The role of temporal, spatial and kin associations in grey seal breeding colonies.

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I, Paula Redman, hereby certify that this thesis, which is approximately 40,000 words in length, has been written by me, that it is the record of work carried out by me and that it has not been submitted in any previous application for a higher degree.

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Contents page

Acknowledgements

Abstract 1

Chapter One: General Introduction

1.1. Benefits and costs of group formation 2
1.2. Membership of the group 4
1.3. Social behaviour 5
1.4. Factors affecting group formation in pinnipeds 5
1.5. Group formation in grey seals 7
1.6. Aims 9

Chapter Two: General Methodology

2.1. Study species – The grey seal (*Halichoerus grypus*) 11
    2.1.1. Geographical range and sub-populations 11
    2.1.2. Breeding behaviour 13
2.2. Study colonies 14
    2.2.1. North Rona 14
    2.2.2. The Isle of May 17
2.3. Fieldwork 19
2.4. Behavioural observations 19
    2.4.1. North Rona 19
    2.4.2. The Isle of May 21
2.5. Pelage identification 23
    2.5.1. Field sketches 23
    2.5.2. Video images 23
    2.5.3. Photographs 25
2.6. Skin sampling 25
Appendix 2.1 26
Appendix 2.2 27

Chapter Three: Pelage identification of grey seals

3.1. Summary 30
3.2. Introduction 30
    3.2.1. Aims 32
3.3. Methodology 32
    3.3.1. Classification of pelage patterns 32
    3.3.2. Assessment of video and photographic images 35
    3.3.3. Assessment of sketches 37
    3.3.4. Factors affecting image quality and female recognition 37
    3.3.5. Data analysis 38
3.4. Results 38

3.4.1. Agreement between judges 38
3.4.2. Classification of pelage types 38
3.4.3. Assessment of video and photographic images 39
3.4.4. Assessment of sketches 40
3.4.5. Affect of image quality on recognition of females 41
3.5. Discussion 42
3.5.1. Pelage pattern classification 42
3.5.2. Image quality 42
3.5.3. Affects of topography, hide location and female behaviour 43
3.5.4. Using quality scores to determine identifiable females 44
3.6. Conclusions 46

Chapter Four: A comparison of female matches using pelage identification and microsatellite analysis

4.1. Summary 48
4.2. Introduction 48
4.3. Methodology 49
4.4. Results 50
4.5. Discussion 54

Chapter Five: Grey seal breeding patterns and site fidelity

5.1. Summary 57
5.2. Introduction 58
5.2.1. Aims 58
5.3. Methodology 58
5.3.1. Resightings of known females 59
5.3.2. Recording female locations 59
5.3.3. Site fidelity, pupping date and time ashore 60
5.3.4. Summer resightings 60
5.4. Results 61
5.4.1. Return rates 61
5.4.2. Site fidelity and pupping date 62
5.4.3. Time ashore prior to pupping 64
5.4.4. Duration of lactation 66
5.4.5. Summer resightings 66
5.4.6. Comparisons with branded females 67
5.5. Discussion 69
5.5.1. Comparisons between years 69
5.5.2. Summer resightings 70
5.5.3. Comparisons with branded females 72
Appendix 5.1 74

Chapter Six: Passive and active association between female grey seals on North Rona

6.1. Summary 76
6.2. Introduction 77
    6.2.1. Aims 78
6.3. Classifying association 79
    6.3.1. Methodology 79
    6.3.2. Results 80
6.4. Inter-annual association 82
    6.4.1.Methodology 84
        6.4.1.1. Observed data 84
        6.4.1.2. Site fidelity model 85
    6.4.2. Results 90
        6.4.2.1. Observed data 90
        6.4.2.2. Model data 91
        6.4.2.3. Comparison of observed and modelled data 92
6.5. Discussion 94
    6.5.1. Frequency of association 94
    6.5.2. Active associations between years 94
    6.5.3. Model limitations 95
    6.5.4. Associations outside of the breeding season 96
6.6. Conclusions 96
Appendix 6.1 97

Chapter Seven: Relatedness of spatially associated female grey seals on North Rona

7.1. Summary 99
7.2. Introduction 99
    7.2.1. Aims 100
7.3. Methodology 100
    7.3.1. Sample collection 100
    7.3.2. Microsatellite analysis 101
    7.3.3. Proximity and relatedness of adult females 101
    7.3.4. Relatedness of females showing inter-annual association 102
7.4. Results 103
    7.4.1. Proximity and relatedness of adult females 103
    7.4.2. Relatedness of females showing inter-annual association 103
7.5. Discussion 104

Chapter Eight: The effect of water availability on grey seal maternal attendance patterns and locomotory behaviour

8.1. Summary 107
8.2. Introduction 107
8.3. Methodology 109
8.4. Data analysis 110
    8.4.1. Calculations of locomotion rate and suckling rate 110
    8.4.2. Tests of observer and location difference 111
    8.4.3. Pseudoreplication 112
    8.4.4. Statistical comparisons and analyses 113
8.5. Results 113
    8.5.1. Affect of pup age on behaviour 113
    8.5.2. Long-distance locomotion 113
    8.5.3. Short-distance locomotion to pools 114
    8.5.4. Short-distance locomotion to conspecifics 114
    8.5.5. Total locomotion to water 115
    8.5.6. Time spent with pup 115
    8.5.7. Suckling rate 115
8.6. Discussion 118
Appendix 8.1 122

Chapter Nine: The affects of maternal attendance patterns and pup age on aggression interactions of lactating grey seals

9.1. Summary 124
9.2. Introduction 124
    9.2.1. Aims 126
9.3. Methodology 126
    9.3.1. Behavioural observations 126
    9.3.2. Duration of pup stages and affect of pup age on female aggression 127
    9.3.3. Levels of aggression 128
    9.3.4. Data analysis 128
9.4. Results 128
    9.4.1. Duration of pup stages 128
    9.4.2. Frequency of aggressive interactions 129
    9.4.3. Female/female interactions 131
    9.4.4. Aggression towards males 132
    9.4.5. Aggression towards alien pups 133
    9.4.6. Aggression towards gulls 134
    9.4.7. Quantification of aggression intensity 135
9.5. Discussion 137
    9.5.1. Aggression towards females 138
    9.5.2. Aggression towards males 139
    9.5.3. Aggression towards alien pups 140
    9.5.4. Aggression towards gulls 141
    9.5.5. Intensity of aggressive interactions 141
9.6. Conclusions 142

Appendix 9.1 143

Chapter Ten: General Discussion

10.1. Female grey seal behaviour and spatial association 144
10.2. Further research 146
    10.2.1. Is site fidelity affected by topography? 146
    10.2.2. Is site fidelity a result of fidelity to conspecifics? 147
    10.2.3. Determining where associations first develop 147

References 149

Abstract

This thesis describes how pelage patterns were used to identify individual female grey seals so that their breeding behaviour could be studied with minimal disturbance to the colony. The data collected included timing and duration of lactation, female locations (hourly and daily), and aggressive and maternal behaviour. Comparisons were made within and between colonies and also between branded and non-branded females on North Rona.

Results showed that pelage patterns were a reliable means of identifying individual females between years and that females on North Rona were highly site faithful, giving birth around the same date each year. When pools were abundant, females on North Rona moved <10m each day, but they travelled up to 300m to the sea when pools were scarce, indicating that access to water is important during lactation. Individuals that were observed within 20m of one another were classed as spatially associated within a year and many females were associated between years. Modelled data indicated that two thirds of inter-annual association could be due to location-based fidelity alone, but that the rest was likely to be active association amongst conspecifics. Summer resightings showed that some adult females that gave birth on North Rona were also present around the island outside the breeding season.

Although females on North Rona generally remained close to their pup, those at Tarbet on the Isle of May often left their pup unattended when travelling to water. A comparison of female/female aggression at these sites showed that the rate of aggression remained constant at Tarbet, but decreased on North Rona as the pup got older. It is hypothesised that female association and conflict reduction have developed on North Rona over the last 150 years through familiarity of females that stay close to their pup, and hence one another, throughout lactation.

Chapter One:
General Introduction

The work presented in this thesis investigates the behaviour of individual female grey seals, *Halichoerus grypus*, at two study colonies, North Rona and the Isle of May, which have been studied for approximately 40 years and 20 years respectively (e.g. Boyd *et al.,* 1962; Anderson *et al.,* 1975; Baker, 1984, 1988; Kovacs & Lavigne, 1986; Kovacs, 1987; Baker & Baker, 1988; Twiss, 1991; Pomeroy *et al.*, 1994, 1999, 2000a, 2000b, 2001; Twiss *et al.,* 1994, 2000a, 2000b, 2001; Allen *et al.*, 1995; Worthington-Wilmer *et al.*, 1999). Previous studies at these colonies provide a detailed background of intra-colony dynamics, against which social structure and behaviour can be viewed. However, the fine-scale behavioural observations of individual females in this study take these investigations a step further by helping to determine whether group membership, at a spatial scale which is relevant to the individual (i.e. within tens of metres rather than hundreds of metres) is consistent or random within and between breeding seasons. This in turn will help define the degree and nature of social organisation between individuals and characterise the role of spatial, temporal and kin associations within colonies of breeding grey seals.

1.1. Benefits and costs of group formation

It is not always possible to give a definitive answer to the question of what drives group formation, as many factors may be involved. For example, group formation may initially be determined by the availability of suitable habitats, as animals will aggregate where resources (e.g. breeding sites, roosting sites, food etc.) are patchy or scarce. Even if availability of resources does not lead to permanent group formation, grouping with conspecifics at certain times may relay specific benefits such as shared warmth (e.g. Arnold, 1990; Takahashi, 1997) and acquisition of information about food sources (e.g. Galef & White, 1997; Laland & Williams, 1997). However, this is only one side of the coin as there are also many costs to sociality, such as competition for mates, food and nest sites (e.g. Alexander, 1974;

Alatalo *et al.,* 1987) and it is difficult to determine why some animals are social and others are not without weighing up the pros and cons of group living.

The two most common answers to the question 'why do animals live in groups?' revolve around the fact that living with conspecifics provides protection from predators and helps in the finding of food. Group living can reduce the risk of predation through increased vigilance and group defence or because the dilution effect of being surrounded by conspecifics reduces the chances that a particular individual is eaten (e.g. Alexander, 1974; Krebs & Davies, 1995). Consequently, animals can spend less time looking for predators and more time feeding or resting. However, predators may also benefit from being part of a group, and sociality in wolves, *Canis lupus*, and lions, *Panthera leo*, has been linked with their ability to hunt co-operatively to kill prey much larger than themselves (Jordan *et al.*, 1967; Alcock, 1993; Stander, 1992). Prey size and availability is important to maintain these groups, and wolf packs that feed mainly on deer are much smaller than those that feed on moose (Jordan *et al.*, 1967). In fact, many canids and felids are generally solitary because they feed on small or sparsely distributed prey that can not support larger groups (Corbett & Newsome, 1975; Nott, 1997; Robinson, 1997).

Although living with others confers many advantages, these are not always distributed evenly within the group. Dominant animals generally obtain more food, better nest sites and have greater reproductive success. Male dominance in particular often coincides with the ability to monopolise copulation attempts where temporal or spatial grouping of females occurs (Emlen & Oring, 1977). This, in turn, leads to polygyny - the most common mating system amongst mammalian species. Within a polygynous species, subordinate animals may have little or no reproductive success. However, it is not just males that suffer. Females are also subjected to reproductive suppression (e.g. badgers, *Meles meles*, Woodroffe & MacDonald, 1995), and in some species, for example with the alpha-pair breeding system of wolves (Packard *et al.*, 1985), both sexes are affected. In the latter case, the cost of reproductive suppression may result in non-alpha wolves leaving a pack to breed elsewhere provided the food supply is abundant (Nott, 1997). Therefore, the degree of sociality observed within and between species reflects a trade-off between the costs and the benefits of group living.

1.2. Membership of the group

Animal aggregations, for example migrating herds, may contain many hundreds of thousands of individuals, but groups that interact socially are generally much smaller and are often kin-based. In species that are solitary most of the time a group consists solely of a parent and their offspring, but in more gregarious species several adults and their offspring may live together and many members of the group may be related. Group size is often regulated by juvenile dispersal from the natal site (Greenwood, 1980), which decreases the likelihood of inbreeding and reduces competition (Dobson, 1982). Juvenile dispersal may occur voluntarily, as in the European wild rabbit, *Oryctolagus cuniculus* (Künkele & von Holst, 1996), or because the offspring are driven out by adult aggression, as in the Columbian ground squirrel, *Spermophillus colombianus* (Wiggett & Boag, 1989). In most cases, one sex disperses and the other remains close to the natal area, with males being the predominant dispersers in mammals, and females being more likely to leave the natal area in birds (for reviews see Greenwood, 1980; Moore & Ali, 1984). Other factors, such as maternal dominance (Jones, 1980; Pope, 1998) or a change in the dominant breeding male (Rudran, 1973) may also affect whether an individual stays in the natal group or is driven away.

Despite the attention directed at kin-based associations, it is not unusual for social groups to contain unrelated animals (Waterman, 1995; Kerth & König, 1999; Burland *et al.,* 2001). This may occur as a consequence of natal dispersal, where the dispersing animals form unrelated same sex groups (e.g. Hoffmann, 1983; Penzhorn, 1984; Rasmussen & Farrington, 1994; Waterman, 1995), or through non-random association and reciprocal interaction. The latter may provide many benefits for animals that group with familiar conspecifics, for example where animals group together in maternity colonies (e.g. Bechstein's bats, *Myotis bechsteinii,* Kerth & König, 1999; brown long-eared bat, *Plecotus auritus,* Burland *et al.,* 2001), or to share warmth at night (e.g. Japanese macaques, Takahashi, 1997)). In these cases, familiarity alone may provide advantages within a group – for example, affiliated though unrelated Japanese macaques huddle more frequently and for longer at night than non-affiliated animals (Takahashi, 1997).

1.3. Social behaviour

The extent and form of social behaviour within a group varies enormously amongst different species - from the altruistic behaviour of eusocial insects, to less extreme conflict reduction/avoidance behaviour, which may take the form of tolerance. Although altruism incurs a cost to the individual performing the act, e.g. the predator alarm warnings of the Belding's ground squirrel, *Spermophilus beldingi*, (Sherman, 1977), many altruistic acts are reciprocal and the individual that performs the act one time will benefit at a later date (e.g. allogrooming (Dobson *et al*., 1998)). Co-operative behaviour is particularly common during contests over limited resources (Parker *et al*., 1995; Gompper *et al*., 1997) and leads to benefits for both participants. However, despite the fact that this appears to be 'for the good of the group', co-operative behaviour may arise solely because each individual is trying to maximise their own fitness.

Not all species have high levels of social organisation and recently it has been suggested that conflict reduction can be regarded as a first step towards sociality (Michod, 1999). Like co-operation, conflict reduction may arise from the selfish behaviour of individual animals – this is thought to be particularly likely in situations where animals frequently meet with the same individuals, for example where habitat is limited. Animals that interact frequently can develop signals to avoid conflict or can determine their relative status through the formation of dominance hierarchies. Although hierarchies may initially be established by agonistic interactions, (e.g. red deer, *Cervus elaphus*, (Clutton-Brock & Albon, 1979); red junglefowl, *Gallus gallus*, (Collias & Collias, 1996)) they may remain stable for many years once the initial period of conflict is over. Dominant individuals are able to maintain their position by ritualised threats and signals because subordinate individuals know the likely outcome of an aggressive interaction and rarely challenge those above them in rank. This benefits each individual by reducing the risk of injury and allowing each animal to spend more time in other activities such as feeding.

1.4. Factors affecting group formation in Pinnipeds

All three pinniped families – the Phocidae (true seals), the Otariidae (fur seals and sea lions) and the Odobenidae (walruses) – have adapted to life in a marine environment but are still tied to land or ice to give birth and raise their pups (Bonner, 1984; Oftedal *et al.*, 1987). It is thought that pinnipeds are often gregarious during the breeding season because of the limited availability of habitat that is suitable for giving birth and because gathering temporally allows individuals that are widely-spaced during the year to come together to mate (Bartholomew, 1970). However, gregariousness varies enormously – from the highly social walrus, *Odobenus rosmarus rosmarus*, which is always seen in large groups (Fay, 1982, as cited in Trillmich, 1996), to the bearded seal, *Erignathus barbatus*, where solitary females give birth on drifting ice floes (Kovacs, 2002).

As a breeding substrate for pinnipeds, ice varies enormously in its temporal and structural stability, the ease of access to water (and hence access to food) and the risk of predation. The maternal behaviour of ice-breeding phocid seals has been reviewed by Lydersen & Kovacs (1999) and they show that the degree of aggregation varies greatly between species. For example, predation by polar bears on bearded and ringed seals affects many aspects of their breeding ecology and neither form large aggregations like the harp, hooded and grey seals (Lydersen & Kovacs, 1999). However, nearest neighbour distances between females of the latter species vary dramatically, and although female harp seals, *Phoca groenlandica*, are found only a few metres apart (Kovacs, 1995), hooded seals, *Cytophora cristata*, are commonly separated by 50-100+ metres (Boness *et al.*, 1988; Kovacs, 1990). The differences in these species stem from the fact that harp seals aggregate around cracks and leads that allow easy access to the water beneath (Stewart, 1987), whilst hooded seals actively move away from the ice edge and do not enter the water during lactation (Boness *et al.*, 1988; Kovacs, 1990).

Although ice is a fairly homogenous substrate, pinnipeds that breed on land experience a wide variety of different habitats. At land-based colonies, topographical constraints can have a large impact on the degree of clumping observed, and animal densities may vary with terrain, ease of access to the sea for foraging, and the availability of pools and shade for thermoregulation (Boyd *et al.*, 1962; Anderson *et al.*, 1975; Costa, 1991; Campagna *et al.*, 1993; Pomeroy *et al.*, 1994, 2000a; Caudron, 1998; Twiss & Thomas, 1999; Twiss *et al.*, 2000a, 2001). Even on homogenous, open terrain, clustering is evident, indicating that social factors such as male harassment and the risk of injury to the female and her pup may also play a role in group formation (Boyd *et al.*, 1962; Boness, 1991; Le Boeuf & Mesnick, 1991; Campagna *et al.*, 1992; Boness *et al.*, 1995).

Another factor that is likely to affect social behaviour in pinnipeds is the lactation strategy used by the female. Pinnipeds have three maternal strategies – one that involves fasting, which is of short duration, and two that involve foraging, where the duration of lactation may be short or long (Boness & Bowen, 1996). The period of social contact between mother and pup is therefore affected by the duration of lactation as well as by whether or not the female leaves the breeding colony to feed. Lactation varies from four days in the hooded seal, *Cystophora cristata* (Bowen *et al.*, 1985; Perry & Stenson, 1992) to as long as three years in the walrus, (Miller & Boness, 1983, as cited in Kovacs & Lavigne, 1992), and whilst hooded seal pups are weaned abruptly and rapidly have to fend for themselves, walrus pups can learn to swim and forage whilst they are still being suckled by their mothers (Kovacs & Lavigne, 1992). Walrus pups may learn these skills directly from their mothers as they frequently join them on foraging trips (Fay, 1982, as cited in Trillmich, 1996). However, otariid pups, whose mothers also forage during lactation, are left at the breeding colony (e.g. Bonner, 1984; Trillmich, 1990; Bowen, 1991) and are unlikely to learn such skills directly from their mothers (Trillmich, 1996)

1.5. Group formation in grey seals

The grey seal is one of only a few pinniped species that breed on both ice and land (e.g. Bartholomew, 1970; Anderson & Harwood, 1985; Tinker *et al.*, 1995), and although similar aggregation patterns can be seen on each substrate, differences in group stability and composition are often evident due to variability in the ease of access to water. Grey seals breed on ice in densities similar to those seen on land (Lydersen & Kovacs, 1999), and in both cases adult males defend small groups of females (e.g. Twiss *et al.*, 1994; Tinker *et al.*, 1995), although the sex ratio of males to females may vary from 1:1.3 (Sable Island, Nova Scotia (Boness & James, 1979)) to 1:9 (North Rona (Anderson *et al.*, 1975)). Whenever there is unrestricted access to the sea, grey seals spend a considerable amount of time in the water during lactation. This is true for ice-breeding individuals (Lydersen *et al.,* 1994; Haller *et al.*, 1996), as well as for those that give birth on land (Davies, 1949, 1956; Hewer & Backhouse, 1960; Fogden, 1971; Hewer, 1974; Kovacs, 1987), although Sable Island is a notable exception (Boness & James, 1979). However at inland sites such as North Rona and some areas on the Isle of May, travelling to and from the sea on a regular basis is both difficult and energetically costly. Many females may therefore remain ashore throughout lactation (Boyd *et al.*, 1962; Anderson *et al.,* 1975; Twiss *et al.,* 2000a). Similarly grey seals breeding on ice may be forced to remain ashore throughout lactation because rafting ice may effectively block access to the sea (Lydersen & Kovacs, 1999).

Differences in maternal attendance behaviour have repercussions for group stability as high levels of disturbance, caused by females travelling to and from the sea, increase the number of aggressive encounters in which a female grey seal is involved. These, in turn, interrupt suckling bouts and increase incidences of mother/pup separation, pup starvation and pup mortality (Fogden, 1971; Hewer, 1974). Individuals that group with familiar conspecifics often benefit from reduced levels of aggression (Parker *et al.,* 1995; Gompper *et al.,* 1997), and it is possible that grey seals that remain ashore throughout lactation could become familiar with their neighbours. Consequently, these females may be involved in fewer agonistic interactions and improve their chances of successfully raising a pup to weaning (Pomeroy *et al.,* 2001; Twiss *et al.*, in press).

Females determine the degree of aggregation within grey seal colonies and new arrivals are generally attracted to areas where animals are already present (Hewer, 1960; Reijnders *et al.*, 1995). However, evidence of non-random patterns of distribution has been reported within some breeding colonies as a result of philopatry and site fidelity (Pomeroy *et al.*, 1994, 2000b; Twiss *et al.*, 1994), thereby indicating that there might be specific benefits for choosing particular sites within a colony. At land-based colonies many causes of pup death (e.g. starvation, drowning, infection, and trauma) are influenced by the topography of the breeding site (Anderson *et al.*, 1979; Baker, 1984, 1988; Baker & Baker, 1988, Twiss *et al.*, in press), and consequently maternal choice of pupping location may be very important for offspring survival. Pomeroy *et al.* (1999) have noted that older females tend to pup earlier in the breeding season (thereby gaining access to the best sites) and are generally more successful at raising their pup to weaning. Therefore, if female hierarchies are present within grey seal breeding colonies, philopatric offspring could benefit from maternal dominance when competing with conspecifics for access to high quality pupping sites.

1.6. Aims

Grey seals have been the subject of many behavioural studies, but these have generally concentrated on determining general patterns of breeding behaviour across whole colonies. This thesis focused on grey seal behaviour at a finer scale, looking at social interactions within small groups of females that were individually recognisable by their pelage markings, studying these individuals both within and between breeding seasons. The aims were as follows:

1) To determine whether female grey seals could be reliably identified by pelage in the field without the aid of computer-matching techniques. This was fundamentally important to the study as individual recognition allows long-term investigations to be carried out on known individuals.
2) To compare the behaviour of animals identified by their pelage markings with data collected from previous studies of animals at these colonies. In the past, studies at these colonies have centred on branded animals (e.g. site fidelity on North Rona (Anderson *et al.*, 1975; Pomeroy *et al.*, 1994; Twiss *et al.*, 1994)), but these animals have been repeatedly captured for long-term studies of maternal investment and may not be representative of the population as a whole. The work presented here compared the breeding behaviour of these two groups to determine whether results from studies of branded animals can be applied to all animals within the colony.
3) To use fine-scale mapping of the daily positions of individual females to determine whether associations exist between animals. Site fidelity alone could account for animals being observed close to one another in successive breeding seasons. However, closer examination of the distances travelled by associated animals should provide an indication of whether some animals actively choose to be close to one another.
4) To look at how the physical environment affects female behaviour. Female grey seals show clear habitat preferences (Twiss *et al.*, 2000a) and the work presented here investigated how short-term temporal changes in the physical habitat (i.e.

within-season, weather-induced changes) affected female locomotion and other behaviours. This work also investigated how differences in maternal attendance patterns and social stability affected aggressive behaviour at the two study colonies.

5) To determine whether associations between females were correlated with levels of genetic relatedness as measured using nine microsatellite loci. Behavioural data was linked with genetic data to determine whether kin association was prevalent at these breeding colonies.

Chapter Two:
General Methodology

This chapter provides an introduction to the study species and a description of the two study colonies. In addition, it presents the methodology common to the behavioural studies performed at both of the colonies; more specific methods, as well as information on data collation and analysis, are detailed in the appropriate chapters.

2.1. Study species - The grey seal (*Halichoerus grypus*)

The grey seal belongs to the family Phocidae (the true seals) and is the only species within the genera *Halichoerus* (Bonner, 1981). It is one of two species of seal native to the UK, the other being the common or harbour seal (*Phoca vitulina*). Grey seals can be distinguished from harbour seals by their 'Roman' nose (more pronounced in males than females), their larger size, and by their pelage. Females and juvenile grey seals are generally dark on the back and lighter underneath with darker spots or patches over the body (Figure 2.1), whereas males frequently have a predominantly dark pelage with lighter patches (Figure 2.2). Grey seals are sexually dimorphic, with males being longer and heavier than females; at the start of the breeding season, males are 195-250cm long and can weigh up to 400kg (e.g. Twiss, 1991), whereas females are 165-210cm long and weigh up to 250kg at parturition (e.g. Baker *et al.*, 1995; Pomeroy *et al.,* 1999).

2.1.1. Geographical range and sub-populations

The geographical range of grey seals is large, with three main sub-populations occurring in the west Atlantic, the Baltic and the east Atlantic (de Jong *et al.,* 1997). Outside of the breeding season individuals of this species are widely dispersed, but they need to return to land or ice to breed, so large numbers often gather together to give birth and mate. Breeding colonies tend to occur in uninhabited locations or areas inaccessible to land-based predators (predominantly humans (Reijnders *et al.*, 1995)), and are found in a variety of habitats. These range from land fast ice (e.g. Amet Island, Nova Scotia (Tinker *et al.*, 1995)), to caves (e.g. S.W. England, Ireland and France (de Jong *et al.*, 1997)), sandy beaches (e.g.

Monach Isles (Anderson & Harwood, 1985)) and grassy inland sites (e.g. North Rona (Anderson & Harwood, 1985)).

Figure 2.1. Female grey seal, displaying a typical pelage pattern.

Figure 2.2. Male (left) and female (right) grey seals. Note the larger size, darker pelage and more pronounced nose of the male.

In addition to their geographic separation, the three sub-populations of grey seals are also separated by differences in the timing of their breeding season. The eastern Atlantic group breeds between September and December, the western Atlantic group breeds from mid-January to mid-February and the Baltic group breeds in late February or early March (Bonner, 1981). The British Isles contains around 50% of the world population of grey seals (most of these breeding on islands in the north and west of Scotland (Hiby *et al.*, 1996)) and even here, there are differences in the timing of the breeding season. Seals in Cornwall breed earliest, with the timing of peak pupping becoming progressively later as one moves clockwise around the British coast. Although the reasons for the differences in timing are not known, they appear to reduce genetic transfer between colonies. Studies on animals from North Rona (NW Scotland) and the Isle of May (E Scotland), for example, show that there is considerable genetic differentiation between the two sites (Allen *et al.,* 1995). These two islands are only ~500km apart (a distance easily within the range of dispersing pups and adult movements (McConnell *et al.,* 1992; Hammond *et al.* 1993)), yet peak pupping dates are one month apart and observations of branded and tagged animals suggest that animals rarely travel from one island to the other to breed (P. Pomeroy, pers. comm.).

2.1.2. Breeding behaviour

The grey seal has an annual reproductive cycle. After mating and leaving the breeding colony the female undergoes three months of delayed implantation and during this time she feeds a lot to regain body condition before hauling out to moult. Implantation is followed by an eight-month gestation period (Hewer & Backhouse, 1968), after which the female returns to the breeding colony to give birth to a single pup. The pup is suckled for between 18-20 days (Bonner, 1972; Pomeroy *et al.,* 2001) and although mothers do not need to eat during lactation, stomach content analyses of ice-breeding grey seals have shown that some mothers do consume small quantities of fish during this time (Baker *et al.,* 1995). In general, however, females rely predominantly, if not exclusively, on stored blubber reserves to sustain their metabolism and provide milk for their pup during lactation (Fedak & Anderson, 1982). Weaning occurs abruptly when the female is mated and returns to the sea (Fogden, 1971; Bonner, 1981); the weaned pup remains on the breeding colony for between one week and one month before going to the sea for the first time (Reilly, 1991).

Although each female is only ashore for around three weeks, large numbers of seals congregate at the breeding colony and a typical breeding season lasts approximately 8 weeks. Adult grey seals display a high degree of site fidelity (Pomeroy *et al.,* 1994; Twiss *et al.,* 1994), with females (which usually give birth for the first time at around 5 years of age (Harwood & Prime, 1978; Boyd, 1985)) using the same breeding colony for 25 years or more (Pomeroy *et al.,* 1999). Males, which tend to become socially mature at around 8 years old, may use the same colony for up to 10-15 years (Twiss, 1991; Worthington-Wilmer *et al.,* 1999). Females have been observed mating with several different males in a single breeding season (Anderson *et al*., 1975; Boness & James, 1979; Twiss, 1991), but multiple copulations with a single individual tend to occur only with a resident male (S.D. Twiss, pers. comm.). Males that are resident for long periods of time gain more successful copulations (Twiss *et al.,* 1994). However, younger or less dominant individuals that do not defend areas of the breeding colony (transient males), may have some success by adopting a 'sneaky' mating strategy where they travel around the periphery of the colony and attempt to mate with females that are moving to and from the sea (Twiss *et al*., 1994).

2.2. Study colonies

The work presented here comes from studies at two island colonies – North Rona (N.W. Scotland) and the Isle of May (E. Scotland) that were visited in three consecutive breeding seasons.

2.2.1. North Rona

North Rona (59°06′N, 05°50′W) is an uninhabited island situated about 75km N.N.W. of Cape Wrath, Scotland (Figure 2.3). The island covers an area of 120 hectares, rising to a height of 108m above sea level, although the majority of seals breed on the relatively low-lying peninsula of Fianuis on the northern side of the island (Boyd *et al.,* 1962). The main area used in this study lies within the southern part of Fianuis, covering an area of 0.38km^2 (Figure 2.4) (Twiss *et al.,* 1994). Access from the sea to this area is largely restricted to a few narrow gullies on the east side of the island. These gullies lead to an open grassy slope where animals are relatively free to move around. A small proportion of animals breed on the rocky outcrop of Sceapull at the southwest point of the island (Figure 2.4).

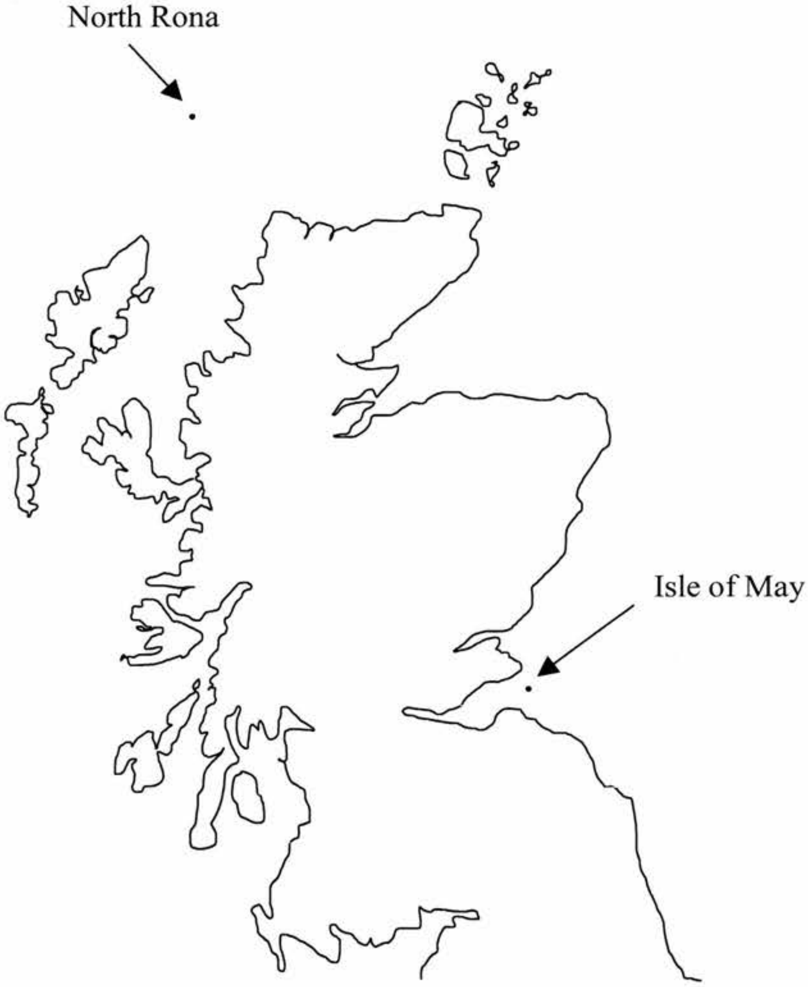

Figure 2.3. Location of North Rona and the Isle of May around the Scottish coast.

