A network analysis approach to understanding shark behaviour

Submitted by David Jacoby to the University of Exeter as a thesis for the degree of
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I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

Signature: ___________________________

ABSTRACT

The mechanisms and functions of shark grouping behaviour have received relatively little scientific attention to date. The current widespread use of social network analysis to study animal groups, in concert with rapid advances in animal tracking technology, now allows us to test specific hypotheses about how and why sharks form groups. This thesis uses replicated laboratory experiments to investigate some of the mechanisms underpinning aggregation in a model species of benthic, oviporous elasmobranch, the small spotted catshark (*Scyliorhinus canicula* L. 1758; Scyliorhinidae). Acoustic tracking of this species in the wild is also conducted to explore how network analyses can be adopted to study the localized movements, habitat connectivity and ranging behaviour of adult sharks.

Groups of juvenile *S. canicula* were characterized by non-random social preferences, crucially, only when individuals were familiar with one another suggesting social recognition is important in young sharks of this species. Genetic analyses of parent and offspring DNA revealed very high levels of multiple paternity in this species, likely due to male sexual harassment and multiple mating, which leads to increased genetic diversity between juvenile sharks. Perhaps unsurprisingly, there was no evidence of kin relatedness structuring social interactions between conspecifics. Furthermore, testing the effects of environment on social behaviour provided evidence that these juveniles aggregated more in structurally complex environments than simple ones. However, at the individual level sharks showed consistency in their social network positions through time and across different habitat types. This result is indicative of personality traits in *S. canicula*.

Using data gathered via passive acoustic telemetry of wild shark behaviour, network analysis provided a useful tool with which to quantify movement between receivers. One chapter has been dedicated to the application of these methods, highlighting a number of different analyses for predicting movement behaviour from such data. Finally, these methods were adopted to address ecological questions in this sexually segregated species. Persistent site fidelity to a localised inshore area by both male and female sharks suggested that segregation occurred at a relatively small spatial and temporal scale. Despite strong evidence of segregation, analyses of movement networks and individual co-occurrences revealed distinct periods of behavioural synchronicity during the months of March, April and May. In addition, habitat complexity appeared to be a significant driver of female behavioural strategy. Enhancing our knowledge of the social and environmental drivers of aggregation and movement in sharks is of great importance given the ecological threat facing many of our ocean’s top elasmobranch predators.

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The work contained in this thesis has involved collaboration with Andrew Griffiths (AG), Edd Brooks (EB), Lauren Fear (LF), Nick Humphries (NH) and Steve Cotterell (SC). Their contributions are indicated below. As my supervisors, Darren Croft (DC) and David Sims (DS) were involved in all chapters at some level and provided comments on the write-up.

Chapter III: NH helped create the null model used to test for assortment in juvenile shark association data.

Chapter IV: I designed the study and carried out the data collection/statistical analysis including a section on behaviour which contains new material not included in the stated publication. AG conducted genetic analyses and provided feedback on earlier drafts.

Chapter V: LF carried out the data collection as part of her undergraduate project at the University of Exeter. I devised and designed the study and carried out full independent and additional analyses.

Chapter VI: EB contributed the data on Caribbean reef sharks and provided insightful discussion of ideas and feedback on drafts. I designed the study and carried out all analyses.

Chapter VII: SC set up the acoustic array in which the sharks were tracked and NH handled the import and cleaning of raw telemetry data prior to use.

In addition, some of the work contained in this thesis has been published:

Chapter I was published in **Fish and Fisheries**

Jacoby, D. M. P., Croft, D. P. & Sims, D. W. (2012) Social behaviour in sharks and rays: analysis, patterns and implications for conservation. *Fish and Fisheries*, **13**(4), 399-417.

Chapter III was published in the **Journal of Fish Biology**

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Chapter IV. The analyses of multiple paternity were published in the **Journal of Heredity** (*Co-first author), however additional, behavioural material has been added and the chapter completely rewritten.

Griffiths, A. M.*, Jacoby, D. M. P.*, Casane, D., McHugh, M., Croft, D. P., Genner, M. J. & Sims, D. W. (2012) First Analysis of Multiple Paternity in an Oviparous Shark, the Small-Spotted Catshark (*Scyliorhinus canicula* L.). *Journal of Heredity*, **103**, 166-173.

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Chapter I:

General introduction

1.1 Introduction

Group living has been documented in animals from a wide range of terrestrial, freshwater and marine taxa (Krause and Ruxton 2002). The formation of social groups may involve both active and passive processes. For example, individuals may actively prefer to associate with conspecifics and orientate to their direction of locomotion (Couzin et al. 2005; Guttal and Couzin 2010). Some fish species for example show both polarised *schooling* behaviour, defined by highly synchronous swimming when moving from one place to another or evading a predator, and less organised, uncoordinated *shoaling* behaviour when aggregating for social purposes (Pitcher 1983). Such patterns of grouping can be maintained by each individual obeying a few simple, localised rules of attraction, orientation and repulsion (Couzin et al. 2002; Sumpter 2006). In contrast many animal aggregations do not involve social attraction and form as a result of animals being drawn to aggregate due to a limited resource such as food or specific habitat requirements (Johnson et al. 2002) or due to synchronised patterns of daily or seasonal activity (Guttal and Couzin 2010). Thus an important distinction needs to be made between aggregations that do not involve social attraction (referred to hereafter simply as aggregation) and those that do (hereafter, social groups). Throughout this introduction and indeed more generally throughout the thesis, 'aggregation' will also be referred to when there is no clear indication or sufficient research to support that grouping is socially derived or under experimental conditions where social interactions are not clearly measured (see experiment 1, *Chapter III* for example).

Animal groups arise from a complex trade-off of costs and benefits associated with both conspecific and heterospecific interaction. Freshwater teleost fishes for example, gain antipredator benefits such as the dilution of risk or the confusion effect when shoaling with group mates (Krause and Ruxton 2002; Hoare et al. 2004). Schooling behaviour in larger fish and, equally, formation flight in some migratory birds, also appear to facilitate a reduction in the energetic costs associated with movement (Cutts and Speakman 1994; Herskin and Steffensen 1998). Furthermore, benefits can be conferred to groups of predators hunting large prey items cooperatively that they would be otherwise unable to tackle alone and, indeed, some predators use both strategies (e.g. Ebert 1991; Hoelzel 1991). Conversely there are costs associated with grouping behaviour, typically a reduction in foraging efficiency or an increased risk of parasite or disease transmission (Johnson et al. 2002; Hoare et al. 2004). As a result the fitness of an individual in a group is likely to vary as a function of both group size and composition and the context under which grouping has occurred. Unsurprisingly, group living has been the subject of intense research by behavioural ecologists with particular focus on optimum group size and the decision to join or leave a group (Caraco 1979; Côté and Poulin 1995; Krause and Ruxton 2002), the genetic consequences of interacting with kin (Hamilton 1964; Hain and Neff 2007), the mechanisms underlying patterns of social organisation (Krause et al. 2000 a; Croft et al. 2005) and those required to support repeated individual interaction such as social recognition and familiarity (Barber and Wright 2001; Tibbetts and Dale 2007; Ward et al. 2007).

Shoaling and schooling behaviour in fish has been highly selected for in extremely variable three dimensional (3D) aquatic environments (Pitcher and Parrish 1993). Some small freshwater teleost fish however, also shoal under laboratory conditions and therefore much of what is known so far about social behaviour in fish can be attributed to research on model teleost species such as the guppy (*Poecilia reticulata*, Magurran et al. 1994; Krause and Ruxton 2002; Croft et al. 2004), the European minnow (*Phoxinus phoxinus*, Barber and Wright 2001; Griffiths et al. 2007) or the three-spined stickleback (*Gasterosteus aculeatus*, Ward et al. 2002; Frommen et al. 2007; Ward et al. 2008).

Sharks and rays (Class Chondrichthyes, Subclass Elasmobranchii; known collectively as elasmobranchs) are also frequently observed grouping in large numbers, however little is known about the mechanisms driving this behaviour. Indeed, quantifying aggregation or social interactions in marine fishes presents a significant challenge in comparison to smaller, freshwater teleost species. Laboratory experiments have repeatedly demonstrated that predator avoidance behaviour constitutes a common driver of shoaling among many teleost fishes (Lachlan et al. 1998; Krause et al. 2000a; Hoare et al. 2004). This idea, although rarely empirically tested (see Guttridge et al. 2012a for exception), is often alluded to in studies of juvenile elasmobranch behaviour (Morrissey and Gruber 1993; Heupel and Simpfendorfer 2005a) whom themselves are likely to be vulnerable to a range of larger predators. Many highly predatory species of shark occupy apex positions within their respective food webs suggesting that there are arguably significant factors other than predator avoidance that dictate elasmobranch grouping behaviour, at least in the adults. Shark aggregations and the physical or environment variables which underpin these events are reasonably well documented in the scientific literature (Economakis and Lobel 1998; Heupel and Simpfendorfer 2005a; Dewar et al. 2008). In contrast there is considerably less known about the occurrence of social groups in wild sharks, although some species have been hypothesised to engage in diel periods of social refuging behaviour (Sims 2003). It is well known for instance that scalloped hammerhead sharks (*Sphyrna lewini*) which are largely solitary foragers, exhibit regular, polarised schooling behaviour associated with specific locations such as underwater seamounts (Klimley and Nelson 1984), and that these social groups may exhibit fission-fusion properties. Refuging behaviour has been observed in a number of other elasmobranchs and includes group resting behaviour in some demersal species, whereby individuals refuge, often in close physical proximity or contact at regular periods throughout the day or year (Sims et al. 2001, 2005; Powter and Gladstone 2009). The proximate causes of such behaviours and the functions underlying aggregation and social grouping in these top predators remain relatively unexplored.

This general introduction aims to synthesize the existing research on aggregation and social grouping in elasmobranch fishes. Drawing on examples from the teleost fish literature for comparison, this chapter will review our current knowledge of the patterns, mechanisms and function of aggregation events in elasmobranch fishes. The latter section of this chapter considers the methodological developments which have both promoted current research into elasmobranch grouping behaviour and which may aid future development of this field. The use of social network analysis as a theoretical framework with which to study repeated interactions in gregarious animals is discussed in light of the benefits of applying such analyses to a *K* selected species of marine predator.

1.2 Patterns of grouping behaviour in elasmobranch fishes

Elasmobranchs, which are subclass of the Chondrichthyans, are a highly diverse, marine vertebrate taxon that has adapted to fill the apex predatory role within estuarine, coastal and oceanic environments. Characterised by a cartilaginous and not bony skeletal composition, this highly successful taxonomic group evolved over 420 million years ago (Compagno et al. 2005). Chondrichthyans are known to utilise six different types of reproductive mode (Compagno 1990) and in general contrast to bony fish, are much slower to attain maturity, produce fewer, more well-developed offspring and regularly live for periods of decades, rather than years. These *K* selected life-history traits are consistent across all species of elasmobranch despite the substantial variation in reproductive mode and behavioural strategy. Some benthic sharks such as catsharks (Scyliorhinidae) or bullhead sharks (Heterodontidae) for example, are typically found within coastal regions (Sims et al. 2001; Powter and Gladstone 2009) foraging on invertebrates and undergoing oviparous reproduction. In contrast, large pelagic species, such as sphyrnid or carcharhinid sharks are highly mobile, viviparous and may forage on other elasmobranchs or large migratory teleost fish (Klimley 1987; Carey et al. 1990). Due to these *K* selected life-history traits, elasmobranchs are particularly vulnerable to anthropogenic exploitation and thus rely heavily on high juvenile survival rates to sustain population stability (Feldheim et al. 2002; Chapman et al. 2009). The evolution of group living in sharks appears relatively common both in the juvenile (e.g. Rowat et al. 2007; Guttridge et al. 2009a) and adult phases (e.g. Economakis and Lobel 1998; Hight and Lowe 2007), presumably acting to maximise individual fitness. In addition, elasmobranchs are also characterised by a high brain mass to body mass ratio (Northcutt 1977; Yopak et al. 2010) and this may be an indication of their potential to develop and maintain complex social behaviours such as dominance hierarchies and stable social bonds (Myrberg and Gruber 1974; Dunbar and Shultz 2007).

In the last 20 years there has been an abundance of research devoted to the exploration of conspecific and heterospecific aggregation in many species of shark and ray (Economakis and Lobel 1998; Semeniuk and Dill 2006; Dudgeon et al. 2008). The distinction has already been made between non-social aggregations through synchronised behaviours or for limited resources and social grouping behaviour; it is noteworthy to mention here that the majority of the literature discussed falls into the former category. Some species may show both aggregation and social grouping and indeed aggregation may well in some cases be an important prerequisite for the development of social groups (Sims et al. 2000; see Fig. 1). A summary of the species documented as demonstrating group behaviours and the context and timings of these events is given in Table 1.

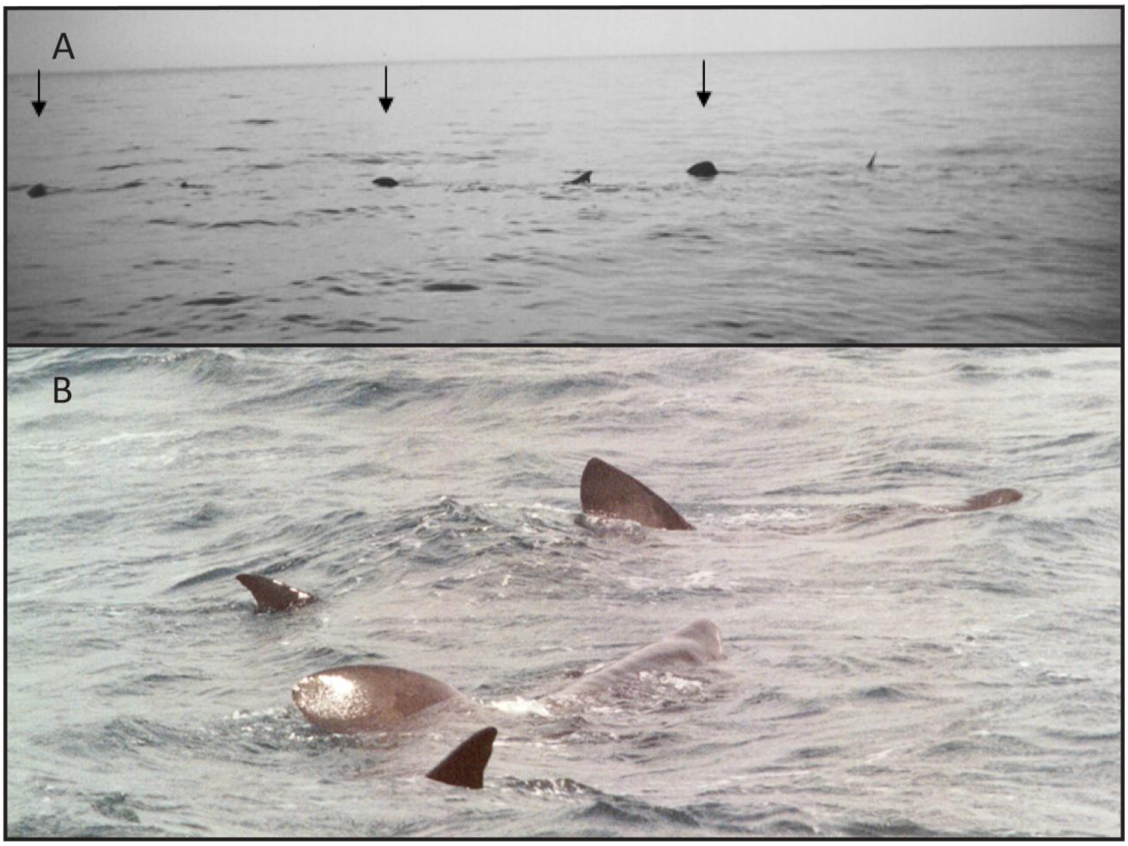

Figure 1. Aggregation for food resources leading to social grouping. (A) Photograph of three basking sharks *Cetorhinus maximus* (arrowed) conducting courtship 'following' behaviour in the western English Channel. Basking sharks are solitary but aggregate in thermal fronts to feed on rich zooplankton patches; it is at this time that they also conduct social behaviour typified by 'following' of females by males, a behaviour which can last for many hours. (B) Close-up of two basking sharks conducting close following behaviour; when one shark turns the other follows. At such times when courtship behaviour occurs, full body breaching is also observed, which may be part of courtship. See Sims et al. (2000). Photographs courtesy of the Marine Biological Association of the UK.

