural and energetic determinants of individual mating success in male grey seals (*Halichoerus grypus*, Fabricius 1791). PhD Thesis, University of Glasgow.

Twiss, S. D. & Franklin, J. (2010). Individually consistent behavioural patterns in wild, breeding male grey seals (*Halichoerus grypus*). *Aquatic Mammals*, 36, 234-238.

Twiss, S. D. & Thomas, C. J. (1998). Fine scale topographic influences on environmental potential for polygamy (EPP) in grey seals. World marine mammal conference. Monaco, 20-24th January.

Twiss, S. D., Cairns, C., Culloch, R. M., Richards, S. A. & Pomeroy, P. P. (2012). Variation in Female Grey Seal (*Halichoerus grypus*) Reproductive Performance Correlates to Proactive-Reactive Behavioural Types. *PLoS ONE*, 7, e49598.

Twiss, S. D., Caudron, A., Pomeroy, P. P., Thomas, C. J. & Mills, J. P. (2000). Finescale topographical correlates of behavioural investment in offspring by female grey seals, *Halichoerus grypus*. *Animal Behaviour*. 59, 327–338.

Twiss, S. D., Culloch, R. & Pomeroy, P. P. (2011). An in-field experimental test of pinniped behavioral types. *Marine Mammal Science*, 28, 280-294.

Twiss, S. D., Poland, V. F., Graves, J. A. & Pomeroy, P. P. (2006). Finding fathers: spatio-temporal analysis of paternity assignment in grey seals (*Halichoerus grypus*). *Molecular Ecology*, 15, 939-1953.

Twiss, S., Anderson, S. & Monaghan, P. (1998). Limited intra-specific variation in male grey seal (*Halichoerus grypus*) dominance relationships in relation to variation in male mating success and female availability. *Journal of Zoology*, 246, 259-267.

Utne-Palm, A. C., & Hart, P. J. (2000). The effects of familiarity on competitive interactions between threespined sticklebacks. *Oikos*, 91 (2), 225-232.

Vahed, K. (1998). The function of nuptial feeding in insects: a review of empirical studies. *Biological Reviews*, 73, 43-78.

Van Oers, K., De Jong, G., Van Noordwijk, A. J., Kempenaers, B. & Drent, P. J. (2005). Contribution of genetics to the study of animal personalities: a review of case studies. *Behaviour*. 142, 1185-1206.

Van Oers, K., Drent, P. J., Dingemanse, N. J. & Kempenaers, B. (2008). Personality is associated with extrapair paternity in great tits, *Parus major*. *Animal Behaviour*, 76, 555-563.

Watkins, J. F. (1990). Observations of an aquatic grey seal (*Halichoerus grypus*) mating. *Journal of Zoology*, 222, 677–680.

Williams, L. J., King, A. J., & Mettke-Hofmann, C. (2012). Colourful characters: head colour reflects personality in a social bird, the Gouldian finch, *Erythrura gouldiae*. *Animal Behaviour*, 84 (1), 159-165.

Wolak, M. E., Fairbairn, D. J. & Paulsen, Y. R. (2012). Guidelines for Estimating Repeatability. *Methods in Ecology and Evolution*, 3 (1), 129-137.

Wolf, M., & Weissing, F. J. (2012). Animal personalities: consequences for ecology and evolution. *Trends in ecology & evolution*, 27 (8), 452-461.

Wood, S. N. (2011). Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. *Journal of the Royal Statistical Society (B)*, 73 (1), 3-36

Worthington Wilmer, J., Allen, P. J., Pomeroy, P. P., Twiss, S. D., & Amos, W. (1999). Where have all the fathers gone? An extensive microsatellite analysis of paternity in the grey seal (*Halichoerus grypus*). *Molecular Ecology*, 8 (9), 1417-1429.

Zuri, I., & Rado, R. (2000). Sociality and agonistic behavior in the lesser white-toothed shrew, *Crocidura suaveolens. Journal of mammalogy*, 81 (2), 606-616.