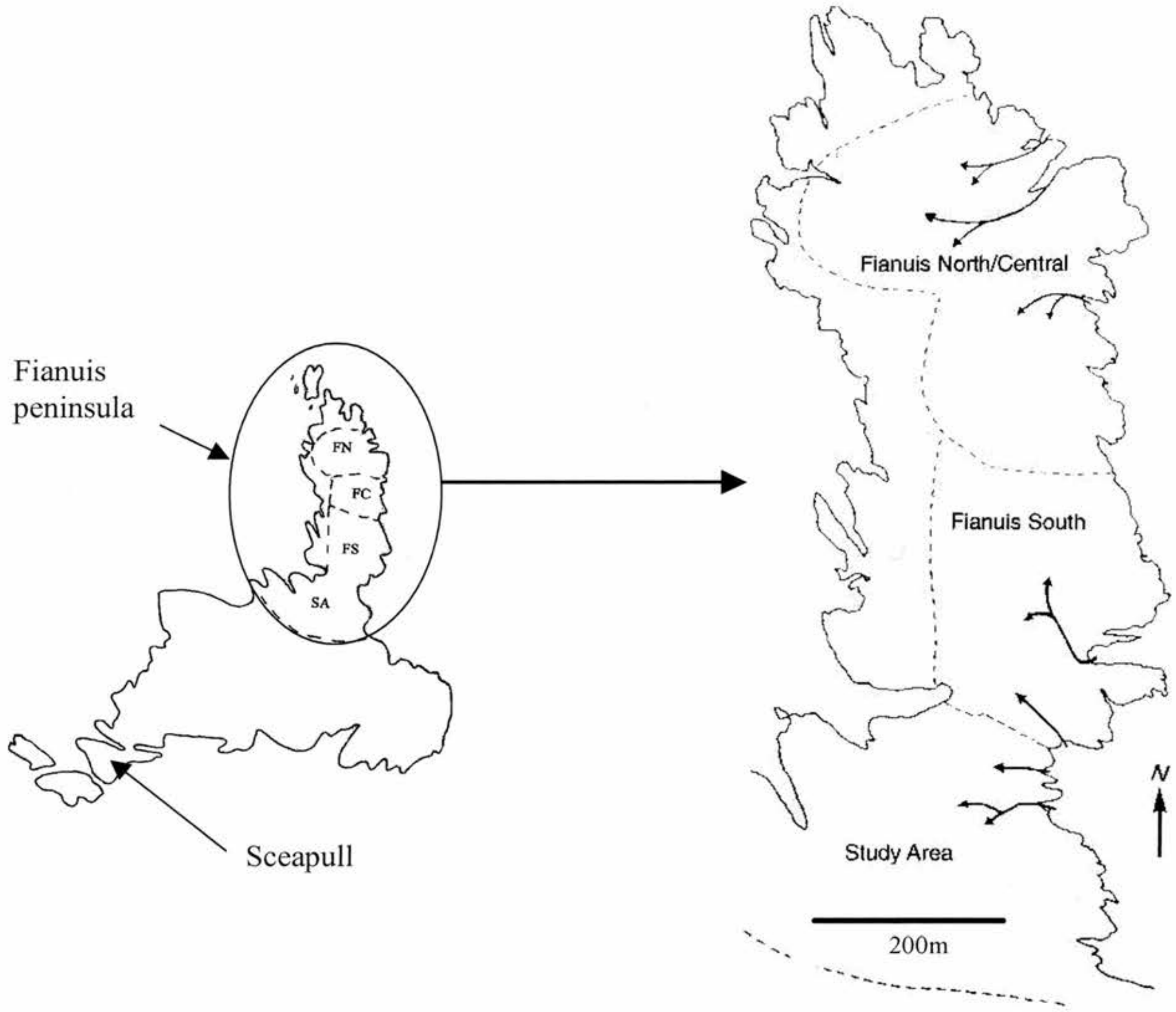

Figure 2.4. Map of North Rona showing the location of the Fianuis peninsula and Sceapull – breeding sites for grey seals. The majority of seals breed on the Fianuis peninsula, gaining access via gullies on the east side (marked by arrows). The study area (SA) is situated at the southern end of the peninsula. FN = Fianuis North, FC = Fianuis Central, FS = Fianuis South.

The breeding season at North Rona spans late September through to late November (Boyd & Laws, 1962) with peak pupping occurring around the 8th October each year (Hiby *et al.*, 1996). At this colony, females aggregate around pools of water and access points, spreading up to 300m inland, and show a tendency to remain close to their pups throughout lactation (Anderson *et al.*, 1975; Pomeroy *et al.*, 1994). The colony is believed to have been present since circa 1844 (Boyd *et al.*,

1962) and currently has a stable pup production, with approximately 1100 pups born in 2000 (C. Duck, pers. comm.).

2.2.2. The Isle of May

The Isle of May (56°11′N, 2°33′W), located in the mouth of the Firth of Forth, Scotland, (Figure 2.3), is 2km long and 0.5km wide, with cliffs rising to 40m above sea level on the south and west sides of the island. Instead of the expansive, gentle sloping terrain found on North Rona, the Isle of May has rocky crags and outcrops that break up the suitable breeding habitat so that it occurs in pockets at several locations around the island. As a result, animals are found in many locations, although they tend to be concentrated at the north end of the island (Figure 2.5). Direct overland movement between suitable pupping areas is difficult, but there is no real need for animals on the Isle of May to travel long distances on land as low-level terrain on the north and east sides of the island, and numerous discrete access points, allow access to many suitable pupping areas directly from the sea. The variable topography on the Isle of May also gives rise to different patterns of maternal attendance (Twiss *et al.*, 2000a). Where females are close to pools, they make short, regular journeys to water but spend most of their time close to their pups – a maternal attendance pattern that is not dissimilar to that observed on North Rona. However, where rocky outcrops and steep slopes make the journey to water more energetically costly, females on the Isle of May make fewer trips and leave their pups unattended for longer periods of time (Twiss *et al.*, 2000a).

The colony on the Isle of May is a more recently established colony than the one on North Rona, and as a result it is likely that the age structure of animals differs at the two islands (Pomeroy *et al.*, 2000a). Seals did not breed on the Isle of May before the 1950s (Eggeling, 1985), but since 1977 the number of pups born there has increased from around 25 (Harwood & Wylie, 1987) to over 2100 in 2000 (C. Duck, pers. comm.). Evidence from recaptures of tagged pups has led to the suggestion that many animals first came to the Isle of May from the Farnes off the Northumberland coast (Eggeling 1985) after the National Trust began to employ methods to deter pupping there in 1977 (Hammond *et al.*, 1993). The Isle of May is now the biggest single island colony for grey seals on the east coast of the UK (Hiby *et al.*, 1996).

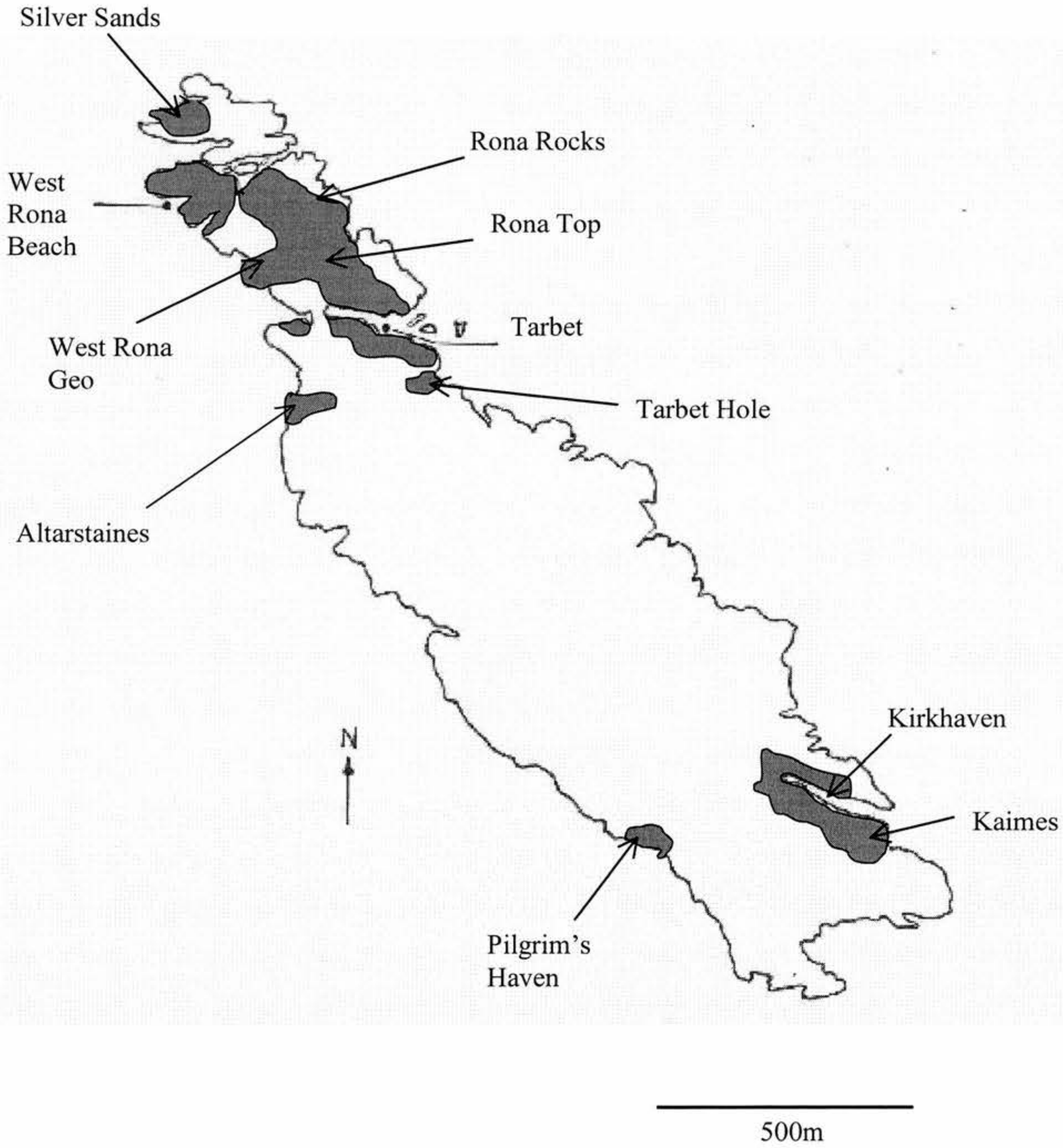

Figure 2.5. Map of the Isle of May – the main breeding areas are marked. The two study areas, Tarbet and West Rona Beach, are at the northern end of the island.

2.3. Fieldwork

Fieldwork was carried out on North Rona and the Isle of May between the following dates (see Appendix 2.1 for a summary of observation hours and data collected).

Year 1:	North Rona – September 29th 1998 to October 25th 1998
	Isle of May – November 4th 1998 to November 24th 1998
Year 2:	North Rona – September 30th 1999 to October 24th 1999
	Isle of May – November 5th 1999 to November 27th 1999
Year 3:	North Rona – September 29th 2000 to November 9th 2000*
	Isle of May – October 29th 2000 to November 29th 2000*

* P. Redman was present on North Rona from September 29th 2000 to November 9th 2000 and then on the Isle of May from November 11th 2000 to November 29th 2000; P. Pomeroy was present on the Isle of May from October 29th 2000 to November 17th 2000.

Pelage images were also collected on North Rona outside the breeding season between May 27th 1999 and May 29th 1999 (by R. Harris) and between June 17th 2001 and June 23rd 2001 (by P. Pomeroy).

2.4. Behavioural observations

2.4.1. North Rona

Focal observations were made on small groups of females and their pups at five locations within the study area on North Rona during the three breeding seasons between 1998 and 2000. Each group was defined according to locality using permanent landmarks that encompassed an area approximately 20m x 20m; the locations of the study groups are shown in Figure 2.6. Focal group size varied from day to day depending on the number of animals in an area, but ranged from three to twenty females, plus their pups and any males in the immediate area. Each group was observed at a distance of approximately 150 metres, from a hide on a ridge that was approximately 40 metres higher than the study area.

Continuous observations were made using binoculars during daylight hours. The first hour of each observation period was used to scan the study area for known females (females identified by pelage that had been assigned an individual name or number), and their positions and the age of their pup were recorded. Subsequently, maps of the study females (known females undergoing behavioural observation) were plotted at hourly intervals on A4 transparency sheets placed over images of the study areas. These images were derived from geo-rectified aerial photographs that had been overlaid with a 10m by 10m grid using a geographical information systems (GIS) database (ARC INFO) (Twiss *et al.,* 2000a; 2000b) and allowed accurate positioning (to within a few metres) of the animals within the study area. These images were also used to map the location of known females and study females on days when behavioural observations were not made.

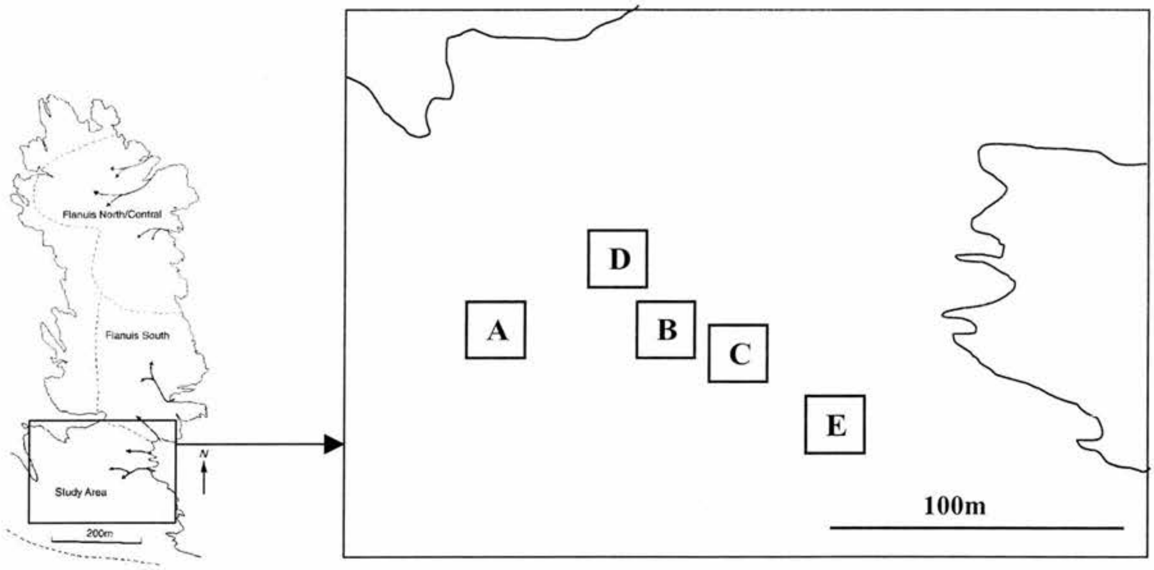

Figure 2.6. Location of study groups on North Rona. A = West pools, B = Castle, C = Keep, D = Arena and E = East pools. Study groups were observed at locations A and B in 1998, C and D in 1999, and B, C, D and E in 2000.

Throughout the observation period the behaviour of study animals was recorded on prepared data sheets. Pup age was recorded using the 'stage' categories of Kovacs and Lavigne (1986), which are modified from the original categories of

Boyd and Campbell (1971). In this system of classification, pups are allocated age groups according to changes in physical characteristics (Appendix 2.2).

2.4.2. Isle of May

Focal observations on the Isle of May were made at two sites, Tarbet and West Rona Beach (Figure 2.5), in 1998 (alternating between sites daily) but only at Tarbet in 1999 and 2000. Behaviour, pup age and female positions were recorded during daylight hours as described above.

Tarbet:

Tarbet is situated on the east side of the Isle of May and is often used as an access point for females that have pupping sites further inland. There is a large tidal inlet, which forms a narrow channel of seawater separating the north and south sides of the study area at high tide; water remains in a large pool when the tide goes out. Females give birth on shallow rocky slopes around this pool and on a plateau on the northern side (Figure 2.7) and they frequently travel between their pup and the pool. A hide was erected on the south side of the pool at a distance of between 20m and 50m from the study animals. Binoculars were used to help see the details of the pelage patterns when drawing the animals and subsequently to identify individuals, but were not required for observations.

West Rona Beach:

West Rona Beach is a low-lying area situated at the north end of the Isle of May (Figure 2.5). A large tidal pool and several gullies allow females easy access to and from the sea (Figure 2.8). A hide was erected to the south of this pool, at a distance of between 5m and 30m from the observed animals.

Figure 2.7. View from the hide at Tarbet, showing the pool at high tide

Figure 2.8. View from the hide at West Rona Beach (photograph by C. Stephenson).

2.5. Pelage identification

Images of the pelage patterns of individual female grey seals were collected in three ways:

1) Sketches on prepared outline sheets in the field
2) Video images - onto Hi-8 tapes (1998 and 1999)
   - using a digital camcorder (2000)
3) Black and white 35mm photographs

2.5.1. Field sketches

Pelage markings and scars of individual females were sketched on prepared outline sheets (Figure 2.9) so that their identification could be confirmed daily. Where possible, video or photographs were also taken of each study animal and linked to the sketches to allow identification in subsequent years. Sketches were also annotated to indicate the percentage of markings captured for each side.

2.5.2. Video images

Hi-8 tapes: a Canon UC8 camcorder with 20x optical zoom and 1.4x tele-converter was used to obtain video images of both the left and right hand sides of study females whilst observers were in the study colony (North Rona) or in the hide (Isle of May). Full-body shots and close-ups of the head were collected of study females and surrounding females where possible. Additional females were videotaped in conjunction with the collection of skin samples (see section 2.6) towards the end of each field season on the two colonies. These females were given an individual identification number that was used to cross-reference the pelage shots, location and skin sample.

Images were obtained from Hi-8 tapes using the 'vidigrab' system developed by P. Lovell and L. Hiby at the Sea Mammal Research Unit (SMRU). This system captures images from the videotape and saves them as digital images that were printed out and used in the field for identification purposes.

Digital: An MV30 digital camcorder with 20x optical zoom and a 1.4x tele-converter was used on the Isle of May during the 2000 field season and on North

Rona in June 2001. Images were obtained using the Pinnacle systems 'Studio DV Plus' digital video-editing software package.

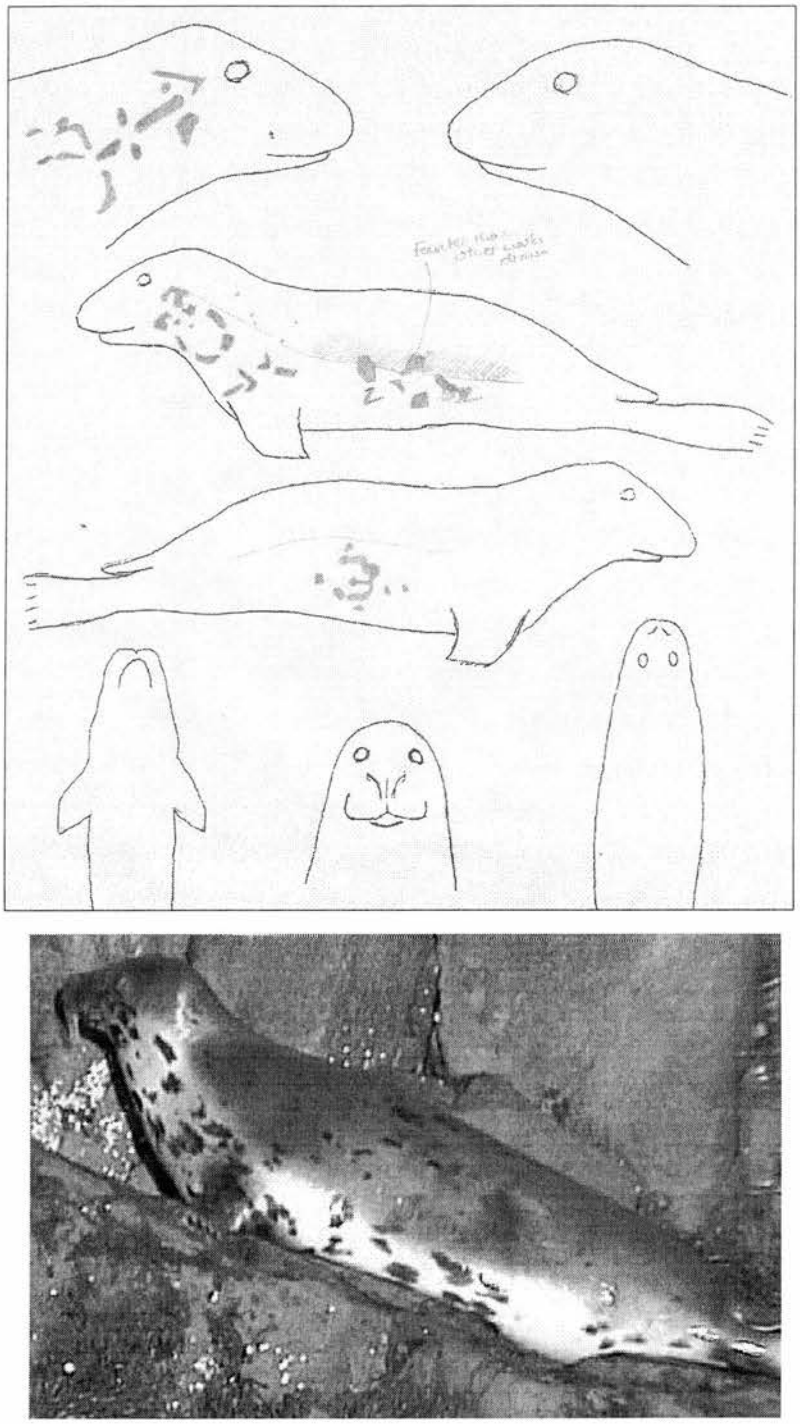

Figure 2.9. Example of field sketch with captured video image for comparison

2.5.3. Black and white 35mm photographs

Photographs of females were taken using a Minolta Dynax 7000 camera with a 300mm auto-focus lens and a 1.4 multiplier, on black and white XP2 400 iso film. Date, location and female ID were recorded along with the film and frame number to allow cross-referencing of photographs in the laboratory. The negatives were scanned using Hewlett Packard Photosmart S20 scanning software and saved as digital files. Images were printed out and used in the field for identification purposes.

2.6. Skin sampling

Two methods were employed to collect skin samples from study females for microsatellite analysis. First, when females were sedated (for procedures not directly related to this project), samples were taken from the inter-digital webbing of a hind flipper using piglet ear-notching pliers; second, when females were not sedated, skin samples were taken from the flank using a biopsy head attached to the end of a pole. The ear notching pliers and biopsy heads were thoroughly cleaned between samples using a dilute solution of chlorohexidine. Skin samples were stored in micro-tubes containing the preservative buffer 20% dimethylsulphoxide (DMSO) saturated with salt (Amos & Hoelzel, 1991) and labelled with an identification number. On returning from the islands these samples were stored in a freezer at minus 20°C until microsatellite analysis was performed. All sampling procedures were carried out under Home Office licence.

Appendix 2.1. Data collected during breeding seasons (1998-2000) and outside the breeding season (May 1999, June 2001 (North Rona only))

North Rona

Year	Days of behavioural observation	Study group locations [a]	Observers [b]	Man-hours of observation	Number of females sketched [c]	Number of females with video/photo images [c]	Number of maps of female positions	Number of skin samples collected
1998	16	A, B	PR, PP	180	79	68	207	124
1999 [d]	-	-	RHs	-	-	24	-	-
1999	16	C, D	PR, PP	184	127	151	259	62
2000	24	B, C, D, E	PR, PP, RHt	231	94	202	267	50
2001	-	-	PP	-	-	75	-	-

Isle of May

Year	Days of behavioural observation	Study group locations [a]	Observers [b]	Man-hours of observation	Number of females sketched [c]	Number of females with video/photo images [c]	Number of maps of female positions	Number of skin samples collected
1998	18	T, W	PR	108	27	58	94	17
1999	20	T	PR	108	54	188	67	28
2000	9	T	PR, PP	45.5 [e]	40	250	17	13

[a] A = West pools, B = Castle, C = Keep, D = Arena, E = East pools, T = Tarbet, W = West Rona Beach

[b] PR = P. Redman, PP = P. Pomeroy, RHs = R. Harris, RHt = R. Harcourt

[c] Numbers given are a maximum, as left and right sides of an animal may not be linked

[d] May 1999

[e] Behavioural observations made by PR only

Appendix 2.2. Descriptive age categories for grey seal pups (based on Kovacs & Lavigne, 1986).

Age class	**Description**
Stage I (Figure A2.1)	At this stage, the pup lacks co-ordination and the ribs, hips and shoulders are clearly visible. The umbilicus looks pink and fresh and the skin forms loose folds around the neck and shoulders; there may be a yellowish tint to the pelage.
Stage II (Figure A2.2)	At this stage, the pup shows improved co-ordination and the ribs become less prominent as a layer of blubber is deposited. The umbilicus is dried and shrivelled (it is lost in the early part of this stage) and the pelage is white.
Stage III (Figure A2.3)	At this stage, the pup shows good co-ordination and the body is barrel-shaped. The pelage is white, although the muzzle may show a slight loss of natal coat. There are no signs, on the body, of moulting to the juvenile pelage.
Stage IV (Figure A2.4)	At this stage, the pup starts moulting to the juvenile pelage; the lanugo is still present in places.
Stage V (Figure A2.5)	At this stage, the pup is completely moulted to the juvenile pelage.

Figure A2.1. Stage I pup – hips, ribs and shoulders clearly visible and skin forms loose folds. Umbilicus is pink and fresh.

Figure A2.2. Stage II pup – ribs less prominent, umbilicus is dried and shrivelled.

Figure A2.3. Stage III pup – barrel shaped body with no signs of moult.

Figure A2.4. Stage IV pup – onset of moult to juvenile pelage.

Figure A2.5. Stage V pup - completely moulted to juvenile pelage.

Chapter Three:

Pelage identification of grey seals

Images of female grey seals (sketches, video and photographs) were captured in the field by P. Redman, P.P. Pomeroy, S.D. Twiss, S.E. Moss, A.F. Helyar, R. Harris, and S. McCulloch

Video footage was digitally captured by P. Redman and R. Harris.

The SMRU pelage database was maintained and updated by L. Hiby, J. Watkins and P. Lovell

3.1. Summary

This chapter describes how female behaviour, hide position and the physical environment affected the quality of photographs and video images of grey seal pelage markings. It also describes how these factors, as well as observer familiarity, affected subsequent recognition of female grey seals. Four broad pelage pattern types are classified here to facilitate future pelage identification of female grey seals.

3.2. Introduction

Studies of behaviour often require the ability to identify individual animals within a population. This can be achieved through the use of artificial marks (e.g. freeze marking, Rood & Nellis, 1980; coloured tape, Gompper *et al.*, 1997; flipper tags and brands, Pomeroy *et al.*, 2000b; leg bands, Chuang-Dobbs *et al.*, 2001; collars and dyes, Lazaro-Perea, 2001) but there may be problems associated with their application. Capture of animals to apply artificial marks is impractical in some species (e.g. cetaceans), and can be expensive, time consuming or disruptive in others (Foster, 1966; Laird *et al.*, 1975; Bretagnolle *et al.*, 1994). Handling, or even the marks themselves, may affect the behaviour that is being studied (Fiske & Amundsen, 1997; Johnsen *et al.*, 1997; Bernard *et al.*, 1999; Broderick & Godley, 1999; Menu *et al.*, 2000), and where animals require sedation, there is an additional risk for both the study animal and the researcher. In addition, the ease of re-identification and longevity of artificial marks must be considered if the individuals are involved in long-term studies – leg rings and tags, for example, frequently become unintelligible or lost (Scott, 1988; annual tag loss rate estimated at 0.24 in grey seals (Pomeroy, unpublished data)).

Individual identification through the use of natural markings (e.g. pelage patterns and scars) can overcome many of these difficulties, and many studies, encompassing a wide range of species (e.g. giraffes, *Giraffa camelopardalis,* Foster, 1966; zebra, *Equus burchelli Gray,* Briand-Petersen, 1972; blunt-nosed minnows, *Pimephales notatus,* Pot & Noakes, 1985; adders, *Vipera berus,* Sheldon & Bradley, 1989; many species of cetacean, IWC, 1990 and references therein; ospreys, *Pandion haliaetus,* Bretagnolle *et al.*, 1994; tigers, *Panthera tigris tigris,* Karanth & Nichols, 1998), have used this method successfully. The main advantages of natural markings are that they do not artificially affect behaviour, animals do not have to be handled, and individuals are effectively 'marked' by taking photographs. However, for pelage identification to work well, several criteria must be met. The markings used must show little or no change over time (Foster, 1966; Sheldon & Bradley, 1989; Agler *et al.*, 1990; Bretagnolle *et al.*, 1994), they must be distinctly different between individuals (Foster, 1966; Briand-Petersen, 1972; Sheldon & Bradley, 1989; Bretagnolle *et al.,* 1994; Karanth & Nichols, 1998) and they must allow reliable identification of a large number of animals (Foster, 1966; Rees, 1981; Sheldon & Bradley, 1989; Stevick *et al.*, 2001). Previous studies have also shown that the ability to re-identify an animal using pelage identification techniques is greatly affected by the quality of the image captured (Whitehead *et al.*, 1997; Wilson *et al.*, 1999; Friday *et al.*, 2000) and is improved if distinctive scars or pigmentation are present (Calambokidis *et al.*, 1990; Mizroch *et al.,* 1990).

Female grey seals have highly variable pelage patterns and it has been demonstrated that they can be individually identified using a computer-aided pelage matching technique (Hiby & Lovell, 1990). In brief, this technique matches images of the head and neck area of seals from a 'pattern cell' derived from a specific area on the neck. The pattern cell is captured from digitised images (which must show the ear, the eye and the nose of the animal as reference points) over which a three-dimensional model has been stretched, and is stored as a matrix of numbers that represents the grey-scale intensities of the pelage in this area. As the model is independently fitted to each image, the numerical description or 'identifier array' is based on the same area of the neck for each animal and is independent of the orientation of the original image. Identifier arrays for individual images are compared by a computer to find possible matches and then compared by eye to confirm that the animal is the same. Such methods of automated screening reduce the number of photographs that have to be compared by eye and are useful when analysing large numbers of photographs in mark-recapture experiments. However, for behavioural studies where fieldwork on remote islands may restrict access to many facilities necessary for photo identification (e.g. film processing equipment), they have a more limited application. In this study, resightings between days and between years were made by comparing animals within the colony with previously captured images. New images could not be captured every day, and it was necessary to expand on Hiby and Lovell's (1990) computer-aided matching technique and use the whole body pelage pattern to allow recognition in the field by eye.

3.2.1. Aims

The main aim of the work presented here was to determine what factors affected capture of good quality pelage images on North Rona and the Isle of May. Factors that affected subsequent recognition of female grey seals in the field were also investigated.

3.3. Methodology

3.3.1. Classification of pelage patterns into types

The pelage patterns of female grey seals vary enormously, but four broad categories were discerned as described below.

Type 1 (Figure 3.1a). Females in this group had a clearly defined counter-shaded 'base' pelage (mid grey over the back and very light grey down the sides and under the belly). Markings were perceived as dark areas of pigment overlying the lighter 'base' pelage. Dark markings covered less than 40% of the body.

Type 2 (Figure 3.1b). Females in this group had a body colour that was predominantly dark, with markings being perceived as lighter areas on the darker background. Counter-shading was not generally visible, although markings on the back tended to be mid grey and markings lower down tended to be very light grey. Light markings covered less than 40% of the body.

Type 3. Females in this group had pelage with light and dark pigment present in roughly equal proportions. There were two sub-categories for this pattern type:

a) Aggregation of pigment occurred in large patches (type 3p – Figure 3.1c (i)), which did not hinder identification, although sketches drawn by different observers may not be matched due to different perception of the pattern (seen as either dark on light or light on dark).

b) Pigment had a mottled appearance (type 3m - Figure 3.1c (ii)), which made individual identification more difficult.

Type 4. Females in this group were a uniform mid grey with no counter-shading (Figure 3.1d (i)). A sub-category (type 4d - Figure 3.1d (ii)) was assigned to females that had darker markings present; females with this pelage pattern type were rare.

Each side of a female was classified separately because the left and right pelage patterns of an individual animal were not always assigned to the same group (with the exception of uniform grey females, as the few that were observed were uniform on both sides). This was not a disadvantage as such, as knowing that the two sides of a female did not have the same pelage type facilitated identification.

The proportion of well-marked animals in the population was determined using a sub-sample of pelage images obtained from North Rona and the Isle of May. All good quality images obtained in 1998 and 1999 that displayed a minimum of 50% of the pelage markings were categorised as described above. In total, 112 images were categorised for North Rona and 107 images were categorised for the Isle of May. To minimise discrepancies, a single judge (PR) classified the pelage type for each image, however, a sub-sample of 80 female images were scored for pelage type by five judges to determine whether or not these categories could be used by other observers.

Figure 3.1a. Type 1 pelage pattern

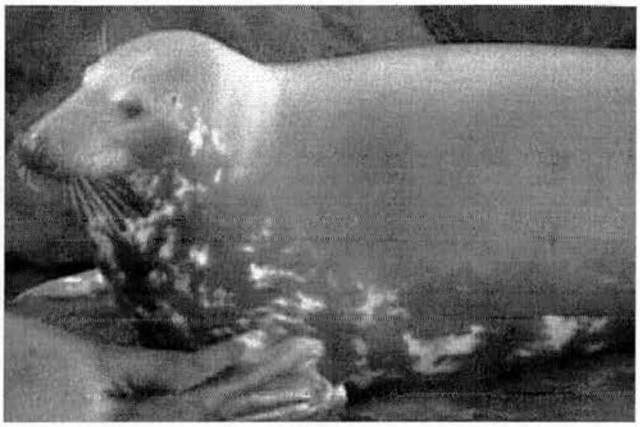

Figure 3.1b. Type 2 pelage pattern

Figure 3.1c(i). Type 3p pelage pattern

Figure 3.1c(ii). Type 3m pelage pattern

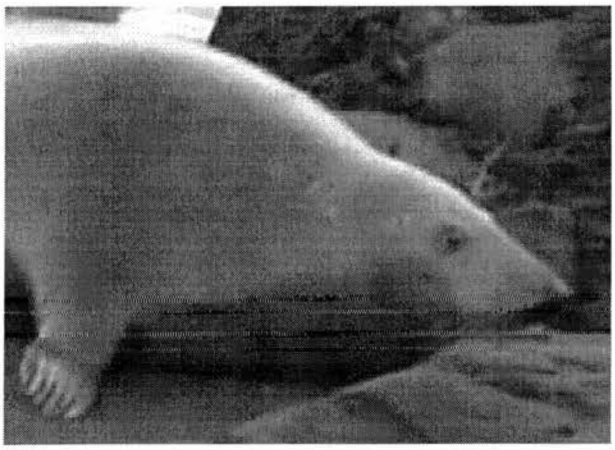

Figure 3.1d (i). Type 4 pelage pattern

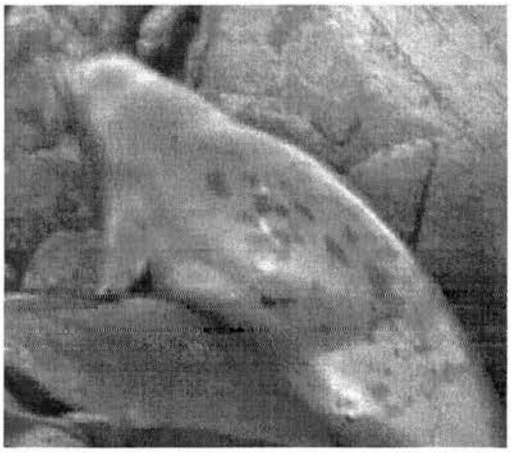

Figure 3.1d (ii). Type 4d pelage pattern

Figure 3.1 a–d. Broad categories of pelage pattern seen on female grey seals.