Table 1. Summary of elasmobranch literature documenting aggregation and social behaviours. Species are listed by taxonomic order and by family within order.

Aggregation /Social	Species	Scientific Name	Predominant Sex	Time of Year	Study/Function	Data Collection Technique	Source
Aggregation	Spurdog	*Squalus acanthias*	Mixed	Not specified	Population distribution and sexual segregation	Fisheries landings	Ford (1921)
	Port Jackson shark	*Heterodontus portusjacksoni*	Female	Aggregate year round, more abundant Jul - Oct, some dispersal	Reproductive strategies and sexual segregation	Observational, fisheries landings	McLaughlin and O'Gower (1971),
					Site fidelity and refuging	Observational, active telemetry	Powter and Gladstone (2009)
	Nurse shark	*Ginglymostoma cirratum*	Mixed (male bias)	Not specified	Reproductive strategies	Observational	Carrier et al. (1994)
	Zebra shark	*Stegostoma fasciatum*	Mixed (female bias)	Nov - Apr	Reproduction strategies	Mark-recapture, photo identification	Dudgeon et al. (2008)
	Whale shark	*Rhincodon typus*	Male	Apr - Jun, plankton blooms/coral spawning	Population distribution	Photo identification	Riley et al. (2010)
	Basking shark	*Cetorhinus maximus*	Not specified	Sept - Oct	Reproductive strategies	Aerial photography	Wilson (2004)
	White shark	*Carcharodon carcharias*	Not specified	Aug - Feb	Philopatry and population distribution	Satellite and acoustic tagging, genetic analyses	Jorgensen et al. (2009)
	Small spotted catshark	*Scyliorhinus canicula*	Single sex groups	Aggregate year round (♀)	Environmental conditions and population distribution	Trawl survey	Rodríguez-Cabello et al. (2007)
			Single sex groups		Movement, philopatry and sexual segregation	Mark-recapture, acoustic telemetry	Sims et al. (2001)

Cont.	Leopard shark	*Triakis semifasciata*	Female	Daytime aggregation (summer months)	Behavioural thermoregulation	Active and passive telemetry	Hight and Lowe (2007)
	Grey reef shark	*Carcharhinus amblyrhynchos*	Female	Daytime aggregation (Mar - May)	Environmental conditions	Observational, passive telemetry	Economakis and Lobel (1998),
			Mixed		Site fidelity and movement	Active telemetry, observational	McKibben and Nelson (1986)
	Blacktip shark	*Carcharhinus limbatus*	Mixed (juveniles)	Daytime aggregation (Jun, Oct & Nov)	Environmental conditions and nursery habitat use	Passive telemetry	Heupel and Simpfendorfer (2005a)
	Lemon shark	*Negaprion brevirostris*	Not specified	Not specified	Activity patterns/habitat use	Active telemetry, observational	Gruber et al. (1988)
	Blue shark	*Prionace glauca*	Single sex groups	Not specified	Sexual segregation and habitat use	Fisheries landings	Litvinov (2006)
	Whitetip reef shark	*Triaenodon obesus*	Mixed	Not specified	Reproductive strategies	Video analysis	Whitney et al. (2004)
	Scalloped hammerhead shark	*Sphyrna lewini*	Female	Daytime aggregation year round (offshore)	Schooling behaviour	Observational /photographic	Klimley (1985)
			Mixed		Population distribution	Observational and passive telemetry	Hearn et al. (2010)
	Southern stringrays	*Dasyatis americana*	Not specified	n/a (anthropogenic influence)	Ecotourism impacts	Photo identification	Semeniuk and Rothley (2008)
	Cowtail stingrays and Whiprays	*Pastinachus sephen* and *Himantura uarnak*	Not specified	Not specified	Mixed species aggregations and predator avoidance	Observational	Semeniuk and Dill (2006)
	Spotted eagle ray	*Aetobatus narinari*	Mixed	Not specified	Activity patterns and behavioural ethogram	Active telemetry, observational	Silliman and Gruber (1999)

Cont.	Giant manta ray	*Manta birostris*	Not specified	Summer (North), Winter (South)	Site fidelity and movement	Passive telemetry	Dewar et al. (2008)
Social	Sevengill shark	*Notorynchus cepedianus*	Not specified	Not specified	Group foraging and social facilitation	Observational	Ebert (1991)
	Basking shark	*Cetorhinus maximus*	Mixed	May - Jul	Environmental conditions and reproductive strategies	Observational	Sims et al. (2000)
	White shark	*Carcharodon carcharias*	Mixed	n/a (anthropogenic influence)	Social ethogram	Observational	Sperone et al. (2010)
	Small spotted catshark	*Scyliorhinus canicula*	Female	n/a captive	Social networks	Captive study	Jacoby et al. (2010)
	Nursehound	*Scyliorhinus stellaris*	Not specified	n/a captive	Social refuging	Captive study	Scott et al. (1997)
	Smooth dogfish	*Mustelus canis*	Mixed	n/a captive	Dominance hierarchies	Captive study	Allee and Dickinson (1954)
	Lemon shark	*Negaprion brevirostris*	Mixed (juveniles)	Interactions predominantly May - Dec	Social organisation Social organisation	Captive study Wild observational	Guttridge et al. (2009a) Guttridge et al. (2011)
	Blacktip reef shark	*Carcharhinus melanopterus*	Mixed	Daytime aggregation year round	Social networks (wild)	Photo identification/ Observational	Mourier et al. (2012)
	Scalloped Hammerhead shark	*Sphyrna lewini*	Not specified	Daytime aggregation year round	Social refuging	Active telemetry	Klimley and Nelson (1984)
	Bonnethead shark	*Sphyrna tiburo*	Mixed	n/a captive	Dominance hierarchies	Captive study	Myrberg and Gruber (1974)

1.2.1 Aggregation

There is widespread evidence of elasmobranchs aggregating in both single- and mixed-sex communities (McKibben and Nelson 1986; Litvinov 2006; Semeniuk and Dill 2006). Such aggregations have been correlated with numerous environmental and biological variables from geographic locations (Klimley and Nelson 1984) and time of year (Heupel and Simpfendorfer 2005a; Robbins 2007) to areas of high prey abundance (Hulbert et al. 2005; Martin et al. 2009). Some of the earliest insights into how shark aggregations may be structured were gained from analysing the catches from commercial trawlers. Landings of spurdog (*Squalus acanthias*) and small spotted catshark (*Scyliorhinus canicula*) in Plymouth, United Kingdom in the early 1900s offered the first indication of the average group sizes and sexual segregation of the individuals occurring on the trawling grounds (Ford 1921). Geographic segregation of the sexes is a widespread phenomenon in sharks (see Wearmouth and Sims 2008 for review) and consequently may lead to differential anthropogenic exploitation of the sexes (Mucientes et al. 2009) through spatially-focused fishing pressure (see *Conservation Implications and future research directions*, *Chapter VIII*).

Research into elasmobranch aggregation includes both the easily accessible coastal and benthic species and also the highly mobile, oceanic migrants (Klimley 1985; Robbins 2007; Riley et al. 2010). An example of the latter is the aggregation of white sharks (*Carcharodon carcharias*), albeit sparse over a large area, during a seasonal migration from the coast of Baja, California, to a region of the eastern Pacific Ocean (Weng et al. 2007; Jorgensen et al. 2009). White sharks frequenting this area have also been extensively catalogued using photo-identification methods outlined in Domeier and Nasby-Lucas (2007), which offers the prospect of monitoring individual co-occurrences at different coastal and oceanic locations to explore ideas about preferential association (see *Social grouping*). The function of these aggregation events in such wide-ranging predators remains largely speculative, although social interactions in white sharks have now begun to be quantified (Sperone et al. 2010). Aggregation behaviour may occur on a much smaller scale, for example, within a coastal environment. Heupel and Simpfendorfer (2005a) studied the movements and interactions of neonate blacktip sharks (*Carcharhinus limbatus*) over a period of three years revealing persistent aggregation during daylight hours and dispersal at night. Sharks demonstrated high site fidelity when aggregating and seasonal patterning of interactions (Heupel and Simpfendorfer 2005a). Although there are significantly fewer studies considering batoid aggregation behaviour, some species such as the spotted eagle ray (*Aetobatus narinari*), exhibit remarkable aggregation and schooling behaviour (see the section on *Social grouping*) typically consisting of between 5 and 50 individuals, arranged in a variety of swimming formations (Silliman and Gruber 1999). It is not yet known whether social factors underpin this behaviour.

Aggregation behaviour of sharks documented in studies to date appears largely motivated by a desire to understand species abundance and distribution within a changing environment. Research on wide-ranging planktivorous elasmobranchs such as the giant manta ray (*Manta birostris*) and the whale shark (*Rhincodon typus*) for example, are generally aimed at describing site fidelity and ranging behaviour of individuals between areas/populations (Dewar et al. 2008) with a view to conserving known aggregation sites (Riley et al. 2010). Knowledge of the spatial and temporal dynamics of these events is extremely important, not least as it contributes to improved conservation and fisheries management practices for these species. It is unclear whether many of these aggregation events may also serve to facilitate some exchange of social information. It is possible though, that species often considered solitary might in fact integrate some aspect of social interaction into their behavioural repertoire to gain some of the benefits associated with group living (described above). In fact recent studies suggest that sharks have the cognitive potential required for such information exchange (see Guttridge et al. 2009b for review) and that this can aid social learning of foraging tasks in some species (e.g. Guttridge et al. 2012b).

1.2.2 Social grouping

Despite burgeoning research on aggregation, there is a distinct paucity of literature on the descriptions and specific patterns of elasmobranch social behaviour (cf. Table 1). Springer (1967) identified this knowledge gap over 40 years ago whilst considering the social organisation of the many species of shark in the Gulf of Mexico. Although unclear at the time due to a lack of empirical evidence, Springer offered the view that "*…some shark populations exhibit complex behaviour that constitutes part of their social organization*", and based on anecdotal evidence from aerial surveys "*…that large sharks (and rays) are often in groups and not randomly distributed*". In this early study Springer observed synchronised, collective behaviours such as echelon swimming, milling and size assortment that have become indicative of social interaction in some shark species (Myrberg and Gruber 1974; Sims et al. 2000). Indeed, assortment by size, colouration, familiarity and kinship are all well documented in teleost fishes (see Krause et al. 2000a for review) and have been shown to confer individual benefits such as minimising predation risk through phenotypic oddity and reducing competition and aggression between size classes (Hoare et al. 2000). Size assortment has also recently been demonstrated as important in the structuring of juvenile lemon shark groups (*Negaprion brevirostris*, Guttridge et al. 2011) in a study representing the first quantification of associative preferences and social organisation in a free-ranging shark population. Subsequent research has built upon these ideas with a study by Mourier et al. (2012) defining statistically differentiated social communities in a population of blacktip reef sharks (*Carcharhinus melanopterus*). It is worth emphasizing for future research the importance of empirical data and hypothesis testing as demonstrated by these studies, because the current information provides largely anecdotal evidence of social organisation in sharks. This is particularly important since it is equally likely that assortment of individuals may occur through passive processes such as variable swimming speeds between different sized individuals or habitat features (e.g. temperature) which are favourable for some but not others (Croft et al. 2003; Wearmouth and Sims 2008).

In contrast to teleost fishes, experimental research on the social interactions of sharks have been few and far between (for exceptions see Guttridge et al. 2009a; Jacoby et al. 2010). The earliest research into elasmobranch social behaviour began with Allee and Dickinson (1954), who conducted the first quantitative analyses of dominance and subordination in captive smooth dogfish, now known as the dusky smoothhound (*Mustelus canis*). Later, Myrberg and Gruber (1974) identified an extensive dominance hierarchy amongst mature bonnethead sharks (*Sphyrna tiburo*). These data revealed a size-dependent dominance hierarchy and an apparent predominance of males over females. Direct observations and passive acoustic tracking of another closely related species, the scalloped hammerhead (*Sphyrna lewini*), showed highly coordinated schooling behaviour within large groups of individuals, made up almost exclusively of females (Klimley and Nelson 1984; Klimley 1987). The authors proposed a social refuging hypothesis during the resting phase of the sharks' diel behavioural cycle as foraging behaviour was never witnessed during schooling events (Klimley and Nelson 1984).

1.3 Mechanisms and functions of grouping

1.3.1 Aggregation

Many elasmobranch species demonstrate high levels of philopatric behaviour to specific habitat locations (Hueter et al. 2005). During early life stages particularly, site fidelity is common in sharks with shallow, coastal waters offering ideal nursery areas for juveniles to aggregate (Simpfendorfer and Milward 1993). Immature lemon sharks (*Negaprion brevirostris*) at a subtropical lagoon in Bimini, Bahamas for example, disperse relatively slowly from their natal breeding grounds with locally born individuals being recaptured within the same areas up to six years after birth (Chapman et al. 2009). The shallow water, mangrove habitat favoured by groups of juvenile lemon sharks offers suitable conditions for individuals to increase somatic growth in the warm prey-abundant waters whilst at the same time avoiding larger elasmobranch predators (Morrissey and Gruber 1993; Guttridge et al. 2012a). This extended opportunity for juvenile *N. brevirostris* to interact has clearly influenced the behavioural strategy of this species with large aggregations observed in both juvenile and adult phases of this species (Gruber et al. 1988; Wetherbee et al. 2007; Guttridge et al. 2009a). Site-attached behaviours, such as mating or refuging in some species, periodically bring elasmobranchs together for a variety of resource requirements. These aggregations are often associated with specific times of day or months of the year.

Preferences for a specific thermal niche for example, appear to favour adult females of some shark species, resulting in single sex aggregation in warm, shallow coastal habitat (Economakis and Lobel 1998; Hight and Lowe 2007). Equally, aggregations of adult scalloped hammerhead sharks (*Sphyrna lewini*) utilise the inshore waters of Kaneohe Bay, Oahu, Hawaii between the months of April and October for mating and delivery of pups (Clarke 1971) but, as discussed previously, are also known to form large daytime social groups (social refuging) around underwater seamounts in the Gulf of California (Klimley and Nelson 1984). This species serves to illustrate the potential overlap between aggregation and social grouping behaviour in some elasmobranch species.

A widespread characteristic of shark aggregation is sexual segregation which may be influenced by a range of factors including sexual dimorphism in body-size or differential activity budgets between the sexes (see Wearmouth and Sims 2008 for review). In many species of elasmobranch, asymmetry in gamete production and thus differential reproductive success from multiple mating events may be sufficient to cause very different behavioural strategies and movement patterns (Sims 2003, 2005). Different life-history traits between the sexes for example may select for male sharks to invest more time in pursuit of mates than female sharks which may themselves allocate a higher percentage of time in search of suitable environmental conditions to aid gestation or egg incubation (Hight and Lowe 2007). Indeed, a high level of male harassment in some teleost fish has been shown to drive spatial segregation of the sexes (Darden and Croft 2008). Shark mating behaviour, however, is notoriously aggressive, with females often sustaining severe wounds and serious abrasions to the body and pectoral fins from bites by males (Carrier et al. 1994). Therefore it is perhaps unsurprising that sexual segregation is relatively common among elasmobranchs (Ford 1921; Klimley 1987; Sims et al. 2001; Litvinov 2006; Mucientes et al. 2009). The need for females to avoid energetically expensive and potentially damaging multiple mating events is a commonly hypothesised mechanistic driver of single-sex, female refuging behaviour in sharks (Economakis and Lobel 1998; Sims et al. 2001) and sexually segregated schooling behaviour in teleost fish (Croft et al. 2006a; Darden and Croft 2008). Furthermore, with no recorded evidence of parental care observed in any species of elasmobranch, it is possible that segregation might occur from a relatively young age (Litvinov 2006).

Despite such widespread sexual segregation, there comes a point when both males and females must find a mate and consequently mating aggregation behaviour might even occur within species which are typically found at very low densities. Group reproductive behaviours have been observed in several species (Carrier et al. 1994; Whitney et al. 2004) and have been closely linked to both transient and permanent environmental conditions such as increases in zooplankton abundance (Sims et al. 2000) and rocky reef habitat (Powter and Gladstone 2009), the latter indicating the possibility of discrete breeding populations even in some species that disperse widely. It seems apparent that there are numerous temporal as well as spatial influences on aggregation and segregation behaviour in elasmobranch fishes. One example is the dispersal of juvenile sharks of different sexes away from nursery habitat. Blue sharks (*Prionace glauca*) are the most widely distributed elasmobranch species in the pelagic ocean environment and they also demonstrate sexual segregation at the earliest age. Male blue sharks appear to move offshore very early in life (at < 70 cm total length) and occupy dense aggregations around oceanic seamounts, seemingly leaving the females in shallower coastal waters until nearly three times this size

(Litvinov 2006). Nearshore environments are commonly utilised by elasmobranch species to aggregate (Knip et al. 2010). It is evidently easier to study this type of behaviour in shallow coastal waters and as such, aggregation at depth is currently best inferred from fisheries data (Girard and Du Buit 1999). However, nearshore habitats are likely to offer high levels of prey diversity and abundance for elasmobranch predators and consequently many species periodically return inshore to forage and/or breed, permitting juveniles a greater chance of survival in the shallow, sheltered waters (Heupel et al. 2004; Chapman et al. 2009). Behaviourally these aggregation events may serve additional functions as well as protection from larger predators, given that some smaller benthic or demersal species occupy coastal habitat for the duration of their life (Sims et al. 2001; Dudgeon et al. 2008). Passive acoustic monitoring techniques have been employed to quantify the levels of site fidelity shown within leopard shark (*Triakis semifasciata*) aggregations. This study suggested that female leopard sharks selectively occupy warmer, inshore refuges as a means of behavioural thermoregulation (Hight and Lowe 2007). It is hypothesised that thermoregulation behaviour enhances gestation and periods of embryonic development in this species, a theory not without support from observations of groups in other shark species. Sims et al. (2001, 2006) used a combination of active acoustic telemetry, archival tagging and laboratory experiments to explain the differential sexual strategies in another benthic predator, the small spotted catshark. They concluded that the apparent spatial separation in this species was due to the females' ability to store sperm and thus avoid male copulation attempts during periods of gestation and egg laying (Sims et al. 2001). As a result, female *S. canicula* in the wild are seen in tightly packed groups lying on top of one another inside shallow-water, rocky openings such as crevices and caves (Sims 2003; see also Fig. 2). This proposed male avoidance appears a significant selection pressure causing disruption among weakly socially-associated females (Jacoby et al. 2010) and forcing them to occupy areas outside of their preferred thermal/metabolic niche (Sims 2003; Wearmouth et al. 2012).

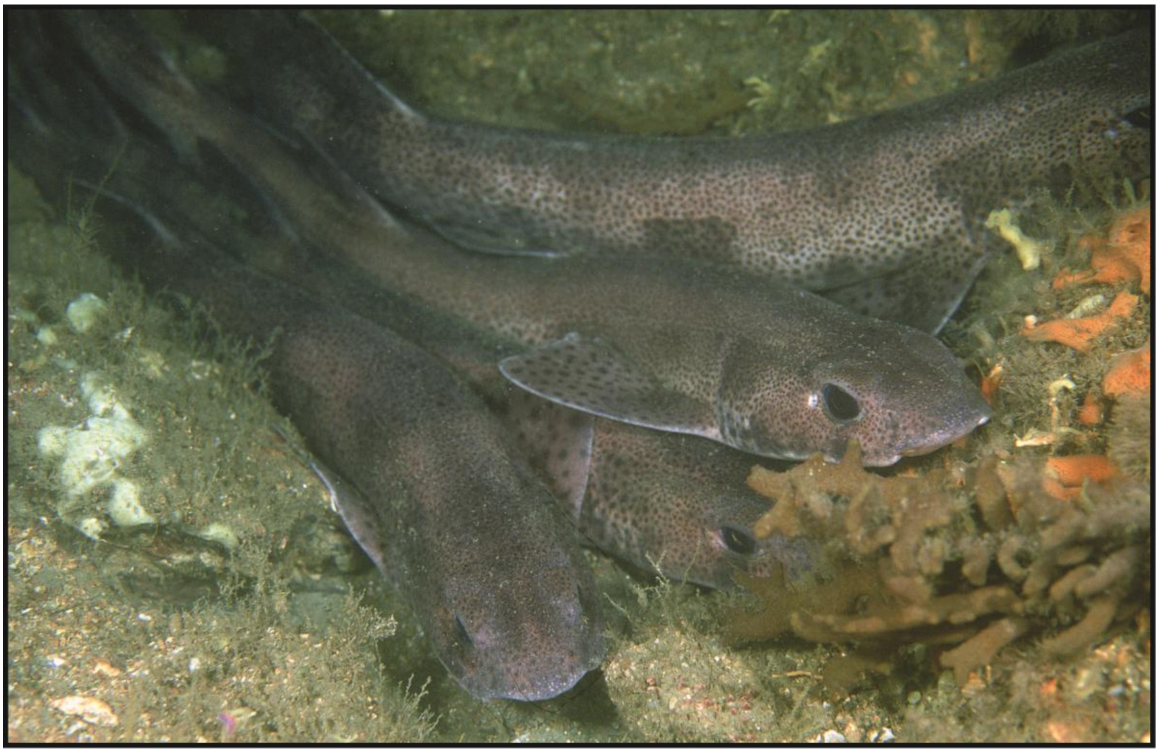

Figure 2. Aggregation of four female small spotted catsharks (*Scyliorhinus canicula*) in a narrow rock gully off Plymouth, UK. Female-only refuging aggregations are common in this species and are thought to arise from the avoidance of males. Photograph courtesy of Paul Naylor.

1.3.2 *Social grouping*

The adaptive significance of elasmobranch social behaviour is poorly understood despite a growing ecological threat to many species globally (Baum et al. 2003; Ferretti et al. 2010). During the juvenile phase of development, avoiding predation is a likely driver of aggregation (Morrissey and Gruber 1993; Economakis and Lobel 1998). Indeed in stingrays, the antipredator benefits of aggregation by cowtail stingrays (*Pastinachus sephen*) are greater in heterospecific groups than single species groups suggesting a social mechanism maintains groups that have formed for protection. Heterospecific grouping is apparently due to the quicker flight responses and thus earlier warning of approaching predators by the reticulate whipray (*Himantura Uarnak,* Semeniuk and Dill 2006). Until recently, the mechanisms and functions underpinning social grouping in elasmobranchs have been speculative. Anecdotal evidence and observational data however, still provide the greatest insight into social interactions particularly in wild sharks (Sims et al. 2000; Sperone et al. 2010) suggesting some unusual adaptive mechanisms in some species. Predatory sevengill sharks (*Notorynchus cepedianus*) appear to utilise multiple feeding strategies depending upon the prey size and type. Sevengills are thought to use social facilitation and pack hunting strategies in order to tackle larger prey items such as Cape fur seals (*Arctocephalus pusillus pusillus*, Ebert 1991). More recently, the burgeoning study of animal social networks (Croft et al. 2008), accompanied by developments in computational and analytical power has resulted in more hypothesis-led investigations of shark social behaviour under semi-wild or captive conditions (Guttridge et al. 2009a; Jacoby et al. 2010).

Active partner preference has long been inferred in teleost fishes using binary choice experimental manipulation (Lachlan et al. 1998; Griffiths and Magurran 1999; Croft et al. 2006b). Whether slow growing, wide-ranging elasmobranchs are also capable of showing active partner preference has remained unexplored until recently. Guttridge et al. (2009a) used similar binary choice experiments to demonstrate that juvenile lemon sharks (*Negaprion brevirostris*) show significant associative preferences for both conspecific over heterospecific groups (nurse shark, *Ginglymostoma cirratum*), and size matched over non size-matched conspecifics, clearly indicating an overall active preference for social behaviour. Active partner preference demonstrates a social mechanism by which some sharks appear to maintain social groups. These social preferences may in fact be driven by population level processes such as intersexual conflict whereby the individuals associating are each attempting to maximise their own reproductive success. The impact of male behaviour upon female small spotted catshark (*S. canicula*) aggregations for example, was recently tested experimentally and showed that the strength of social bonds between females within aggregations were non-random (i.e. social groups were exhibited), but were also unevenly distributed, and that weakly associated females were more susceptible to disruption by male behaviours that were costly to females (Jacoby et al. 2010). Inferring this level of detailed information in wild populations is clearly more challenging.

In an attempt to summarise the relatively scarce literature surrounding the functions of elasmobranch social groups, it is important to draw again on comparisons with their teleost counterparts. The benefits of schooling behaviour in teleost fishes have been shown to include a variety of antipredatory functions (Magurran 1990; Krause and Ruxton 2002), reduced energetic demand and oxygen consumption (Herskin and Steffensen 1998), information transfer and cooperation (Croft et al. 2006b) and more efficient collective decision making (Ward et al. 2008). It is unknown which of these, if any, apply to apex predatory sharks despite a number of species which have been documented schooling in large numbers (Klimley 1985; Wilson 2004). In a comparative study of teleost and elasmobranch schooling behaviour, Klimley (1985) observed that scalloped hammerhead shark (*Sphyrna lewini*) schools comprised predominantly of females with larger individuals aggressively vying for central positions within the school. This study hypothesised that social refuging behaviour served to facilitate information exchange between resting sharks

(Klimley 1985). It has been discussed that sharks are capable of active partner choice (Guttridge et al. 2009a; Guttridge et al. 2011) implying some form of recognition or phenotype matching mechanism. If so, there are probable benefits to associating with unfamiliar conspecifics, as these individuals are arguably more likely to possess information about resources outside an individuals' home range (Goodale et al. 2010). Thus, these refuge aggregations, particularly in facultative schoolers like the scalloped hammerhead may serve to disseminate novel information throughout a population via fission-fusion behaviour and social learning, something which has been qualified in free-ranging French grunts (*Haemulon flavolineatum*) in a coral reef habitat (Helfman and Schultz 1984). Within these social groups it is also probable that there is some degree of hierarchy and/or assortment between individuals (Allee and Dickinson 1954; Myrberg and Gruber 1974).

Alternatively, social groups may be rather brief and sporadic, functioning merely as a means of finding and mating with the opposite sex (Sims et al. 2000). The basking shark (*Cetorhinus maximus*) is the world's second largest fish and they appear to conduct annual social behaviours associated with tidal-shelf and oceanic fronts. These behaviours include close following behaviour and parallel and echelon swimming in what are thought to be courtship displays that include full body breaching, although mating has never been observed conclusively in this species (Sims et al. 2000; see also Fig. 1).

To conclude this section on the patterns, mechanisms and function of aggregation and social grouping behaviour in elasmobranchs, it is important to reiterate that evidence of social interaction among sharks is, in part, likely to result from common behavioural strategies such as migration and collective movement or aggregation around a specific resource. These may include thermal habitat for gestation or incubation (Hight and Lowe

2007), suitable topographic or environmental features to avoid male harassment (Sims et al. 2001, 2005) or oceanographic attributes which provide a familiar 'way-point' on a migratory route (Jorgensen et al. 2009). Determining the relative influence of social interaction on the initial formation of shark aggregations is still a relatively new endeavour, but will surely prove an interesting and challenging area for future research. This thesis will attempt to address this gap in knowledge by testing hypotheses relating to the mechanisms which may underpin social behaviour in sharks.

1.4 Methods for studying shark social behaviour

1.4.1 Tracking and telemetry

The spatial and temporal dynamics of gregarious animal interactions are highly complex. Tracking these interactions through time requires extensive records of specific individuals, something which has, perhaps unsurprisingly, proven extremely difficult to obtain for free-ranging elasmobranchs. Acoustic telemetry has developed considerably in the past 25 years, particularly in its application to monitoring the movements and behaviours of marine fishes such as sharks (Sims 2010). As a functional yet rather more labour intensive predecessor to passive telemetry, active tracking using ultrasonic telemetry relies on following an acoustic transmitter tagged shark from a boat using a directional hydrophone. This technique has proved successful in revealing polarized schooling behaviour in several large elasmobranch predators (Klimley and Nelson 1984; McKibben and Nelson 1986). More recently, passive acoustic and satellite telemetry techniques have shed light on the movements and interactions of elasmobranch species at a variety of spatial scales (e.g. Eckert and Stewart 2001; Sims et al. 2006). Although expensive at the outset, the continual development of smaller and cheaper acoustic tags has promoted the widespread use of passive acoustic telemetry as a means of tracking large numbers of teleost and elasmobranch species as they interact through space and time (Sims 2010). Chapters *II*, *VI* and *VII* provide more information on deploying and analysing data gathered via acoustic biotelemetry technology. As an example of how this technology can be used to study the behaviour of wild sharks, Heupel and Simpfendorfer (2005a, b) used omnidirectional passive acoustic receivers to study the movement and interactions of juvenile blacktip sharks (*Carcharhinus limbatus*). Nearest neighbour analysis of the telemetry data revealed that shark aggregations were more common in the late summer during which there were strong diel patterns to aggregation events (Heupel and Simpfendorfer 2005a). This serves to demonstrate that it is now possible to monitor continuously multiple individual sharks and their movements within a designated area, provided the receivers encompass at least a good proportion of the activity space (or home range) of the species in question. Given that arrays of acoustic receivers are often spatially limited, this often means studies are restricted to those on juvenile or neonate sharks that have smaller core activity spaces. However, large scale deployments for long periods in deep water areas are now being made to track adult movement patterns (see *Chapter II* for detail on the telemetry techniques adopted during this project). Regardless of spatial scale this technique provides one of the few means to determine round-the-clock habitat use and home range dynamics of sharks (Heupel et al. 2004), in addition to their tendency to form large daily aggregations (Economakis and Lobel 1998).

Determining the precise occurrence and scale of social interactions from presence/absence data on omnidirectional acoustic receivers is still problematic without high levels of range overlap. Field of view overlap of receiver stations is often traded off against increased area coverage within acoustic arrays and consequently new technology that accounts for proximity of individuals is required to facilitate analyses of social behaviour in wide ranging animals (Krause et al. 2011). Novel studies on both Galapagos sharks (*Carcharhinus galapagensis*, Holland et al. 2009) and juvenile lemon sharks (Guttridge et al. 2010) employed specialised, prototype transmitter/receiver tagging techniques or proximity loggers to test the reliability of coded data exchange between sharks within a given range of one another. The devices used in the lemon shark study were capable of detecting individuals within 4 m (~4 body lengths) of each other, but rarely when at 10 m distance, emphasising the value of this technique for recording close interactions between individual sharks (Guttridge et al. 2010). Once developed further, these techniques offer huge scope for advancing our understanding of the ways in which animals interact in the wild. It seems likely that significant, rapid progress will be made by combining this new technology with social network analyses (Krause et al. 2011).

Passive acoustic telemetry, widely used in elasmobranch studies of behaviour, has been used both to infer and now directly to record social interactions between sharks (Holland et al. 2009; Guttridge et al. 2010). From a management and conservation perspective, however, it is important to monitor not only where and when these animals are aggregating, but also to understand individual and group-based movements between habitats within the home ranges of the species in question. As such, it can be useful to view specific habitat features as locations interconnected by the animals that move between them creating a complex movement structure, the dynamics of which undoubtedly has implications for the transmission of disease or parasites between animals (e.g. Fortuna et al. 2009). Equally, such movement structures or 'movement interaction networks', as they will be referred to throughout this thesis, are likely to prove important predictive indicators for sharks occupying threatened habitat such as nearshore areas (Knip et al. 2010). In many ways these movement interactions closely resemble the complex structures observed in studies of animal social networks and therefore are highly amenable to similar analytical approaches (see *Chapter VI*).