3.3.2. Assessment of video and photographic images

Video images and photographs were graded according to their clarity, the proportion of markings visible and the distinctiveness of pelage markings in the image (Table 3.1, see also Figure 3.2 a-c). Individual scores for each category were totalled and the overall quality of the image was classified as good if the total = 12-15, fair if the total = 7-11, and poor if the total = 3-6. Although a single judge (PR) assigned scores to all images used in these analyses, five judges tested concurrence of scores assigned to 'clarity' and 'proportion of markings captured' (as thirds of the body) using a sub-sample of 80 female images captured on the Isle of May.

Table 3.1. Table detailing how images were awarded scores for clarity, proportion of markings visible and distinctiveness.

Score	Clarity	Proportion of markings visible	Distinctiveness of pelage markings
5	Sharp image, in focus with good contrast.	At least half of the body markings visible.	Very distinctive pattern, many bold markings that are easily recognised.
4	Sharp image, in focus with good contrast; a little glare or mud may be present.	At least one third of the body markings visible.	Distinctive pattern, bold markings easily recognised.
3	Image slightly blurred or some markings lost through glare or poor contrast.	At least one third of the body markings visible, but body may be twisted making them hard to position accurately.	A few distinctive markings.
2	Blurred image or one that is subject to a lot of glare, poor contrast or markings covered by mud.	At least 10% of body markings visible. Body may be severely twisted.	Poor, indistinct pattern.
1	Poor quality image, out of focus, poor contrast, markings obscured by mud or glare.	Few (if any) markings visible.	Unable to determine distinctiveness due to poor quality of image.

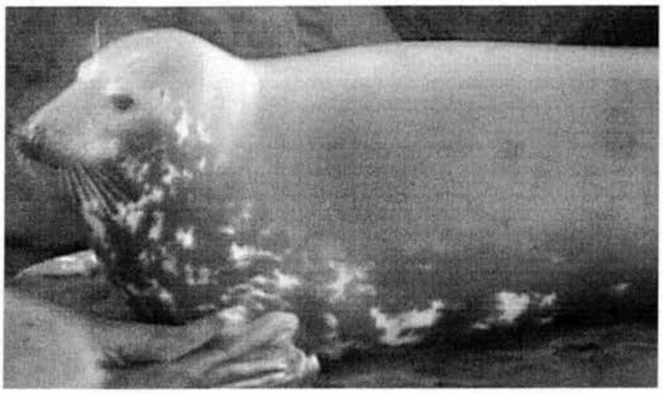

Figure 3.2a.
Clear image with good contrast, in focus (score 5 for clarity), >50% markings (score 5 for proportion of markings visible), bold pattern with some easily recognised markings (score 4 for distinctiveness).

Total score = 14. Classified as good quality image.

Figure 3.2b
Poor contrast with some markings obscured (score 3 for clarity), >10% of markings visible (score 2 for proportion of markings visible), a few distinctive markings (score 3 for distinctiveness).

Total score = 8. Classified as fair quality image.

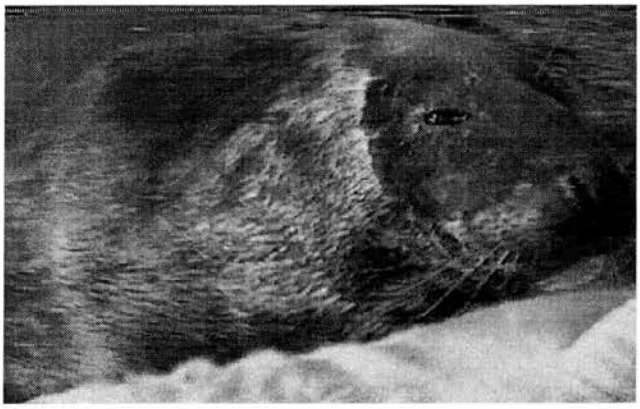

Figure 3.2c.
Poor contrast, markings obscured by mud (score 1 for clarity), no markings clearly visible (score 1 for proportion of markings visible), unable to determine distinctiveness of markings due to poor quality of image (score 1 for distinctiveness).

Total score = 3. Classified as poor quality image.

Figure 3.2 a-c.
Examples of video and photographic images of female grey seals of varying quality.

3.3.3. Assessment of sketches

Assessment of sketches was difficult because of their incomplete nature, but they were rated good, fair or poor based on the proportion of markings captured on the outline sheets.

3.3.4. Affect of female behaviour, location and observer familiarity on image quality and female recognition

One problem with pelage identification is that each side of an animal has different natural markings, and if the two sides are not linked, a single animal may be assigned two identities. This could be a particular problem with grey seals because they spend a lot of time inactive, and it is common for only one side of the body to be visible for much of an observation period. It was anticipated that failure to link the two sides was most likely to occur if there was a consistent difficulty in capturing good quality images of one particular side. To determine whether this occurred, images obtained on North Rona and the Isle of May in 1998 and 1999 were rated for clarity separately for left and right sides - the distribution of scores was then compared for each side.

Unfamiliarity with females may also lead to false negatives, particularly when sketches are used as the main method of identification. This is because different observers may pick out different patterns within the pelage as being distinctive. During each breeding season on North Rona, two observers (PR and PP) drew sketches of animals observed in study groups within the colony. Over a period of time, each observer became familiar with the animals they had drawn during behavioural observations, but was generally unfamiliar with animals that they did not observe regularly. It was anticipated that this would influence the recognition of individual animals in subsequent years independent of image quality, so resights made by PR were analysed with respect to the observer that drew each female (PR or PP). To minimise the effect of sketch quality on the resighting of individual females, only images that were classified as 'good' were used in this analysis.

3.3.5. Data analysis

Kappa tests were carried out as described in Siegel and Castellan (1988) and G tests were carried out as described in Fowler and Cohen (1992); all other statistical analyses were performed using Minitab for windows version 10.5.

3.4. Results

3.4.1. Agreement between judges

Kappa tests of concurrence (performed on a sub-sample of 80 images from the Isle of May) showed that there was significant agreement between all judges for scores assigned for clarity ($z = 8.01$, $p<0.001$), proportion of pelage captured (as thirds of the body) ($z = 11.86$, $p<0.001$) and pelage type ($z = 15.86$, $p<0.001$).

3.4.2. Classification of pelage types

The number of females classified as each pelage type on North Rona and the Isle of May is shown in Table 3.2. This table also shows the percentage that was considered to be easily distinguishable from others of the same pattern type.

Table 3.2. Number of images classified as each pelage type on North Rona and the Isle of May (see also Figures 3.1a to d).

	Type 1	Type 2	Type 3p	Type 3m	Type 4	Type 4d	Total
North Rona							
Number of images	32	16	49	13	1	1	112
Number that were easily distinguished	32 (100%)	16 (100%)	49 (100%)	7 (54%)	0 (0%)	1 (100%)	105 (94%)
Isle of May							
Number of images	35	11	36	19	4	2	107
Number that were easily distinguished	35 (100%)	11 (100%)	36 (100%)	6 (32%)	2 (50%)	2 (100%)	92 (86%)

Type 4/4d pelage images were omitted from statistical analyses due to the low numbers observed, so only types 1, 2, 3p and 3m pelage were compared between the two study sites. A G-test showed that there was no significant difference in the distribution of these pelage types between North Rona and the Isle of May (G = 3.8, d.f. = 3, NS). Images of pelage types 1 (dark on light), 2 (light on dark), 3p (equal proportions – patchy distribution) and 4d (uniform with dark marks) were easily distinguished from one another but type 3(m) pelage images (equal proportions – mottled appearance) were more difficult to differentiate from one another. Type 4 pelage images (uniform mid grey) were only identifiable between years if prominent scars were visible. Overall, it was calculated that around 90% of the 219 images classified were easily distinguishable.

3.4.3. Assessment of video and photographic images

The clarity scores assigned to left and right video and photographic images are shown in Table 3.3. The frequency of each score was significantly different between North Rona and the Isle of May (G test, G = 51.4, d.f. = 4, p <0.01), with more images on the Isle of May being assigned a score of 5 or 1, and more images on North Rona being assigned a score of 4, 3 or 2.

Table 3.3. Clarity scores assigned to left and right images of female pelage obtained from video and photographs (5 = good, 1 = poor)

	score	5	4	3	2	1	total
North Rona							
	left	25 (15%)	36 (22%)	70 (42%)	29 (17%)	7 (4%)	167
	right	12 (8%)	40 (27%)	62 (42%)	29 (20%)	5 (3%)	148
	total	37 (12%)	76 (24%)	132 (42%)	58 (18%)	12 (4%)	315
Isle of May							
	left	33 (49%)	8 (12%)	20 (29%)	4 (6%)	3 (4%)	68
	right	22 (31%)	8 (11%)	18 (25%)	16 (22%)	8 (11%)	72
	total	55 (39%)	16 (11.5%)	38 (27%)	20 (14.5%)	11 (8%)	140

When the left and right images from each site were compared, there was no significant difference in the allocation of each score for animals on North Rona (G test, G = 4.55, d.f. = 4, NS). However, there was a significant difference in the scores assigned to left and right sides at Tarbet on the Isle of May (G test, G = 12.27, d.f. = 4, $p < 0.05$). At this site, more left images were given the highest score (5) than right images, and fewer left images were rated poorly (i.e. given a score of 1 or 2); roughly equal numbers were assigned scores of 3 or 4.

3.4.4. Assessment of sketches

Table 3.4 shows the number of sketches drawn at the two study sites (1998 and 1999 combined) and the number classified as good, fair or poor.

Table 3.4. Table showing the scores allocated to left and right sides of sketches drawn on North Rona and the Isle of May.

	Number of sketches	sketches rated good	sketches rated fair	sketches rated poor
North Rona (n = 206 females, 116 females (56%) had both sides sketched)				
left	167	78 (47%)	65 (39%)	24 (14%)
right	155	70 (45%)	66 (43%)	19 (12%)
Isle of May (n = 81 females, 43 females (53%) had both sides sketched)				
left	62	39 (63%)	17 (27%)	6 (10%)
right	62	28 (45%)	21 (34%)	13 (21%)

The overall proportion of sketches rated good, fair and poor was not significantly different between North Rona and the Isle of May (G test, 2df, G = 5.38, NS), nor was there a significant difference in the scores assigned to left and right sides at each site (G test, G = 1.11, 2df, NS for North Rona; G = 4.87, 2df, NS for Isle of May). Resightings of females with good sketches on North Rona were also assessed in relation to the observer that drew them (Table 3.5). PR made all the resightings in the field from one year to the next, therefore females sketched by PR were considered familiar, and females sketched by PP were considered unfamiliar. It was found that significantly more resightings were made of familiar females than unfamiliar ones (G test, G = 8.45, 1df, $p < 0.01$ for females sketched in 1998; G = 8.43, 1df, $p < 0.01$ for females sketched in 1999).

Table 3.5. Number of females sketched by two observers on North Rona and their subsequent resightings. Resightings of females sketched in 1998 occurred in 1999 and 2000 (each resighting is only represented once here). Resightings of females sketched in 1999 were only possible in 2000. All resightings were made by PR, hence sketches made by PR were familiar and sketches made by PP were unfamiliar.

Year sketched	Observer	Number of good sketches	Number of females resighted from good sketches	Percentage resighted
1998	PR	22	20	91%
	PP	21	11	52%
1999	PR	39	27	69%
	PP	27	9	33%

3.4.5. Affect of image quality on subsequent recognition of females

The percentage of females recognised in subsequent years decreased when females with poorer quality images were included in the dataset (Table 3.6). There appeared to be a substantial decrease in the number of females resighted when images with a score of 7-11 were added to the dataset. Therefore chi-squared tests (with Yates correction applied) were used to compare the number of females resighted/not resighted if only good quality images (score 12-15) were included in the dataset, with the number of females resighted/not resighted if poorer images were included in the dataset (score 7-15). Data from 1998 and 1999 were pooled for each island. There was a significant difference in the numbers of females resighted/not resighted on North Rona when images with a score of 7-11 were added ($\chi^2 = 30.71$, d.f. = 1, $p<0.005$), but not on the Isle of May ($\chi^2 = 2.94$, d.f. = 1, NS).

Table 3.6. Number of females captured (photographically or on video) and resighted in the field in relation to the best quality image of each female. Resightings from images captured in 1998 were made in 1999 and 2000; resightings from images captured in 1999 were made in 2000 only.

Score of best quality image	12-15 (good)	7-11 (fair)	3-6 (poor)
North Rona 1998			
Captured	30	35	12
Resighted	30	6	0
	(100%)	(17.1%)	
North Rona 1999			
Captured	44	43	15
Resighted	37	6	0
	(84.1%)	(14%)	
Isle of May 1998			
Captured	9	9	7
Resighted	5	0	0
	(55.6%)		
Isle of May 1999			
Captured	34	15	5
Resighted	17	0	0
	(50%)		

3.5. Discussion

3.5.1. Pelage pattern classification

There was no significant difference in the proportion of each pelage pattern type present at the study sites on North Rona and the Isle of May, which indicates that the proportion of each pelage type seen here probably reflects the pattern variability within the species. These patterns types should provide a useful method of sub-dividing pelage images of female grey seals that will aid pelage identification in future studies.

3.5.2. Image quality – Sketches versus video and photographic images

Although sketches have been used to help identify individuals (e.g. Bewick's swans, Rees, 1981; ospreys, Bretagnolle *et al.,* 1994), photographs are the preferred method for pelage identification in most, if not all, species (e.g. giraffes, Foster, 1966; zebra, Briand-Petersen, 1972; cetaceans, IWC, 1990; tigers, Karanth & Nichols, 1998). Undoubtedly, this is because photographs are a more accurate, and often easier, method of capturing the pelage pattern. In addition, sketches may be considered less useful for individual recognition if there are different observers within and between years. The results presented here suggest that observer familiarity aids identification (also observed by Sheldon & Bradley, 1989) – this is likely to occur because different observers perceive different patterns as being distinctive.

Many sketches were drawn for the work presented here, however, around 20% of them were judged to be of poor quality. There were many reasons for this, including dry pelage (which made the markings harder to see and hence draw), the female being covered in mud, or insufficient time or opportunity to obtain a good sketch (e.g. the female was lying on her back for the entire study period). Despite these problems, sketches were a necessity for this work, as they were the only means of identifying new study females from day to day - video and photographic images could not be processed in the field and were therefore unavailable for identification purposes in the first field season that a female was subject to behavioural observations. Furthermore, sketches were sometimes used to identify animals on North Rona between years because photographs and video images could not be captured from the hide and bad weather conditions prevented image capture whilst working in the colony on several occasions. The same problems were not experienced on the Isle of May, because video images could be captured from the hide in bad weather.

3.5.3. The affect of topography, location of hide and female behaviour on the clarity of captured images

The significant difference in the clarity of left and right images captured at Tarbet on the Isle of May, showed that many of the left images were better than the right images at this site. This can be explained by the topography of the area and the affect that this had on female locomotion between the pup and the tidal inlet/pool. There is little low-lying land at Tarbet and many females gave birth on a plateau to the north of the inlet which could be accessed by a long gradual slope or several short, steep slopes (Twiss *et al.* 2000a). In general, females chose the long slope (situated to the left of the study site as observed from the hide) over the steep slopes (in the middle and to the right of the study site as observed from the hide) when travelling from the pool to their pup. To get to the longer, less steep slope, females moved along a narrow shelf of rock at the waters edge that presented their left side to the hide, giving a perfect opportunity to capture the pelage on video or as a sketch. In addition, the pelage of females leaving the pool was clean, and this allowed good quality images to be captured. On their return to the pool, however, females chose either route – the reverse journey down the slope and along the narrow shelf, which presented the right-hand side for capture (although this time the female was usually covered in mud), or down the steeper rock face, which allowed only an angled view of the pelage or a view of the back to be captured. The return journey to the pool also provided less time to obtain a good image as females generally moved much quicker downhill than they did uphill.

A comparison of all images between the two study sites also showed that more females on the Isle of May had images classed as good than females on North Rona. The most likely reason for this is that the hide was much closer to the females on the Isle of May (around 10-40 metres away), making them easier to draw and allowing video images to be captured whenever the opportunity arose. Sketches on North Rona were made (using binoculars) from a distance of approximately 200 metres, and video and film images could only be collected whilst amongst the animals in the colony. Under these circumstances, there was often a short time-span in which to capture video/photographic images and images were taken irrespective of whether the female was wet or dry, clean or dirty. However, as both sides were captured within a few minutes of one another, the clarity of the left and right images of an individual female were generally similar, hence the reason that there was no significant difference in the clarity ratings of left and right images on North Rona.

3.5.4. Using quality scores to determine females that should be identifiable in the field

Cetacean studies that use natural markings for identification in mark/recapture experiments generally conclude that only the best quality images should be used (e.g. Whitehead *et al*., 1997; Wilson *et al.,* 1999; Friday *et al*., 2000) but that animals should not be selected on the basis of distinctiveness (Hammond, 1986). However, the overall aim of the work presented here was to see whether natural markings could be used in long-term studies of grey seals during the breeding season. In these circumstances it is an advantage to have distinctive markings that increase the likelihood that a female will be recognised, hence the reason why the overall quality scores used here included a score for distinctiveness.

The results show that adding poorer quality images to the dataset may increase the number of false negatives (females recorded as absent even when they are present) because fewer females with fair or poor quality images were resighted in a subsequent year. Although animals with good quality images were easier to recognise, the proportion of animals resighted between years was influenced by various factors at the two study sites. This was demonstrated by the fact that, although a greater proportion of the females at Tarbet had good quality images, a higher percentage of females were recognised in subsequent years on North Rona. The position of the hides and the large difference in the area of the island that could be observed from these hides were major factors influencing this. At Tarbet, females could easily move to an area that was not visible from the hide due to the rocky topography. The hide on North Rona, however, was situated on a high ridge that overlooked most of the study area, which allowed females to be resighted even if they moved 100m or more from their previous pupping location. It is also possible that the left and right sides of females at Tarbet were less likely to be linked because of their movement to and from the pool. Females that used Tarbet as an access route between the sea and a pupping site further inland were only seen for short periods of time as they travelled between their pup and the pool, making it difficult to photograph or video both sides of their body. On North Rona, females remained close to their pups and showed little movement within or between days allowing a long period of time in which body sides of the body could be photographed or identified.

3.6. Conclusions

The work presented here provides several useful guidelines for future work that uses pelage patterns to identify female grey seals on the breeding colony. These are summarised below:

1) Subdividing females by their pelage pattern type can be considered a useful means of assisting field identification where access to computer-aided matching techniques is not available.
2) Both image quality and female distinctiveness should be used when choosing which animals should be used in long-term studies of grey seal breeding behaviour. It is better to use markings from the whole body (rather than just the head and neck as in computer-aided pelage matching techniques (Hiby & Lovell, 1990)) when using pelage identification in the field as these provide a more comprehensive opportunity for identification.
3) Video and photographic images capture the pelage markings of an animal independent of individual perception whereas sketches are affected by what the observer considers to be distinctive within the pattern. However, the usefulness of video and photographic images is largely determined by their clarity. Sketches were extremely useful for identifying individuals within a season, but should only be used for resighting animals between years by observers that are familiar with the individuals in question (unless video or photographic images are available as a back-up check).
4) The topography and behaviour of females can affect the quality of captured images. This needs to be considered when calculating the number of animals resighted at a particular location.

Chapter Four:

A comparison of female matches using pelage identification and microsatellite analysis.

Images of female grey seals and skin samples were collected by P. Redman, P.P. Pomeroy, S.D. Twiss and S.E. Moss.

Video images were digitally captured by P. Redman and R. Harris.

4.1. Summary

Microsatellite analysis and pelage identification were used in a double-marking experiment to detect matches between females sampled (genetically and through capture of pelage markings) on North Rona in two consecutive years. A total of 14 matches were initially detected between the two years by independent assessment of the two methods. Eight of these were detected by microsatellite analysis (where identity was assigned to pairs that had a relatedness (R) value of 1) and 13 were detected visually from natural and artificial markings.

A comparison of the matches obtained by each method identified a number of errors within the relatedness matrix that was derived from skin sample genotypes. These errors were corrected and a new relatedness matrix was produced in which all visually matched females had R values >0.87. Less than 1% of pairs visually classed as 'non-matches' had R values >0.5, and none had R values >0.75 (n = 6109 pair-wise comparisons). Therefore the relatedness value to which identity should be assigned was re-appraised and individuals were classed as the same if they were assigned an R value of 0.8 or higher. Using this value for identity, 17 matches were detected by microsatellite analysis using the new matrix; 14 of these were confirmed by comparing images of natural markings or brands between years.

4.2. Introduction

There are two types of error that may occur when resighting individual animals on an annual, or even daily, basis. These are 'false positives', where different animals are mistakenly identified as being the same, and 'false negatives', where images of the same individual are not matched. For studies involving population estimates, both types of error have important consequences, as false positives reduce the population estimate and false negatives increase it. However, the work presented in this thesis was concerned with the behaviour of individual females rather than the colony as a whole. False positives were therefore of greater concern than false negatives because incorrectly assigning the same identity to two separate individuals would result in different animals being compared between years. This, in turn, would lead to inaccuracies in the results obtained (e.g. time ashore and pupping date). Failure to match images of the same individual would only reduce the sample size for inter-year comparisons.

It has already been discussed in chapter three how individuals of many different species can be distinguished by their pelage patterns and scars, and how the error rate for resighting animals using natural markings is generally reported to be low provided that good quality images are used. To my knowledge, however, only one published paper (Stevick *et al.*, 2001) has used microsatellite loci scores from genetic samples in a double-marking experiment with pelage identification. This chapter presents a small-scale double-marking experiment to compare the ability of microsatellite analysis and pelage identification to match the identities of female grey seals that were sampled on North Rona in 1998 and 1999. This chapter was written using results from a collaborative study with Dr. Bill Amos at the University of Cambridge. Dr. Amos and his research team conducted all the microsatellite analyses for skin samples collected on North Rona in 1998 and 1999, and provided the combined relatedness matrices that were used for this work.

4.3. Methodology

Skin samples and pelage images were collected on North Rona from 124 females in 1998, and 62 females in 1999, as described in chapter two. Individual identification numbers (allocated in the field) were used to cross-link skin samples with their pelage images to allow a comparison between genetic matches detected by microsatellite analysis and image matches detected by eye. The identification numbers gave no prior indication of possible matches between years to ensure that genetic matches and matches using natural or artificial markings were carried out blind.

Skin samples were genotyped for up to nine polymorphic microsatellites (Allen *et al.*, 1995) by Dr. Amos and his research group at the University of Cambridge as described in Worthington-Wilmer *et al.* (1999). A relatedness matrix, combining the samples from the two years, was calculated as described by Queller and Goodnight (1989) using the program Kinship (Goodnight software, http://bioc.rice.edu/Keck2.0/ labs/). Females that were genotyped for seven or more microsatellites were included in the combined relatedness matrix, resulting in a comparison of 120 females that were sampled in 1998 with 55 females that were sampled in 1999. The combination matrix assigned identity (i.e. samples being from the same individual) where the relatedness (R) value of a pair of samples from different years was 1.

Pelage images were obtained for 92 of the sampled females in 1998 (an additional 24 females were identifiable through individual brands) and 42 of the sampled females in 1999 (an additional 8 females were identifiable through individual brands). Pelage images and brands were compared by eye (PR) to detect females that had been sampled in both years.

4.4. Results

Prior to receiving the combined 1998/1999 relatedness matrix, eight skin sampled females were identified from pelage markings as being sampled in both 1998 and 1999 (see Figure 4.1 for examples). A further five females that were identifiable by brands were also skin sampled in both years. This gave a total of 13 females matched by eye from natural and artificial markings, which could be compared to the R values obtained from comparisons of skin sample genotypes (Table 4.1). The relatedness matrix returned an R value of 1 (identity) for 7 of the 13 females matched by eye or by brands, plus an additional female that had not been detected by eye (Table 4.1, column 3). Examination of the images for this female indicated that a match was not made because of the poor quality of the image captured in 1999, in which the markings were blurred.

The six females whose natural markings or brands were matched between years but who had R values <1 were reported to the research group at Cambridge. A number of unspecified errors were detected and a new matrix was produced that assigned identity to 10 of the 13 females whose natural markings or brands had been matched between years. The three remaining females all had R values >0.9 (Table 4.1, column 4). Identity was assigned to a further pair of samples (after the matrix had been adjusted) with an addition two pairs of samples returning high R values of 0.87 and 0.93. One of the latter pairs was confirmed as being the same female when the pelage images were checked, but the other two could not be confirmed due to poor or missing images.

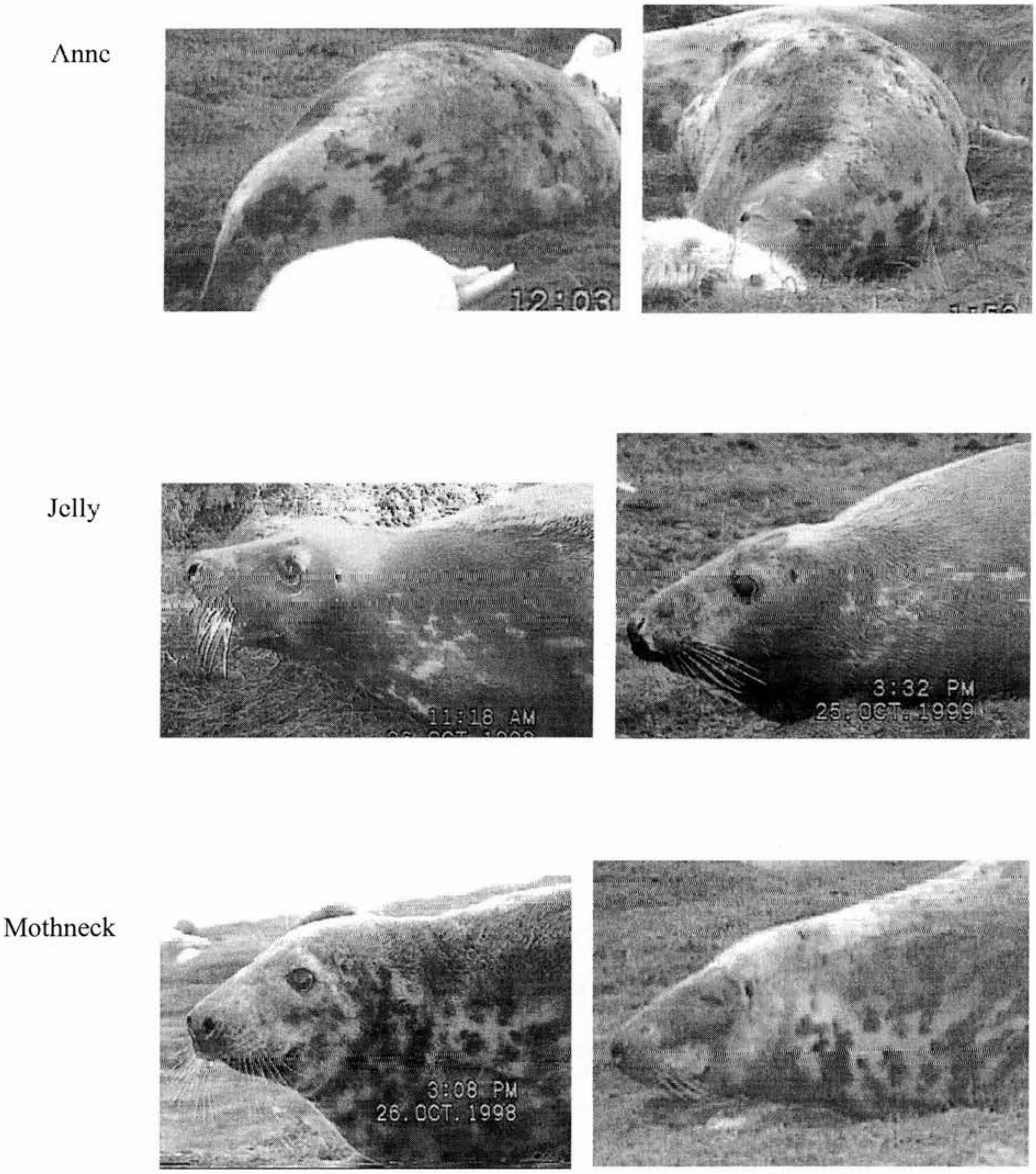

Figure 4.1. Examples of females (skin sampled in both 1998 and 1999) that were matched visually using pelage markings.

Table 4.1. Comparison of matches made by eye (using pelage markings and brands) with matches made using the 1998/1999 combined relatedness matrix. Matrix adjustment occurred after matches made using pelage images drew attention to errors in gel band scoring.

Female ID	Matched by eye	Relatedness (R) value (1998/1999 combined matrix) – initial matrix	R value after matrix adjustment for errors in scoring
7A	Yes – branded	1	1
TA	Yes – branded	0.913	0.913
TD	Yes – branded	1	1
TH	Yes - branded	1	1
TL	Yes - branded	0.863	0.927
H right (cohort brand)	Yes	0.774	1
Anne	Yes	1	1
Female 11	Yes	0.846	1
Fem V-BH	Yes	1	1
Jelly	Yes	1	1
Mothneck	Yes	0.912	0.912
Rose	Yes	No value present	1
Spotty 1	Yes	1	1
Fem 55-7	No – poor image	1	1
Fem 82-35	Not initially – matched after reassessment	No value present	0.87
Fem 83-21	No – poor image	0.93	0.93
Fem 84-12	No – missing image	No value present	1

Using the new matrix, the mean R value of females matched by eye was 0.97 (st. dev. = 0.0459, n = 14, Figure 4.2). The mean R value of pairs of images that were classed as 'non-matches' was –0.007 (st. dev. = 0.196, n = 6109 pair-wise comparisons, Figure 4.2). The R values of pelage 'matches' and 'non-matches' were significantly different (T = 78.38, $p < 0.001$, df = 14), with less than 1% of 'non-matches' having R values greater than 0.5 and none having R values greater than

0.75. Therefore, paired samples that were given an R value greater than 0.8 were considered to be the same individual. Using this value to assign identity, microsatellite analysis detected 17 matched pairs (after correction of gel scores); 14 of these were confirmed by matching pelage images between years. Two pairs matched by microsatellite analysis had poor images (rated 5 using the quality scores from chapter three) and therefore could not be matched by eye; a further pair matched by microsatellite analysis only had a pelage image available in one year. Assuming that no false positives were recorded using microsatellite analysis, this means that 13/16 matches using images of pelage patterns or brands were successfully detected by eye at the first attempt (14/16 overall), and 8/17 matches were successfully detected by microsatellite analysis at the first attempt (when identity was given for pairs where R = 1).

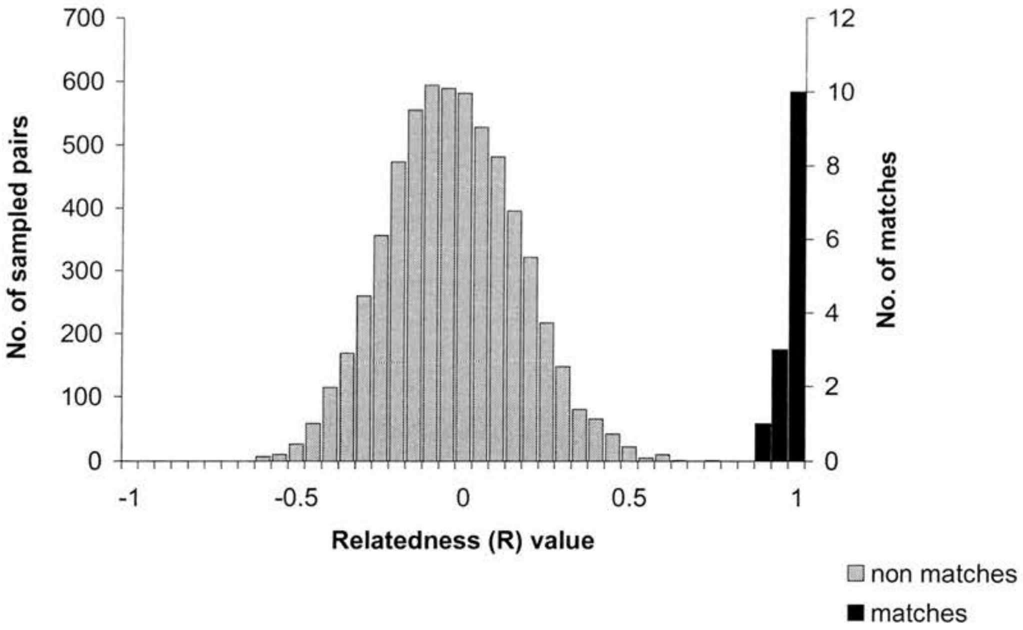

Figure 4.2. Relatedness values between samples taken in two years for 'matches' (identified as the same individual using pelage ID and brands) and 'non matches' (sampled pairs classed as different individuals using pelage ID). The data presented here use the R values from the corrected matrix; false negatives (i.e. matches detected using microsatellites that were not detected by eye) are not included in this data set.

4.5. Discussion

The relatedness values of visually matched pairs showed no overlap with the relatedness values of non-matched images. It was therefore considered reasonable to assume that no false positive matches were scored using this method. Poor quality images resulted in three false negatives (assuming that the matches made by microsatellite analysis were correct), however, given previous knowledge on how image quality affects pelage identification (Whitehead *et al.*, 1997; Wilson *et al.*, 1999; Friday *et al.*, 2000; see also chapter three), these were not unexpected and would reflect a cautious approach to assigning matches.

Conversely, the large number of false negatives for matches made using microsatellites (initially, 9 out of 17 matches were missed) was cause for concern. Prior to the comparison of the results obtained here, it was assumed that microsatellite analysis would be more accurate than pelage identification at detecting matches between years, and therefore provide a test of the reliability of the latter. The results, however, indicated that the method of microsatellite analysis used by the research group at Cambridge was prone to errors in band scoring (6 out of 17 values changed between the initial matrix and the revised matrix) and that the values used to assign identity may need to be revised. In many cases, the errors in band scoring could have been detected by double-checking sample pairs that had a relatedness value greater than 0.5. This would provide an easy check for future work, as less than 1% of non-matched pairs had R values greater than 0.5. Lowering the value for identity to 0.8 would also ensure that all matches made by eye were detected genetically and would cause no overlap with the relatedness values of non-matched pairs.

One reason that false negatives may have been common for matches made by microsatellite analysis is that females were included in the matrix if they were successfully scored for seven out of nine microsatellites. It is possible that two samples that were each scored for seven microsatellites may actually have had only 5 microsatellites in common when compared between years. This may account for matches that returned a relatedness value of less than 1. However, it was not possible to check this, as Dr. Amos only provided completed relatedness matrices that did not indicate which individual microsatellites were successfully scored for each animal.

These results suggest that pelage identification was as good as, if not better than, microsatellite analysis for identifying female grey seals between years.

Although pelage identification cannot replace microsatellite analysis for studies such as determining paternity of pups, looking at mate fidelity or determining the degree of relatedness between individuals, it can be used to identify animals that have been sampled before. Depending on the data that is being collected, these females can then be targeted in subsequent years for long-term studies, or avoided to prevent duplication of samples.

Chapter Five:

Grey seal female breeding patterns and site fidelity

Although this chapter concentrates on data collected from North Rona, similar data was also collected on the Isle of May. The latter was more limited due to reduced observation time at each study area and is therefore included only as an appendix (Appendix 5.1)

P. Redman, P.P. Pomeroy and R. Harcourt carried out the behavioural observations that are included in this chapter.

5.1. Summary

Previous studies of grey seal breeding behaviour on North Rona have been obtained from branded and tagged females undergoing long-term studies of maternal investment. However, repeated capture and handling of these females means that results obtained from these studies might not be representative of the colony as a whole. The work presented here focused on females identified by natural markings, which had not been disturbed by frequent capture, and therefore provided a comparison with previous studies at this colony.

In total, 67/74 females with good quality pelage images were resighted. The median pupping site fidelity of females that were observed with a pup in two consecutive years was 32m ($n = 51$). This was significantly less than the pupping site fidelity obtained in previous and current studies at this colony using females involved in long-term studies (median distances = 55m, $n = 103$ (Pomeroy *et al.*, 1994), and 44m, $n = 34$ (Pomeroy *et al.*, unpublished) for previous and current studies respectively). On average, females identified by natural markings gave birth on the same date in consecutive years and suckled their pups for 18 days. These behaviours were not significantly different to those recorded for females involved in long term studies over the same time period (parturition date: $T = 0.28$, $p= 0.781$, $n_1 = 51$, $n_2 = 44$, duration of lactation: $T = 0.26$, $p = 0.793$, $n_1 = 27$, $n_2 = 42$, two sample T-tests). Branded females were first seen ashore (prior to giving birth) significantly earlier than females identified by natural markings (Mann-Whitney, $W = 2110.5$, $p < 0.001$, medians = 4 days and 2 days before parturition respectively, $n_1 = 50$ and $n_2 = 54$). This difference might be explained by the fact that brands focus attention and are easier to identify than pelage markings.

5.2. Introduction

Behavioural studies of lactating grey seals on North Rona have, up to now, relied on observations of branded or tagged animals to provide information on aspects of grey seal breeding biology such as pupping site fidelity, pupping date and duration of lactation (e.g. Pomeroy *et al.,* 1994; 1999). However, the animals involved in these studies have also been captured and handled in successive years for long-term studies of female reproductive success (P. Pomeroy, pers comm.). Furthermore, many females captured and branded at this site were peripherally located animals that were chosen because they were easy to capture and handle (M. Fedak, pers. comm.). As a result of these procedures and the disturbance involved each year, the researchers involved in these studies have always been concerned that measurements of pupping site fidelity, pupping date and duration of lactation obtained from branded animals on North Rona might not truly represent the population as a whole. Therefore, the work presented here investigated female breeding behaviour using females identified by their pelage markings, which had not experienced the same degree of disturbance as those involved in long-term studies.

5.2.1. Aims

The aims of this study were to record pupping site fidelity, pupping date, time spent ashore before parturition and the duration of lactation for females identified by natural markings on North Rona, and to compare these results to those previously recorded for branded and tagged animals at the same colony.

5.3. Methodology

Throughout this chapter, ‘resighting’ of an individual refers to recognition of a known female in the field using previously captured images (photograph, sketch or video). This term is used for both within and between year recognition of females. However, where the overall return rate is reported, it must be remembered that resightings of females first identified in 1998 were possible in two years (1999 and 2000) whereas resightings of females first identified in 1999 were only possible in 2000. In addition, for datasets pertaining to pupping date, pupping location and time ashore prior to parturition, females were included only if their parturition date was known to an accuracy of within one day. This meant that only females that were observed giving birth or were seen with a stage I pup were included in these datasets (pups generally being recorded as a stage I pup for the first two days after birth (Kovacs & Lavigne, 1986)). Females that were first observed when their pup was a stage II or older were not included in these datasets, although they were counted as resightings for calculations of return rate.

5.3.1. Resightings of known females

In chapter three, it was discussed how using poor quality images to identify females between years was likely to result in false negatives (i.e. recording a female as absent when in fact she was present), and lead to an underestimate of the proportion of females returning to the colony each year. This means that to provide the most accurate estimate for site fidelity and return rate of female grey seals, only individuals with good quality images and distinctive markings should be included in the 'known female' datasets. Therefore, only females that had good quality images were included in these analyses.

Resightings were made in the field during daily scans of the study area and opportunistically throughout the observation period. The daily scans were performed during the first hour of each day (around 0900-1000hrs BST) from a hide on a high ridge overlooking the study area (see chapter two for details). PR compared females observed in the field with pelage images of known females from previous years; the first resighting of each female was verified by a second observer (P. Pomeroy or S. Twiss).

5.3.2. Recording female locations

Female positions were recorded using digitised, geo-rectified maps as described in chapter two; the age of each female's pup (if she had one and it could be seen) was also noted. Females seen coming ashore were observed to ascertain if they had been identified before, and the route taken by known females through the colony was monitored to determine their location at the end of the day. When known females were observed giving birth, the location and time were also recorded.

5.3.3. Site fidelity, pupping date and time ashore

Pupping site locations were recorded accurately for all known females that were observed giving birth or were seen with a stage I pup (stage I pups did not generally move very far from the birth site, personal observation). The x,y co-ordinates of pupping locations were used to calculate the Euclidean distance between pupping sites in consecutive years. These were then compared to a null distribution of distances between actual pupping sites on the colony in consecutive years (using a randomised sample of 1000 values to give a conservative test) to see if females were more site-faithful than expected by chance. The null distribution was computed from stage I pup locations marked on daily maps over a period of four years (1997 to 2000) by P. Pomeroy and S. Twiss. The difference between pupping dates in consecutive years was also calculated for each known female that was observed giving birth or with a stage I pup in two years. Daily maps and observations of pregnant females coming ashore provided information on the minimum time spent ashore prior to parturition and allowed the total duration of lactation to be calculated. The distance travelled from the location where a female was first seen ashore to where she later gave birth was also calculated using these data.

5.3.4. Summer resightings

In addition to the three main fieldtrips to North Rona, two trips were made to this island outside of the breeding season in 1999 and 2001. Video and photographs were taken of seals that were hauled out on rocks around the island and in the water close to the island (within photographic range) on two days in May 1999 (by R. Harris) and on three days in June 2001 (by P. Pomeroy). As the animals spent most of their time in the water, the majority of the photographs and video images consisted of head and neck shots only. Images of hauled-out females were also obtained, but the angle of the body in the frame resulted in most of these being discarded due to poor quality.

Photographic and video images were captured and processed as described in chapter two and examined for duplicates, with only the best image of each side of a female being kept. Images identified as being of adult males were removed from the dataset, but it is possible that some of the images included were sub-adult males, as it was very difficult to distinguish between females and juvenile males when the animals were in the water. All images were assessed for clarity as described in chapter three and those with a poor score (i.e. 4 or 5) were excluded from the dataset.

All data were tested for normality and then analysed with appropriate parametric or non-parametric tests using Minitab for Windows, version 13. The first sections of the results deal with observations from this study only; observations from this study are compared with studies of branded animals in the final section (5.4.6).

5.4. Results

5.4.1. Return rates in consecutive years

Table 5.1 shows the number of females (with captured images rated good) that were resighted in subsequent breeding seasons. This table shows that all females whose images were captured in 1998 and classified as good were identified again, but that not all of them were seen every year. Three females were seen with a pup on North Rona in 1998 and 2000 but not in 1999, and it was assumed that they skipped a year (although they may have pupped elsewhere and been missed in sighting surveys).

Table 5.1. Number of females, with good images, that were resighted on North Rona in subsequent years. Resightings for females identified in 1998 occurred over two years (1999 and 2000) whereas resightings for females identified in 1999 occurred in 2000 only.

Year first identified	Number of females with good images	Number resighted (1999)	Number resighted (2000)	Total number resighted
1998	30	27 (90%)	28 (93%)	30 (100%)
1999	44		37 (84%)	37 (84%)

The median distance travelled in consecutive years by resighted females was 33m (range 0 - 565m, n = 89 although some females were present in both years so this represents 67 females, Figure 5.1)

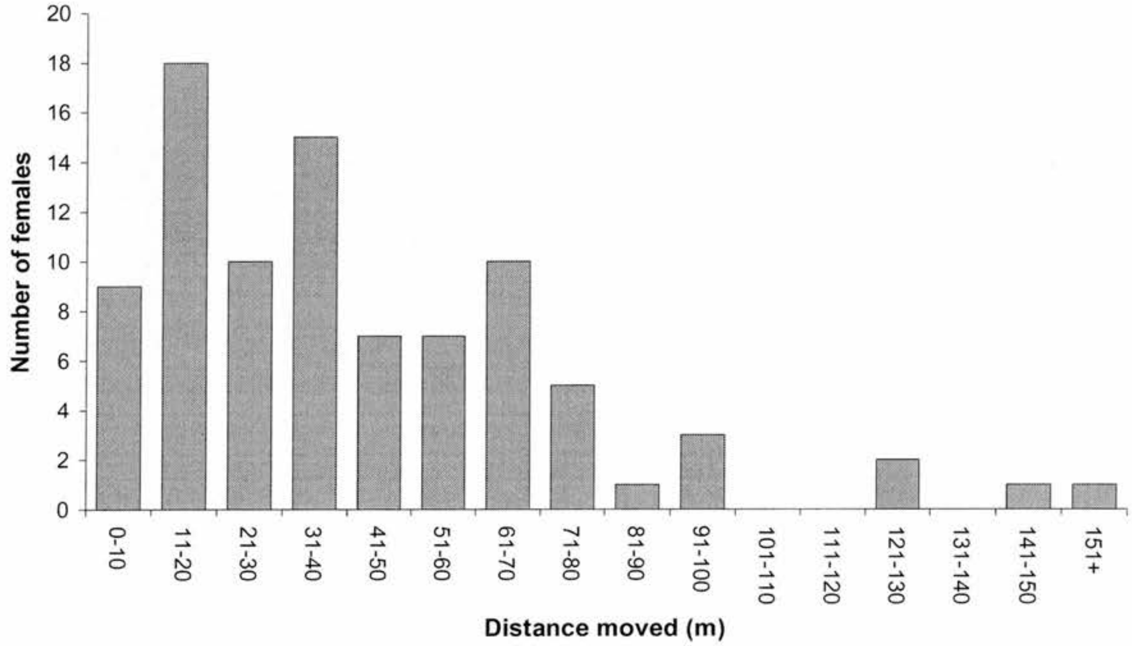

Figure 5.1. Distance moved in consecutive years for all females resighted from good quality images on North Rona.

5.4.2. Pupping site fidelity and pupping date of pelage identified females in consecutive years

Pupping sites were recorded for 15 females that were present in both 1998 and 1999, and 36 females that were present in both 1999 and 2000. There was no significant difference in the median site fidelity between years ($W = 425.5$, $p = 0.47$, NS, Mann-Whitney, median distance travelled = 31m and 32m in 1999 and 2000 respectively, range = 9m to 81m in 1999 and 0m to 140m in 2000 (Figure 5.2)). Although there is a mode at 11-20m in the 2000 data, the distribution of site fidelity in this year was not significantly different from the distribution seen in 1999 ($D_{max} = 0.28$, $n_1 = 15$, $n_2 = 36$, $p>0.05$, NS, Kolmogorov-Smirnov two sample test). The median distance between consecutive pupping sites was smaller than would be expected if seals were randomly assigned using actual pupping sites ($W = 7213$, $p < 0.001$, Mann-Whitney, observed site fidelity = 32m, Q1 = 15m, Q3 = 58m, n = 51;

expected site fidelity = 102m, Q1 = 62m, Q3 = 156m, n = 1000). The distribution of these two samples also differed significantly ($D_{max} = 0.603$, $p<0.001$, Kolmogorov-Smirnov two sample test).

The mean difference between pupping dates in consecutive years was not significantly different between breeding seasons ($T = 0.26$, $p = 0.801$, $n_1 = 15$, $n_2 = 36$, two sample T-test) with females giving birth on or around the same day each year (Figure 5.3).

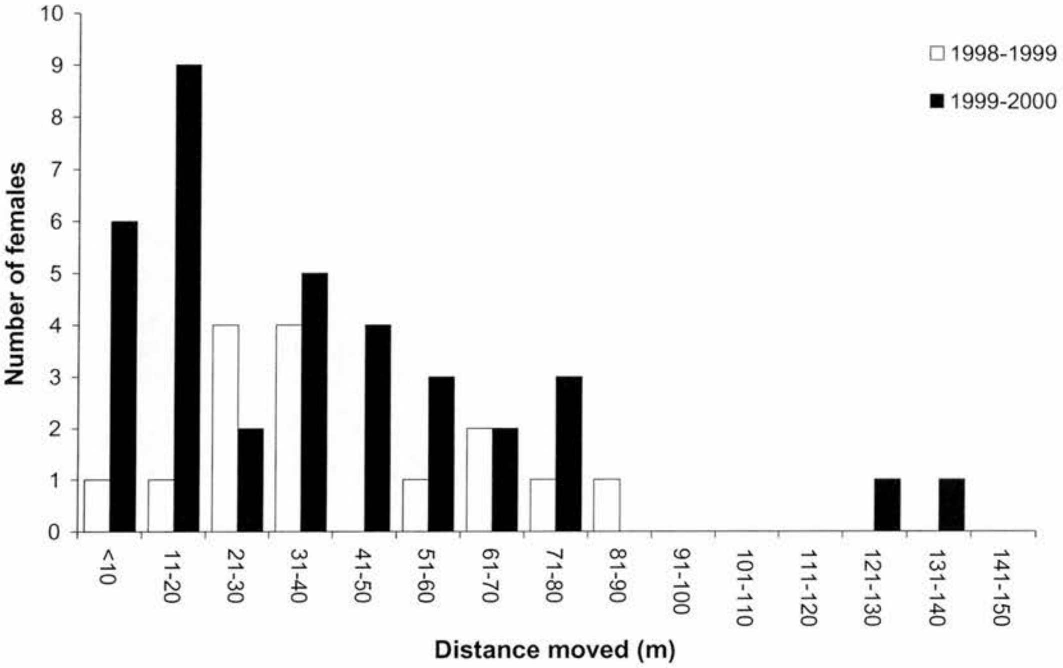

Figure 5.2. Distance moved between pupping sites for females seen in consecutive years on North Rona.

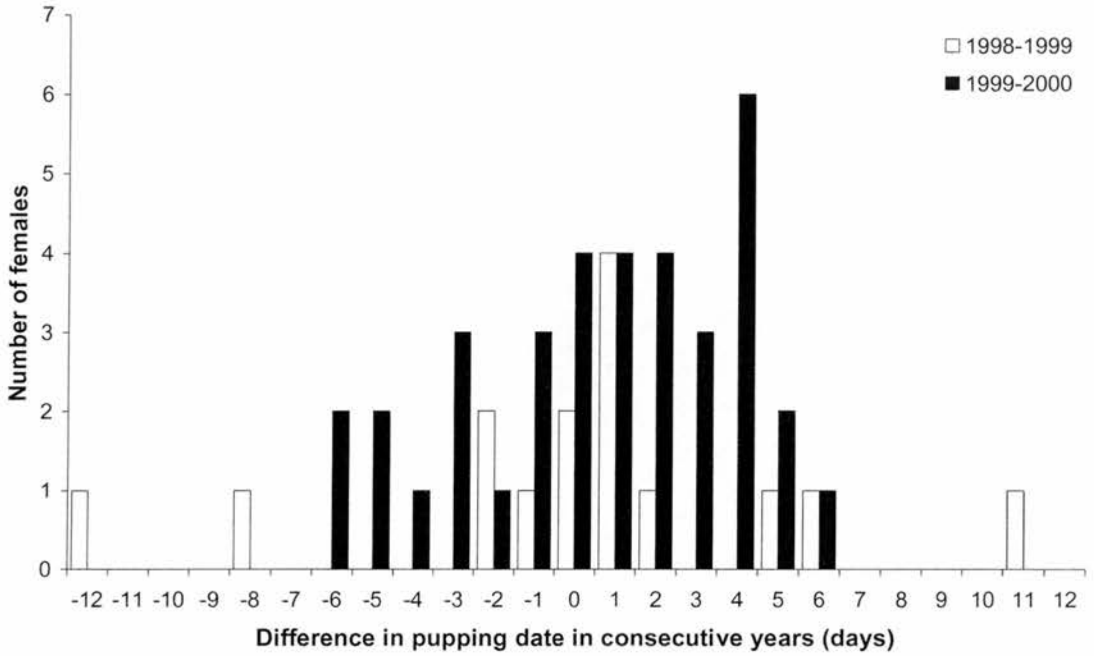

Figure 5.3. Difference in pupping dates in consecutive years on North Rona.

5.4.3. Time ashore prior to pupping

In 1999, three 'known' females (i.e. recognised from pelage markings) were first seen ashore on the day that they gave birth and 8 known females were first seen ashore $\geq$ 1 day before parturition. The latter were a median distance of 23m from their subsequent pupping site (range = 4m-101m, Figure 5.4). In the following year, 10 females were first seen ashore on the day that they gave birth and 29 females were seen ashore $\geq$ 1 day before parturition – the latter were a median distance of 31m from their subsequent pupping site (range = 0m-118m, Figure 5.4). These distances were not significantly different between years (Mann-Whitney, W = 120.5, n_1 = 7, n_2 = 26, p = 0.96, NS), so data were pooled to give a median distance of 30m (range = 0m-118m). The median time ashore before pupping was 2 days in both years – these results were not significantly different in the two years (Mann-Whitney, W = 271, n_1 = 11, n_2 = 39, p = 0.83, NS) (Figure 5.5).

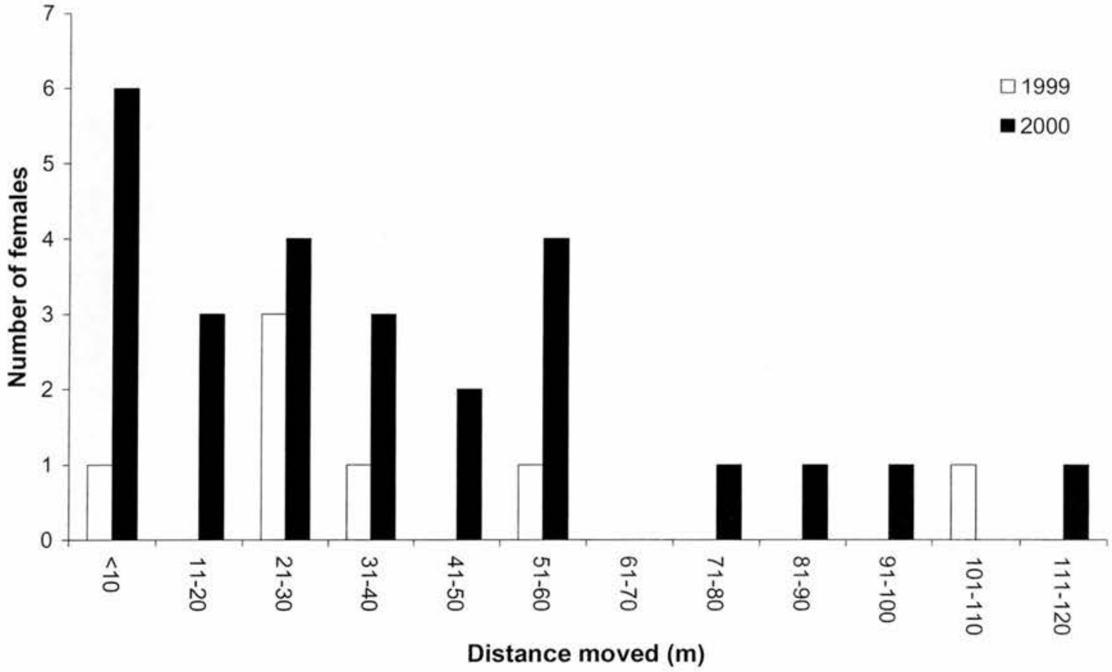

Figure 5.4. Distance moved from site first seen to pupping site for females seen at least one day before parturition on North Rona in 1999 and 2000.

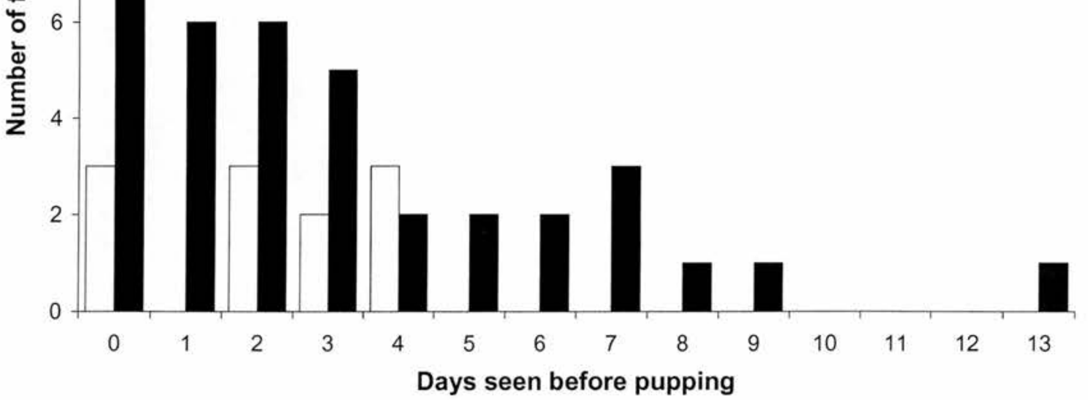

Figure 5.5. Number of days that females were seen ashore before parturition on North Rona in 1999 and 2000.

5.4.4. Duration of lactation

The median duration of lactation was recorded as 19.5 days in 1998 (n = 4, range 18-21 days), 17 days in 1999 (n = 12, range 14-21 days) and 17 days in 2000 (n = 11, range 15-25 days) (Figure 5.6). The data were not normally distributed in all years, so a Kruskal-Wallis test was used to compare the data (H = 3.95, d.f. = 2, p = 0.139, NS). The data were then pooled (resulting in a normal distribution) and the mean duration of lactation calculated as being 18 days (Figure 5.6).

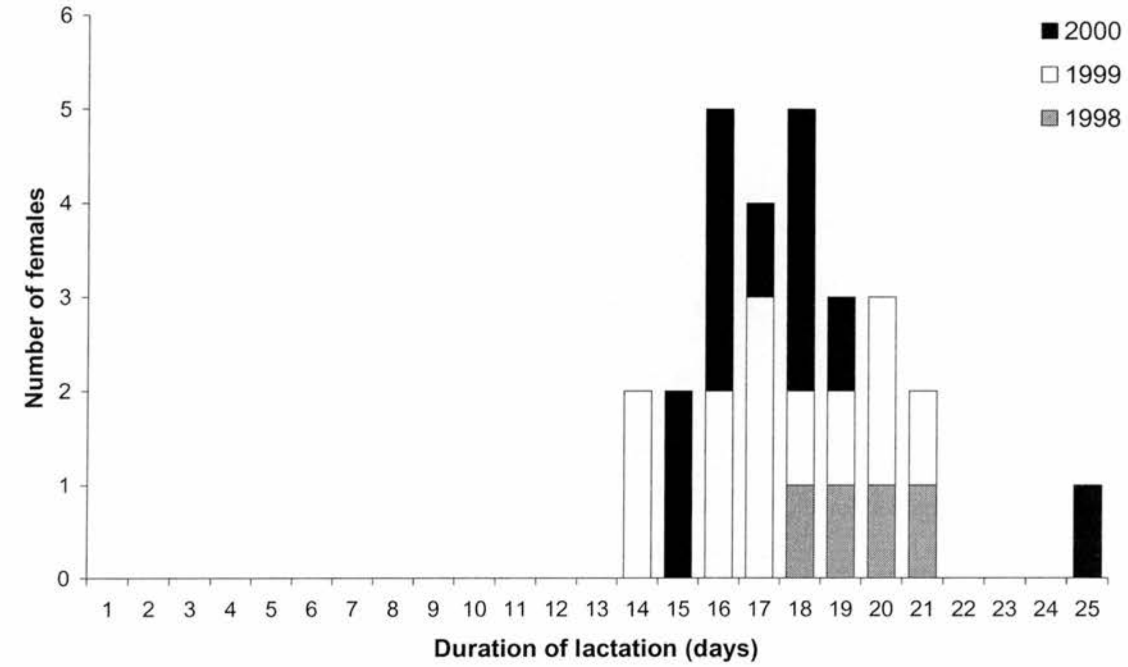

Figure 5.6. Duration of lactation for females on North Rona.

5.4.5. Summer resightings

In May 1999, 18 good quality left images and 19 good quality right images were captured on North Rona. Thirteen animals were known to have both left and right sides captured, resulting in a minimum of 19 and maximum of 24 individual animals. In June 2001, 30 left images and 48 right images were captured and judged to be of sufficient quality for resightings to be made (by eye) with images of previously identified females. Three animals were known to have had both left and right sides captured, so the overall number of animals captured was between 48 and 75.

Four females whose pelage markings were videoed or photographed in June 2001 were identified as females that had bred on North Rona in 2000; three of these were known study animals. A further female (photographed in June 2001) was identified as one that was present at North Rona in May 1999.

5.4.6. Behavioural comparison of females identified by pelage markings and females identified by brands

Return rate

The results from this study were compared with those collected from 67 females branded in 1985 (whose breeding behaviour was observed from 1985 to 1989, Pomeroy *et al.,* 1994) and with data from 28 branded females observed between 1998 and 2000 (Pomeroy, unpublished data). Table 5.2 provides a summary of the results from these studies. The return rates of females identified by pelage markings in 1998 and 1999 (and so resighted in 1999 and 2000 respectively) were between 3% and 30% higher than the return rates recorded in consecutive years by Pomeroy *et al.* (1994), and between 7% and 29% higher than the return rates of branded females between 1998 and 2000 (Pomeroy, unpublished data). No compensation for mortality was applied to the non-branded females identified in 1998 and resighted in 1999, because it was known that all these females were alive in 1999 (refer to Table 5.1). However, a 5% annual mortality rate was used to compensate for possible mortality of females that were resighted in 2000. The return rate of females identified by pelage markings in this year was a maximum of 93%. This was between 8% and 33% higher than the return rate for branded females observed in consecutive years between 1986 and 1989, and up to 32% higher than the return rate of branded females observed between 1998 and 2000.

Site fidelity, pupping date and duration of lactation

Median pupping site fidelity in consecutive years (1998 to 2000) for non-branded females was calculated as being 32m (section 5.4.2). This was significantly less than the median site fidelity of 55m recorded for branded females observed between 1985 and 1989 by Pomeroy *et al.* (1994) (Mann-Whitney: $W = 3107$, $p =$

Table 5.2. Table comparing breeding behaviour of female grey seals identified by pelage markings (this study) and female greys seals identified by individual brands (Pomeroy *et al.* 1994; unpublished).

	Females identified by pelage markings	Branded females
Comparisons with branded females observed between 1985 and 1989 (Pomeroy *et al.* 1994)		
Percentage returning (consecutive years)	90% from 1998-1999 88% from 1999-2000 (93% from 1999-2000 if assume 5% annual mortality)	60%-85%[#]
Median pupping site fidelity in consecutive years	32m (n = 51) (all data combined)	55m (n = 103) (all data combined)
Comparisons with branded females observed between 1998 and 2000 (Pomeroy, unpublished)		
Percentage returning (consecutive years)	As above	81% in 1998[$] 66% in 1999[$] 64% in 2000[$]
Median pupping site fidelity in consecutive years	As above	44m (n = 34) (all data combined)
Mean difference in pupping date in consecutive years	Less than one day (n = 51) (all data combined)	Less than one day (n = 44) (all data combined)
Median time ashore before pupping	2 days (n = 50) (all data combined)	4 days (n = 54) (all data combined)
Mean duration of lactation	18 days (n = 27) (all data combined)	18 days (n = 42) (all data combined)

[#] calculated as a percentage of the number of females expected to be alive each year (assuming an annual mortality of 5%) from data presented in Pomeroy *et al.* 1994.

[$] based on number of branded females known to be alive in 1997 and correcting for 5% mortality each year

0.0012, $n_1 = 51$, $n_2 = 103$). It was also significantly less than the median site fidelity of 44m recorded for branded females observed between 1998 and 2000 (Mann-Whitney: $W = 624.5$, $p = 0.03$, $n_1 = 51$, $n_2 = 34$, Pomeroy *et al.* unpublished). On average, non-branded females and branded females gave birth on the same date in consecutive years between 1998 and 2000 ($T = 0.28$, $p = 0.781$, $n_1 = 51$ (non-branded), $n_2 = 44$ (branded), NS, two sample T-test). The mean duration of lactation was not significantly different for these two groups of females ($T = 0.26$, $p = 0.793$, $n_1 = 27$, $n_2 = 42$, NS, two sample T-test), being an average of 18 days for both non-branded females and branded females. However, branded females were first seen ashore (prior to giving birth) significantly earlier than non-branded females (Mann-Whitney, $W = 2110.5$, $p < 0.001$, medians = 2 days and 4 days before parturition for non-branded and branded females respectively, $n_1 = 50$, $n_2 = 54$).

5.5. Discussion

5.5.1. Comparison of female breeding behaviour between years

The breeding behaviour of females identified by pelage on North Rona was virtually identical between years. These females were generally seen ashore 2 days before giving birth in an area close to their subsequent pupping site (although females that were seen ashore more than a week before parturition were generally only seen for a day or two, suggesting that they returned to the sea before giving birth (see also Pomeroy *et al.,* 1994)). Furthermore, the median pupping site fidelity of these females was the same between years (31m and 32m in 1999 and 2000 respectively), with females giving birth on the same date in consecutive years and having a similar duration of lactation over the three breeding seasons.

There was initial concern that the site fidelity of females identified by their pelage markings would be biased towards those that returned close to their previous pupping site, as it was not possible to survey areas outside of the main study area from the hide - the distances involved were too great to allow identification of pelage markings. In 2000, one female was sighted, opportunistically, at a distance of 565m from her previous pupping site with a stage three pup. However data from branded females suggests that it is rare for females to move this far (Pomeroy *et al.,* 1994, 2001). Furthermore, 88% of females with good images obtained in 1998 were resighted in 1999, and 93% of the females with good images obtained in 1999 were resighted on North Rona in 2000 (after accounting for an annual mortality of 5%). This high resight rate, which was greater than that recorded for branded females, suggests that the results obtained here were an accurate reflection of the site fidelity of non-branded females and were not biased by recognition of females that returned to a location where they were more likely to be observed (i.e. close to the hide).

5.5.2. Summer resightings

The results obtained here provide evidence that adult females that use North Rona as a breeding colony also use the island during the summer months. It is possible to make a very rough estimate of the percentage of breeding females that did this by using simple mark-recapture calculations. A total of 113 females were 'marked' as 'known' animals (i.e. classed as having good quality photographic/video images) over the three years of this study and the estimated number of breeding adult females on North Rona in 2000 was 1100 (C. Duck, pers. comm.). During the short stay on the island in June 2001, a maximum of 75 different animals were photographed (assuming that all of these were adult females and all unlinked left and right images were of different individuals). Knowing that ~10% of the breeding females were 'marked', seven or eight of the 75 females were expected to have been photographed previously if all the females around North Rona in the summer used the island during the breeding season. The actual number of 'known' females recaptured was three, suggesting that a minimum of 38% of the females that bred on North Rona in 2000 were there in June 2001.