1.4.2 Introduction to social network analysis

There are a variety of interactions between group living animals that have the potential to greatly influence population structure and dynamics. Social animal systems may be highly complex and dynamic, involving hundreds and sometimes thousands of individuals but can still be successfully interpreted by understanding the influence of individuals or groups at different spatial and temporal scales. Social network theory therefore offers a useful tool with which to study in detail, social organisation on a multitude of levels, from individual behaviours to population-level processes (Krause et al. 2009). Central to this approach is the idea that individuals differ in their importance within a network and thus their ability to influence group decisions (information transfer) or indeed pathogen transmission. Network analysis is therefore becoming increasingly popular amongst behavioural ecologists (Krause et al. 2007; Croft et al. 2008; Sih et al. 2009).

Social network analysis provides a means of analysing the global properties of a system based on a variety of dyadic interactions (an interaction or relationship between two individuals) from aggression bouts and dominance hierarchies to cooperation and reciprocal altruism (Krause et al. 2000a; Croft et al. 2005, Fig. 3).

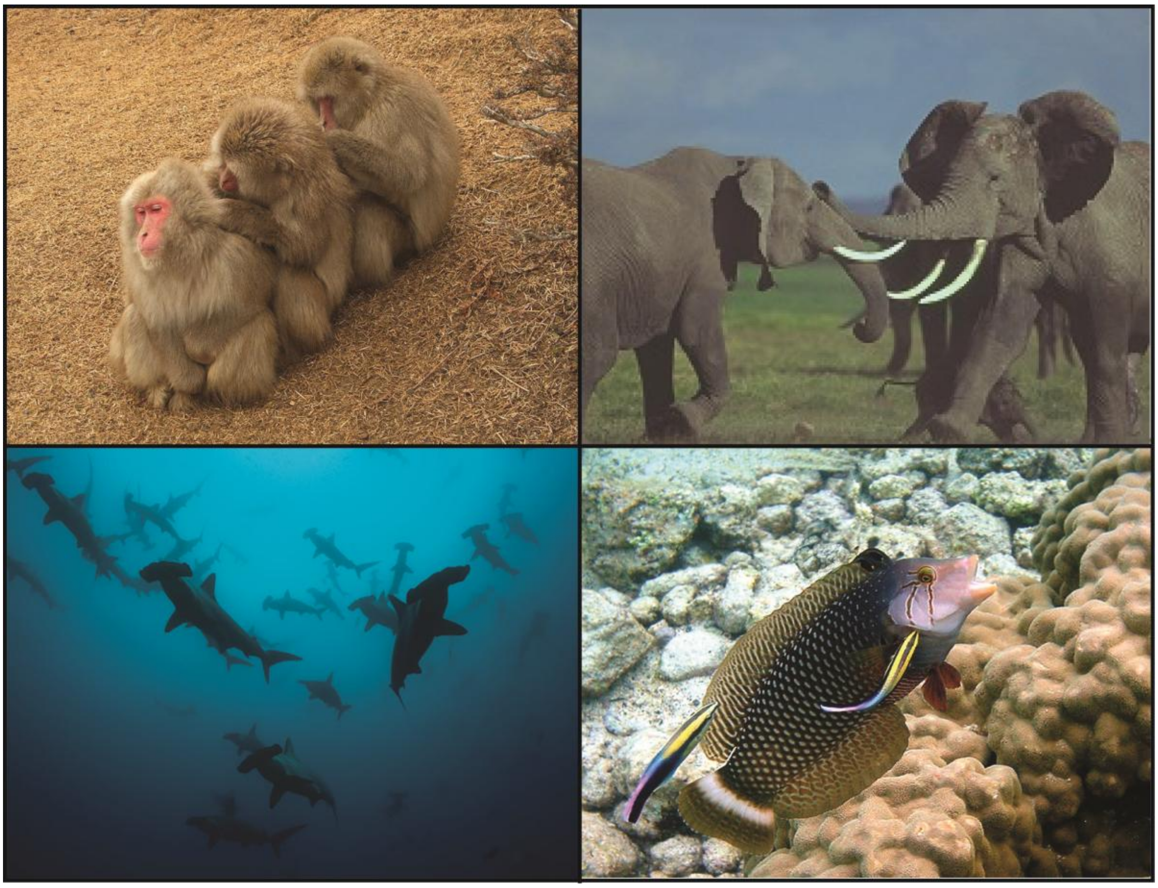

Figure 3. Examples of social interactions from the animal kingdom. Social networks can be constructed from dyadic interactions such as grooming behaviour (Japanese macaques, *Macaca fuscata*; top left, credit Noneotuho), aggression bouts (African elephants, *Loxodonta africana*; top right, credit Maksim), schooling (scalloped hammerhead sharks, *Sphyrna lewini*; bottom left, credit Xvic) and cleaner/client interactions (rainbow cleaner wrasse, *Labroides phthirophagus* bottom right, credit Brocken Inaglory). Photographs courtesy of Wikimedia.

Equally, these dyadic interactions might represent the movements of an animal between two different areas or habitat types, in which case we would be dealing with a movement interaction network (see *Chapter VI*). Networks can be represented through simple graphical format where each individual in a group (or location) is represented by a node and a tie or edge between two nodes represents some form of interaction (Fig. 4a). Network diagrams may be enhanced by representing the edges between nodes as *directional* indicating an asymmetry in the observed dyadic interaction (Fig. 4b), or *weighted*, pertaining to the frequency an interaction is observed between individuals or a movement made between locations (Fig. 4c). Furthermore, accompanying every descriptive network diagram is a corresponding $n \times n$ association matrix upon which statistical analyses of the association data may be performed, such as testing for non-random associative preferences or clustering within the network.

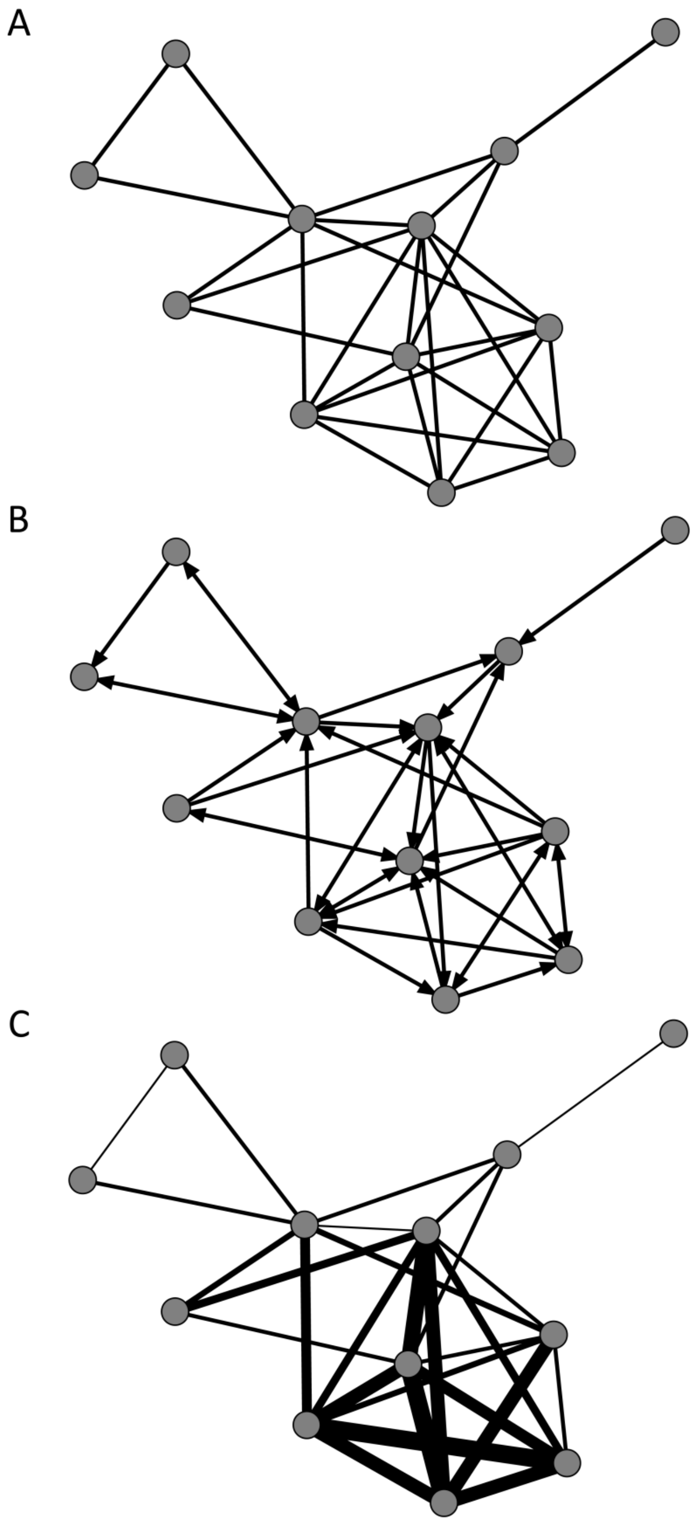

Figure 4. Illustration of a simple (a), directed (b) and weighted (c) network. Circles represent nodes (i.e. individual organisms) and the ties between them represent interactions (i.e. aggressive displays, social grooming etc).

Perhaps one of the major benefits to studying interaction behaviour in a network format is the ability to overlay large amounts of biological data regarding attributes of individuals on top of a social network. Substantial insight can then be gained by highlighting, within the network data, those individuals of a particular size, sex, colouration or age class. For example we may ask if the social connectivity of an individual can be predicted by its attributes or if there is positive or negative assortment in the network based on phenotypic traits. By integrating attribute data within the social network, the biological relevance of aggregation behaviour becomes clearer (Wolf et al. 2007; Croft et al. 2008). As such, foraging behaviour (Morrell et al. 2008), sexual harassment (Darden et al. 2009) and size assortment (Croft et al. 2005) have all been explored in teleost fish using a social networks approach. Furthermore, it might be of particular interest to compare directly matrices of social interaction with data on the genetic relatedness of individuals within a group, giving potentially important insight into the benefits, or indeed costs, of associating with kin (*Chapter IV*; Hain and Neff 2007; Frére et al. 2010). Statistical analyses may then be performed on either the complete network or on data restricted to specific attribute or kin groups allowing considerable flexibility for specific hypothesis-driven research. A more extensive discussion of the issues associated with sampling and analysing network data and detailed description of network-related metrics can be found in subsequent chapters (specifically *Chapters II* and *VI*). First and foremost, social network analysis is used throughout this thesis to consider the importance of social interaction in juvenile and adult shark behaviour. It is, however, also adapted to facilitate the interpretation of shark movements from data gathered using passive acoustic telemetry techniques (see *Chapter VI*). These applications are not necessarily mutually exclusive (see *Chapter VII*), but rather serve to demonstrate how network analyses of complex systems should become integral to the analysis of telemetry data (Krause et al. 2011; see also Fig. 5).

Many of the studies cited in this section serve to reiterate the pivotal role teleost fish have so far played in providing a model for understanding the ecological and evolutionary processes dictating the development of sociality. Similar analyses, however, have also been adopted to help reveal the complex community structuring in a number of wide-ranging marine mammals (Connor et al. 2001; Gowans et al. 2001; Lusseau 2003) using photo-identification techniques that are now being adopted to study elasmobranch aggregations (Domeier and Nasby-Lucas 2007; Riley et al. 2010). The use of such photo-identification in concert with social network analyses might enable more detailed understanding of shark aggregation events which could prove important in the future management and conservation of many vulnerable elasmobranch species. The complexities of conserving elasmobranch populations through a combination of scientific research and conservation strategies go well beyond the scope of this review. Discussion of the implications and future directions of this type of research is given at the end of this thesis in the general discussion (*Chapter VIII*).

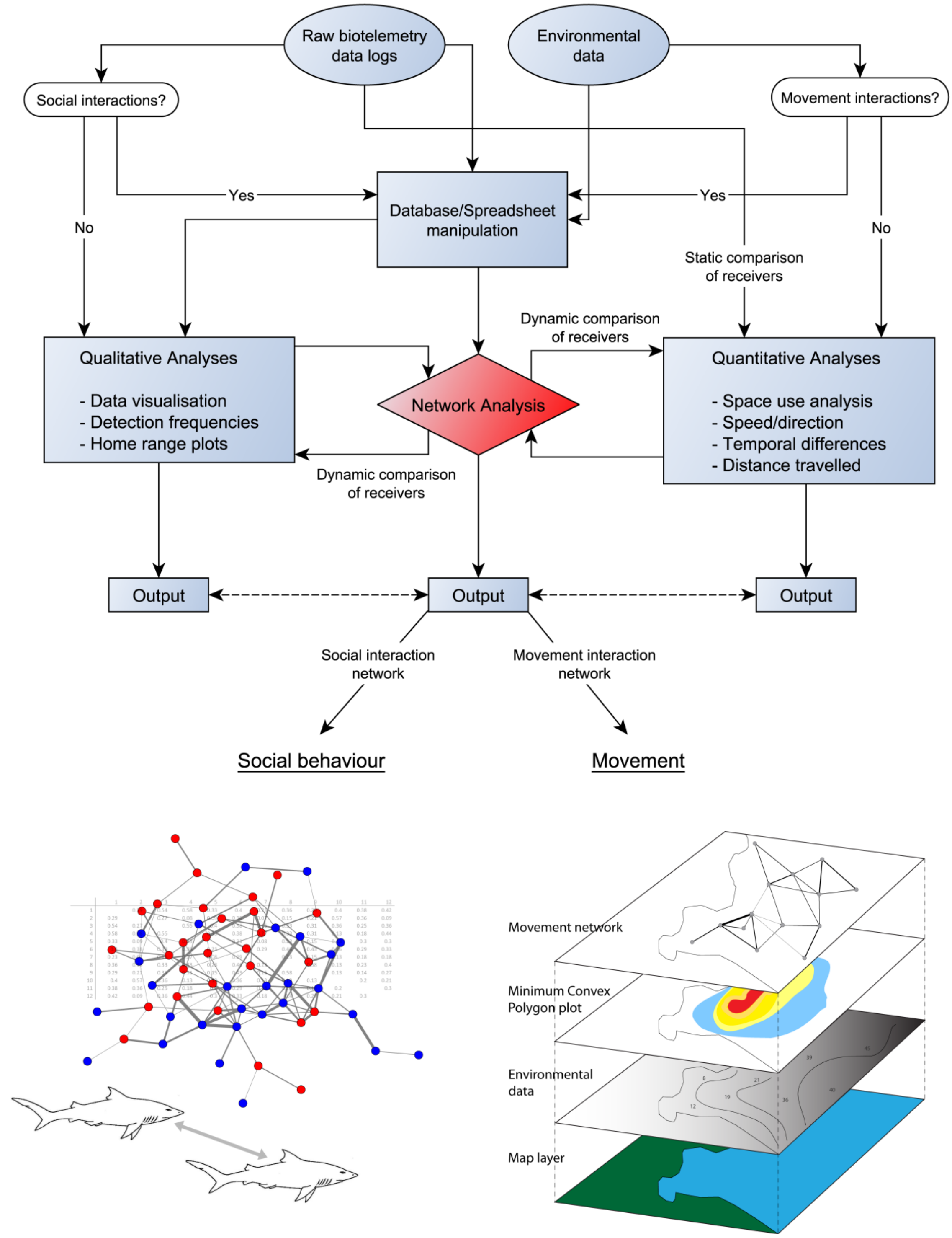

Figure 5. Conceptual model illustrating a networks approach to analysing telemetry data. Here network analysis is used to support other, more traditional, analyses of both movement and social behaviour in sharks. As illustrated in *Chapters VI* and *VII* telemetry data containing simple information on the presence of individuals at specific times and locations can be used to reveal interesting patterns of movement and co-occurrence using a networks approach.

1.5 Conclusions

Studying the temporal interactions of large wide-ranging sharks is clearly a challenge. However, there are a number of intermediate sized species which still fulfil elevated predatory functions within the food web and are also highly tractable to being maintained in groups under laboratory conditions (Sims 2003). Benthic sharks, such as the widely distributed small spotted catshark (*S. canicula*) used here throughout this thesis, are known to demonstrate site philopatry and single-sex refuging behaviour and thus are likely to prove a useful species with which to tackle some of the questions this review highlights.