There are a number of reasons why this is likely to be an underestimate. Firstly, it is possible that not all the left and right images captured during the summer were of different animals. Using the minimum estimate of animals photographed in June 2001 (i.e. 48), five females from the breeding season would be expected and the three that were seen would represent 60% of the breeding population. Secondly, there was the problem of correctly differentiating between males and females during the summer trips to North Rona when animals were photographed whilst they were in the water. It is possible that some sub-adult males were mistaken for adult females, which would artificially increase the number of images included as females during the summer and therefore reduce the number of animals that were identified as being present in the breeding season. Thirdly, the waters around North Rona may have been used as a feeding ground in the summer by animals that did not breed there in the autumn – this would 'dilute' the proportion of females recaptured that did use the island as a breeding colony. Lastly, many of the females identified by their pelage patterns on North Rona during the breeding season were not easily recognisable, by eye, from the markings on their head and neck alone. During the breeding season, observations were made from a hide at a distance of approximately 150 metres from the study animals and pelage identification was based on natural markings that could be seen using a pair of binoculars at this distance. Females were therefore more readily identifiable from markings on the main part of their body rather than those on the head and neck, i.e. the markings that were captured when females were photographed in the water. Of the three 'known' females resighted in June 2001, only one had distinctive neck markings that allowed her identity to be confirmed; the other two were recognised from their body markings because they happened to be hauled-out on rocks when their pelage was photographed. It is possible that more females photographed during the summer would have been recognised if their body markings had been captured as well.

One reason that the total number of animals photographed during the summer was low would be that animals observed in the waters around North Rona during the summer were most likely feeding in the surrounding area. Studies on foraging grey seals have shown that individual animals generally spend less than half of their time close to a haul-out area (McConnell, 1986; Thompson *et al.,* 1996), and that they may frequently travel between different haul-outs (McConnell *et al.*, 1992). In addition, grey seals may be submerged for more than 80% of the time whilst resting in the water close to a haul-out (Thompson *et al.,* 1991). Therefore only a very small fraction of the seals present around North Rona during the summer months would have been close enough to the island for an image of their pelage to be captured, with many animals foraging at sea during the short time period when photographs were taken. In addition, it was not possible to check all potential haul-outs during the summer, as sheers cliffs made access to some of these areas impossible. This means that many females present during the summer would not have been observed, much less photographed.

5.5.3. Comparisons between females involved in long-term studies and those identified by pelage markings

Around 88% to 93% of females with good quality photographs or video images of pelage markings were resighted in consecutive years (1998 to 2000) compared with only 60%-85% of the branded females observed between 1985 and 1989 (Pomeroy *et al.*, 1994) and 64%-81% of the branded females observed between 1998 and 2000 (Pomeroy, unpublished data). The high resighting rate for females identified by pelage markings means that the median site fidelity calculated for these females is likely to be an accurate reflection of the range of distances they moved, and as such, the females observed in this study showed a higher degree of site fidelity than branded females involved in long-term studies. These results might indicate that repeated capture and disturbance of female grey seals affected their return rate to North Rona as well as their degree of site fidelity. However, the return rate might also be affected by the age of the females – many of the females involved in long-term studies were captured and branded in the 1980's (Pomeroy *et al.*, 1994) and were therefore at least 20 years old in 1998. The lower return rate of these females might reflect a naturally higher mortality due to their age.

It is also possible that the difference in the site fidelity of branded females and those identified by natural markings occurred as a result of the different locations observed in these studies. The work presented here focused on females in the study area, Fianuis South (see map in chapter two) as these females could be easily observed from the hide; females further north, within Fianuis Central and Fianuis North, could not be identified by their pelage markings because of their greater distance from the hide. Pomeroy *et al.* (1994) observed females from all locations on the Fianuis peninsula and their effort expended on resighting females away from the study area was not as great as that for animals that were close to the hide. This may have influenced the degree of certainty with which female positions were recorded and may have affected the likelihood of resighting an individual. Conversely, it is also possible that animals within the Study Area/Fianuis South were more site faithful than animals in other regions. If this was the case, the study sites chosen for non-branded females may have inadvertently contained animals that were more site faithful than the colony as a whole. Therefore branded animals (with their greater variety of pupping locations) could be considered more representative of the colony than the females observed in this study.

Using data that was obtained from females observed between 1998 and 2000, branded females were first seen ashore significantly earlier than females identified by natural markings (four and two days before parturition respectively). Although this might reflect differences in the animals themselves, it is perhaps more likely that it reflects the fact that many branded females were identified coming ashore via the access gullies, whereas most females identified by their pelage markings were already ashore when they were recognised. This indicates that branded females were easier to detect within the colony, although this is not particularly surprising as recognition of an individual with a large artificial mark is much easier than recognition of a particular pattern amongst many seals that all have pelage markings. Comparisons of the difference between pupping date in consecutive years and the duration of lactation in 1998, 1999 and 2000 showed no significant difference between branded females and those identified by natural markings.

In summary, these results show that branded females were not significantly different to non-branded females with respect to their pupping dates in consecutive years and the duration of lactation – behaviours which are difficult to change on arrival at the colony because they are controlled by intrinsic factors such as the timing of oestrous, implantation and gestation. However, behaviour that could be modified by the individual, such as the difference in pupping site location between years, was significantly different between the two groups. Further comparisons are necessary to determine whether such differences are due to the behaviour of the animals or are artefacts caused by comparing animals in areas with different topographies.

Appendix 5.1: Data obtained on the breeding behaviour of female grey seals on the Isle of May

Return rate in consecutive years

Females seen in 1998 that had good images captured = 9, number resighted in 1999 = 3 (33%), number resighted in 2000 = 4 (44%), total number resighted = 5 (56%).

Females seen in 1999 that had good images captured = 34, number resighted in 2000 = 17 (50%).

Median distance travelled in consecutive years = 14m, range = 0-33m, n = 15 measurements (from 13 females). Two females appeared to skip a year on the Isle of May; these moved 4m and 31m respectively between 1998 and 2000.

Median distance between consecutive pupping sites

Few females were seen giving birth, but rocky topography restricted pup movement, and locations of resighted females were therefore used as a rough estimate of pupping site fidelity

1998-1999: pupping site fidelity = 14m, range = 10-20m, n = 3

1999-2000: pupping site fidelity = 14m, range = 0-33m, n = 12

Pupping date could only be estimated in two years for one female – this female gave birth two days earlier in the second year. The amount of time ashore before pupping could not be assessed for this female.

Median duration of lactation

Duration of lactation = 15 days, range 14-19 days, n = 3.

Chapter Six:

Passive and active associations between female grey seals on North Rona

P. Redman, P.P. Pomeroy and R. Harcourt collected the data included in this chapter.

6.1. Summary

A high degree of site fidelity and the annual return of females to the breeding colony at around the same date each year means that non-random associations are likely to occur amongst adult female grey seals. However, it is not known if these occur at a spatial scale that is relevant to the individual seal, i.e. one where females regularly come into contact with one another. Similarly, it is not known whether these associations occur solely due to site fidelity, or if females actively choose to be close to one another. The work presented here used measurements of female daily movement to define the spatial scale at which individual females were likely to come into contact with one another on the breeding colony. Hourly maps of female positions revealed that the maximum daily displacement from the female's starting position was 10m or less for 88% of females in 1999 and 97% of females in 2000. Females were therefore considered unlikely to come into contact with one another if they were separated by a distance of >20m. Using this distance (20m) to establish a definition for association, 226 female-pairs (from a total of 67 lactating mothers) were defined as being associated within a breeding season on North Rona; 45 of these pairs were associated in two years. A simple model was used to calculate the likelihood of female association in two years as a result of site fidelity. The results showed that passive association could account for inter-annual association between females that returned close to their previous pupping site (<30m). However, the observed number of female-pairs that showed inter-annual association after a displacement of >30m, >50m and >70m were, respectively, 6, 15 and >30 times greater than that expected using the assumptions of the site-fidelity model. This suggests that active association might also occur between adult females on North Rona.

6.2. Introduction

Large groups of animals often occur where suitable habitats (e.g. nesting sites, over-wintering sites, etc.) are scarce, where food is abundant but patchy in its distribution, and where large groups provide protection from predators (Alcock, 1993; Krebs & Davies, 1995). Within these groups, non-random associations – which occur because animals spend long periods of time in close proximity – may generate 'reciprocity in behaviour' which in turn strengthens the bond between animals and leads to active association and conflict reduction (Michod, 1999). This process has been suggested as a first step towards sociality (Michod, 1999).

Higher levels of social organisation (e.g. co-operation and reciprocal altruism) are determined by interactions between individuals (Hinde, 1976) and consequently allies/associates can be determined by looking at the behaviour between group members – for example, measuring incidences of allogrooming between individuals (e.g. Sugiyama, 1988; Dobson *et al.*, 1998). However, it is much more difficult to measure conflict reduction, especially if animals have few interactions with one another. In many species (e.g. marine mammals, nocturnal animals) it is not possible to observe all the animals within a group or the interactions between them. Researchers have therefore used alternative means of defining association (see Whitehead & Dufault, 1999). One of the simplest, and most common, is to classify animals as being associated if they are present within the same group or seen in close proximity. The assumption here is that spatial association is necessary before behavioural association can occur – the former is therefore used as a proxy for the latter. The scale of spatial association must be defined for each study species, as species-specific traits (e.g. methods of communication, size of home range, etc.) affect the scale at which spatial association may be present. Animals classified as associates may therefore be separated by anything from a couple of metres (e.g. greater white-toothed shrew, Cantoni & Vogel, 1989; captive Japanese macaques, Corradino, 1990) to a hundred metres or more (e.g. giraffes, Leuthold, 1979; coyote, Andelt, 1985; white tailed mongoose, Waser & Waser 1985). Measures of association based on proximity may also vary with the activity being observed – for example Arnold *et al.* (1981), classed individual sheep as associated if they were within an area of $50m^2$ when they were resting, but animals within an area of $400m^2$ were considered associated when they were grazing.

Previous studies (Pomeroy *et al.,* 1994, 2001, see also chapter five) have shown that female grey seals display a high degree of site fidelity to previous pupping locations and give birth on or around the same date each year. This means that females could come into contact with the same individuals in consecutive years, which in turn could lead to non-random associations and potential conflict reduction amongst neighbours that are familiar with one another (Michod, 1999). However, it is not known whether associations between female grey seals actually exist at a spatial scale that will affect their behaviour. Although individual female grey seals show a high degree of site fidelity on North Rona - within the range of 30m (chapter five) to 55m (Pomeroy *et al.*, 1994) - observations of female movement have shown that females on North Rona remain close to their pup (e.g. Anderson *et al.,* 1975; Twiss *et al.*, 2000a) and generally move less than 10m within a day (Aust, unpublished). In addition, overt interactions tend to occur only between female seals that have pups and which come within 3-4m of one another (pers. obs.). It may therefore be assumed that the formation of associations to reduce aggression would occur at a very fine spatial scale – i.e. between nearest neighbours. However, females often travel between their pup and a nearby pool (chapter eight) and this movement might bring them into close contact with a larger number of females (depending on the distance to the pool and the density of females in the area). This could result in conflict reduction between females over a greater area than would at first be expected. Any definition of association for these animals must therefore encompass their general daily movement – i.e. movement that brings them close enough to one another that they are likely to interact.

6.2.1. Aims

This study used a fine scale temporal and spatial approach to estimate the likelihood of associations between breeding female grey seals. Accurate hourly maps of female positions on North Rona were used to measure the maximum displacement of lactating grey seals from their daily starting position (i.e. the location where a female was first mapped each day). These measurements were used to determine the spatial scale within which female grey seals might regularly come into contact with one another, therefore providing a definition of association for female grey seals breeding on North Rona. The aims of the work presented here were as follows:

1) To measure the frequency and extent of inter-annual association on North Rona using the daily positions of known females in successive breeding seasons.
2) To create a model that would estimate the probability of passive association between females in successive breeding seasons on North Rona.
3) To compare the probabilities derived from this model with observations of known females to determine whether passive association could account for the frequency and extent of inter-annual association observed at this colony.

The following chapter is split into two sections – the first (section 6.3) deals with the classification of spatial association for female grey seals on the breeding colony of North Rona. The results of this section precede the section on the modelling of passive association (section 6.4), as it was not possible to determine inter-annual association without first defining spatial association.

6.3. Classifying spatial association for lactating grey seals on North Rona

Although daily locations were recorded in all three years (1998 to 2000), female movement was affected by the limited availability of pools during the start of the 1998 breeding season (Redman *et al.,* 2001, chapter eight) with many females travelling long distances from their pup (>20m) to gain access to water. The exact positions of these females were not recorded, which means the maximum displacement of females from their daily starting position could not be calculated. Data from 1998 have therefore been excluded from these analyses.

6.3.1. Methodology

The daily starting positions (x,y co-ordinates) of study females were recorded during the first hour of each observation period using digitised, geo-rectified maps overlaid with a 2m by 2m grid; the positions of these females were also recorded at hourly intervals throughout the observation period. Hourly displacement from the daily starting location was calculated for each female, using equation 6.1, from the x,y co-ordinates recorded each hour. Only females seen with a pup were included, as pregnant and non-parous females did not have the same constraints as lactating females.

$$D_n = \sqrt{(x_s - x_n)^2 + (y_s - y_n)^2} \qquad \text{Equation 6.1.}$$

Where:

D_n = distance moved from starting position at time n
x_s = x co-ordinate of daily starting position
x_n = x co-ordinate at time n
y_s = y co-ordinate of daily starting position, and
y_n = y co-ordinate at time n.

The maximum hourly displacement from the daily starting location was recorded as the maximum daily displacement for each female. This was repeated separately for each day that the female was observed. The values obtained from all females were then used to determine the separation distance within which associations should be investigated.

6.3.2. Results

A total of 650 measurements of maximum daily displacement were calculated over two breeding seasons (1999 and 2000) involving 149 females. In 1999, 88% of measurements involved a maximum displacement from the daily starting position of <10m; in 2000, 97% of measurements were within 10m (Figure 6.1). Given these results and the fact that daily positions of known females (not including study females which were mapped using finer-scale 2m by 2m maps) were recorded as the mid-point of a 10m by 10m grid square (chapter two), a separation distance of 20m was considered an appropriate cut-off point to look for association. Females were therefore described as associated within a breeding season if:

a) they were within 20m of one another on at least 50% of the days that their positions were recorded, or
b) they gave birth within 20m of one other.

Classification 'b' was included because the pupping site may represent the behavioural decision of the female in terms of where to pup, or possibly who to pup near, but a female's daily location may be influenced by the locomotion of her pup. Pups become more mobile as they get older (Boness & James, 1979; Kovacs, 1987) and a female that follows her pup as it moves within the colony may not remain close to conspecifics that she originally chose to be near.

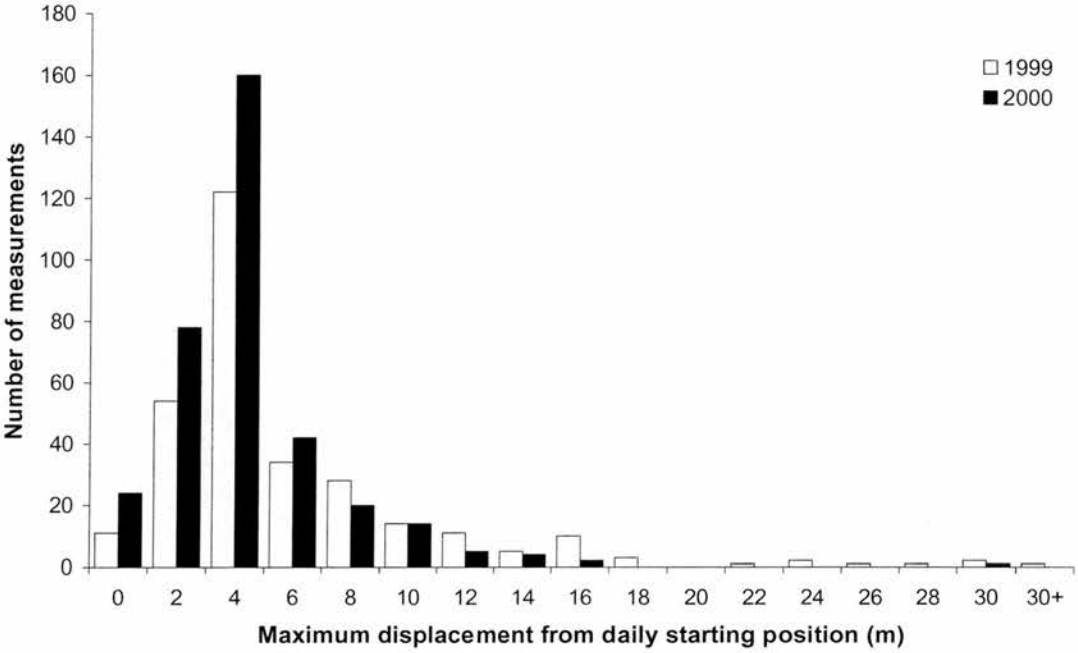

Figure 6.1. Maximum displacement from the daily starting position for females on North Rona.

6.4. Inter-annual association

Having chosen the spatial scale at which associations might be present amongst female grey seals on the breeding colony, it was then possible to look at whether inter-annual association occurred passively due to site fidelity, or actively through females choosing to be close to one another. Figure 6.2 demonstrates how females that are highly site faithful, moving only a few meters between consecutive pupping sites, are extremely likely to be associated in consecutive years simply due to site fidelity alone. In fact, if they are recorded within the same grid cell in year 1, and each moves 10m or less between pupping sites (section 6.2), these females will be recorded as associated in year 2 regardless of the direction in which they travel. If each female moves more than a few metres, however, the direction in which they travel is important if they are to remain associated in year 2.

The separation distance between the two females in year 1 will also affect the likelihood of association occurring passively, and it is possible for females that move in opposite directions to remain associated in subsequent years even if they move more than 10m (Figure 6.3). Figure 6.3 (example 1) shows two associated females (A and B) separated by 15m in year 1 (A_1 and B_1); examples 2-4 represent possible female positions in year 2 (A_2 and B_2 respectively) if each female moves 15m from her previous pupping site. Examples 2 and 3 show how movement in opposite directions can either result in a separation distance of 45m (not associated) or 15m (associated) in year 2. Example 4 shows movement of the two females in the same direction - these females are also 15m apart in year 2 and therefore spatially associated. However, although this example appears to show co-ordinated movement (and therefore active association), this scenario is just as likely to occur by chance as the scenarios depicted in examples 2 and 3.

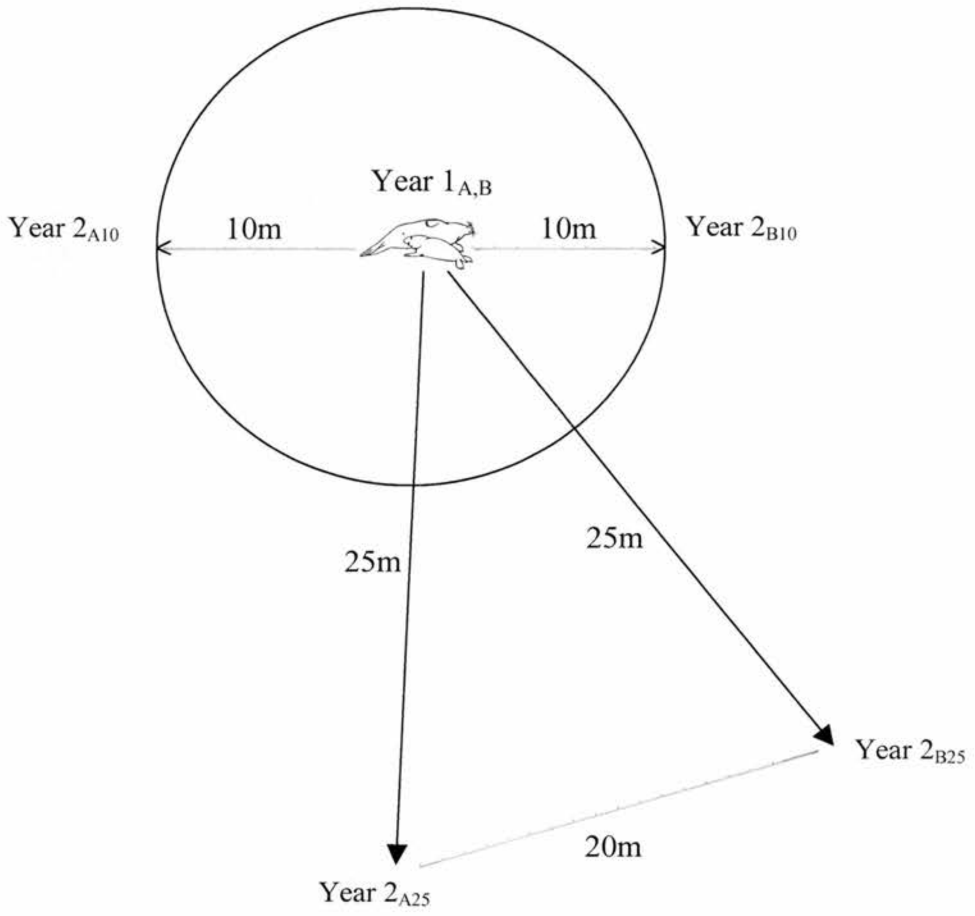

Figure 6.2. Figure demonstrating how the distance moved between years affects th probability that passive association will occur. If two females, A and B, (at the sam location in Year 1) each show an inter-annual site fidelity of <10m in year 2, they wil be associated (i.e. within 20m of one another) irrespective of what direction they mov in. This spatial association will occur in year 2 by passive means even if they happen t move in opposite directions (e.g. Year 2_{A10} and Year 2_{B10}) as all points on or within th circle are within 20m of one another. However, if each female moves more than 10m i year 2, for example 25m as shown here, the two females will only be associated if the travel in roughly the same direction (e.g. Year 2_{A25} and Year 2_{B25}); if they travel i opposite directions, they will be more than 20m apart.

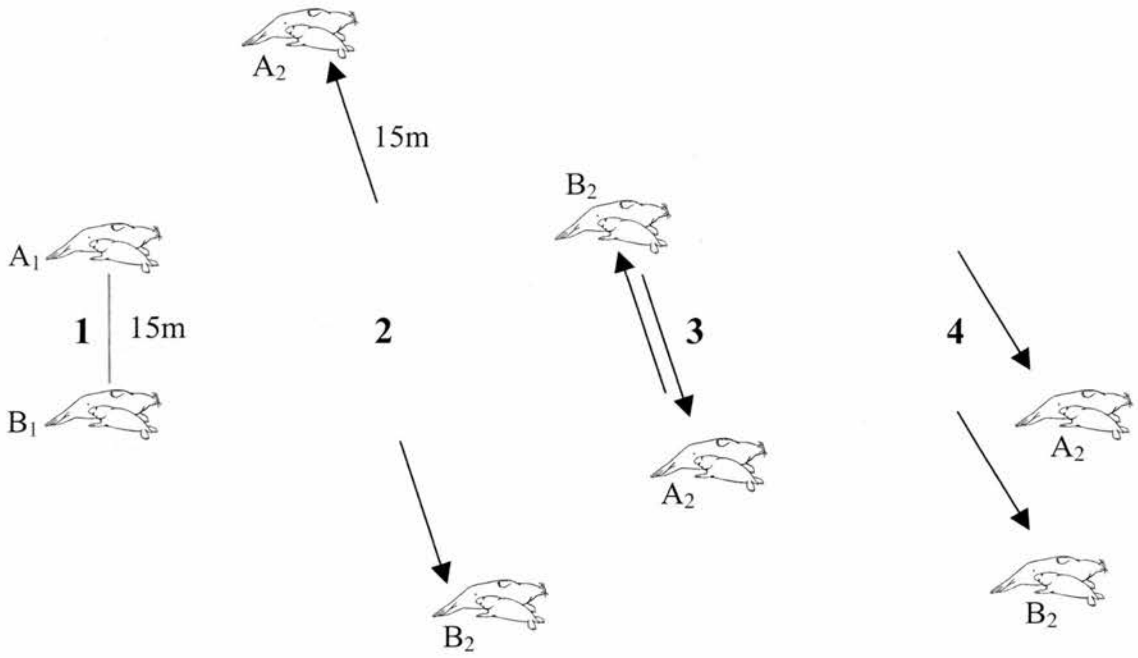

Figure 6.3. This figure demonstrates how the initial distance separating a pair of female and the direction of their movement between years may affect spatial association i subsequent years. Example 1 shows the position of two females (A_1 and B_1) in year 1. Examples 2-4 represent possible positions in year 2 (A_2 and B_2 respectively). The arrow indicate the direction of movement, with each female moving 15m in these examples.

6.4.1. Methodology

6.4.1.1. Observed data - Measuring spatial association between female grey seals on the breeding colony

Dr Sean Twiss converted the daily x,y locations of known females into a single ARC-INFO (ESRI)[1] GIS point coverage[2] which was incorporated into an established GIS database of the North Rona colony (Twiss *et al.,* 2000a, 2000b, 2001, in press). For each female's location, all other females within a radius of 20m were identified using the POINTDISTANCE procedure in ARC-INFO. These data were downloaded to SPSS, and data from individuals that were present (with a pup)

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[1] ESRI = Environmental Systems Research Institute Inc., 380 New York Street, Redlands, California USA. ARC-INFO version 7.0.3.

[2] a file that topologically links geographical features with their associated descriptive data. In this case, seal locations on North Rona were linked with the individual identity of the seal.

on the same date were extracted. The data were sorted by female identity and the number of times that specific female-pairs were within 20m of one another was calculated. Females were then classed as associated using the protocol in section 6.3.2.

6.4.1.2. A model for calculating the probability that a pair of females will be associated in two years due to site fidelity alone

The site fidelity model presented here used assumptions derived from the empirical data obtained in chapter five and section 6.3 to determine the probability that a pair of females (A & B), recorded as associated in year 1, would be associated in year 2 as a result of site fidelity alone (i.e. passive association). Figure 6.4 shows a flow diagram that outlines the steps taken in the model.

Given the start location for each female (which represents her pupping location in year 1), the displacement distance between years (site fidelity) and the direction taken from the start location (bearing from north), the model calculated the end location (pupping location in year 2) for each female by following the steps below (see also Figure 6.5):

1. calculate change in y co-ordinate: $f = \cos E \times d$
2. calculate change in x co-ordinate: $e = \tan E \times f$
3. calculate new x co-ordinate: $x_2 = x_1 + e$
4. calculate new y co-ordinate: $y_2 = y_1 + f$

Where:
d is the displacement distance between consecutive pupping sites,
E is the bearing travelled along (from north),
e is the change in x location,
f is the change in y location,
x_1,y_1 is the location in year 1, and
x_2,y_2 is the location in year 2.

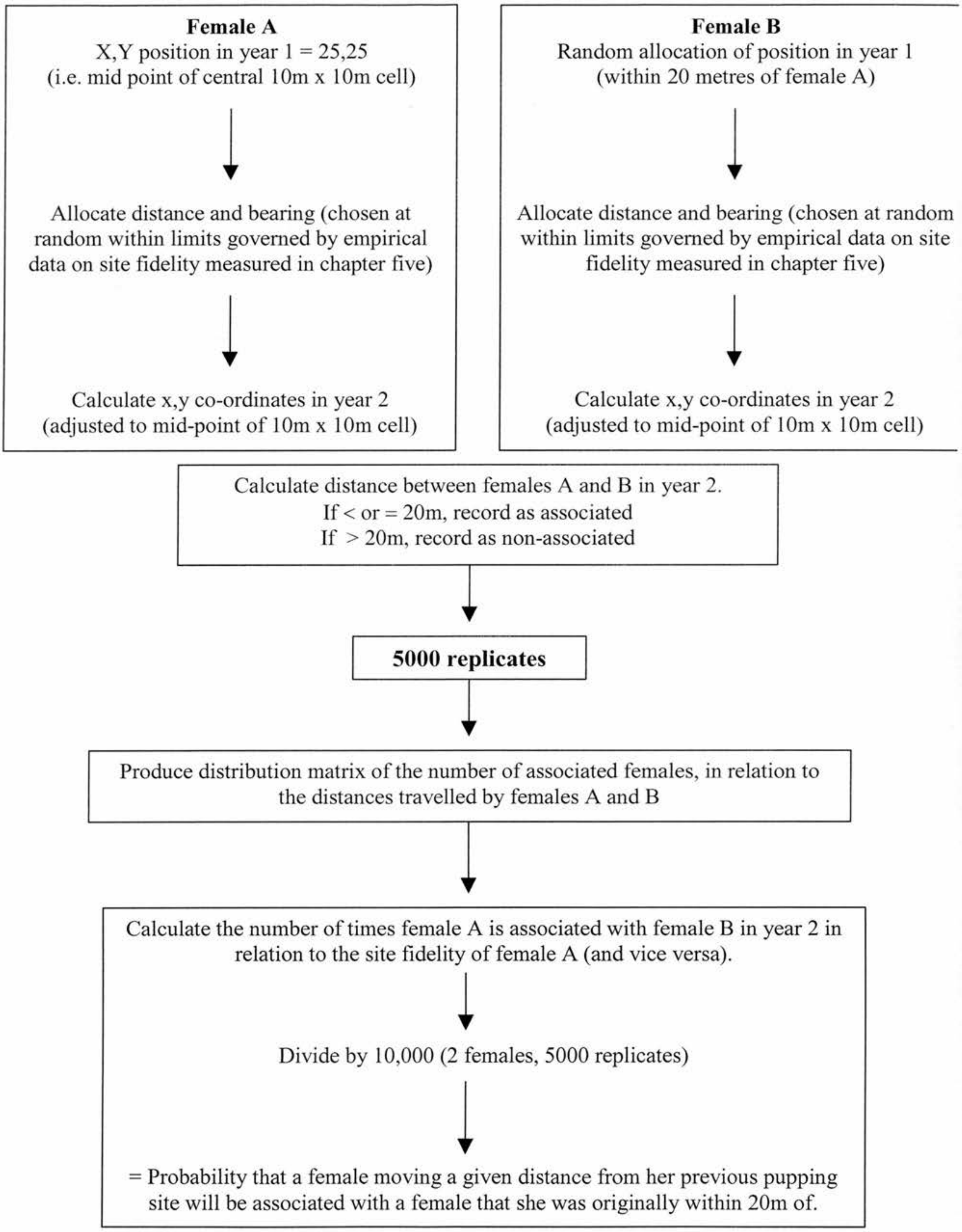

Figure 6.4. Flow diagram representing the stages within the site fidelity model, using females and B as examples. The initial x,y co-ordinates (e.g. 25,25) refer to locations in Figure 6.6.

The end locations (x_2,y_2) for females A and B were adjusted to correspond with the mid-point of their new grid cell location and the distance between the new co-ordinates of the two females calculated using Equation 6.2.

$$\text{Distance separating A \& B in year 2} = \sqrt{(x_a - x_b)^2 + (y_a - y_b)^2} \qquad \text{Equation 6.2}$$

Where:

x_a = x co-ordinate of female A in year 2,

x_b = x co-ordinate of female B in year 2,

y_a = y co-ordinate of female A in year 2, and

y_b = y co-ordinate of female B in year 2.

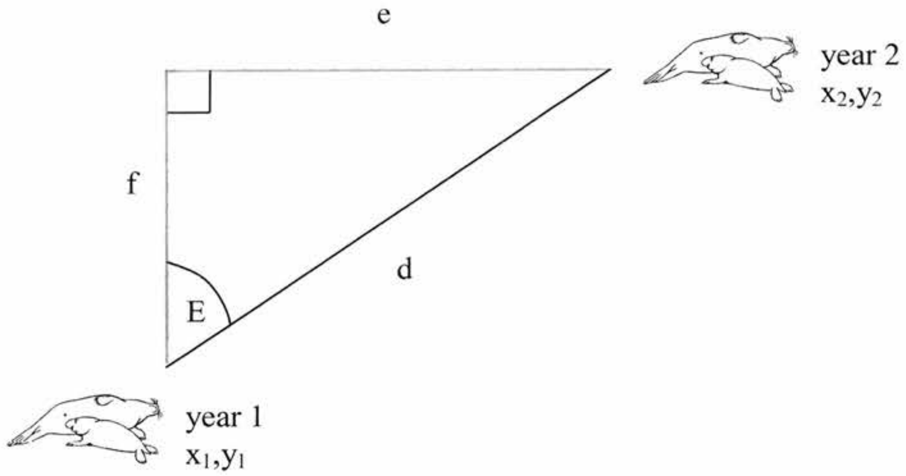

Figure 6.5. Calculation of female x,y location in year 2, where d = distance moved E = direction of travel (bearing from north), and e and f are the changes in x and co-ordinates respectively. North is assumed to be at the top of the figure.

Each step of the site fidelity model reflects the accuracy with which data were collected in the field, and is based on observations of female movement and site fidelity during three breeding seasons on North Rona (chapter five) as outlined on the following pages.

1) *Initial locations for associated females (year 1)*

Observed data: Daily positions of known females were recorded using a map of the study area overlaid with a 10m x 10m grid – female locations were recorded as the mid-point of the grid cell in which each female was first observed. Most females did not move more than 10m from their initial position on any given day (section 6.3) and it was assumed that they were unlikely to have regular encounters (or form associations) with individuals that were greater than 20m away.

Site fidelity model: The model used the mid-point of cells on a 10m x 10m grid to assign female location; females were not considered associated if they were more than 20m apart. Figure 6.6 is a schematic diagram that represents part of the study colony, with a female (A) being mapped in the centre cell (x,y co-ordinates 25,25) in year 1.