Developments in marine tracking technology and visual identification methods continue to enhance our knowledge of site philopatry and refuging behaviour in elasmobranchs. Determining what drives predatory elasmobranchs to group is important, not only for understanding interactions at lower trophic levels but also for the future conservation and management of populations and areas associated with their fundamental life-history traits. During this review I offer an appraisal of where and when such groups have been documented to occur and in the current research described in this thesis I use controlled and replicated laboratory experimentation, in combination with wild acoustic tracking of sharks, to explore some of the possible mechanisms and functions of this behaviour.

Furthermore, social network theory is appealing in its relevance to our own behaviour and the idea of animal social networks is both engaging and accessible. Detailed knowledge of how sharks associate in the wild will provide insight into population distribution and assortative behaviour such as sexual segregation, which can substantially influence fishing mortality (Wearmouth and Sims 2008; Mucientes et al. 2009). It is hoped therefore that this will encourage a more fine scale and hypothesis-driven approach to studying grouping and movement behaviour in sharks and rays.

Following this general introduction, I introduce the model species *S. canicula*, studied in this research and discuss some of the methodological intricacies and specific issues associated with using social network analysis to study behaviour in sharks (*Chapter II*). For social interactions to occur, however, individuals must first come together to form groups and in *Chapter III* I quantify juvenile aggregation and social behaviour in this species whilst assessing the effects of conspecific familiarity on gregariousness. In *Chapter IV* the link between female reproductive strategy and juvenile social behaviour is explored. This collaborative chapter uses genetic techniques to determine the level of female polyandry, a phenomenon where a single female mates with multiple males, exhibited by this species. This was necessary for, and prior to the rearing individuals of known relatedness for experiments on kin association. The following chapter (*Chapter V*) investigates the potential mechanisms driving sociality, this time specifically testing for consistencies in social interactions that might be indicative of personality traits. Here I address the extent to which juvenile sharks might maintain or indeed alter their social behaviour under increasing levels of habitat complexity. *Chapter VI* is a methods chapter in which I demonstrate how important recent advances in telemetry technology for large marine predators can be combined with social network analysis to provide new insights to shark movement behaviour. The final experimental chapter (*Chapter VII*) draws upon previous chapters by applying a holistic approach to the understanding of free-ranging shark behaviour. Here I apply the methods outlined in the previous chapter to determine periods of behavioural synchronicity between the two sexes which generally show marked sexual segregation in the wild. Long-term telemetry data and environmental sampling of the area allow predictions to be made about the spatial and temporal dynamics of this population while network analyses are utilised to study both movement behaviour and individual co-occurrences. These final two chapters are intended as a look towards the future direction of shark socio-ecological research, reflecting the final section of this review with specific focus on how elasmobranch populations might be managed under ever more intensive fishing pressure (Baum et al. 2003; Myers et al. 2007).

Chapter II.

General methodology, study species and key concepts

2.1 Introduction

The previous chapter provided a detailed exploration of elasmobranch aggregation and sociality. Broad examples of social behaviour in sharks were compared and contrasted with our current understanding of teleost fish behaviour. Furthermore, analyses of social and movement based networks were introduced as a means of elucidating complex data on association or movement interactions. To avoid repetition throughout the thesis this chapter will outline some of the more general methodological considerations that are central to the subsequent data chapters. It will start by introducing the model species of shark, the small spotted catshark (*Scyliorhinus canicula,* L., 1758) used throughout the study. The conditions and ethical considerations under which this work was conducted and the area in which this species was tracked acoustically are then discussed, finally concluding with a discussion on the sampling and analysis of non-orthogonal, network data.

2.2 Model species

2.2.1 Background

Chondrichthyes, of which elasmobranchii are a subclass, have radiated into a wide variety of forms in order to exploit most available marine habitats and in some cases some freshwater habitats as well. Small spotted catsharks (*S. canicula*, Fig. 6) belong to a diverse order of Carcharhiniformes and have a broad distribution in the Northeast Atlantic from as far north as Norway, down to Senegal on the west coast of Africa, also occurring in the Mediterranean and Adriatic Seas (Compagno et al. 2005, Fig. 7). These sharks are primarily benthic, occupying coastal shelf habitat and are found at depths ranging from just a few metres to approximately 400 m (Rodríguez-Cabello et al. 2007). This species is abundant in UK coastal waters and is regularly caught in the Western English Channel (Ford 1921; McHugh et al. 2011) where specimens for this study were obtained. Once a stable fishery (Ford 1921), the demand for *S. canicula* has significantly reduced resulting in high levels of discard as by-catch. This species however, shows very high levels (98%) of survivorship following discard (Revill et al. 2005) which potentially supports stable population sizes.

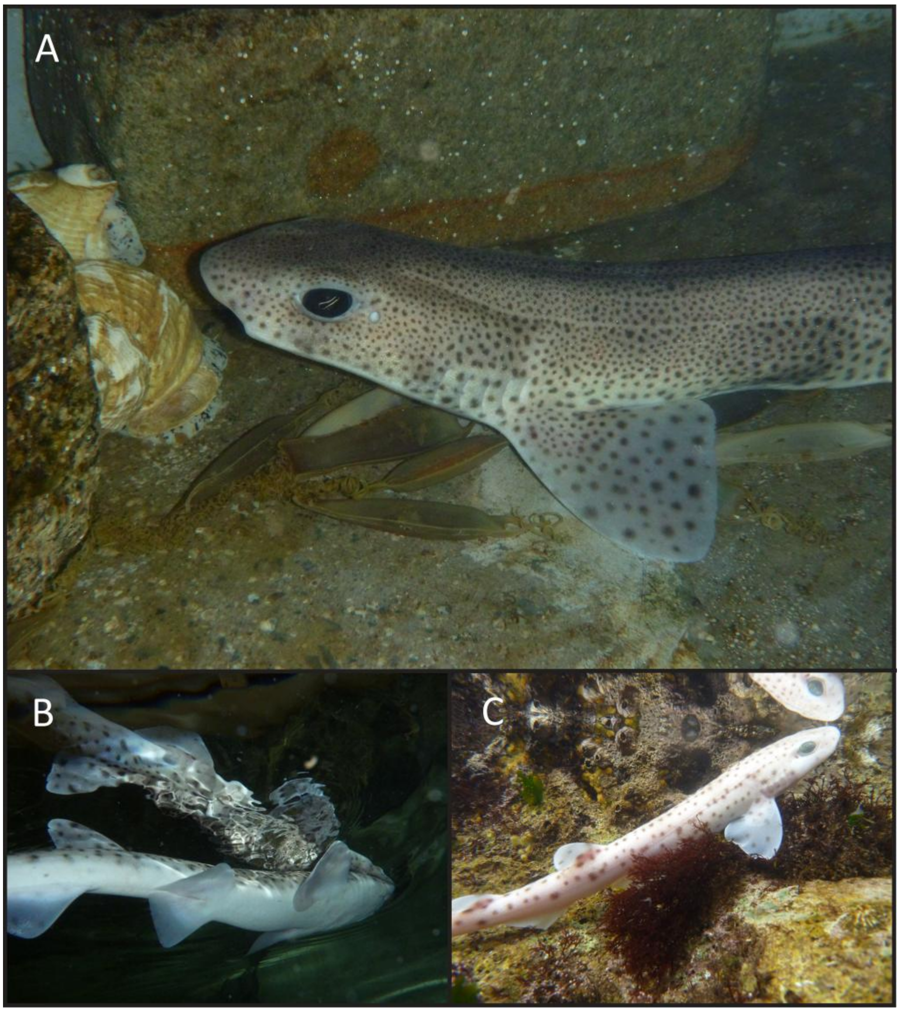

Figure 6. Adult small spotted catshark (*Scyliorhinus canicula*) in lateral view beside egg cases (A), ventral view (B) and as a juvenile (C).

Figure 7. The geographic distribution of *Scyliorhinus canicula*. Maximum total length (L_T) in this species is considerably smaller in the Mediterranean Sea (Max L_T: 0.6 m) than in the North Sea (Max L_T: 1.0 m; Compagno et al. 2005). Map courtesy of Jonathan Hornung.

2.2.2 Biology

The intermediate size of this shark at maturity (Male L_T: 490 – 540 mm, Female L_T: 520 – 640 mm; Ellis and Shackley 1997) and its propensity to survive and breed well in captivity have led to widespread studies on the biology, physiology and behaviour of this animal (Ballard et al. 1993; Bozzanao et al. 2001; Kimber et al. 2009), making it a model species for both field- and laboratory-based elasmobranch research (Sims 2003; Sims et al. 2006: Jacoby et al. 2010). In the wild, adult *S. canicula* demonstrate distinct sexual segregation spatially (Wearmouth and Sims 2008) attributed to substantial differences in behavioural strategy and home ranges between the sexes despite being sexually monomorphic in body size (Sims et al. 2001; Wearmouth et al. 2012). Females refuge in small aggregations during the day in warm, shallow-water, rocky crevices venturing into deeper water every few days to feed (Sims et al. 2001). By contrast, male *S. canicula* undertake crepuscular, diel vertical migration (DVM) from colder, deeper waters where they spend the daylight hours, up into shallow, prey rich waters at night (Sims et al. 2006). These alternative behaviours are thought to result largely from aggressive male mating and mobbing behaviour (Dodd 1983), which drive females to aggregate in single sex groups as an avoidance strategy (Sims 2005; Wearmouth and Sims 2008). Whilst the social factors hypothesis (male sexual harassment of females) arguably plays a significant role in both sexual segregation and aggregation in this species, reproductive benefits are also likely to be gained by females occupying warmer waters (Economakis and Lobel 1998). Aggregation behaviour is also thought to occur in both adult male catsharks and juveniles, although this remains speculative to date (D.W. Sims *pers. comm.*) and social preferences have not been quantified in either before. Given what is already known about behavioural strategies in this species, this thesis seeks to quantify empirically specific hypotheses about aggregation behaviour and identifies potential mechanisms driving such events.

2.2.2.1 Reproductive biology

S. canicula is an oviparous shark, laying a pair of eggs, on average, every two to three weeks during a protracted breeding season that peaks between November and July (Metten 1939; Sumpter and Dodd 1979; Griffiths et al. 2012). Egg cases are generally deposited on complex habitat structures such as macroalgae (Ellis and Shackley 1997) and therefore it is likely that neonate sharks hatch alongside others, some of which might be related. It has been hypothesised by Sims (2003) that in addition to male avoidance, female refuging behaviour might also serve to facilitate increases in the rate of egg development due to the higher water temperatures in shallow habitat. Given the burgeoning evidence of male sexual harassment in this species (Sims et al. 2001; Jacoby et al. 2010; Wearmouth et al. 2012) and well documented evidence for sperm storage (Metten 1939) there are probable consequences for the reproductive output of female *S. canicula.*

2.3 Capture and husbandry

2.3.1 Trawl survey data and experimental shark capture

The Marine Biological Association (MBA) has been conducting monitoring of the marine fish assemblage off the coastal of Plymouth in Devon, UK for over a century (McHugh et al. 2011). This long-term monitoring program provides detailed time-series data on *S. canicula.* From this data Ebenezer Ford carried out his detailed survey of catshark landings between 1911 and 1921 with subsequent research continuing up to the present day (Ford 1921; McHugh et al. 2011). Since the beginning of this recording period efforts have been made to retain as similar trawl gear as possible, with current gear consisting of an otter trawl with a 12 m head rope and a 50 mm (mesh) cod end. All adult experimental sharks used in this study were captured aboard an MBA research vessel using this technique with the majority of these individuals captured during 20 min trawls at Whitsand Bay, Cornwall (Inshore: 50° 20.44′ N, - 4° 16.38′ W to 50° 19.17′ N, - 4° 14.20′ W), and a few captured during 40 min trawls around the L4 monitoring station (Offshore: 50° 15.0' N; 4° 13.0' W). The location of these sites is given in figure 8. Once aboard the vessel, sharks were maintained in flow-through tanks before being transported, on land, to the MBA laboratory (ca. 15 min journey).

Figure 8. Capture locations of experimental sharks, Cornwall, UK. Sharks were caught using an otter trawl predominantly between the two points in Whitsand Bay or occasionally around the L4 Monitoring Station.

2.3.1.1 Wild catch size distribution

During 44 years of trawling between 1913 and 2011, a total of 1024 trawls were conducted at L4, 774 of which contained *S. canicula*. With aggregation behaviour forming the crux of this thesis, the longevity of this dataset provided an interesting opportunity to investigate the distribution of catch size in the wild stock population of the focal species. It is unlikely that complete groups are retained within a single trawl or that individuals participating in refuging behaviour are captured, however, the data at least provides a sub-sample of the numbers present out in open areas at the time of fishing. Taken in its entirety, these data reveal that catch sizes fit a heavy-tailed or power law distribution (Fig. 9a) with an exponent of $\alpha = 1.8$ (Fig. 9b). Power law distributed group sizes, although not consistent with stable group size theory (Beauchamp and Fernández-Juricic 2005) do occur frequently in nature (Niwa 2003) in addition to power laws predicting other aspects of animal behaviour (Sims et al. 2008). These distributions are characterised by a small number of very large group sizes and often cause excitement when found in biological systems due to the potential link between the mathematical assumptions which underlie power laws and the behaviour of the individuals participating in group related behaviours (see Sumpter 2009 for a review).

The mean catch size of these sharks during this 98 year period was 32.9, however, in the last 10 years that number has increased to 74.0 which likely reflects a recovery in stocks due to reduced demand for this species and the high survivorship following discard (Revill et al. 2005). Since 2008, the sexes of individuals caught have also been recorded for each trawl. Given the female propensity to refuge, it was expected that a greater number of males might be captured in areas suitable for trawling (i.e. relatively flat seabed topography). Indeed, a significantly higher mean number of males (Mean ± SE: 40.61 ± 6.18) than females (22.62 ± 4.15) were captured per trawl at L4 between 2008 and 2011 (Mann Whitney test: $U = 5621$, $z = -2.367$, $p = 0.018$). The result supports trends revealed by tracking of this species discussed in a subsequent chapter (*Chapter VII*).

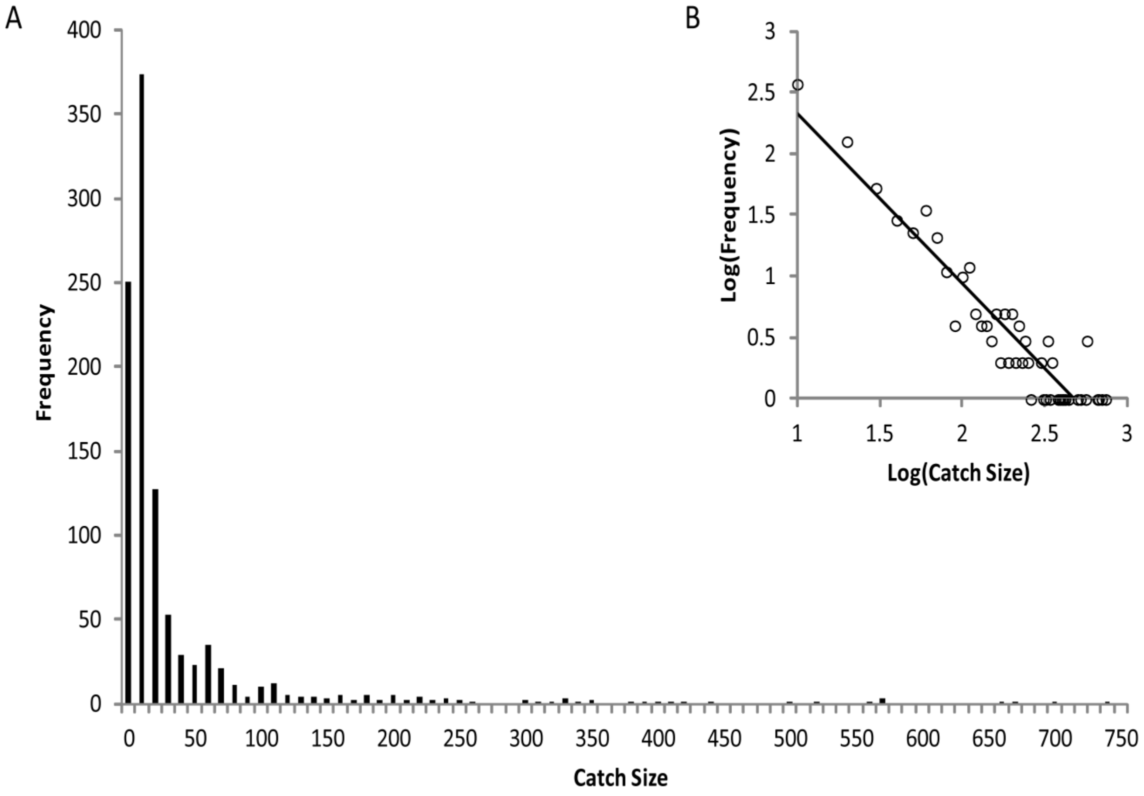

Figure 9. Catch size frequency distribution of *S. canicula* from 774 trawls at L4 spanning 98 years. Evidence of a power law distribution is shown in a heavy-tailed histogram (A) and as a log-log plot (B) with a scaling parameter of α = 1.8.

2.3.2 Husbandry

Throughout the study adult and juvenile catsharks, which were maintained in the Seawater Hall laboratory at the MBA, were monitored on a daily basis. This was in addition to frequent assessment by our resident Named Animal Care and Welfare Officers (NACWO). All research was carried out in accordance with Home Office regulations under the following licences: Personal Licence PIL 30/8940 and Project Licence PPL 30/3442. All animals were fed on a twice weekly discrete feeding schedule of approximately 2.5% wet body mass per individual per feed (Sims and Davies 1994). Food comprised a combination of white fish (mixed species), squid (*Alloteuthis subulata*) and queen scallop (*Aequipecten opercularis*) cut into small pieces and mixed with liposome enrichment and a commercial pellet. Weight and length change was closely monitored in all experimental animals and additional food provided wherever weight gain was not observed. During experimentation all feeding occurred at the end of the day post data collection. All sharks were checked and certified by a NACWO prior to release either into Plymouth Sound or Whitsand Bay.

The water supply to all holding and experimental aquaria came from a semi-open recirculation system in which seawater was drawn through a course filter in the sublittoral zone of Plymouth Sound and pumped into a 67500 L reservoir. Seawater from the reservoir passed through a commercial filtration system before entering the aquaria. Water was tested regularly for pH, ammonia, and nitrate concentration, and temperature control was maintained by passing the water through a chiller twice a week. Seawater was subject to seasonal variation in temperature despite partial temperature control with the chiller; ambient water temperatures in MBA aquaria vary from 13 ^{o}C in winter to 19 ^{o}C in the summer. All holding and experimental aquaria were subject to consistent, year round diel light cycles (12 h light/12 h dark).

2.3.2.1 Individual identification

Whilst in captivity, all adult sharks were tagged with coloured T-bar anchor tags (FD94; Floy Tag, Seattle, WA) on the posterior dorsal surface just above the lateral line and level with the pelvic fin emargination. Individual identification not only enhanced the welfare of these sharks during captivity but also helped to determine the maternal heritage of the eggs laid by different females. For identification of the substantially smaller juvenile and neonate *S. canicula*, individuals were tagged with a unique, fluorescent visible implant elastomer tag

(VIE – Northwest Marine Technology, WA, USA) which was injected subcutaneously on the dorsal surface in any one of 10 different positions or orientations. While difficult to see under natural light conditions, due to the nature and thickness of shark skin/dermal denticles, tags fluoresced brightly under a VI light source (Fig. 10). There was no evidence that this light source influenced behaviour and individuals did not move when illuminated.

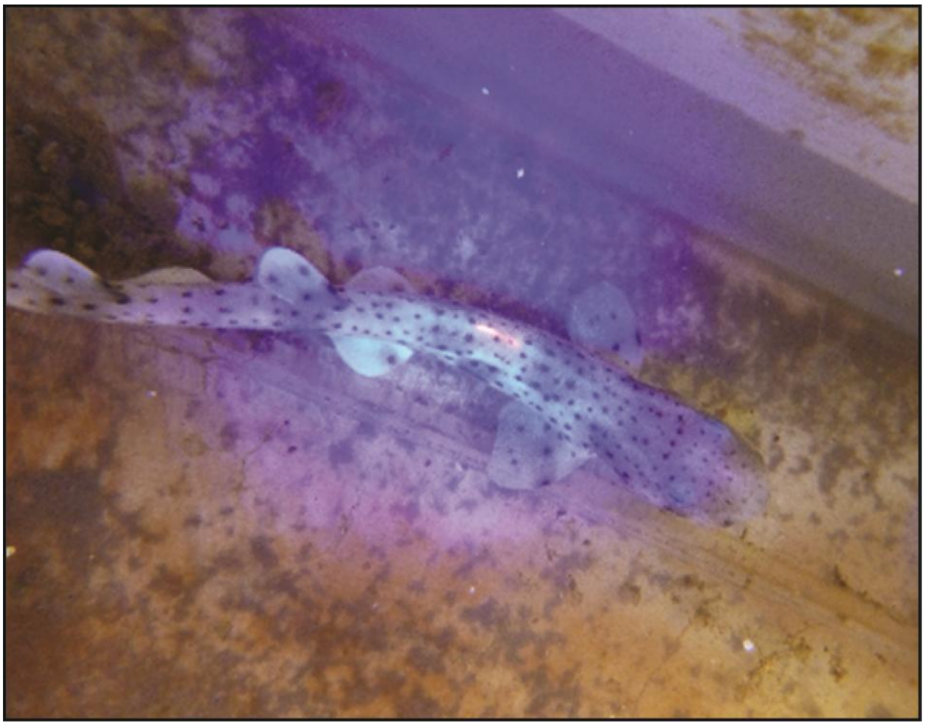

Figure 10. Visible implant elastomer (VIE) identification tag fluorescing under VI light. VIE tags are gradually shed backwards out of the skin as the shark grew.

2.3.2.2 Experimental aquaria and hatchery programme

Approximately 20 adult females were maintained in the laboratory for extended periods (between four and 10 months) with these individuals providing virtually all eggs for the subsequent studies on juvenile sharks. Adults were housed in large, circular aquaria which were 1.83 m in diameter by 0.43 m in depth with a capacity of 2242 l of seawater. An additional 48 adults were brought into captivity in order to surgically implant acoustic transmitters and to monitor recovery, prior to release into the acoustic array (see *Chapters VI* and *VII* for details). Experiments involving neonate or juvenile sharks were conducted in large rectangular aquaria (1026 l capacity, 1.8 × 1.0 × 0.6 m) with some small groups of individuals being transferred from smaller 'familiarisation' tanks (35 l capacity, 0.3 × 0.4 × 0.3 m, see *Chapter III* for more detail). All aquaria were connected to the semi-open, recirculation system.

Eggs were removed from captive females when tendrils were observed trailing from the cloaca and thus maternal heritage was determined (see *Chapter IV* for more detail). Eggs were then removed from the aquaria and were transferred to an egg rack in a separate tank where they were housed individually until they hatched (Fig. 11). The incubation period was negatively correlated with seawater temperature with a mean of 177.7 days (range: 128 – 226 days). During the course of the project a total of 355 individual sharks, of known maternal heritage, were reared in this manner, all of which were measured and weighed upon hatching and a small fin clip taken for DNA analysis (see *Chapter IV*). Additional individuals from unknown eggs ($n \approx 400$) were also used for laboratory experiments. Following the post-hatching measurements, individuals were moved into specific holding environments, depending on the experiment and details of these are outlined in each chapter. Sex of the individuals was determined at birth with an approximate sex ratio of 1:1. As there were no significant effects of sex on social behaviour in juveniles (see *Chapter IV*), for all laboratory chapters the sex of the individuals were randomly selected.

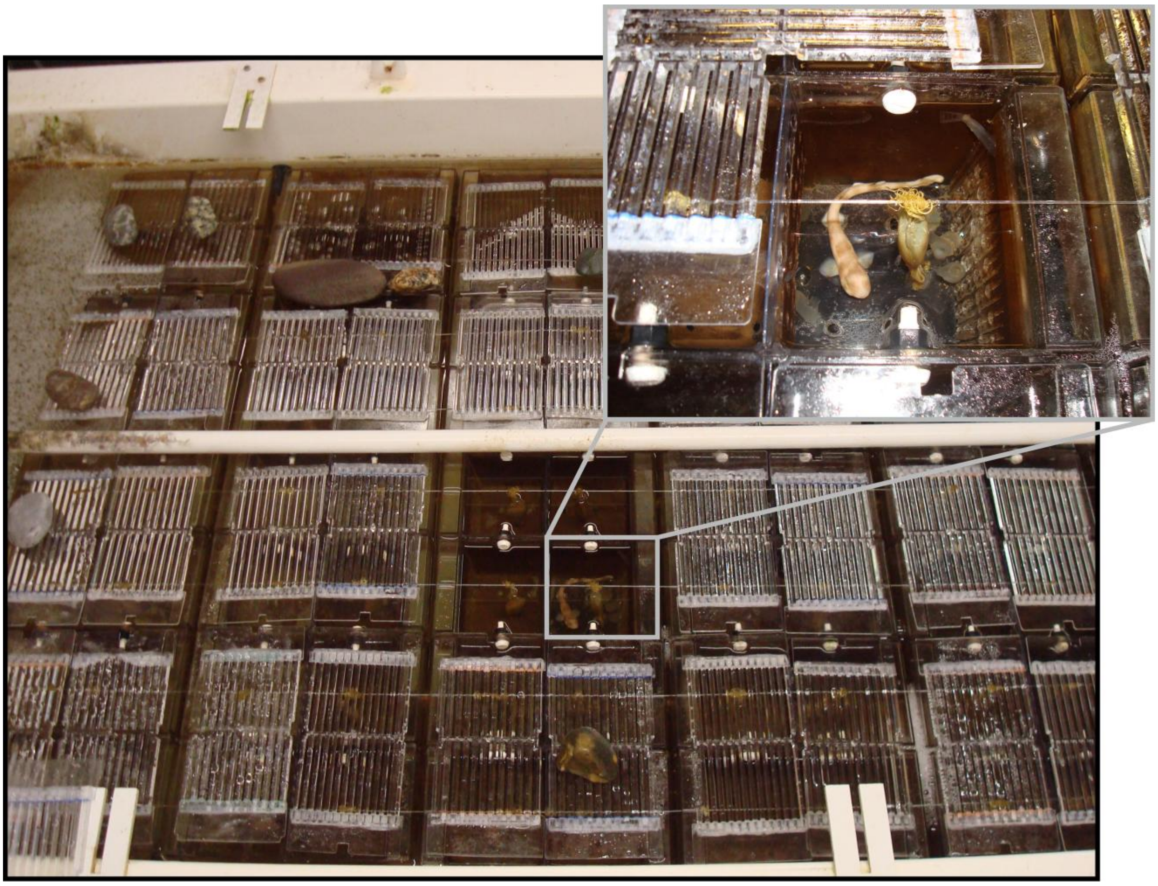

Figure 11. Purpose built egg hatchery with hatchling (inset). Individual social environments were manipulated from the day sharks hatched.

2.4 Analytical considerations

Throughout this thesis a number of different statistical and analytical programs were used. Unless otherwise stated, data manipulation was conducted in Microsoft Excel and Access and all traditional parametric and non-parametric statistics were carried out in PASW Statistics 18 (IBM Corp., Somers, NY, USA) and R (R Development Core Team). Network analyses were largely conducted in SOCPROG 2.4 (Whitehead 2009) and UCINET (Borgatti et al. 1999). Throughout this thesis the term 'aggregation' is used to define group resting behaviour that may or may not be underpinned by social preferences. Aggregation is therefore quantified by determining mean group sizes or group numbers within an experiment. 'Social groups' therefore refer to aggregations where social preferences have been determined and explicitly tested. In order to do this a social networks approach was adopted, the ideas and benefits of which were introduced in *Chapter I*. Before social networks can be constructed and analysis undertaken however, there are a number of important issues to address.

2.4.1 Network construction

Social interactions, where one animal is observed directing behaviour towards another animal (e.g. grooming or fighting), are often difficult to record within wild animal populations due to the speed or brevity of some behaviours, the number of animals involved or the location and timing of these events. Due to some of these constraints social networks are often constructed from symmetric, association data based upon who is in close proximity to whom. What constitutes a social interaction or even alliance to a specific group and at what spatial scale we might expect these to occur, largely depend on the species in question and these are all important considerations when determining data collection techniques. Group resting behaviours, such as those observed in some benthic elasmobranchs that rest either in tactile contact or within a single body length (Sims et al. 2001; Hight and Lowe 2007) command a different set of associative parameters at a different scale, than those required for studying wide ranging facultative schoolers where there might be a distance of 10-15 m between individuals for example (Klimley 1985). To be of use therefore, a social network must account for at least some of these species specific behaviours and it is therefore crucial to be mindful of incorporating both biological relevance and scale when defining interaction/association parameters (Lusseau et al. 2008). With these considerations in mind, an implicit understanding of these interactions demonstrates how sociality may influence individual and group level behaviours (Wey et al. 2008). The formation of weighted social networks (Fig. 4c, *Chapter I*) ultimately relies upon the truncation of multiple static or binary networks representing each sampling period and provides a 'snapshot' of social interactions across all of these periods at once. As such there is the potential to lose a lot of information during this process; the sampling frequency and duration of data to be included in the final network used to illustrate and analyse social behaviour in the study system, must therefore be given considerable thought. Recent research into time-aggregated and time-ordered networks are beginning to address this issue offering statistical methods for determining suitable sampling regimes (Haddadi et al. 2011; Blonder et al. 2012).

2.4.1.1 Collecting association data

Data on animal associations can be gathered in several different ways depending on the study system and the objectives of the experiment. Scan sampling of a population whereby animals within a predetermined distance of one another are deemed to be grouping ('gambit of the group' – GoG) is just one way in which association data is often accumulated (Whitehead 2008; Franks et al. 2010). Alternatively, egocentric networks can be constructed by using focal follows of individual animals over a given time period. The network is established based on the individuals with whom the focal animal interacts with during that time (e.g. Edenbrow et al. 2011). In an attempt to reduce the potential for subjectivity in both of these commonly used methods, established protocols are now being developed to calculate the associative distances and minimum sampling frequencies required to accurately construct a social network (Haddadi et al. 2011). Given the controlled and replicated conditions under which the laboratory experiments were carried out for this current project, GoG scan sampling was used throughout with a sampling frequency determined by comparing preliminary network structures across a range of sampling intensities.

For all experiments where aggregation or association measures were calculated, the experimental protocol remained practically the same and is outlined here. Any differences in the data collection methods are included in the methods in the later chapters. The propensity of my model species to spend long periods resting, either in small groups or individually, allowed aggregation and social grouping to be quantified based on scan samples of who was resting next to whom (Fig. 12). Indeed the social networks of adult female aggregations have been quantified using the same methodology previously (Jacoby et al. 2010). All sharks were free to be solitary during a sample and any swimming sharks were recorded to determine levels of activity. Consequently group size and group number were not expected to co-vary. During each scan sample individuals were considered aggregated or associating, if the centre point of the shark (a predefined point halfway along the length of the body) was estimated to be within one body length of a conspecific. Body lengths were based on the mean body length of the individuals within the experimental group. This simple measure reduced the complication associated with individual orientation and was considered a sufficient metric for aggregation based on the large size of arenas relative to shark size, and the few individuals used per trial.