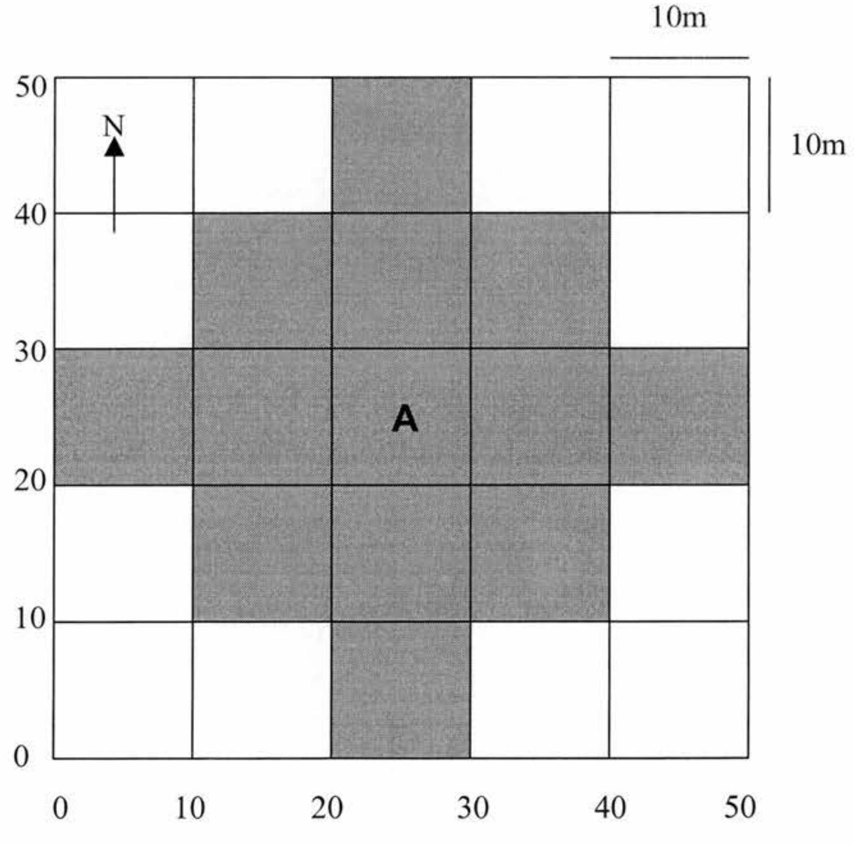

Figure 6.6. Schematic diagram showing the location of a female (A) on the study colon and the area around her in which associates may be found (shaded cells). The location of female was recorded as the mid-point of the cell within which she was first seen each day hence the x,y co-ordinates of female A in this example are 25,25. The mid-point of eac shaded cell is within 20 metres of the x,y co-ordinates of female A.

The mid point of each shaded cell is within 20m of the centre cell, so a female within one of these cells is classed as being associated with female A. In this example, the associated female is called female B. Female B was allocated a starting position within one of the shaded cells by numbering the cells from 1 to 13 and using the random number generator in Microsoft Excel 2000 to pick a number from this range; each cell had an equal probability of being chosen. The x,y coordinates for the chosen cell were recorded as the start position for female B in year 1.

2) *Distance travelled by each female*

Observed data: The displacement distance between pupping locations in consecutive years was measured for all study females (n = 55) that were present on North Rona for two or more years. To avoid pseudoreplication, each female was only included once, therefore, if a female was seen in 1998, 1999 and 2000, only the distance between the 1998 and 1999 pupping sites was used. These distances were used to produce a distribution curve of site fidelity (Figure 6.7).

Site fidelity model: The random number generator in Microsoft Excel 2000 was used to assign displacement distances for females A and B from the distribution curve derived above. The distances were measured in 10m bins (i.e. 0-10m, 10-20m...etc.) to be consistent with the accuracy of the 10m x 10m grid used to record female positions. The mid point of each bin (i.e. 5m, 15m...etc.) was therefore recorded as the distance moved in the model.

3) *Direction of travel*

Observed data: The bearing between consecutive pupping sites was measured to the nearest 5° for the same 55 females as above (Appendix 6.1). To determine whether the distribution was random, regular or clumped, an index of dispersion was calculated using the ratio of the variance to the mean (see Fowler & Cohen, 1992, p62-65). The ratio was calculated to be 0.97, which indicated that the distribution was random.

Site fidelity model: The model assigned a random bearing from north (5°-360°, at 5° intervals) using the random number generator in Microsoft Excel 2000; each 5° bearing had an equal probability of being chosen.

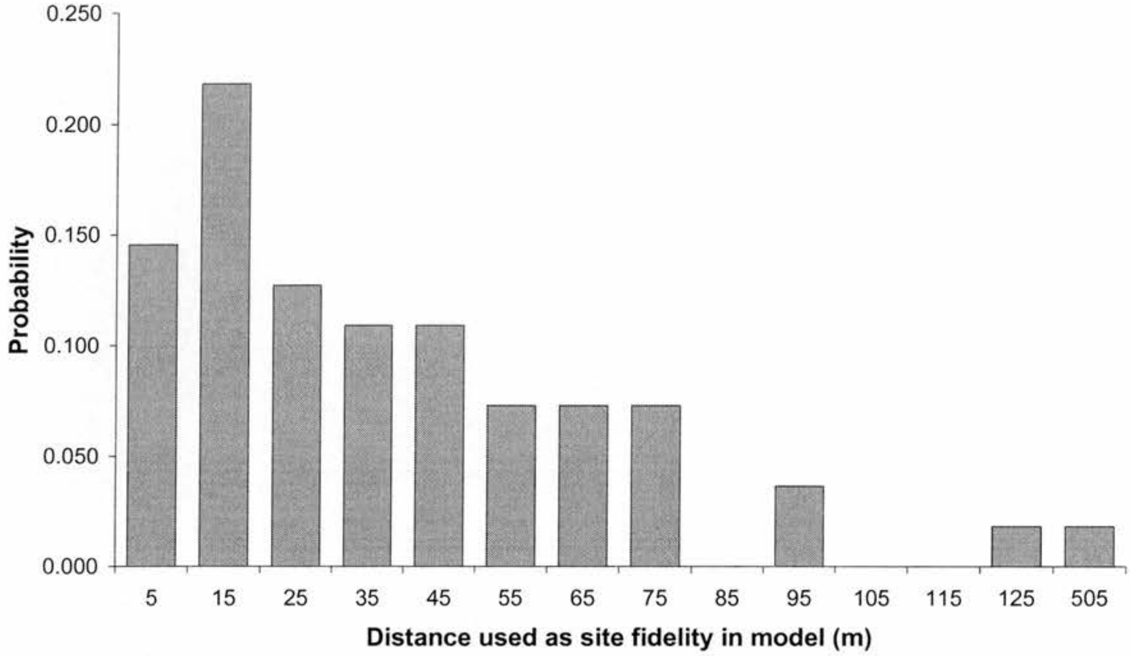

Figure 6.7. Probability curve of site fidelity used in model – the curve is based o observational data of consecutive pupping site fidelity on North Rona between 199 and 2000.

4) *Running the site fidelity model*

Five thousand replicates were performed, with the start location of female B and distances and bearings for each female chosen at random as previously described. The model always allocated the starting position of female A to the centre cell (as indicated in Figure 6.6) as her position relative to the associated female (female B) was more important for these calculations than her exact position in the colony. The outcome of each replicate was scored as positive if the two females were calculated to be within 20m of each other in year 2, and negative if they were not.

6.4.2. Results

6.4.2.1. Observed data – frequency of inter-annual association

A total of 67 mothers were mapped in two or more years on North Rona, giving rise to 226 female-pairs that were associated in at least one year – the large number of associated pairs occurred because many females were within 20m of several other females within a breeding season. From these 226 female-pairs, 45 female-pairs were associated in two years; these females are represented in Table 6.1. These 45 female-pairs included 44 of the original 67 females, which means that a minimum of 66% of the females that were mapped with a pup in two or more years had at least one associate that was present over two years.

Table 6.1. North Rona observed data.
Table showing the displacement distance from previous pupping sites for the 45 female-pairs that were associated in two years. The highlighted example shows that there were 10 cases where one female moved 10-20m and the other moved 20-30m.

Distance moved (m)	0-10	10-20	20-30	30-40	40-50	50-60	60-70	70-80	80+
0-10	**3**								
10-20	**6**	**1**							
20-30	**4**	**10**	**2**						
30-40	0	**2**	**2**	**1**					
40-50	0	0	**1**	0	**1**				
50-60	0	0	0	**1**	**2**	**1**			
60-70	0	0	0	0	0	0	0		
70-80	0	0	0	0	**2**	0	**2**	0	
80+	0	0	0	0	0	0	**1**	0	**3**

6.4.2.2. Modelled data – calculation of inter-annual association due to site fidelity

Table 6.2 shows the pair-distance combinations (i.e. displacement distances of the modelled female-pair) that were derived from 5000 runs of the site fidelity model. Two examples are highlighted in these tables to demonstrate that the probability of each female being 10-20m from her previous pupping site was greater than the probability of each female being more than 80m from her previous pupping site – this is to be expected given the known distribution of site fidelity (Figure 6.7). The number of positive outcomes from these replicates (i.e. the two females being within 20m of one another in year 2) is shown in Table 6.3. The same two examples are highlighted in this table, demonstrating that 113 of the 234 female-pairs where both animals travelled 10-20m were associated in the year 2 (i.e. 48%,) whilst none of the 24 female-pairs where both animals travelled >80m were associated in the second year.

Table 6.2. Site fidelity model data: Table showing the modelled distribution of pair-distance combinations after 5000 replicates. The table represents the numbers of times two females travelled the distances shown between consecutive pupping sites due to assumptions of the site fidelity model.

Distance moved (m)	0-10	10-20	20-30	30-40	40-50	50-60	60-70	70-80	80+
0-10	94								
10-20	331	234							
20-30	192	296	85						
30-40	153	264	136	74					
40-50	161	235	130	115	55				
50-60	93	167	79	72	83	28			
60-70	119	154	113	76	74	53	30		
70-80	102	169	79	63	77	53	64	15	
80+	129	148	91	80	67	43	49	51	24

Table 6.3. Site fidelity model data: Table showing the number of positive outcomes for association (i.e. females being within 20m of one another due to site fidelity alone) from female-pairs that travelled the distances presented in Table 6.2. For example, approximately half of the female-pairs had a positive outcome (i.e. 113/234) if both females travelled 10-20m between years, but no positive outcomes were recorded if both females moved more than 80m.

Distance moved (m)	0-10	10-20	20-30	30-40	40-50	50-60	60-70	70-80	80+
0-10	**86**								
10-20	**185**	**113**							
20-30	**56**	**58**	**37**						
30-40	**5**	**25**	**28**	**14**					
40-50	**2**	**5**	**17**	**7**	**8**				
50-60	0	0	**4**	**2**	**2**	**2**			
60-70	0	0	0	**3**	**1**	**2**	**3**		
70-80	0	0	0	0	**1**	**2**	0	0	
80+	0	0	0	0	0	0	0	**2**	0

6.4.2.3. Comparison of observed data and site fidelity model data

Using Tables 6.2 and 6.3, it was possible to work out the probability that a pair of females, displaced by a given distance from their previous pupping location, would be associated in the second year. Table 6.4 gives a number of examples.

The first example in Table 6.4 shows that the probability of both females being within 10m of their previous pupping site is low (0.02), but for those that show this degree of site fidelity the probability of the two females being associated is high

(0.91). Therefore, the overall probability of any female-pair being associated in the second year, after both females return to within 10m of their previous pupping site, is 0.02 x 0.91 = 0.018. The observed number of associated female-pairs that returned to within 10m of their pupping site was 3/226 = 0.013. This indicates that these associated pairs could be explained purely due to the assumptions in the site fidelity model. The examples in the last three columns, however, indicate that females with a greater displacement distance between years can not be so easily explained by the site fidelity model. Female-pairs that were displaced by >30m were observed six times more frequently than expected using this model, female-pairs that were displaced by >50m were observed 15 times more frequently than expected, and female-pairs that were displaced by >70m were observed over 30 times more frequently than expected. These data therefore suggest that active association might also occur between adult female grey seals on North Rona.

Table 6.4. Table showing the probability, calculated from modelled data, of two females remaining associated after moving the distance shown. The last two rows show the observed data from North Rona.

Site fidelity	<10m	<20m	<30m	>30m	>50m	>70m
Modelled data						
Both move distance shown	0.02	0.13	0.25	0.25	0.08	0.02
Probability that females remain associated	0.91	0.58	0.43	0.04	0.03	0.02
Probability of both females moving distance shown and remain associated due to assumptions of site fidelity model	0.018	0.075	0.108	0.010	0.002	0.0004
Observed data						
Number of female-pairs that remained associated after travelling distance shown	3/226	10/226	26/226	14/226	7/226	3/226
Probability	0.013	0.042	0.115	0.062	0.031	0.013

6.5. Discussion

6.5.1. Frequency of association

The results obtained here demonstrate that most female grey seals did not move more than 10m from their recorded position at the start of each day. It was therefore considered unlikely that associations would develop between individuals that were more than 20m apart, and this distance was chosen as a cut-off point when looking for associations between females on North Rona. Using this measure of proximity, around 66% of females that were mapped with a pup in two or more years showed inter-annual association with at least one other female. However, this might not represent the true number of associates for all the females studied, as pelage markings were not captured for every female in the study area - consequently it is likely that there were other associated pairs that were not detected between years. This is most likely to be true for females that gave birth towards the middle of the pupping season (which corresponded with the end of each field season in this study), as these females were only observed for a couple of days each year. In addition several females, although present on the colony, were not seen with a pup in two consecutive years. These females were not included in the analyses, thereby reducing the amount of data available on possible between-year associations. Despite this, the results obtained here suggest that a large number of females were associated with at least one other female from year to year.

6.5.2. Active associations between years

Although it was not possible to use the site fidelity model to distinguish between passive and active association for females that showed a high degree of site fidelity (i.e. returned to within 30m of their previous pupping site), the incidence of inter-annual association observed amongst females that were less site-faithful was much greater than expected using the assumptions of the site fidelity model. This suggests that active association might occur amongst grey seals (at least on North Rona) with specific females choosing to be near one another. Although, there has been little evidence of active association within grey seal breeding colonies before, Pomeroy *et al.* (2000b) recorded co–occurrence of a breeding mother/daughter pair at a distance of 110m from the daughter's natal site, in a study of philopatry and site fidelity using branded and tagged females. One of the most likely reasons that there has been little evidence of active association in the past could be related to the difficulty of identifying large numbers of individual seals. Previously, long-term identification of grey seals has been achieved through the application of brands or tags. However, capture of large numbers of animals in localised areas to apply artificial marks would be extremely disruptive and time consuming. By using natural markings to identify individuals this difficulty has been removed, allowing the current study to provide insights into the social structure of female grey seals on the breeding colony.

6.5.3. Model limitations

When using models, a balance between biological realism and simplification has to be struck. The major elements of the model presented here were based on methods used in the field and results from available data on lactating females. However, as with all models, there were limitations regarding what could be incorporated. For example, the model itself did not take into consideration the fact that females might not return every year, and even if they did, that they might not give birth. For this reason, the expected results were calculated from pairs of females where both individuals were observed with a pup in two or more years. This selection process also used animals from the data set with distinct pelage markings and it is possible that this artificially increased the proportion of resightings made. Assumptions that females not seen during the breeding season were absent from the colony might not always be valid as individuals can, and do, travel long distances between pupping locations (chapter five). However, studies of branded animals (Pomeroy *et al.,* 1994) and high resight rates for females identified by pelage markings (chapter five) suggest that this is rare.

There may also have been a problem of non-independence in the observed data, for example if females returned as associated triads. The model estimated the likelihood of association using 5000 replicates of a single pair of females. As a result, a single associated triad would be recorded as three separate incidences of associated pairs. Examination of female identity amongst those that showed inter-annual association indicated that there was one possible triad amongst the females observed in this study. However, as this involved females that returned close to their previous pupping sites (<20m), it was not possible to distinguish between passive and active association. It is thought unlikely that this one incidence would have a significant effect on the results obtained here, even though, theoretically, a triad of associated females would be less likely to occur passively than a pair of associated females.

6.5.4. Are associations observed on North Rona likely to persist outside of the breeding season?

Information on the social systems of pinnipeds outside of the breeding season is scarce due to the difficulty in locating and studying animals at sea and the low numbers of individuals on which tracking has been carried out (e.g. McConnell, 1986; Thompson *et al.*, 1991,1996; McConnell & Fedak, 1996). However, recent findings indicate that animals that are grouped together in one location may travel to similar areas outside of the breeding season, providing an opportunity for associations to develop and persist outside of the breeding colony. Satellite tracking of recently weaned elephant seal pups departing from Macquarie Island has revealed that pups do not set off in random directions, but that many follow similar routes, even though individuals may leave the colony several days apart (Fedak *et al.*, 1998). In addition, evidence from northern fur seals indicates that a high degree of site fidelity exists for foraging areas as well as for breeding colonies, with animals from nearby breeding sites foraging in the same areas (Robson *et al.*, 1998). Summer resights on North Rona (chapter five) also suggest that at least one third of the adult females present during a breeding season at this colony use the island as a summer haul-out. This would provide the opportunity for associations, observed between grey seal females during the breeding season, to persist throughout the year.

6.6. Conclusions

This study provides a definition for association between female grey seals and presents results that suggest that active inter-annual associations might occur between pairs of breeding females on North Rona – something that has not been noted before in grey seals. This may have implications for future studies of social behaviour in this species, as active associations will influence where and when females pup within the colony.

Appendix 6.1. Direction of travel (bearing from north to the nearest 5°) between consecutive pupping sites for a sub-sample of 55 females whose pupping site was recorded in two years.

Bearing	5°	10°	15°	20°	25°	30°	35°	40°	45°
No. of females	1	1	0	0	1	0	2	1	3
Bearing	50°	55°	60°	65°	70°	75°	80°	85°	90°
No. of females	1	1	0	0	2	1	0	0	2
Bearing	95°	100°	105°	110°	115°	120°	125°	130°	135°
No. of females	0	3	0	2	0	1	1	3	2
Bearing	140°	145°	150°	155°	160°	165°	170°	175°	180°
No. of females	1	2	0	0	1	1	1	0	2
Bearing	185°	190°	195°	200°	205°	210°	215°	220°	225°
No. of females	0	0	1	1	0	1	0	1	1
Bearing	230°	235°	240°	245°	250°	255°	260°	265°	270°
No. of females	0	2	0	0	0	0	1	0	0
Bearing	275°	280°	285°	290°	295°	300°	305°	310°	315°
No. of females	1	2	0	2	0	1	0	0	0
Bearing	320°	325°	330°	335°	340°	345°	350°	355°	360°
No. of females	0	0	1	0	0	0	0	1	1

Chapter Seven:

Relatedness of spatially associated female grey seals on North Rona

Skin samples were collected by P. Redman, P.P. Pomeroy, S.D. Twiss and S.E. Moss.

7.1. Summary

Microsatellite analysis of female skin samples was used to investigate whether kin clustering occurred amongst adult females on North Rona during the 1998 and 1999 breeding seasons. The relatedness of females that showed inter-annual association in two or more years (from 1998-2000) was also investigated. Regression analysis revealed no significant relationship between relatedness and Euclidean distance in 1998 or 1999, and there was no significant difference in the relatedness of females that showed inter-annual association compared to those that were not associated between years.

7.2. Introduction

Adult grey seals show a high degree of site fidelity (Pomeroy *et al.*, 1994; Twiss *et al.*, 1994; chapter five in this thesis), with females breeding at a colony for 25 years or more (Hewer, 1960; Pomeroy *et al.*, 1999) and the most successful males using a colony for up to 10-15 years (Twiss, 1991; Worthington-Wilmer *et al.*, 1999). Long-term studies of cohort-branded and tagged pups on North Rona and the Isle of May have also revealed evidence of male and female philopatry at these colonies (Pomeroy *et al.*, 2000b). Such a combination of site fidelity and philopatry could provide a mechanism by which groups of related individuals might occur at these breeding colonies. Indeed, recent studies on North Rona, have shown that females in prime locations (low elevation land which has good access to pools) are more related to the colony as a whole than would be expected by chance (Pomeroy *et al.*, 2001), suggesting that the offspring of these females are more likely to enter the breeding population. This could occur as a result of increased pup survival, with subsequent natal philopatry and site fidelity over a number of years (Pomeroy *et al.*, 2001), or it might be influenced by preferential recruitment of related individuals (e.g. Pope, 1998; Piertney *et al.*, 1999). It is possible that female grey seals are more tolerant of related females, allowing them to share prime pupping areas whilst aggressively repelling unrelated females. Similarly, dominant males may 'allow' closely related males access to mates in prime areas, increasing relatedness on the paternal side rather than the maternal side (Worthington-Wilmer *et al.*, 2000). If related conspecifics are less likely to be repelled from prime locations within the colony, microsatellite analysis should reveal a higher degree of relatedness between individuals in these areas.

7.2.1. Aims

The aim of the work presented here was to investigate whether kin clustering or inter-annual kin association were prevalent amongst breeding females on North Rona, and if so, whether they were affected by female location within the colony.

7.3. Methodology

This chapter was written using results from a collaborative study with Dr. Bill Amos at the University of Cambridge. Dr. Amos and his research team conducted all the microsatellite analyses for skin samples collected on North Rona in 1998 and 1999, and provided the relatedness matrices that were used for this work.

7.3.1. Sample collection

Skin samples were obtained as described in chapter two, and labelled with an individual identification number that was used to cross-reference details of location with photographs or video images of natural markings of each female. The location of each female was recorded using a detailed map of the breeding colony (see chapter two), with the x, y co-ordinates of each female being recorded as the centre of the 10m by 10m grid cell in which she was present.

There was a difference in sampling regimes between 1998 and 1999. In 1998, 124 females were skin sampled from the study area on North Rona (Figure 7.1), with the majority being sampled over a period of two days during the peak of the breeding season. In 1999, small groups of females were sampled at intervals throughout the breeding season in a more focused sampling protocol (Figure 7.1). Study females and their neighbours were specifically targeted in this year, and a total of 62 females were sampled. All sampling procedures were carried out in accordance with Home Office regulations.

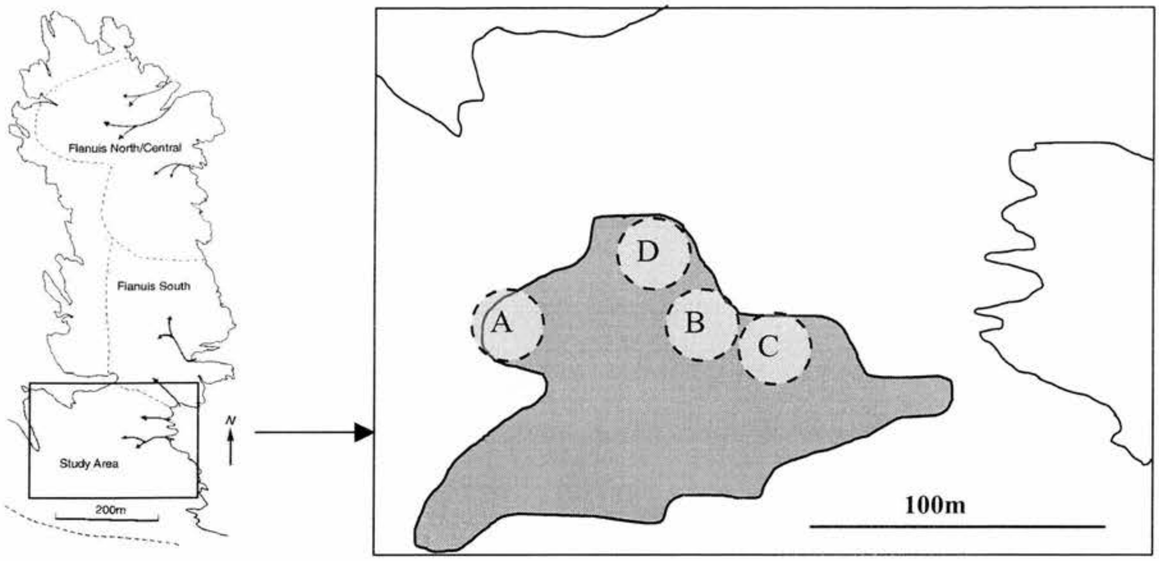

Figure 7.1. Areas where skin samples were collected on North Rona in 1998 and 1999 A broad area was sampled in 1998 (dark grey area, solid line) whereas specific stud females and their neighbours were targeted in 1999 (light grey areas, dashed lines). A = West Pools, B = Castle, C = Keep and D = Arena.

7.3.2. Microsatellite Analysis

Skin samples were genotyped for up to nine polymorphic microsatellite loci (Allen *et al.*, 1995) at the University of Cambridge. Relatedness matrices were calculated as described by Queller and Goodnight (1989) using the program Kinship (Goodnight software, http://bioc.rice.edu/Keck2.0/labs/). Females that were typed for seven or more microsatellite loci were included in these matrices, resulting in an 'all against all' comparison for 120 females in 1998 and 55 females in 1999.

7.3.3. Proximity and relatedness of adult females on North Rona

The Euclidean distance separating each pair of females was calculated from the x, y co-ordinates recorded when skin samples were collected. As females in 1999 were sampled opportunistically over a number of days, only pairs of females judged to be present at the same time (calculated from their parturition dates and allowing for an average lactation of 18 days) were included in the dataset for this year. Linear regression was then performed to determine whether there was a significant relationship between Euclidean distance and the relatedness of a pair of females. As recent studies have revealed that females are more highly related to the colony as a whole in prime locations on North Rona (Pomeroy *et al.*, 2001), separate regression analyses of Euclidean distance and relatedness were also performed for each targeted area observed in 1999 (refer to Figure 7.1). Regression analyses were performed using female-pairs, which artificially increased sample size, so all significance levels were corrected for the actual number of females sampled. The Local Spatial Autocorrelation (LSA) approach used by Pomeroy *et al.* (2001) was not repeated here because this study lacked the large-scale sampling necessary for LSA analysis.

7.3.4. Relatedness of females showing inter-annual association

In chapter six, 45 female-pairs (involving 44 individual females) were found to show inter-annual association, i.e. they:

a) were seen (with a pup) within 20m of one another in two or more years on at least half of the days that both animals were mapped, or
b) had birth sites that were within 20m of each other in two or more years.

Twenty-two of these females were included in the 1998 and 1999 relatedness matrices, providing R values for 17 of the female-pairs that showed inter-annual association (Table 7.1). Normality tests revealed that the relatedness values of non-associated females (i.e. all female-pairs for which relatedness could be determined that were not classed as associated using the definition above) were normally distributed; the relatedness values of females that showed inter-annual association had a distribution that approached normality. Therefore a two-sampled T-test was used to test whether female-pairs that showed inter-annual association were more closely related to one another than non-associated female-pairs. Only known females (those identified by pelage) were used in this comparison, as their positions had been mapped daily, thereby allowing social contact (or lack of social contact) between pairs to be confirmed. It was not possible to determine whether females were associated or not during the breeding season if they had not been mapped on a regular basis.

All statistical analyses presented here were performed using Minitab version 10.5 or SPSS version 9.

7.4. Results

7.4.1. Proximity and relatedness of adult females on North Rona

Regression analysis revealed no significant relationship between relatedness and Euclidean distance in 1998 ($F_{1,119} = 0.07$, $p > 0.05$, NS) or in 1999 ($F_{1,54} = 3.53$, $p > 0.05$, NS). There were also no significant relationships for three of the sub-regions targeted in 1999 - Arena ($F_{1,54} = 0.00$, $p > 0.05$, NS), Castle area ($F_{1,54} = 2.47$, $p > 0.05$, NS) and Keep ($F_{1,54} = 0.24$, $p > 0.05$, NS). There was a significant relationship for the West Pools area ($F_{1,54} = 4.40$, $p < 0.05$, Regression equation: distance = 43.8 - 11.5 relatedness, adjusted $R^2 = 0.07$ (Figure 7.2)).

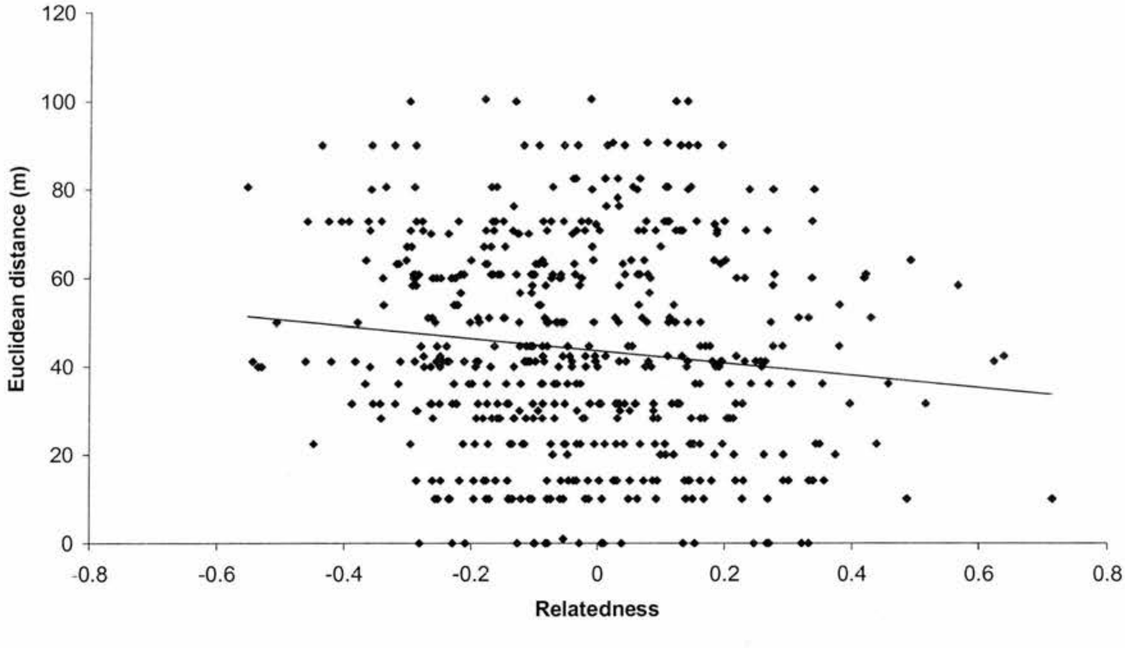

Figure 7.2. Graph showing Euclidean distance between female-pairs of known relatedness for females that were skin sampled in the West Pools area.

7.4.2. Relatedness of females that showed inter-annual association

There was no significant difference between the relatedness of female-pairs that showed inter-annual association and female-pairs that were not associated (T = 1.34, d.f. = 16, p = 0.20, NS, two-sample T-test). The average relatedness between associated female-pairs was 0.051 (range -0.31 to 0.38, n = 17 female-pairs); the average relatedness between non-associated female-pairs was -0.013 (range -0.74 to

0.64, n = 913 female-pairs). Despite this, four of the 17 female-pairs that showed inter-annual association had R values of >0.2 (which might indicate that they were half-siblings) with two of these being ≥0.35 (which might be full-siblings or mother/daughter pairs) (Table 7.1). It was not possible to determine whether these R values were likely to occur by chance as the sample size was very small.

Table 7.1. Relatedness values for female-pairs that showed inter-annual association.

Female pair		R value
Anne	Disney	0.22^{b}
Anne	Female 3a	$0.38^{a,b}$
Anne	Zeb	-0.04
B5	O8	0.30^{b}
Barbie	Female C	-0.15
Barbie	Rose	-0.02
Barbie	XLY	$0.35^{a,b}$
Dice	Olive	-0.15
Female 13-1	Si-fi	-0.12
Female 5-3	Jodie	0.10
Female 5-3	Olive	-0.31
Female Y	Olive	0.05
Female Y	O4	0.10
H right	O8	-0.13
H right	Spotty 1	0.16
Olive	Totem	0.12
Punch	Totem	0.00

[a] = R values that may indicate full-sibs or mother/daughter pairs.
[b] = R values that may indicate half-siblings.

7.5. Discussion

The results presented here suggest that there were few (if any) kin-based associations between females on the breeding colony on North Rona. There was no relationship between relatedness and Euclidean distance in either year or within three of the sub-regions targeted in 1999. Females in one area, West Pools, did show a significant negative relationship between relatedness and Euclidean distance, but the R^2 was incredibly low, indicating that relatedness explained very little of the variance seen. Furthermore, females that were within the same grid square (Euclidean distance = 0m) were not highly related. In fact, only a very few highly related females were within 20m of one another – the distance within which females were classified as being associated (chapter six). Therefore, it is assumed that the relationship detected at West Pools, despite being statistically significant, was not biologically significant.

Females that showed inter-annual association were not, on average, more highly related to one another than non-associated females. Four associated pairs did have R values >0.2, indicating that they could be half-siblings (using the assumption that half-siblings have an average R value of 0.25) and two of these pairs had values >0.35, which might indicate that they were full-siblings or mother/daughter pairs. However, these R values are not conclusive evidence of associations between first-order relatives and as such, this study does not provide any further clues as to when active associations may develop.

Unfortunately, due to the substantial number of errors that were detected in the microsatellite analysis carried out by Dr. Amos's research group for the work presented in chapter four, it is felt that the accuracy of the relatedness matrices provided by his laboratory for the work presented here are also questionable. Therefore, although it appears likely that kin clustering and kin association were not prevalent on North Rona, it is possible that the results obtained here do not truly reflected the relatedness of the sampled females. Further study will be necessary before any definite conclusions can be made.

The results of this study also suffered from a reduced sample size compared to the number of skin samples actually collected. This was due to the fact that microsatellite analyses of skin samples collected in 2000 were not available at the time of writing - relatedness could therefore only be investigated for 17 pairs of associated females. It is also unfortunate that Dr. Amos did not make available the results of extensive DNA sampling carried out in the Study Area on North Rona in 1997. This would have provided a large background sample of individually located females that would have allowed the 1998-2000 samples to be put into a larger scale context. The larger sample size would also have provided a clearer picture of the relatedness between female-pairs, allowing the chance probability of high relatedness between associates to be investigated.

Chapter Eight:

The effect of water availability on grey seal maternal attendance patterns and locomotory behaviour

P. Redman and P.P. Pomeroy collected the behavioural data used in this chapter; S.D. Twiss collected the field notes of weather conditions.

A version of this chapter has been published as: Paula Redman, Paddy P. Pomeroy and Sean D. Twiss. 2001. Grey seal maternal attendance patterns are affected by water availability on North Rona, Scotland. Canadian Journal of Zoology, 79: 1073-1079

8.1. Summary

Previous studies on grey seals have shown that pools of water influence female distribution within inland breeding colonies. This study revealed that availability of pools also affected maternal attendance patterns and might have implications for breeding success. An atypical dry period at the start of the 1998 breeding season on North Rona, Scotland, followed by more typical, wetter weather, provided a natural experiment that allowed examination of female behaviour in relation to the availability of pools. During the dry period, lactating grey seals (1) travelled long distances to gain access to water; (2) had significantly greater rates of locomotion towards water and as a result of interactions between conspecifics; and (3) spent significantly less time close to their pups. Long distance locomotion and reduced time with the pup often lead to permanent mother/pup separation, resulting in starvation of the pup. However, the immediate need to gain access to water for thermoregulation or to maintain a positive water balance outweighed the potential costs of reproductive failure. This study emphasises the importance of water for lactating grey seals even during the relatively cold and damp UK breeding season.

8.2. Introduction

Grey seals breed in a variety of habitats: on land-fast and floe ice, in caves, along sandy beaches or rocky shores, and at grassy inland sites (see Hewer, 1960; Bonner, 1981). These differing environments influence broad scale aspects of their breeding biology such as female distribution, social systems and the amount of time females spend at sea (Stirling, 1975; Anderson & Harwood, 1985; Caudron, 1997). Around the UK, grey seals use two of these habitats predominantly; open beaches (e.g. the Monach Islands) and inland sites (e.g. North Rona). At open-beach colonies, access to the sea is unrestricted and females may spend more than 50% of their time away from their pup, resting at sea within sight of the beach (Fogden, 1971; Hewer, 1974; Anderson & Harwood, 1985; Kovacs, 1987). As grey seals often fast throughout lactation and do not need to go to the sea in order to feed during this time, it has been suggested that mothers exhibit this behaviour to minimise the risk of predation, mainly from humans, and reduce aggressive encounters between conspecifics (Fogden, 1971).

In contrast, grey seals at inland colonies often have very restricted access to the sea. Females at these sites breed far inland, remain close to their pups throughout lactation and are found clustered around pools and streams (Boyd *et al.*, 1962; Anderson *et al.*, 1975; Twiss *et al.*, 2000a). While these different maternal attendance patterns indicate that access to fresh or salt water is important, the explanations offered by Fogden (1971) cannot explain why females at inland sites behave as they do. The use of isolated islands as breeding colonies affords grey seals protection from terrestrial predators, but clustering around small pools (often only large enough to accommodate a single seal) is not an effective way to reduce predation risk. Furthermore, clustering around pools increases local density, which is likely to increase the number of aggressive encounters between conspecifics rather than decrease them. Therefore the reason for the importance of water to lactating grey seals remains unclear.

Factors that influence maternal attendance patterns in different environments include energy conservation, thermoregulation and water balance. Beach breeding seals have continual access to a nearby source of water, which can be reached by moving over low elevation land with a low associated cost of locomotion. At inland sites, the high cost of travelling over difficult terrain to the sea, interactions with conspecifics and the risk of permanent mother/pup separation, explains why mothers remain with their offspring throughout lactation (Twiss *et al.*, 2000a). Mothers using inland sites usually remain in areas that contain pools and have a tendency to return to the same pupping site year after year (Pomeroy *et al.*, 1994). However, when water availability is limited, mothers must choose between attendance patterns that favour either direct contact with their pup or access to water.

Manipulations of seal breeding habitat are difficult to carry out, but this study took advantage of an atypical weather pattern to conduct a natural experiment, allowing investigation of changes in maternal behaviour in response to water availability on the breeding colony. In 1998, the early autumn and first few weeks of the breeding season were unusually dry on North Rona, with the result that pools were extremely scarce and small throughout the breeding colony. About half way through the study period, the weather returned to normal wet conditions and pools became abundant. Female locomotion and behaviour were compared during consecutive periods where access to freshwater pools was first restricted and later unrestricted. The hypotheses were that females would travel further to water when pools were scarce, and that females travelling long distances to water would spend less time with their pups (Twiss *et al.*, 2000a).

8.3. Methodology

Female grey seals were observed on North Rona between September 29th 1998 and October 25th 1998, from a hide overlooking the study area. Females were watched continuously for periods of 50 minutes, which were separated by 10-minute breaks during which female positions were mapped; behavioural observations were not made during these breaks. Two groups of seals were observed (simultaneously on each day) over 16 days within a period of 26 days (a total of 172 man-hours). The two groups were within areas A and B (West Pools and Castle respectively (see chapter two, Figure 2.6)) and observations were carried out by two observers who alternated between groups each day. Individual females were identified from pelage markings and scars (see chapters two and three) and female identity, time, distance moved and reason for locomotion (where it could be determined) were recorded each time a female was seen moving within the study area. The start and finish of each observed suckling bout was also recorded.

Two distinct distance categories for locomotion were recorded: long-distance and short-distance. 'Long-distance' locomotion consisted of movement over a distance greater than ten adult body lengths (approximately 20m) – this cut-off point was used as it was the maximum distance a female could travel and still remain within the study group location. Long-distance locomotion generally involved movement of the mother between the pup and the sea or a pool outside of the study group location (females were observed returning from these excursions with wet pelage). 'Short-distance' locomotion was typically less than two adult body lengths, although movements up to five adult body lengths were recorded. In the majority of cases, a cause was identified for these movements. Therefore short-distance locomotion was classified further - as movement made in relation to local pools, or as movement related to interactions between conspecifics. Maternal behaviour was measured as the number of suckling bouts observed per female each day, and the percentage of time each female spent in close proximity (within two adult body lengths) to her pup.

8.4. Data analysis

For each observation day, females were included in the data set if they conformed to the following criteria:

1) The female was individually identifiable, so that if she travelled to the sea she could be recognised on her return. Very few females were excluded by this criterion alone.
2) The female was observable for the duration of the observation period. Mothers that travelled to the sea were also included in the data set if their pup remained within the study group location, as this allowed information to be obtained about the length of time each female left her pup unattended.
3) The female had a live pup. Females that remained in the study group after their pup had died were excluded from the data set as they were not subject to the same constraints as lactating females.
4) The pup was not born during the observation period. These females were omitted from the day's data set as they frequently spent a prolonged period of time in behaviours associated with birth.

8.4.1. Calculation of locomotion rate and suckling rate

To compensate for differences in observation effort per day, the rate of locomotion for each female was standardised as a rate per hour. For each female fulfilling the four criteria above, the rate of locomotion per observation hour (L) was calculated using equation 8.1.

$$L = (n/t)*60 \qquad \text{Equation 8.1}$$

Where

n = number of movements observed, and

t = length of observation period in minutes.

L was calculated separately for four categories of locomotion:

1) long distance (>10 adult body lengths)
2) short distance to local pools
3) short distance in relation to interactions between conspecifics
4) total locomotion in relation to water (1 and 2 combined).

The number of suckling bouts observed for each female was also calculated as a rate per observation hour and the amount of time spent in close proximity to the pup was calculated as a percentage of the observation period.

8.4.2. Tests of observer and location difference

As the main aim of this work involved looking at the difference in locomotion between the dry and wet periods, the data was first divided into two groups (see Appendix 8.1 for a more detailed description of weather conditions during the study period):

1) Observations during the dry period (30th September to 7th October, n = 6 observation days). Little or no rain fell on each observation day and there were few pools within the study areas; mean daily temperature averaged 11.1°C (range = 10.1°C to 11.9°C).
2) Observations during the wet period (10th October to 25th October, n = 10 observation days). Showers, often frequent and heavy, occurred on each observation day and pools were numerous; mean daily temperature averaged 8.1°C (range = 5.0°C to 11.5°C).

These two time periods were tested separately for observer and location differences. Data were not normally distributed and were therefore analysed using non-parametric tests. The statistics package Minitab for windows (version 10.5) was used for all analyses, except for Wilcoxon signed-ranks tests, which were performed according to Sokal and Rohlf (1995).

A series of Mann-Whitney U tests were used to test the standardised data sets collected by each observer. There were no significant differences between the two observers for any category of locomotion, for the number of suckling bouts per hour or for the percentage time spent near to the pup. Therefore the results from the two observers were pooled.

Differences between the two study locations were also tested using Mann-Whitney U tests. There were no significant differences within any category during the wet period. However, during the dry period, the rate of movement (per hour) to local pools was different at the two study group locations (Mann-Whitney U test: $U = 87$, $n_1 = 12$, $n_2 = 6$, $p = 0.008$). This can be explained by differences in the availability of local pools in each study group location. In area A (West Pools), two small pools were present throughout the dry period and each female was within 10 adult body lengths of one of these pools. In area B (Castle), there were no pools within the study group location. However, a few females at the eastern edge of this area could travel to pools that were within 10 adult body lengths. Movement to pools by the rest of females in area B involved travelling a greater distance and was classified as long distance locomotion. All other categories of locomotion were similar in the two study locations during the dry period. Data were not pooled for data sets pertaining to the rate of locomotion to local pools, although data were pooled for all other categories of locomotion. Suckling bout rate (per hour) and the percentage time spent with the pup were not significantly affected by study location, and these data sets were also pooled.

8.4.3. Pseudoreplication

The manner in which observations were conducted resulted in many females being observed over a number of consecutive observation days. To avoid the problem of pseudoreplication and obtain independence, the average rate (per hour) for each behavioural category (four categories for locomotion, one for suckling behaviour and one for time spent in close proximity to pup) was calculated for each female over the period in which she was observed. Before this was done, a series of Friedman tests for randomised blocks were performed for each category using a sub-sample of females that were present over a period of several days. The tests indicated that individual day within the dry period or the wet period did not significantly affect behaviour for these females.

8.4.4. Statistical comparisons and analyses

The process described above, resulted in a data set with n=19 females during the dry period and n=34 females during the wet period. Of these, eight females were present in both the dry and wet periods. The behavioural categories for these eight females were analysed separately using Wilcoxon signed-ranks tests to compare paired data (dry period vs wet period for each female). The remaining data, which consisted of independent data points (n = 11 females during the dry period and n = 26 females in the wet period), were analysed using Mann Whitney U tests (dry period vs wet period).

8.5. Results

8.5.1. Affect of pup age on behaviour

Due to the temporal scale on which behaviour was observed, it was considered possible that an increase in pup age between the dry and wet periods may have influenced behaviour rather than the absence or presence of pools. During the dry period at the start of the breeding season, all pups were classified as stage 'I' or 'II', whereas during the wet period, pups were classified as stages 'I' through to 'V' (see Appendix 2.2). As data were not normally distributed, a general linear model could not be used to determine whether pup age had a greater effect on behaviour than the availability of water. Therefore female behaviour according to pup age, was compared for each of the dry and wet periods using a series of Kruskal-Wallis tests. Pup age was not found to significantly affect locomotory behaviour of any kind, or the time spent in close proximity to the pup. However, older pups had a greater median number of suckling bouts than younger pups during the wet period (Kruskal-Wallis test: $H_4 = 19.86$, $p = 0.001$).

8.5.2. Long distance locomotion

Females made more long-distance movements per hour during the dry period than during the wet period (Mann-Whitney U test: $U = 320$, $n_1 = 11$, $n_2 = 26$, $p < 0.001$, Table 8.1). There were insufficient data to perform the Wilcoxon signed-ranks test for females that were present during both the dry and wet periods because four females did not make any long distance movements. The remaining females made long-distance movements during the dry period but not during the wet period.

In total, 13 out of 19 mothers travelled >20m at least once during the dry period, with six mothers travelling in excess of 200m to the sea on one or more of their trips. During the wet period, three out of 34 mothers made long-distance movements. Only one of these females did so on a regular basis, travelling to a pool within one of the study group locations from an outlying area that had no pool until late in the wet period. None of the mothers travelled between their pup and the sea when pools were abundant.

8.5.3. Short-distance locomotion to local pools

Location influenced the median rate of locomotion towards local pools, therefore the two study groups could not be pooled. In area A (where all females were within 10 adult body lengths of a pool during the dry period) the median rate of locomotion to pools was significantly lower in the wet period than in the dry period (Mann-Whitney U test: $U = 25$, $n_1 = 2$, $n_2 = 11$, $p = 0.035$, Table 8.1). N_1 was very small as most of the females present in area A during the dry period were included in the subset of females present during both the dry and wet periods. In area B (where a few females were within 10 adult body lengths of a pool during the dry period) there was no significant difference between the median rates of locomotion to local pools during the dry and wet periods (Mann-Whitney U test: $U = 82.5$, $n_1 = 9$, $n_2 = 14$, NS, Table 8.1). It is likely that the result obtained in area B was confounded by the fact that, during the dry period, most females in this group had to travel more than 10 adult body lengths (classified as long-distance locomotion) to their closest pool.
There were insufficient data to perform Wilcoxon signed-ranks tests for females that were present during both the dry and wet periods after the two study groups had been separated.

8.5.4. Short-distance locomotion due to interactions between conspecifics

The median rate of locomotion due to interactions between conspecifics was significantly greater during the dry period than during the wet period (Mann-Whitney U test: $U = 283$, $n_1 = 11$, $n_2 = 26$, $p = 0.012$, Table 8.1). This was also the case with females that were observed in both the dry and wet periods (Wilcoxon signed-ranks test: T = 2, n = 8, p = 0.025, Table 8.2).

8.5.5. *Total locomotion to water*

The median rate of locomotion to water (long distance and to local pools combined) was significantly greater during the dry period than during the wet period (Mann-Whitney U test: U = 293, n_1 = 11, n_2 = 26, p = 0.005, Table 8.1). Females that were present during both the dry and the wet period also showed a significantly greater median rate of locomotion to water during the dry period (Wilcoxon signed-ranks test: T = 3, n = 8, p = 0.025, Table 8.2).

8.5.6. *Time spent with pup*

Females spent significantly more time in close proximity to their pups (within two adult body lengths) during the wet period than during the dry period (Mann-Whitney U test: U = 108.5, n_1 = 11, n_2 = 25, $p < 0.001$, Table 8.1). When pools were scarce, females spent a median of 40% of their time in close proximity to their pups. This increased to a median of 100% of their time when pools were abundant. Females that were present during both the dry and wet periods also spent significantly more time close to their pups during the wet period than during the dry period (Wilcoxon signed-ranks test: T = 0, n = 8, p = 0.01; medians = 81% and 97% for dry and wet periods respectively, Table 8.2).

8.5.7. *Suckling rate*

The median number of suckling bouts per hour was the same during the dry and wet period (Mann-Whitney U test: U = 194.5, n_1 = 11, n_2 = 24, NS, Table 8.1). Females that were present during both periods also showed no significant difference in the median number of suckling bouts per hour (Wilcoxon signed-ranks test: T = 13, n = 8, NS, Table 8.2).

Table 8.1. Summary and comparison of frequencies of locomotion (rate per hour), suckling bouts (rate per hour) and time spent with pup for lactating female grey seals during dry and wet periods. Each female was observed either in the dry period or in the wet period, not both.

	Dry		Wet		Difference between dry and wet periods[1]
	median	Q1-Q3	median	Q1-Q3	
Long-distance locomotion (rate per hour)					
	0.20 (n = 11)	0.13-0.27	0.0 (n = 26)	0-0	P<0.001
Short-distance locomotion (rate per hour)					
To local pools (area A)	0.33 (n = 2)		0.06 (n = 11)[a]	0-0.13	P=0.035
To local pools (area B)	0.0 (n = 9)	0-0.07	0.11 (n = 14)[a]	0-0.14	NS
Interactions with conspecifics	0.21 (n = 11)	0.07-0.27	0.04 (n = 26)	0-0.13	P=0.012
Total locomotion to water (rate per hour)					
Long distance and to local pools combined	0.27 (n = 11)	0.14-0.44	0.11 (n = 26)	0-0.14	P=0.005
Pup Attendance					
Number of suckling bouts per hour	0.14 (n = 11)	0-0.24	0.13 (n = 24)[b]	0-0.33	NS
Time spent with pup (%)	40% (n = 11)	26-82	100% (n = 25)[c]	97-100	P<0.001

[1] Differences tested by Mann-Whitney U.

[a] one female was omitted from the data set because she moved from area A to area B during the wet period.

[b] two females were omitted from the data set – one female was partially hidden from view which meant suckling was difficult to observe; the other adopted a second pup.

[c] one female was omitted from the data set because she adopted a second pup.

Rates were calculated for each female over the total time period in which she was observed. Note: area A had two pools throughout the dry period whilst area B only had pools near its eastern edge during the dry period. N = number of females. 'Area' had no significant effect on locomotion rates (other than to local pool), number of suckling bouts per hour or time spent with pup, so areas were pooled for all other comparisons between the dry and wet periods.

Table 8.2. Summary and comparison of frequencies of locomotion (rate per hour), suckling bouts (rate per hour) and time spent with pup for lactating female grey seals observed during dry and wet periods. Each female was observed in both periods.

	Dry		Wet		Difference between dry and wet periods[1]
	median	Q1-Q3	median	Q1-Q3	
Long-distance locomotion (rate per hour)					
	0.07 (n = 8)	0-0.19	0.0 (n = 8)	0-0	Insufficient data due to ties
Short-distance locomotion (rate per hour)					
To local pools (area A)	0.08 (n = 5)	0.05-0.20	0.0 (n = 5)	0-0.04	Insufficient data due to low 'n'
To local pools (area B)	0.0 (n = 3)	0-0.20	0.04 (n = 3)	0-0.08	Insufficient data due to low 'n'
Interactions with conspecifics	0.21 (n = 8)	0.10-0.25	0.06 (n = 8)	0.03-0.11	P=0.025
Total locomotion to water (rate per hour)					
Long distance and to local pools combined	0.24 (n = 8)	0.06-0.33	0.03 (n = 8)	0-0.07	P=0.025
Pup Attendance					
Number of suckling bouts per hour	0.20 (n = 8)	0.14-0.38	0.24 (n = 8)	0.17-0.42	NS
Time spent with pup (%)	81 (n = 8)	66-92	97 (n = 8)	93-100	P<0.01

[1] Differences tested using Wilcoxon signed-ranks test.

Rates were calculated for each female over the total time period in which she was observed. Note: area A had two pools throughout the dry period whilst area B only had pools near its eastern edge during the dry period. N = number of females. 'Area' had no significant effect on locomotion rates (other than to local pool), number of suckling bouts per hour or time spent with pup, so areas were pooled for all other comparisons between the dry and wet periods.

8.6. Discussion

The availability of pools of water has been suggested as an important factor in determining the distribution of females within grey seal breeding colonies (Boyd *et al.*, 1962; Anderson *et al.*, 1975; Pomeroy *et al.*, 2000a; Twiss *et al.*, 2000a). Here, evidence is provided for a direct link between the spatial and temporal availability of pools and daily maternal behaviour patterns. Female grey seals on North Rona moved more frequently, travelled greater distances and left their pups unattended for longer periods of time when pools were scarce.

Long-distance locomotion and short duration pup attendance are potentially costly for female grey seals at inland sites, as such behaviour may affect their chances of raising a pup to weaning successfully. Both behaviours may increase the risk of permanent mother/pup separation, which results in starvation of the pup (Anderson *et al.*, 1979; Baker & Baker, 1988; Pomeroy *et al.*, 1994). Furthermore, unprotected pups are prone to attacks from adult conspecifics, and bites and trauma may lead to fatalities, either from the injuries themselves, or from infection of the wounds (Anderson *et al.*, 1979; Baker & Baker, 1988). Infection and starvation are the major causes of pup mortality on North Rona (Baker, 1984, 1988) and at least one of the pups in this study died after its mother travelled to the sea, because the two failed to reunite on her return. As grey seals on North Rona normally fast during lactation, it is assumed that this female travelled to the sea because inland pools were scarce at the beginning of the breeding season. During a typical season on North Rona, most females give birth within a few metres of a pool, and long-distance movements are unusual. Females generally remain within 10m of their pupping site (Aust, unpublished data; see also chapter six in this thesis) and rarely travel to the sea during lactation (Pomeroy *et al.* (1994) recorded only three instances of lactating females travelling to the sea between 1985 and 1989). During the present study, females did not travel to the sea during the wet period when pools were abundant, and most movements took them less than five adult body lengths (approximately 10m) from their pup.

Other studies provide evidence that long distance movement is costly for lactating grey seals. Pomeroy *et al.* (1994) observed female movement for 140 lactating females on North Rona between 1985 and 1989. They recorded 17 instances of a female moving more than 20m from her pup, with nine cases of permanent mother/pup separation. At this colony, greater black-backed gulls (*Larus marinus*) attack young pups, particularly when mothers fail to protect them, and the injuries that gulls inflict can result in pup mortality (personal observations; Seddon *et al.*, 1998). Long-distance locomotion also affects the female directly, as movement through the colony leads to increased harassment from males and a greater number of aggressive interactions with conspecifics (Caudron, 1998; Twiss *et al.*, 2000a). The energy used for locomotion and during interactions is effectively 'lost', and this may have repercussions on the weaning weight of the pup or the extent to which a female depletes her body reserves. Whereas the former may affect the pup's chances of survival, the latter may influence whether or not the female has a pup the following year (Pomeroy *et al.*, 1999). Therefore, the distances that these females travelled in order to obtain access to water, and the potential costs of their behaviour, suggest that water is of critical importance during lactation.

There are two major reasons why water may be important for lactating grey seals – firstly, to maintain a positive water balance and secondly, as an aid to thermoregulation. Grey seals must conserve water during the breeding season as they can spend around 20 days ashore during lactation without eating. Fasting phocids are believed to obtain all their water requirements from the metabolism of fat reserves (Irving *et al.*, 1935; Ridgway, 1972 and references therein; Ortiz *et al.*, 1978), at the same time utilising physiological adaptations to help conserve water (Huntley *et al.*, 1984; Folkow & Blix, 1987; Baker, 1990; Reilly, 1991; Skog & Folkow, 1994). Evidence from a study on grey seals breeding on Sable Island, Canada, suggests that fat metabolism provides enough water to meet the added demands of milk production (Schweigert, 1993). However, Reilly *et al.* (1996) showed that female grey seals on North Rona undergo a negative water balance whilst feeding pups. Furthermore, lactating females have been observed drinking from fresh water pools at this colony (personal observations, this study; Reilly *et al.*, 1996). The mean daily temperature at Sable Island during the January breeding season is around 7°C colder than the mean daily temperature during the autumnal breeding season on North Rona[1]. This is a conservative comparison as wind chill and the longer nights at Sable Island probably

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[1] Sable Island data obtained from http://www.wunderground.com/global/stations/71600.html; North Rona data obtained from the British Atmospheric Data Centre (http://www.badc.rl.ac.uk/)

produce a much harsher temperature regime than in the UK. It is likely that this temperature difference between the breeding seasons at the two colonies is sufficient to affect the water requirements of lactating seals.

In addition to their physiological adaptations, pinnipeds can alter their behaviour to help conserve water and aid thermoregulation. Phocids often spend large periods of time apparently asleep (up to 80% of the time in female grey seals (Anderson & Harwood, 1985; Twiss *et al.*, 2000a)) and during sleep, apneustic breathing reduces oxygen consumption, and hence heat output, by as much as 50% (Worthy, 1987; Boily & Lavigne, 1996). Grey seals at inland colonies also regularly spend long periods of time immersed in pools (personal observations, this study, but see Hewer, 1960; Twiss *et al.*, 2000a for examples at other sites). This behaviour is common as a method of thermoregulation for pinnipeds in warmer climates (e.g. Gentry, 1973; Campagna & Le Boeuf, 1988) and even fur seals move into water if the ambient temperature gets too warm (Bartholomew & Wilkie, 1956; McCann, 1980). In this study, mothers only made trips to the sea when pools were scarce during the dry period, and although it is physiologically possible for phocids to restore their water balance by mariposia (Reilly, 1991), it is likely that they travelled to the sea for thermoregulation. Fresh water pools may serve a dual purpose, providing an important source of water for restoring water balance, as well as being used for thermoregulation.

Although the thermoneutral zone of adult grey seals has not been measured, in pups it extends from around -7°C to 23°C (Hansen & Lavigne, 1997). The upper critical temperature would appear to be substantially higher than the mean daily temperatures experienced by the animals on North Rona during our study. However, these laboratory measurements do not take into account the affects of solar radiation, which can have a major impact on thermal balance (Watts 1992). In addition, the heat output of lactating grey seals has been calculated to be about 2.3 times BMR (Reilly *et al.,* 1996). Therefore, the unique combination of heat output due to lactation, an unusually warm and sunny start to the breeding season and limited availability of water once ashore may have brought the females in this study close to their upper thermal limit, producing the stimulus to seek water.

The fact that lactating grey seals require access to water gives rise to a situation where the habitat contains a defendable resource. Higher rates of movement in relation to conspecifics during the dry period may arise due to competition for pools, whilst variation between animals is likely to reflect their ability to monopolise such resources. Although the number of suckling bouts observed for each female was not significantly affected by pool availability, time spent with the pup and long-distance locomotion were highly variable. These results demonstrate that all females fulfilled the essential demands of nursing their pup, even when restricted access to water meant they spent less time close to their offspring. As a result, differences in pup attendance may not lead to detectable differences in pup growth rates. However, they might be reflected by chance events, which lead to mother/pup separation, pup injury and even death.

Appendix 8.1. Precipitation strength, frequency and mean air temperature for observation days at North Rona during the 1998 breeding season

Observation day	Precipitation strength[1]	Precipitation frequency[1]	Mean Temperature[2] (°C)	Abundance of pools[1]
30th September 1998	Light	Occasional	11.9	Area A = Scarce Area B = None
1st October 1998	Dry	None	11.7	Area A = Scarce Area B = None
2nd October 1998	Dry	None	11.6	Area A = Scarce Area B = None
4th October 1998	Light	Occasional	10.1	Area A = Scarce Area B = None
6th October 1998	Dry	None	10.9	Area A = Scarce Area B = None
7th October 1998	Dry	None	10.5	Area A = Scarce Area B = None
10th October 1998	Moderate	Occasional	8.7	Water present in most hollows
11th October 1998	Heavy	Frequent	8.7	Water present in most hollows
13th October 1998	Moderate	Constant	10.1	All hollows filled – pools abundant
15th October 1998	Light	Occasional	7.2	Abundant
17th October 1998	Moderate	Occasional	5.2	Abundant
19th October 1998	Moderate	Occasional	5.0	Abundant
21st October 1998	Moderate	Frequent	11.5	Abundant
22nd October 1998	Heavy	Frequent	10.6	Abundant
24th October 1998	Moderate	Constant	8.3	Abundant
25th October 1998	Moderate	Occasional	5.6	Abundant

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[1] Precipitation frequency, precipitation strength and pool abundance obtained from field observations.

[2] Mean temperature calculated as the average of hourly values over 24 hours. Data obtained, with permission from the British Atmospheric Data Centre, from a weather station on North Rona.

Chapter Nine:

The affects of maternal attendance pattern and pup age on aggressive interactions of lactating grey seals

Behavioural data included in this chapter was collected by P. Redman, P. Pomeroy and R. Harcourt

9.1. Summary

Female aggression towards other females, males, alien pups and gulls was recorded during the breeding seasons of 1998-2000 for study groups on North Rona and the Isle of May. The rate of female/female aggressive interactions was around three times greater on the Isle of May than on North Rona but the rate of female/male and female/pup interactions were not significantly different between the two colonies. It was not possible to determine whether hierarchies were present between females, but pooling of data allowed the affect of pup stage on aggressive behaviour to be examined. The frequencies of female/pup and female/male interactions were not influenced by pup stage at either colony, but it is possible that the sample sizes were too small to detect a difference. The frequency of female/female interactions was affected by pup stage on North Rona but not on the Isle of May. On North Rona females with young pups initiated more interactions than females with progressively older pups. It is hypothesised that this decrease in aggression occurred through conflict reduction between familiar neighbours – familiarity occurring because females on North Rona remained close to their pup, and hence one another, throughout lactation. It is thought that conflict reduction did not occur on the Isle of May because females frequently travelled to and from their pup to a tidal pool and therefore did not become familiar with their neighbours.

9.2. Introduction

It is common for females of many species to show increased aggression after giving birth and throughout lactation (e.g. Wilson & Boelkins, 1970; Koskela *et al.,* 2000; Maestripieri & Megna, 2000; Figler *et al.*, 2001), and as a result, female aggression is often considered a means of offspring protection (e.g. Wolff & Schauber, 1996; Koskela *et al.,* 2000). However, aggression between conspecifics may have many functions, such as food or territory defence (Koskela *et al.*, 1997), maintenance of a dominance hierarchy (Clutton-Brock & Albon, 1979; McCann, 1981; Twiss *et al.,* 1998; Cote, 2000) or as a means of promoting offspring dispersal (Jones, 1980; Wiggett & Boag, 1989; Pope, 1998). Consequently, offspring protection may not always be the primary cause of maternal aggression (Maestripieri, 1992; Koskela *et al.*, 1997; Cote, 2000).

Agonistic interactions are commonly observed between adult pinnipeds (e.g. grey seals (Boyd *et al.*, 1962; Hewer, 1974; Kovacs, 1987); Northern elephant seals (Ribic, 1988); South American fur seals, *Arctocephalus australis*, (Harcourt, 1992)), especially during the breeding season. As pinnipeds come ashore to breed, it is often assumed that female aggression is related to pup protection rather than defence of a territory that provides food. This theory may be further supported by the fact that females are highly aggregated when they are pregnant at the breeding colony, but are more regularly spaced when they have pups. Furthermore, females initiate and win more aggressive interactions after their pup is born (Christenson & Le Boeuf, 1978; Boness *et al.*, 1982; McCann, 1982; Ribic, 1988; Harcourt, 1992) and aggression occurs more frequently when pups are close to their mother (Fogden, 1971; Francis, 1987, as cited in Maestripieri, 1992) or when densities are high (Hewer, 1974). Within grey seal colonies, female aggression also varies with the sex of the approaching animal. Males are threatened more than females, they are threatened regardless of pup position, and they are more likely to invoke fighting from the female (Boness *et al.*, 1982; Kovacs, 1987). In contrast, threats towards females are more common when the pup is between its mother and an approaching female, and female/female interactions rarely involve fighting (Fogden, 1971; Boness *et al.*, 1982; Kovacs, 1987). These findings have led to the suggestion that there are other reasons for female aggression, such as spacing and mate choice (Cox & LeBoeuf, 1977; Boness *et al.*, 1982).

Female grey seals show a wide range of agonistic behaviours, ranging from open mouth displays, erect whiskers and vocalisations, to lunging, biting and chasing. However, they use low intensity threats more frequently than high intensity threats and actually spend very little time involved in agonistic behaviour (Kovacs, 1987). This might indicate an evolution of female agonistic behaviour to reduce physical conflict, possibly related to the formation of non-random associations within and between years (Michod, 1999; see also chapter six). Low intensity threats could act as warning signals between individuals to prevent full-scale fights, which are energetically costly and may result in injury either to the female, her pup, or both. If female aggression serves to defend resources (e.g. pools) as well as to protect the pup, quantification and characterisation of these interactions might also allow determination of whether the dominance hierarchies seen amongst male grey seals (Twiss *et al.,* 1998) also exist between females of this species.

9.2.1. Aims

The aim of this work was to investigate the frequency and intensity of aggressive interactions initiated by female grey seals at two contrasting breeding colonies – North Rona, where females generally remain with their pup throughout lactation, and the Isle of May, where many females travel between their pup and tidal pools – to determine the possible functions of female aggression at these two sites.

9.3. Methodology

9.3.1. Behavioural observations

Behavioural observations were recorded from a hide overlooking the study area using binoculars to identify individual females. The animals were observed continuously for periods of 50 minutes, which were separated by 10-minute breaks during which female positions were mapped; observations were not made during these 10-minute breaks. For each aggressive interaction observed, the following information was recorded:

1. time of interaction
2. initiator (individual identity of female initiating aggressive interaction (if known))
3. recipient (female (including identity if known), male, pup or gull). NB: recipient pups were not the female's own pup
4. age of pup of initiator (if pup present)
5. type of aggression observed (e.g. open mouth threat (omt), flippering, lunge, chase, bite – see Appendix 9.1), from which the level of aggression was determined
6. observed outcome

The interactions between females were examined for possible dominance hierarchies using the UNIX based FORTRAN program 'DOMTIES' which assigns cardinal dominance indices based on the method described by Boyd and Silk (1983). This program was used as it can determine dominance ranks even where draws and ambiguous relationships are recorded between individuals. It has been used previously to determine dominance hierarchies between male grey seals (Twiss *et al.*, 1998). For each female/female interaction observed in this study, a clear outcome that allowed winner/loser status to be assigned was recorded if one female displaced the other by one or more body lengths; if neither female was displaced, the outcome was recorded as a draw. Successive interactions between the same pair of animals were recorded as a single interaction if they were separated by less than one minute and there was no discernable change in the behaviour of either individual between interactions.

To compare the frequency of aggression at the two study colonies, an overall rate of aggression (per hour) was calculated for each colony using Equation 9.1.

$$A = I/(n \times t) \qquad \text{Equation 9.1}$$

Where

A = rate of aggressive interactions (per female per hour)

I = total number of aggressive interactions observed

n = median number of females observed per day

t = total observation time (in hours)

The rate of aggression was calculated for each category of interaction, i.e. female/female, female/male, female/pup and female/gull interactions.

9.3.2. Duration of pup stages and the affect of pup age on the frequency of female aggression

The average duration of each pup stage (for stages I-III) was calculated using data from known females on North Rona to compensate for the fact that the two to six week study periods did not allow every female to be observed for her entire lactation period. Most females left when their pup was a stage IV, so the duration of this stage was only recorded up to female departure. Taking the average period of lactation to be 18 days (Bonner, 1972; see also chapter five), the length of time that females were present whilst their pup was a stage IV (t_{IV}) was calculated using Equation 9.2.

$$t_{IV} = T - (t_I + t_{II} + t_{III}) \qquad \text{Equation 9.2}$$

where:

T = total period of lactation (18 days)

t_I = average time as stage I

t_{II} = average time as stage II

t_{III} = average time as stage III

9.3.3. Levels of aggression

As mentioned in section 9.3.1, the type of aggressive behaviour observed during each aggressive encounter was also recorded (i.e. omt, flippering, lunge, bite, chase). Omt and brief flippering bouts were categorised as low level aggression, prolonged omt and flippering bouts (combined behaviours) and lunging were categorised as mid level aggression and interactions than involved biting or chasing were categorised as high level aggression. The number of low, mid and high level aggressive interactions directed towards females, males, and pups were compared, using G-tests, at the two colonies in 1998 and 1999. Complete records of female/male and female/pup interactions were not collected in 2000 due to a change in the priorities for data collection in this year.