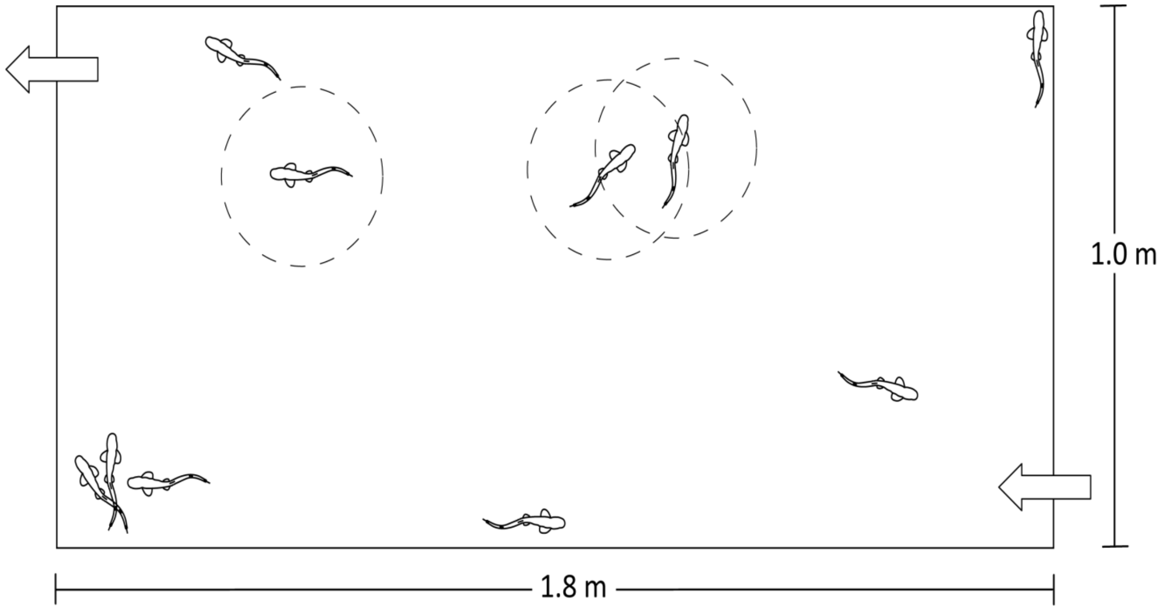

Figure 12. Illustration of juvenile sharks as they rest in small groups or individually at the bottom of an experimental arena. Water flow into and out of the arena (arrows) was at the surface to reduce the influence of current on resting behaviour. Circular zones of association (ZOA), with a radius of one body length are shown (dashed lines) with an overlap of centre points representing individuals in the same group.

2.4.1.2 Association indices

Once collected, association data must then be converted to a matrix format whereby every possible dyadic interaction is assigned either a zero or a one in a binary network or a value between zero and one for a weighted network. There are a number of factors which potentially introduce bias into the data at this stage and thus an appropriate association index must be used to control for these factors (Whitehead et al. 2005; Croft et al. 2008). Inevitably, during studies on free ranging animals, some individuals within a population are more likely to be sampled at a higher frequency than others due to numerous biological factors such as individual differences in home range, age classes and/or dispersal. The benefits of applying different association indices to social data are discussed at length by

Cairns and Schwager (1987). For the purposes of this thesis, I shall focus on the two most commonly used indices, the Simple Ratio Index (SRI) and the Half-Weight Index (HWI), both of which are used during the analysis of catshark data in subsequent chapters. The SRI (Equation 1) provides an absolute measure of the amount of times individual *a* was seen with individual *b*, relative to the amount of times these individuals were observed separately (Cairns and Schwager 1987). The SRI, however, assumes that within every sampling period the whereabouts of each individual is accounted for and thus the use of this index is often restricted to captive studies (Cairns and Schwager 1987). In the wild, it is impossible to track the location and interactions of all of the animals in a population and bias is often introduced due to the increased likelihood of some individuals occurring in a sample over others, as a simple by-product of group composition (Whitehead 1997; Lusseau et al. 2006; Croft et al. 2008). The HWI (Equation 2) is therefore frequently used to account for this bias. The assumptions for this index are that during social network studies of wild populations, individuals are more likely to be identified when associated together than when apart in separate groups. The HWI particularly is useful for when individuals are more likely to be observed apart than together for example, when there is no *a priori* hypothesis about strong and consistent temporal associations between group mates (Whitehead 2008).

Equation 1:

$$\frac{x}{x + y_{ab} + y_a + y_b} \qquad \text{(Simple Ratio Index)}$$

Equation 2:

$$\frac{x}{x + y_{ab} + \tfrac{1}{2}(y_a + y_b)} \qquad \text{(Half-Weight Index)}$$

where x is the number of observations in which *a* and *b* were observed together in the same group, y_{ab} is the number of sampling periods in which *a* and *b* were observed in separate groups, y_a is the number of sampling periods in which only *a* was observed, and y_b in which only *b* was observed (Ginsberg and Young 1992).

When dealing with movement interaction data, that is the movement of a single or group of animals between locations in a movement network context, many of the above considerations still apply. However, the adjacency matrix is constructed from interaction instead of association data and is thus directional as well as weighted. Simple ratio and simple counts of movements can therefore be used. The analysis of movement interaction data is addressed in detail in *Chapter VI.*

2.4.2 Social network metrics

The use of social network analysis facilitates both the exploration of an animal's or a location's role within the overall network structure but also the global properties of a network as a whole which can then be compared across contexts. This can be achieved by calculating a wide array of node-based and global network metrics. Measures of individual centrality for example are useful for highlighting nodes (i) which are likely to be of key importance to the structure of the network. Social cohesion in networks of yellow-bellied marmots (*Marmota flaviventris*) for example, is disproportionately maintained by young, often yearling individuals (Wey and Blumstein 2010). Such measures of centrality include *degree* (k_i), which describes simply the number of edges attached to an individual node (i.e. its network neighbours). Degree can be further defined as In-degree (k_i^{in}) and Out-degree (k_i^{out}) within a directed network in order to determine the amount of behaviour received from conspecifics relative to the amount of behaviour directed towards others. Global properties of a network might include the *edge density* which quantifies the number of network edges relative to the total possible number of edges for a network containing *n* nodes. These and many other social network metrics are discussed at length by Croft et al. (2008) and are expanded upon within subsequent data chapters where specific metrics have been used during the analysis.

2.4.3 Analysing non-independent network data

By definition, relational data points, such as those found in social network data, are inter-dependent upon one another. The social interactions of one individual are ultimately dictated by the social behaviour of others in the population. This must be addressed therefore when testing hypotheses using social network data as many common statistical approaches assume independence (see Croft et al. 2011 for a discussion and a review of approaches to overcome this issue). A popular method and one used frequently throughout this thesis, is to use null models against which observed social or movement data can be compared (Manly 2006). The finer detail of the null models used here are discussed where necessary within each chapter as null models are often data-specific and dependent upon the hypotheses being tested (Whitehead 2008; Croft et al. 2011). More broadly null models offer a tool for randomising one component of our observed data (e.g. group size, group composition), whilst constraining another (e.g. number of groups) before calculating an experiment-specific test statistic. By permuting these random iterations, often between 5,000 and 50,000 times dependent on when the resulting *P* values stabilise, a null distribution can be obtained which reflects the distribution of the test statistic expected from random or chance associative behaviour. The observed test statistic can then be compared to this null distribution and significance values can be obtained.

There remains significant interest in developing and enhancing the ways in which network data is analysed. For more detail on the lively discussion surrounding the appropriate parameters and techniques for permuting social network data refer to Manly (1995), Bejder et al. (1998), Whitehead et al. (2005), Manly (2006), Whitehead (2008), Croft et al. (2008) and Croft et al. (2011). The following data chapters offer worked examples of some of the methods discussed in this chapter and illustrate the need to tailor these analyses and models to the data in question.

Chapter III.

The effect of familiarity on aggregation and social behaviour in juvenile small spotted catsharks, *Scyliorhinus canicula*

3.1 Abstract

Animals that devote time to aggregating with conspecifics may stand to benefit from repeated associations with familiar conspecifics. While the development of familiarity has been demonstrated in many teleost fishes, there is limited information on whether sharks (Elasmobranchii) are capable of, or known to benefit from the recognition of familiar individuals. This study was designed to examine whether juvenile small spotted catsharks, *Scyliorhinus canicula* aggregate and to determine whether aggregation is underpinned by social preferences for conspecifics. Using controlled and replicated experiments, the role of familiarity as a potential mechanism driving aggregation and social behaviour in this species was examined. Replicated familiar and unfamiliar groups (n = 10 for each) were assessed for social structure based on group resting behaviour. Observed *S. canicula* association data compared to null model simulations of random distributions, revealed differences in aggregation under different social contexts. Only familiar juvenile sharks aggregated more than would be expected from random distribution across their habitat. Familiarity increased the mean number of groups but did not significantly affect mean group size. Significant preference and avoidance behaviour across all groups was also observed. Furthermore, the strength of social attraction, quantified by the mean association index, was significantly higher in groups containing familiar individuals. Interestingly there was a high degree of variation across replicates at the individual level, with some groups assorting by familiarity and others not. This study, to my knowledge, is the first to examine experimentally the influence of conspecific familiarity on aggregation behaviour in sharks. These results not

only imply a functional benefit to aggregation but also suggest persistent social affiliation is likely to influence dispersal following hatching in this small benthic elasmobranch.

3.2 Introduction

In an aquatic environment, as on land, the neonate or juvenile life history phase is a particularly vulnerable time for many animal species. Survivorship within the first year is often low (Hall et al. 2001) and consequently juveniles may adopt a number of different strategies in an attempt to find sufficient food for growth, avoid predation and/or resist exposure to potentially lethal environmental conditions (Swaney et al. 2001; Brown 2003; Lürling and Scheffer 2007). One strategy that is commonly adopted is to aggregate with conspecifics, a strategy which might occur through both active and passive processes (Krause and Ruxton 2002). Such behaviour is likely to have implications for the timing of dispersal from natal areas. For species that demonstrate little or no parental care, such as elasmobranchs, these behavioural strategies might be particularly important.

Group living in both the form of active social grouping (schooling and shoaling) and aggregation behaviour, has been highly selected for in both teleost (Pitcher and Parrish 1993) and elasmobranch (see *Chapter I*) fishes. Aggregation behaviour implies a lack of social attraction between individuals and results from a common requirement for shared resources which are clustered in space (Johnson et al. 2002). In contrast social grouping arises through active social preferences between individuals (Krause and Ruxton 2002; Croft et al. 2008). The mechanisms and functions of both aggregation and social grouping in teleost fishes have received considerable attention (Brown and Warburton 1999; Swaney et al. 2001; Krause and Ruxton 2002). Given that one of the main aims of this thesis is to explore aggregation and social behaviour in a model species of shark, aggregation in the context of the thesis refers to the resting behaviour of a social group where social preferences have not yet been explicitly tested for. Research on both adult and juvenile sharks has so far been largely restricted to the structure of aggregations of predominantly large, coastal and often tropical species, typically belonging to the families Sphyrnidae or Carcharhinidae (Klimley, 1985; Economakis and Lobel 1998; Heupel and Simpfendorfer 2005a). Juveniles of many such species have been found to utilise warm shallow, inshore waters for breeding and parturition (Morrissey and Gruber 1993). The relatively safe and food abundant waters associated with tropical lagoons offer ideal nursery grounds in which juvenile sharks can develop and, when large enough, they are known to venture offshore into deeper, more productive waters (Simpfendorfer and Milward 1993; Heupel et al. 2007; Chapman et al. 2009). An interesting consequence of these shark nursery areas is the potential for repeated individual encounters between sharks. Juvenile lemon sharks (*Negaprion brevirostris*) for example, form social groups that have been shown to preferentially assort by body size, with larger individuals showing stronger leadership tendencies (Guttridge et al. 2011).

The development of social recognition or familiarity in teleost fishes is well documented (Griffiths and Magurran 1997; Barber and Wright 2001; Darden et al. 2009) and has been shown to confer benefits in a range of species (Höjesjö et al. 1998; Swaney et al. 2001; Ward and Hart 2003; Jordan et al. 2009). There are distinct advantages in being able to discriminate between kin or familiar conspecifics (see Ward and Hart 2003 for a review), ranging from a reduction in competition and inbreeding, to enhanced antipredator behaviour and inclusive fitness benefits (Griffiths and Magurran 1999; Frommen et al. 2007; Hain and Neff 2007). This ability and the mechanisms maintaining social recognition vary widely depending on the life history of the species (Mateo 2004). While familiarity is key in the shoaling and schooling of freshwater teleost fishes, little is currently understood of the role of familiarity in structuring interactions in social elasmobranchs.

The current trend in analysing animal social networks (Wey et al. 2008; Krause et al. 2009; Sih et al. 2009) is beginning to be applied to elasmobranch social behaviour (see *Chapter I* for review), with recent studies, for example, using this approach to examine the impact of captive male behaviour on females of differential social status in a sexually segregating benthic shark (Jacoby et al. 2010). Social network analysis has also been used to explore the social and spatial component of shark aggregations in the wild (Mourier et al. 2012). Recently the wild social interactions of juvenile *N. brevirostris* were quantified demonstrating non-random assortment of individuals by body length and potentially relatedness within a shallow water tropical nursery area (Guttridge et al. 2011). Understanding the mechanisms, functions and temporal dynamics underpinning shark social behaviour is vital for assessing the vulnerability of different elasmobranch species, many of which require urgent conservation efforts due to persistent anthropogenic threats.

The size and behaviour of large coastal sharks (even as juveniles) renders them difficult subjects in which to carry out experiments testing explicit hypotheses (see Guttridge et al. 2009a for an exception). Indeed, virtually all of our current knowledge on juvenile shark behaviour to date, comes from the tracking and direct observation of viviparous species in shallow, warm water habitat (Holland et al. 1993; Heupel and Simpfendorfer 2005b; Guttridge et al. 2009a). For obvious reasons, data on the behaviour and aggregation of juvenile sharks in oviparous, deeper dwelling species are exceptionally rare (Powter and Gladstone 2009) and hypothesis testing, even more so. With profound differences in life-history traits and different habitat utilisation, oviparous sharks are likely to experience different selection pressures to their live-bearing relatives during development (Sims 2005). Whether aggregation and social behaviour plays a greater role in the early survival of these sharks in deeper water, remains unknown. With eggs often deposited in large numbers on complex habitat features (Sims 2003; Powter and Gladstone 2009), neonates are likely to hatch very close spatially to one another, potentially occupying rocky crevices and other hiding places for some time. It seems pertinent then to determine, not only the likelihood or importance of aggregation and social behaviour, but also the mechanisms which might underpin juvenile behaviour in a model species of benthic, oviparous shark hatched from localised clutches of eggs.

The small spotted catshark (*Scyliorhinus canicula*) is a small benthic elasmobranch common to UK coastal waters (Compagno et al. 2005). As adults, the females particularly, spend a significant proportion of their diel cycle refuging (group resting) in single sex groups in shallow water rocky crevices (Sims 2003; Wearmouth et al. 2012). More detail on the biology of this species is given in *Chapter II*. Equally, neonate *S. canicula*, reared in the laboratory (approximately 100 mm total length upon hatching), spend long periods resting on the bottom (e.g. only 0.6 min h^{-1} are spent active during daytime; Sims et al. 1993). This experiment was designed to test the hypothesis that juvenile (> 6 month old) *S. canicula* aggregate more than would be expected from random space use. How familiarity between conspecifics influenced this aggregation behaviour, was then explored. The specific aims of this study were to determine whether (i) recently hatched benthic sharks show a tendency to form aggregations, (ii) aggregations are underpinned by non-random social preferences between individuals (i.e. they demonstrate social groups) and (iii) familiarity influences both group size/group number distribution and individual social preferences through assortative interactions. Using a relatively small, oviparous elasmobranch species offered a rare opportunity to manipulate the social environment of each individual from hatching (see *Chapter II*). A combination of analytical approaches, including social network analysis (SNA), were used to consider both aggregation, the formation of resting groups that may or may not demonstrate social preferences and social behaviour and the quantification of social interactions within resting groups under controlled, captive conditions. Network replication achieved here, remains relatively rare in studies of animal social behaviour (Croft et al. 2011) and is used (albeit in low numbers) to consider trends across treatments.

3.3 Materials and Methods

3.3.1 Experimental sharks

All *S. canicula* were reared from egg cases maintained in the laboratory and laid by wild female sharks (*n* = 13) temporarily housed at the Laboratory of the Marine Biological Association, Plymouth, UK between April and December 2009 (see *Chapter II* for more detail). Upon hatching, sharks used in *Experiment 2* were tagged with a fluorescent visible implant elastomer tag (VIE; Northwest Marine Technology, WA, USA) in order to identify which specific individuals were associating. A total of 392 neonate and juvenile sharks were used in this study, all of which were held in either a large hatchery/holding aquarium (1026 l capacity, 1.8 × 1.0 × 0.6 m) or in small numbers in isolated familiarisation aquaria (35 l capacity, 0.3 × 0.4 × 0.3 m) in accordance with the experimental design described below. All aquaria were fed from the same circulatory seawater system described in *Chapter II*. Tracking *S. canicula* by individual identification post hatching ensured that all individuals used were exposed to the same social environments prior to experimentation. The ethics and husbandry for this study fall under the general methodology outlined in *Chapter II*.