9.3.4. Data analysis

G tests and chi-square tests were carried out using the procedures in Fowler and Cohen (1992). Friedman tests and Kruskal-Wallis tests were carried out using Minitab for Windows, version 13.

9.4. Results

9.4.1. Duration of pup stages

The average duration of each pup stage was (to the nearest whole day) 2, 7 and 6 days for stages I, II and III respectively (Table 9.1). Using Equation 9.2 the duration of time that pups were classed as a stage IV was calculated as being 3 days. This encompassed the time during which the female was present with the pup on the colony, not the total length of time that the pup remained a stage IV. Stage V pups were not included in these analyses as most females left the colony before their pup was fully moulted.

Table 9.1. Table showing the number of days that pups were classified as stages I, II and III on North Rona.

	Stage I	Stage II	Stage III
Duration of stage			
1 day	1		
2 days	51		
3 days	6		2
4 days			1
5 days		11	5
6 days		13	11
7 days		14	6
8 days		16	6
9 days		8	2
Average duration	2.1 days	7.0 days	6.3 days

9.4.2. Frequency of aggressive interactions

A summary of the number and class of aggressive interactions (female/female, female/male, female/pup and female/gull) observed on North Rona and the Isle of May is presented in Table 9.2. The rate of each class of aggressive interaction (per female per hour) is presented in Table 9.3. The rate of female/female interactions was significantly greater on the Isle of May than on North Rona (Kruskal-Wallis: $H = 4.50$, d.f = 1, $p = 0.034$), but there was no significant difference between the rates of female/pup interactions (Kruskall-Wallis: $H = 3.16$, d.f. = 1, $p = 0.076$) or female/male interactions (Kruskal-Wallis: $H = 0.79$, d.f. = 1, $p = 0.374$) at the two colonies. The difference in the rates for female/pup interactions was close to significance, which may indicate that the sample size was too small for a significant difference to be detected.

Table 9.2. Summary of the number of aggressive interactions observed on North Rona and the Isle of May. The number of female/female interactions where both females were identified and one was assigned as a clear winner is given in brackets. A change in priorities for data collection in 2000 meant that only female/female interactions were recorded in this year.

	Hours observed	Median no. of females	Female/female interactions	Female/male interactions	Female/pup interactions	Female/gull interactions
North Rona						
1998	180	8	250 (35)	225	36	57
1999	184	15	409 (65)	277	27	Not recorded
2000	231	12	316 (27)	Not recorded	Not recorded	Not recorded
Isle of May						
West Rona Beach						
1998	67	9	141 (2)	90	45	0
Tarbet						
1998	41	8	145 (8)	58	21	0
1999	108	13	639 (34)	223	68	1
2000	45.5	10	164 (14)	Not recorded	Not recorded	Not recorded

Table 9.3. Rate of aggressive interactions per female per hour on North Rona and the Isle of May. Rate was calculated per female per hour, using the median number of females observed per day and the number of observation hours (see Table 9.2).

	Female/female	Female/male	Female/pup	Female/gull
North Rona				
1998	0.17	0.16	0.03	0.04
1999	0.15	0.10	0.01	Not recorded
2000	0.11	Not recorded	Not recorded	Not recorded
Isle of May				
West Rona Beach 1998	0.23	0.15	0.07	0
Tarbet 1998	0.44	0.18	0.07	0
Tarbet 1999	0.46	0.16	0.05	0.0007
Tarbet 2000	0.36	Not recorded	Not recorded	Not recorded

9.4.3. Female/female interactions

In total, 975 female/female interactions were observed on North Rona over the three field seasons; in 273 of these both females were identified, but a clear winner and loser was only assigned in 127 cases (see Table 9.2). For the Isle of May, 948 female/female interactions were recorded over three field seasons at Tarbet and 141 female/female interactions were recorded at West Rona Beach in 1998. Overall, 173 of these were between two identified females, but a clear winner was assigned in only 58 cases (Table 9.2). The maximum number of interactions between a specific pair of females where a clear winner could be defined was four, and the median was one. The low number of interactions between specific pairs of females and the difficulty in assigning winner/loser status meant that it was not possible to determine hierarchies amongst adult females using the data collected.

Data from each colony was pooled within each field season and analysed with respect to the pup stage of the female initiating the aggressive interaction using Friedman tests blocked by year. The number of attacks directed towards other females was related to pup stage on North Rona (Friedman test: S = 9.0, d.f = 3, p = 0.029, Table 9.4) with females that had younger pups showing more aggression than females with progressively older pups. There was no significant relationship with pup stage on the Isle of May (S = 7.4, d.f. = 3, p = 0.06, Table 9.4). Due to the variable weather conditions on North Rona in 1998 (chapter eight) and a short field season on the Isle of May in 2000, only the 1999 data was directly compared between the two colonies. The frequency of female/female interactions was corrected for the number of observation hours and the median number of study females at each colony and then analysed using a G-test. There was a significant difference in the distribution of female/female interactions between the two colonies (G-test: G = 78.9, d.f.= 3, p <0.001, Figure 9.1). Females with stage I pups initiated more aggression on North Rona than on the Isle of May but this decreased as pups got older; the rate of aggression on the Isle of May remained relatively constant and was therefore greater at this colony for females with stage II to IV pups.

Table 9.4. Number of female/female aggressive interactions observed on North Rona and the Isle of May in relation to pup stage. All values have been corrected for the duration of each pup stage using the values in Table 9.1.

	North Rona			Isle of May		
	1998	1999	2000	1998	1999	2000
Stage						
I	37.5	41.5	25	9.5	24.5	0.5
II	8.14	21.43	8.14	15.71	26.29	8.57
III	6.33	9.83	5.83	10.33	29.5	7.17
IV	1	3	0.33	4.67	20	4.33

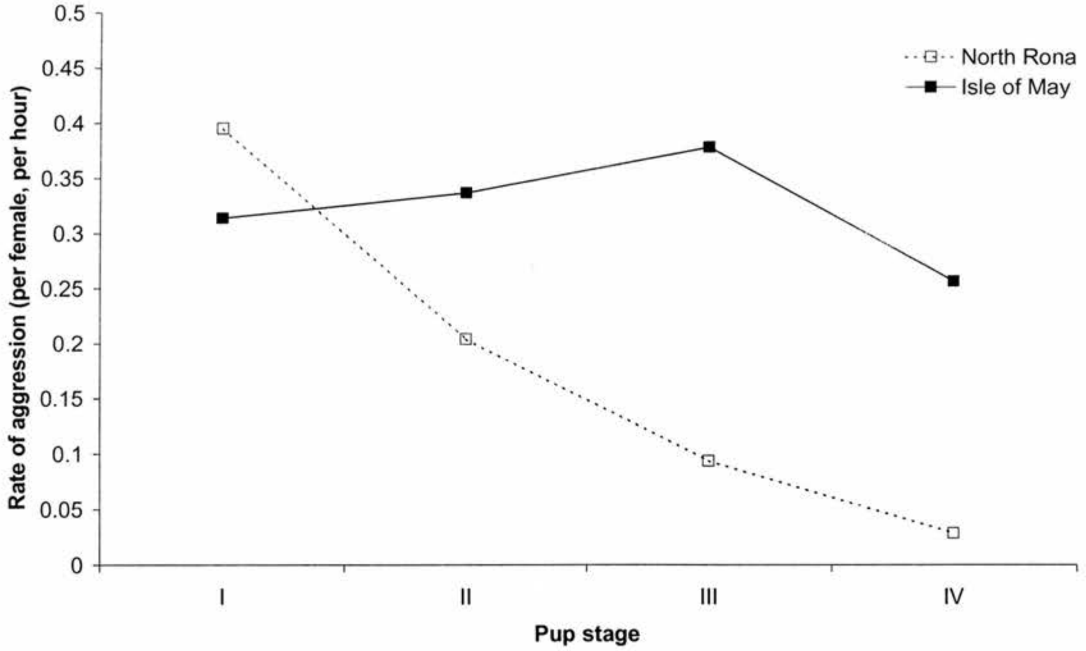

Figure 9.1. Rate of female/female aggression (per female, per hour) in relation to pup stage on North Rona and the Isle of May in 1999.

9.4.4. Aggression towards males

The number of attacks directed towards males was not significantly affected by the stage of a female's pup on either North Rona or the Isle of May (Friedman test: S = 4.89, d.f. = 3, p = 0.18 for North Rona, and S = 2.37, d.f. = 3, p = 0.50 for the Isle of May (Table 9.5)).

Table 9.5. Number of female/male aggressive interactions observed on North Rona and the Isle of May in relation to pup stage. All values have been corrected for the duration of each pup stage using the values in Table 9.1.

	North Rona		Isle of May	
	1998	1999	1998	1999
Stage				
I	23	12.5	1.5	3
II	9.29	13	6.86	7.29
III	7.33	12.5	6.17	12
IV	3	3.33	0.67	12

9.4.5. Aggression towards alien pups

The number of attacks directed towards alien pups was not significantly affected by the stage of a female's pup on either North Rona or the Isle of May (Friedman test: S = 4.20, d.f. = 3, p = 0.24 for North Rona, and S = 4.89, d.f. = 3, p = 0.18 for the Isle of May (Table 9.6)).

Table 9.6. Number of female/alien pup aggressive interactions observed on North Rona and the Isle of May in relation to pup stage. All values have been corrected for the duration of each pup stage using the values in Table 9.1.

	North Rona		Isle of May	
	1998	1999	1998	1999
Stage				
I	5.5	2.5	2	3
II	1.29	1.29	3.43	4.14
III	1.5	0	3	3.67
IV	0	0.33	3	2.33

9.4.6. Aggression towards gulls

The majority of aggression directed towards gulls on North Rona was initiated by females with very young pups or by females whose pups had died (Figure 9.2). When females with live pups were considered separately, pup stage significantly affected the number of attacks made towards gulls ($\chi^2 = 8.33$, $p < 0.01$, df = 1, Yates correction applied), with females with young pups initiating more attacks than females with older pups. Females with stage I and II pups were pooled for analysis as were females with stage III and IV pups because of the low number of expected interactions (Table 9.7).

Only one incident of aggression towards gulls was recorded on the Isle of May – this was by a female with a stage I pup (Table 9.7).

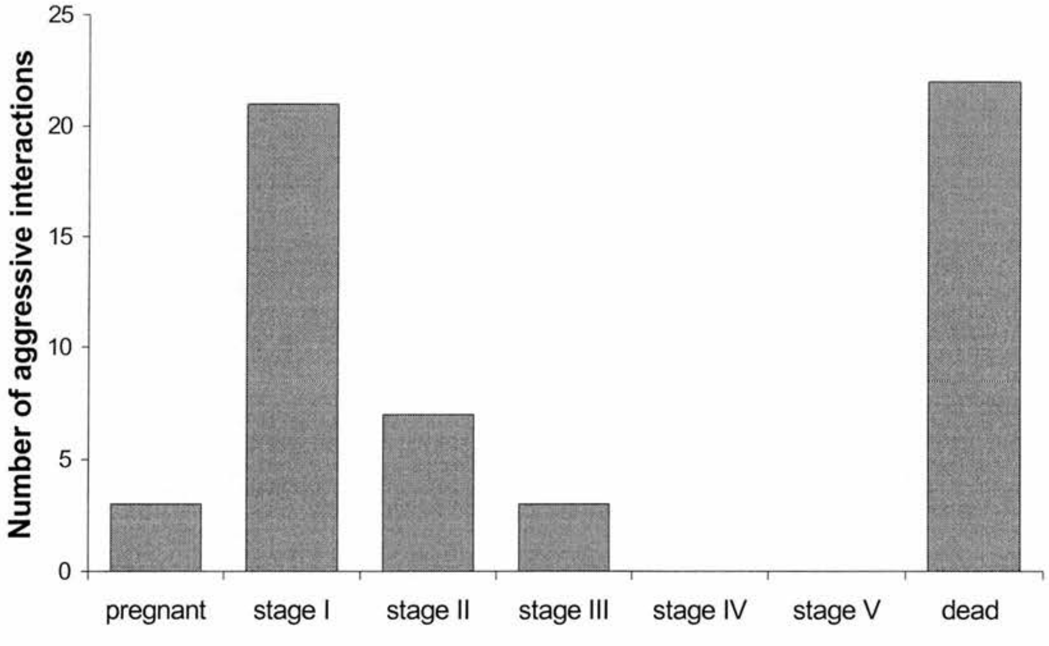

Figure 9.2. Number of aggressive interactions directed towards gulls by females on North Rona. This figure shows that the majority of interactions were initiated by females with stage I pups or dead pups.

Table 9.7. Number of female/gull aggressive interactions observed per day on North Rona and the Isle of May in relation to pup stage. All values have been corrected for the duration of each pup stage using the values in Table 9.1.

	North Rona	Isle of May
Stage I	10.5	0.5
Stage II	1	0
I & II pooled	**11.5**	
Stage III	0.5	0
Stage IV	0	0
III & IV pooled	**0.5**	

9.4.7. Quantification of aggression intensity

Table 9.8 shows the number of low, mid and high-intensity interactions directed towards females, males and pups at the two study colonies. Data from each colony was analysed separately in each year (1998 & 1999) to determine whether females showed different levels of aggression towards females, males and pups.

There was no significant difference in the proportion of low, mid and high-intensity aggression directed towards females and males within each year (see Table 9.9 for analyses), so aggression directed towards females and males was pooled within a year before being compared to aggression directed towards pups. There was a significant difference in the proportion of each type of aggression directed toward pups when compared to that directed toward adults. Most aggressive interactions directed toward adults were of low intensity, whilst at least 50% of the aggression directed toward pups was of high intensity (see Table 9.9 for analyses). Most aggressive encounters with gulls (North Rona 1998) involved lunging at birds that approached the pup (see Table 9.8).

Table 9.8. Number of low, mid and high-intensity aggressive interactions directed at conspecifics (and gulls) on North Rona and the Isle of May.

	Level of aggression		
Recipient	Low	Mid	High
North Rona 1998			
Females	205	32	19
Males	174	26	29
Pups	16	2	20
(Gulls	9	35	13)
North Rona 1999			
Females	333	57	20
Males	227	35	15
Pups	10	3	14
North Rona 2000			
Females	274	28	14
West Rona Beach, Isle of May 1998			
Females	127	8	6
Males	73	11	6
Pups	10	5	30
Tarbet, Isle of May 1998			
Females	112	22	11
Males	45	7	6
Pups	6	5	10
Tarbet, Isle of May 1999			
Females	495	44	47
Males	201	10	12
Pups	17	8	43
Tarbet, Isle of May 2000			
Females	142	15	7

Table 9.9. Table of results from G-tests that compared the relative proportion of low, mid and high-intensity aggression directed towards females, males and pups on North Rona and the Isle of May. These analyses were performed using the data presented in Table 9.8.

	G	df	P value
Comparisons of female/female and female/male intensities of aggression			
North Rona 1998	3.76	2	NS
North Rona 1999	0.30	2	NS
Tarbet, Isle of May 1998	0.65	2	NS
Tarbet, Isle of May 1999	4.73	2	NS
West Rona Beach, Isle of May 1998	3.89	2	NS
Comparisons of female/adult and female/pup intensities of aggression			
North Rona 1998	38.57	2	<0.01
North Rona 1999	43.87	2	<0.01
Tarbet, Isle of May 1998	24.00	2	<0.01
Tarbet, Isle of May 1999	131.26	2	<0.01
West Rona Beach, Isle of May 1998	90.24	2	<0.01

9.5. Discussion

In this study, the aggressive behaviour of grey seal mothers on North Rona and the Isle of May was compared. The females studied on North Rona gave birth far inland, aggregated near small pools of water and tended to remain close to their pup throughout lactation. By contrast the females studied on the Isle of May spent long periods of time in large tidal pools leaving their pups unattended for several hours at a time. The results showed that mothers on North Rona were involved in fewer aggressive interactions with conspecifics than mothers on the Isle of May. This occurred predominantly through a reduced frequency of female/female aggression, as the rates of female/male interactions and female/pup interactions were not significantly different between the two colonies. The following sections look at these findings in more detail and discuss the possible functions of maternal aggression at these two sites.

9.5.1. Aggression towards females

During each breeding season on North Rona (1998-2000), females with young pups initiated more aggressive interactions towards other females than females with progressively older pups. There are two possible explanations for this. Firstly, the frequency of female/female interactions might have decreased because females became accustomed to one another and therefore were more tolerant of one another with time. This could occur through the development of a dominance hierarchy (albeit a temporary one) or through mutual conflict reduction (Michod, 1999). It was not possible to determine whether dominance hierarchies existed at these colonies due to the low number of interactions between females and the high proportion of outcomes that were counted as ties. This suggests that either there were no hierarchies, or that signals that indicated the relative dominance between a pair of females were too subtle to be observed. It is possible that conflict reduction might be expressed by subordinate females simply getting out of the way of dominant females, in which case there would be no conflict to observe. The second possible explanation for the lower frequency of aggression of females with older pups is that the frequency of aggression reflected the vulnerability of the pup. Aggression therefore decreased as the pup became more able to defend itself or was able to move away from conspecifics that threatened it (Giovenardi *et al.*, 2000).

The frequency of aggression between females on the Isle of May was not affected by the age of the initiator's pup - this agrees with previous findings at this colony (Kovacs 1987). The results from this island were very variable between years and it is difficult to say whether this was due to variation between the seasons or was an artefact of the different observation procedures in each year. The results obtained in 1998 might have been affected by the fact that two study sites (West Rona Beach and Tarbet) were observed in this year. Observations were made alternately at these two sites but observation days were interspersed by days working in the colony, resulting in patchy data collection in 1998. In addition, many females that gave birth at Tarbet did so later in the season than at West Rona Beach (Pomeroy *et al.*, 2000a), which might have resulted in a disproportional representation of younger or older stage classes at one site compared to the other. In 2000, the study period on the Isle of May only encompassed two weeks at the end of the breeding season so fewer young pups were present. Therefore, the data collected on the Isle of May in 1999 was probably the most representative of female behaviour during the breeding season at this colony.

Female aggression is also known to be associated with female movement at grey seal breeding colonies (Caudron, 1998; Twiss *et al.,* 2000a), with females interacting only with others that come within 2-3 body lengths (S.D. Twiss, pers. com.). The fact that many females regularly travelled between their pup and a tidal pool on the Isle of May could therefore explain why females at this colony were involved in around three times as many aggressive interactions as females on North Rona. This movement might also provide some explanation as to why pup age did not affect female/female interactions on the Isle of May. Mothers on the Isle of May came into contact with large numbers of other females, with whom they were unfamiliar, either near their pup, whilst moving between their pup and the pool, or when they were in pools. Consequently, females spent very little time close to specific individuals and were less likely to become accustomed to their neighbours, unlike females that remained close to their pup on North Rona. The decrease in aggression with pup age on North Rona may therefore indicate that conflict reduction occurred at this colony due to the familiarity between neighbours. Females at Tarbet on the Isle of May were also frequently observed in aggressive encounters at the edge of the pool as one female tried to enter the water where other females were already present. In most cases the pups of these females were not located close to the pool. This shows that not all of the aggressive behaviour observed on the Isle of May was related to pup protection, and suggests that aggression might have a role in resource defence (with the resource being access to water).

9.5.2. Aggression towards males

Female/male interactions were not significantly affected by pup age on North Rona or the Isle of May. These results were inconsistent with previous findings (Hewer, 1974; Boness *et al.,* 1982) that have recorded a decrease in aggression towards males towards the end of lactation. This has been interpreted as a sign that females are more willing to mate as they came into oestrus. The frequency of aggression towards males on North Rona did appear to be higher for females with young pups and decrease for females with older pups, particularly stage IV pups - it is therefore possible that the sample size was too small to detect a difference at this site. There did not appear to be a similar trend on the Isle of May.

Female aggression towards males is likely to have at least two functions, namely protection of the pup and repelling males that she is not willing to mate with. Movement to and from the tidal pool at Tarbet on the Isle of May could result in a female passing through several male "territories". The frequency of female/male aggression at this site might therefore be affected by a number of factors which are unrelated to pup age. These factors – e.g. the female's distance from the pool, the number of times she travels between the pool and her pup, the route taken and the number of males she encounters – would be different for each female and any overall affect of pup age on female/male aggression could be masked by the variability between individuals.

9.5.3. Aggression towards alien pups

Female aggression towards alien pups was not affected by the stage of the female's own pup. This is perhaps not surprising given that a female that allows an alien pup to suckle is reducing the milk available for her own pup, and this could affect its chances of survival. Although not statistically significant here (probably as a result of the small sample sizes available), the overall rate of aggression towards alien pups showed a tendency to be higher on the Isle of May than on North Rona. At both Tarbet and West Rona Beach on the Isle of May, females spent a large proportion of their time in tidal pools, leaving their pup unattended for long periods of time. This meant that females travelling to and from the pool were likely to be approached by hungry pups and would attack these pups because they had no mother nearby to protect them (Fogden, 1971). This is likely to be exacerbated by the fact that there were more starvling pups (pups which had become separated from their mother at an early age) on the Isle of May (pers. obs.). These pups constantly tried to feed from any female that came nearby, and although sometimes successful, often got chased away and bitten. As mothers remained close to their offspring on North Rona, pups were less likely to approach strange females for food and consequently were less likely to be attacked than pups on the Isle of May.

9.5.4. Aggression towards gulls

Aggression towards gulls was common on North Rona, especially by females with very young pups (newborn/stage I) or by females that remained near a pup that had died. In contrast, aggression towards gulls was very rare on the Isle of May, with only one incident being recorded over the three breeding seasons. On North Rona, gulls sometimes disembowelled newborn pups (after pecking at their umbilicus) if the mother was inattentive or had moved away from the pup in search of water (chapter eight), and it has been suggested that the aggressive behaviour of females towards gulls has arisen because the gulls on this island attack pups (Seddon *et al.*, 1998). At the same time, this behaviour has been limited to females with stage I or dead pups because gulls are most likely to attack these pups. It is possible that attacks on newborn pups have developed from the scavenging behaviour of gulls on placenta and dead pups, both of which are easy and relatively abundant sources of food during the grey seal breeding season. It is possible that this behaviour has not developed on the Isle of May because there are other food sources nearby (e.g. refuse tips on the mainland, dead rabbits and small birds on the island) which do not incur a risk of being attacked by an adult seal.

9.5.5. Intensity of aggressive interactions

The majority of interactions directed at males were low intensity aggressive behaviours such as open mouth threats and short bouts of flippering. Similarly, the majority of interactions directed at other females were low-intensity threats, although prolonged periods of high intensity aggression between females were observed on the Isle of May when two females appeared to be fighting over the same pup. This occurred when a female mistook the identity of her pup, resulting in two females displaying protective behaviour towards the same pup.

In contrast, despite the fact that female/pup aggressive interactions were relatively rare, at least half of the aggression directed at pups was of a high intensity, with females biting or chasing pups. Post-mortem reports on grey seal pups indicate that although injuries caused by adults (crushing, trauma or bites) are less significant than starvation or infection as a cause of death at most breeding colonies, they may result in pup mortality (Coulson & Hickling, 1964; Baker, 1984; Baker & Baker, 1988; Anderson *et al.,* 1979). Bite wounds are common on many pups that have died from other causes, e.g. starvation (Baker, 1984) and in the majority of cases, are predominantly observed on the front half of the carcass (head & upper neck). This pattern of bite marks is similar to that seen on starvling northern elephant seal pups, where alien pups are bitten around the head and neck when they attempt to suckle from a females that is not their mother (Le Boeuf & Briggs, 1977).

9.6. Conclusions

The main aim of the work presented here was to investigate how pup age and maternal attendance behaviour affected the frequency and intensity of female aggression towards conspecifics. There was no significant effect of pup stage on the frequency of female/male or female/pup aggression at either colony, nor was there an effect of pup stage on the frequency of female/female interactions on the Isle of May. On North Rona, however, females with older pups initiated fewer interactions with other females. These results, along with observations of the locations where aggression occurred on the Isle of May (relative to the pup) indicate that female/female aggression might be used to defend access to pools as well as for pup protection. It is possible that conflict reduction or tolerance occurs because familiar females are less likely to fight over resources or because familiar females are not perceived as a threat to the pup. Females on North Rona could become familiar with one another because they remain close to their pup, and hence one another, throughout lactation. It is thought that this is unlikely to occur on the Isle of May because females regularly travel greater distances to and from a tidal pool and come into contact with a larger number of animals.

Appendix 9.1: Ethogram of aggressive behaviours

- *Open mouth threat (OMT):* performed with mouth open and whiskers erect; usually accompanied by vocalisations (see also Figure A9.1).
- *Flippering (to male/female):* an aggressive response often accompanied by an OMT. The animal lies on one side and waves a front flipper vigorously; the flipper may move in the air or make contact with the other animal or the ground depending on the orientation of the animal (see also Figure A9.2).
- *Lunge / Bite:* aggressor lunges neck at recipient; classed as a bite if teeth of attacker make contact with recipient.
- *Chase*: animal moves quickly towards another, which flees. Attacking animal follows second animal for a distance of at least 1 body length.

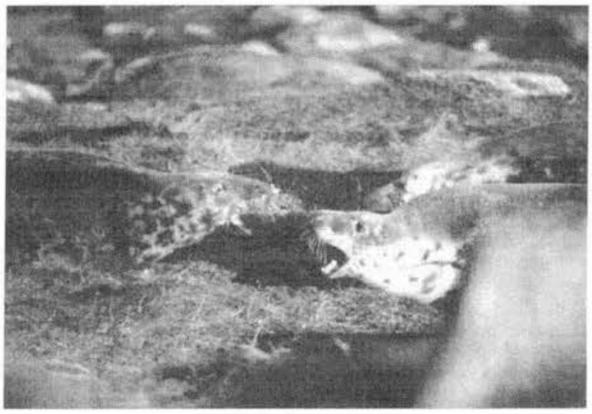

Figure A9.1. Open mouth threat

Figure A9.2. Flippering between females

Chapter Ten:
General Discussion

This thesis investigated how temporal, spatial and kin associations affect the behaviour of female grey seals on the breeding colony. Each chapter has been discussed separately, so the general discussion will be used to highlight the most important points and suggest areas where further research may extend the findings made here. As the data collected on North Rona was more extensive and more comprehensive than that obtained on the Isle of May, the former forms the backbone of this thesis and this is reflected in the general discussion. However, data obtained from the Isle of May provides an important comparison, particularly with respect to the different maternal attendance patterns at the two sites, and will therefore be referred to where appropriate.

10.1. Female grey seal behaviour and spatial association

There were five main aims to this thesis, the first being to determine whether female grey seals could be reliably identified by their pelage markings in the field. Chapter three details the methods used and demonstrates that pelage identification by eye worked well with good quality images of well-marked animals. In fact, this method of identification was more accurate for matching individuals that had been sampled twice than the matching of microsatellite loci carried out by the research group at Cambridge (chapter four).

Using pelage markings to identify individuals allowed large numbers of females to be marked and observed with minimal disturbance. This permitted the second aim - a comparison between the behaviour of branded and non-branded females – to be investigated. Observations of non-branded females on North Rona showed that these individuals had a high return rate between years, a high degree of pupping site fidelity and gave birth on virtually the same date each year (chapter five). Not only did this corroborate previous findings for branded animals and demonstrate the widespread nature of these behaviours, but it also showed that non-random association is likely to occur between females on North Rona from year to year.

Individual identification of a large number of individuals also allowed the spatial association of females to be measured in consecutive years (the third aim of this thesis), revealing that 66% of the females observed on North Rona in two or more years showed inter-annual association with at least one other female (chapter six). Although inter-annual association of female-pairs that moved short distances from previous pupping sites could occur passively due to site fidelity alone, female-pairs that were displaced by more than the median site fidelity showed more inter-annual association than expected using the assumptions of the site fidelity model. Therefore, it is thought that active association between females also occurred at this colony – the first evidence of this kind for adult grey seals.

In chapter eight, female behaviour was examined with respect to short-term, weather-induced changes in the physical environment (aim four). The results from this chapter stressed the importance of access to water for lactating grey seals and demonstrated how restricted access to inland pools could dramatically affect female behaviour. The differences in topography and water availability at the two study sites, along with the corresponding differences in maternal attendance behaviour, provided one of the most interesting findings of this study. On North Rona, females with older pups initiated fewer aggressive interactions with other females than females with younger pups; in contrast, female/female aggression did not decrease with pup age on the Isle of May (chapter nine). This was interpreted as an indication of conflict reduction between females on North Rona that became familiar with one another because they remained within small groups throughout lactation. This reciprocal decrease in aggression, facilitated through the development of some sort of hierarchy or through females becoming tolerant of one another, may also provide some explanation for the inter-annual association seen on North Rona. It is assumed that conflict reduction did not occur at Tarbet on the Isle of May because the females at this site moved backwards and forwards between their pup and the tidal pool. Although these females might have become familiar with their immediate neighbours, they often came into contact with other animals with whom they were unfamiliar and the lack of social stability meant that levels of aggression remained high.

The final aim - to determine whether associations between females were kin-based - was investigated using microsatellite analysis of skin samples from females that were close to one another within a season. Many species gain benefits from grouping with kin (e.g. Sherman, 1977; Brown & Brown, 1993; Petit & Thierry, 1994; Gompper *et al.*, 1997; Dobson *et al.*, 1998), and in situations where site fidelity and philopatry occur together, overlapping of generations may be expected (Chesser, 1998). Hence the system of polygyny and female philopatry seen in grey seals could result in localised groups where individuals are highly related. However, contrary to expectations, microsatellite analysis showed that grey seals did not specifically aggregate with kin on North Rona. In fact, the results indicated that most associated females were unrelated, although a few female-pairs had relatedness values that were indicative of half-siblings. However, the accuracy of the genetic data provided for this work was questionable and as such it was not possible to determine whether the results obtained were a true reflection of the relatedness of associated female-pairs.

10.2. Further research

10.2.1. Is site fidelity affected by topography?

The site fidelity of non-branded females on North Rona was greater than that of branded females at this colony. Although this might indicate that repeated capture of animals subsequently influenced their choice of pupping sites, it is also possible that non-branded animals were more site faithful than the colony as a whole. This could happen if female grey seals use landmarks to orientate themselves on the breeding colony - a behaviour that has frequently been reported in insect and rodent species (Collett, 1992, 1996; Alyan & Jander, 1994, 1997; Salo & Rosengren, 2001). Grey seals on the Isle of May, which has a highly variable topography with many rocky outcrops, are generally more site-faithful than those on North Rona, which is homogeneous by comparison (Pomeroy *et al.*, 2000a), suggesting that landmark use may be a possibility. The sites used for observations of non-branded females on North Rona were chosen because their boundaries could be identified using permanent landmarks, and this may have inadvertently resulted in a choice of study animals that were more likely to be highly site faithful. Branded females, which were distributed over a much wider area, may have shown a greater variability in site fidelity and so be more representative of the colony as a whole. It would therefore be interesting to look at non-branded females from different locations to see whether the presence of landmarks, for example steep slopes and large rock formations, affects site fidelity. Such studies are likely to be possible over the next few years using data that is currently being collected.

10.2.2. Is site fidelity a result of fidelity to conspecifics?

Evidence of active associations between female-pairs that were not highly site-faithful indicates that grey seals may not be site faithful to a location *per se*, but rather site faithful to familiar conspecifics. Although it has been assumed that site fidelity is location based, it is possible that females are actually returning to an area because they know that particular individuals will be there. This could be investigated further by looking at female site fidelity in relation to the location of known males, thereby examining whether inter-annual association occurs as a result of mate fidelity. If the results of such studies suggest that female site fidelity is driven by the desire to be near known conspecifics, a model that calculates passive association based on a fully random distribution of inter-annual movement might be a more appropriate method of examining active association.

10.2.3. Determining where associations first develop

The question of where and when associations form between female grey seals could not be tackled here as relatedness was only investigated for 17 pairs of associated females. This was due to the fact that microsatellite analyses of the skin samples collected in 2000 were not available at the time of writing. Unfortunately, results from extensive DNA sampling carried out on North Rona in 1997 were unavailable for this study. Co-operation from the research group at Cambridge on this matter would have provided a large background sample of individually located females, thereby allowing the 1998-2000 samples to be put in a larger scale context. The larger sample size would also have provided a clearer picture of the relatedness between females that chose to return to sites next to one another as the chance probability of high relatedness between associates could have been investigated.

In addition to continued investigation of relatedness as outlined in chapter seven, further research on inter-annual associations would benefit from the capture of associated females to determine their age. Grey seals can be aged from cross-sections of their incisors (Bernt *et al.,* 1996) and a high prevalence of same-age associates would indicate that associations formed during the post-weaning fast or as juveniles. This could also be investigated through a long-term study of captured pelage images of weaned pups and juveniles. Pelage patterns of female grey seals appear to be stable over time, although they tend to become darker and clearer as juveniles mature (Vincent *et al.*, 2001). It might therefore be possible to use images of pups to investigate association between adult females in future years. This would require an intensive study involving photography of pelage markings over a prolonged period of time. Double-tagging the study pups would help with resightings in subsequent years as the tags would draw attention to animals, ensuring that they were photographed as adults.

This study shows how fine-scale behavioural observations of individual female grey seals may serve as a link between reproductive performance and intra-colony dynamics. The work presented here shows that some female grey seals form associations that persist between years and that familiarity between neighbours might reduce aggression within the breeding colony. Together these are indicative of a relatively rapid behavioural adaptation on North Rona, which is likely to have consequences for the stability and genetic make-up of this colony. These consequences may already be evident, as females in prime locations on North Rona are more related to the colony as a whole, suggesting that their pups are more likely to enter the breeding population (Pomeroy *et al*., 2001). Prime areas on North Rona are likely to be colonised first because of their desirable qualities (low lying land, access to water and away from main access routes) and the behavioural adaptation of reduced aggression amongst familiar neighbours would be focused in such areas; increased pup growth and hence survival could therefore be an advantage of these associations and locations.

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