3.3.2 General methodological procedure

During a trial, experimental sharks were removed from specific holding environments and transferred to experimental arenas (1026 l capacity, 1.8 × 1.0 × 0.6 m) for a 24 h acclimation period prior to data collection. Although the sex of an individual to be used was selected randomly (*Chapter IV* demonstrates that no evidence was found for sex effects on group structure), size-matched individuals were specifically chosen for all trials to control for potential size assortment on aggregation behaviour [mean total length, $L_T \pm SD = 171.92 \pm 8.68$ mm for juveniles (> 6 months old) and 103.51 ± 4.57 mm for neonates (< 2 months old)]. Wild data on the activity patterns in adults of this species reveal that both males and females frequently spend daylight hours resting and that females particularly become more active at night (Sims et al. 2001; Wearmouth et al. 2012). Furthermore, juvenile *S. canicula* have been shown to show significant increases in activity rate and oxygen consumption (VO_2) during hours of darkness (Sims et al. 1993). Data collection therefore comprised four scan samples per experimental day, each three hours apart (0830, 1130, 1430 and 1730 h) which, given the time of year (winter), was sufficient to encompass crepuscular and daylight aggregation behaviour. Data were collected on the number of groups (including zero values when all individuals were resting alone), the number of individuals per group with a minimum group size of two individuals and in *Experiment 2* the identity of each group participant.

3.3.3 Experiment 1 – Familiarity and aggregation behaviour

Experimental juveniles were selected from a large holding aquaria and were measured and weighed (L_T between 150 and 180 mm, mean weight: 13.94 g) before being transferred on Day 0 of the experiment to small familiarisation aquaria (35 l capacity, 0.3 × 0.4 × 0.3 m). Here they were kept in groups of 10 individuals, with each familiarisation aquaria both visually and chemically isolated from one another. Preliminary studies using a series of binary choice experiments revealed an apparent trend towards an associative preference for familiar conspecifics after just four days of familiarisation, although this trend was marginally non-significant (D.M.P. Jacoby, unpublished data). Therefore, 10 days of familiarisation were allowed prior to experimentation. Following assignment to these holding conditions, juveniles within the same holding aquaria were deemed 'familiar' and those of different tanks 'unfamiliar'.

Following familiarisation, whole familiar groups were transferred to the experimental arena for acclimation (24 h) before data collection on aggregation behaviour commenced. Unfamiliar groups, also acclimatised for 24 hours, were constructed by taking one individual from 10 familiarisation aquaria. Using four experimental arenas, two familiar and two unfamiliar treatments were run simultaneously until 10 replicates of each had been achieved. Aggregation data, as defined in the introduction and the general methods (*Chapter II*), were collected over two days providing eight samples per trial. Group sizes and number of groups were averaged across the eight samples for each replicate and a multivariate general linear model (GLM) was then carried out to determine the influence of familiarity (independent variable) on the dependent variables of mean number and mean size of shark groups. These observed values were then compared to randomised distributions of group sizes calculated in a null-model simulation program coded in C#. This facilitated testing of whether juvenile *S. canicula* aggregated more than would be expected from chance distributions within the behavioural arena. Parameters for the simulation model, such as arena size, number of sharks, sampling protocol and mean size of sharks, were standardised to mirror the empirical experimental regime. Association parameters for the null model were based on a circular zone of association (ZOA) surrounding each individual which equated to one body length from the centre of the randomly assigned virtual shark. Individuals (n = 10) were then randomly assigned to the virtual arena space using a uniform, random distribution of x y coordinates. Associations were deemed to have occurred when the ZOA of one individual overlapped the centre point of another individual. Thus, group sizes and group numbers were recorded and averaged across eight samples, which was the same as that of the experimental protocol. Null models were constructed for each sample group which allowed each null model to be tailored to the exact mean size of sharks within a particular treatment, therefore accounting for any potential bias imposed by the ratio of shark size to arena size. This procedure was replicated 10,000 times for each observed group and P values were calculated from frequency distributions of the observed test statistic for randomised data (Manly 2006). Pilot runs of the randomisation tests demonstrated that 10,000 iterations were sufficient for P values to stabilise. Stouffer's method for combining P values was used to produce an overall level of significance (Piegorsch and Bailer 2005).

3.3.4 Experiment 2 – Social preferences and assortment

To determine whether aggregation behaviour was underpinned by social preferences at the individual level, another experiment was carried out in the same experimental arenas, this time using smaller neonate catsharks (< 2 months old). During this experiment, data on social behaviour was collected over five days (20 samples in total) for 16 replicated groups, again divided into familiar and unfamiliar treatments (*n* = 8, per treatment). Each trial comprised 12 size-matched experimental sharks which were selected from small familiarisation/hatching aquaria, each containing four individuals. Experimental groups therefore consisted of either three groups of four or one individual from 12 separate aquaria, thus allowing testing of whether individuals assort based on familiarity within their smaller conspecific groups.

Social network analysis (SNA) was used to test for preferential association between individuals and to provide individual metrics of sociality across networks. Manipulation of the association data was carried out in SOCPROG 2.4 (Whitehead 2009) whereby data on group membership for each trial was converted to a matrix of association using the Simple Ratio Index (SRI; Cairns and Schwager 1987). Each possible dyadic or paired association was therefore assigned an association index (AI) value between 0 and 1 based on the frequency with which those two individuals were observed together across the 20 samples (i.e. a weighted network measure). A Monte Carlo permutation test, outlined in Whitehead (2009) was conducted on each of the 16 replicated groups addressing the null hypothesis of random preference and avoidance of within-group associations. For this test, row and column totals of the incidence matrix were maintained, preserving group sizes whilst individual group membership was randomised. For each of the 16 groups, a null frequency distribution was produced from 20,000 permutations, each with 1000 'flips' of the data between columns and rows, a revised version of the Manly/Bejder procedure (Manly 1995; Bejder et al. 1998; Whitehead 2009). Significant dyads of preference and avoidance were also calculated by highlighting those dyads with a significantly higher (AI > 97.5%) or significantly lower (AI < 2.5%) value compared to the randomly generated distribution of AIs.

Finally, patterns of assortment by familiarity within these neonate groups were quantified using a null simulation model that was designed to test whether within-group association (i.e. between familiars) was significantly higher than between-group association (i.e. between unfamiliar individuals). The mean value for between-group association was subtracted from the within-group association providing a difference which was used as the test statistic for this experiment. For each of the groups containing familiar conspecifics ($n = 8$) participants were randomly permuted into the observed group sizes for each of the 20 observed samples. This process was iterated 10,000 times to create a null distribution against which the observed values were compared. All traditional data analyses (i.e. excluding network analyses and null modelling) were conducted in PASW Statistics 18 (IBM Corp., Somers, NY, USA) and R (R Development Core Team).

3.4 Results

3.4.1 Experiment 1 – Familiarity and aggregation behaviour

Across all replicates within the familiar treatment there were significantly higher mean numbers of groups than expected by random space use (Stouffer's test: $n = 10$, $p < 0.001$).

However, groups differed in their patterns of aggregation. Of the 10 familiar treatment replicates, six demonstrated significantly higher mean numbers of groups than random (two tailed test: $p < 0.025$), whilst patterns of aggregation did not differ from random for the remaining four groups (two tailed test: $p > 0.025$). Two of the six significant groups also showed significantly higher mean group sizes (two tailed test: $p < 0.025$).

Table 2. Summary of the null model simulation for aggregation behaviour (*Experiment 1*) of familiar (F) and unfamiliar (UF) shark groups.

		Mean Number of Groups			Mean Group Size		
Group	Treatment	Observed	Expected	*P*	Observed	Expected	*P*
1	F	1.13	1.63	0.8972	2.22	2.35	0.7595
2	F	2.38	1.63	**0.0017**$^{+}$	2.37	2.35	0.4497
3	F	2.13	1.63	**0.0188**$^{+}$	2.82	2.35	**0.0194**$^{+}$
4	F	2.50	1.63	**0.0004**$^{+}$	2.80	2.35	**0.0227**$^{+}$
5	F	2.38	1.63	**0.0017**$^{+}$	2.68	2.35	0.0634
6	F	1.25	1.63	0.8045	2.20	2.35	0.7956
7	F	2.25	1.63	**0.0075**$^{+}$	2.78	2.35	0.0285
8	F	1.38	1.63	0.6650	2.36	2.35	0.4520
9	F	2.00	1.63	0.0533	2.50	2.35	0.1920
10	F	2.75	1.63	**0.0001**$^{+}$	2.36	2.35	0.4520
1	UF	0.75	1.63	**0.9954**$^{-}$	2.33	2.35	0.5029
2	UF	1.13	1.63	0.8972	2.00	2.35	**0.9895**$^{-}$
3	UF	1.38	1.63	0.665	2.55	2.35	0.1537
4	UF	2.38	1.63	**0.0017**$^{+}$	2.26	2.35	0.4520
5	UF	1.63	1.63	0.3496	2.23	2.35	0.7410
6	UF	0.88	1.63	**0.9821**$^{-}$	2.29	2.35	0.6191
7	UF	0.88	1.63	**0.9821**$^{-}$	2.43	2.35	0.3185
8	UF	1.13	1.63	0.8972	2.44	2.35	0.2986
9	UF	1.13	1.63	0.8972	2.56	2.35	0.1506
10	UF	0.75	1.63	**0.9954**$^{-}$	2.40	2.35	0.3713

Two tailed test: (+) Result is significantly greater than expected. (-) Result is significantly smaller than expected. Significance is indicated by bold lettering.

Conversely the unfamiliar treatment overall showed significantly lower mean group numbers than would be predicted under random space use (Stouffer's test: $n = 10$, $p = 0.999$). Of the 10 replicates for this treatment, only one group showed significantly higher mean group numbers ($p < 0.025$) with four of the remaining nine groups showing significantly lower mean numbers of groups than would be predicted under random space use (two tailed test: $p > 0.975$). Furthermore, all 10 groups showed randomly distributed mean group sizes ($0.025 < p < 0.975$). A summary of the results comparing the familiar and unfamiliar treatments is given in Table 2.

Data on the mean number of groups and the mean group sizes of juvenile *S. canicula* in both familiar and unfamiliar treatments were normally distributed (Kolmogorov-Smirnov test with Lilliefors correction, $p > 0.050$) and satisfied the assumption of equal variance (Levene's test: $p > 0.050$). As predicted from the previous analysis, the multivariate GLM revealed that conspecific familiarity significantly increased the mean number of juvenile shark groups (mean: Familiar = 2.02; Unfamiliar = 1.20; $F_{1,18} = 11.570$, $p = 0.003$, Fig. 13) but did not significantly influence the mean size of these groups (mean: Familiar = 2.51; Unfamiliar = 2.35; $F_{1,18} = 2.965$, $p = 0.102$), suggesting a greater number of samples where all individuals were resting alone in the unfamiliar treatments.

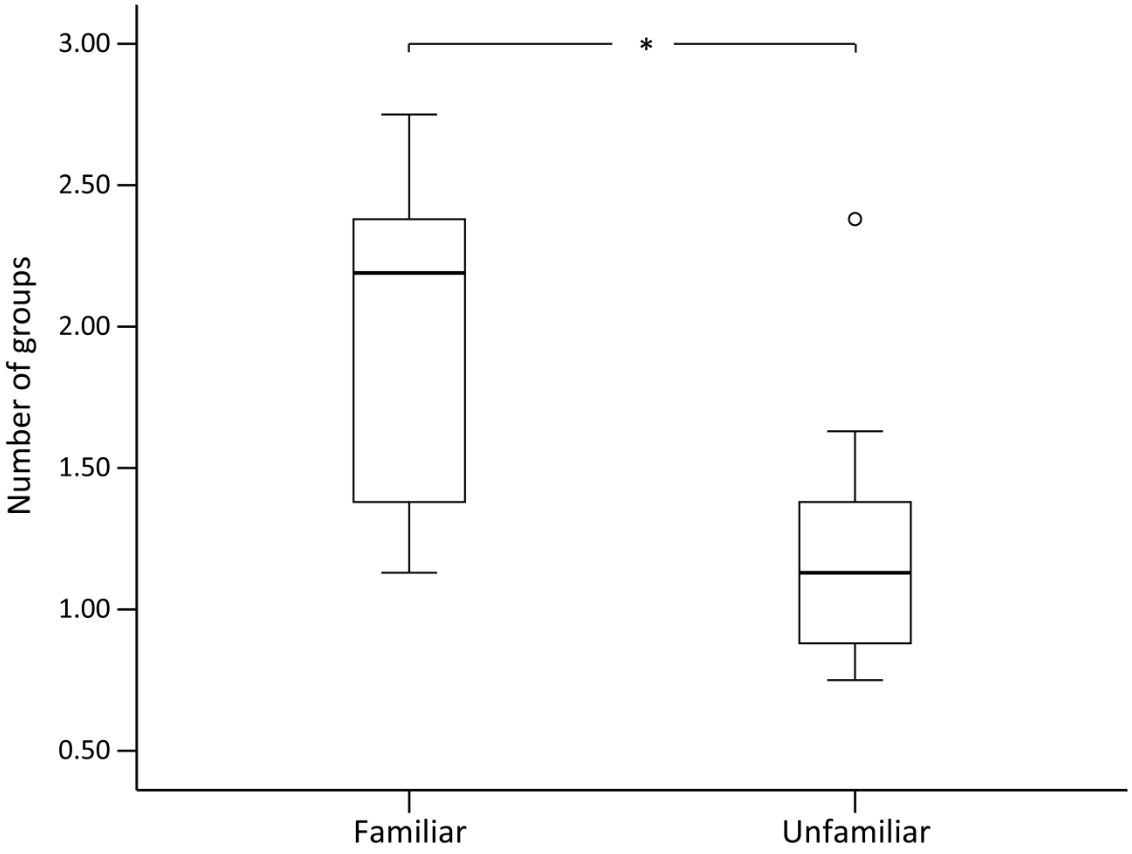

Figure 13. Median box-plot with inter-quartile range of number of groups formed under familiar and unfamiliar aggregation experiments. * Denotes significance (p < 0.01) and ° an outliner.

3.4.2 Experiment 2 – Social preferences and assortment

The permutation test for non-random association revealed that each of the 16 networks showed preference and/or avoidance behaviour more than would be expected by chance (p < 0.050, Table 3) with high variation between groups in the number of preferred and avoided dyads. Non-random social preferences therefore appeared to underpin the patterns found in the aggregation analysis (*Experiment 1*). How then did familiarity affect the social behaviour of individuals? There was a significantly higher mean association index for networks containing familiar individuals ($t_{(14)}$ = 2.757, p = 0.015, Fig. 14), suggesting familiarity promoted associative behaviour through the persistent aggregation of familiar sharks into several small groups. Interestingly, familiar treatments showed significantly higher levels of individual avoidance when compared to the unfamiliar treatments (mean rank: Familiar = 12.13; Unfamiliar = 4.88; $U_{(14)} = 3.00$, $z = -3.106$, $p = 0.002$, Table 3), despite very similar levels of preferential association (mean: Familiar = 3.25; Unfamiliar = 3.38; $t_{(14)} = -0.116$, $p = 0.910$, Table 3). This may reflect the fact that individuals in this particular experiment were housed in groups of four during familiarisation, so were familiar with only three out of a possible 11 other conspecifics, choosing to associate largely within treatment groups. To test this idea a null model was used to test for assortment in the social networks by familiarity. Only two of the eight groups containing familiar individuals showed significantly greater within-group association than between-group ($p < 0.050$) and thus assortment by familiarity. There were high levels of network variation with the overall effect being non-significant (Stouffer's test: $n = 8$, $p = 0.305$). In fact in some networks, individuals showed marginally non-significant, negative preferences for familiar association suggesting high individual variation in social preferences.

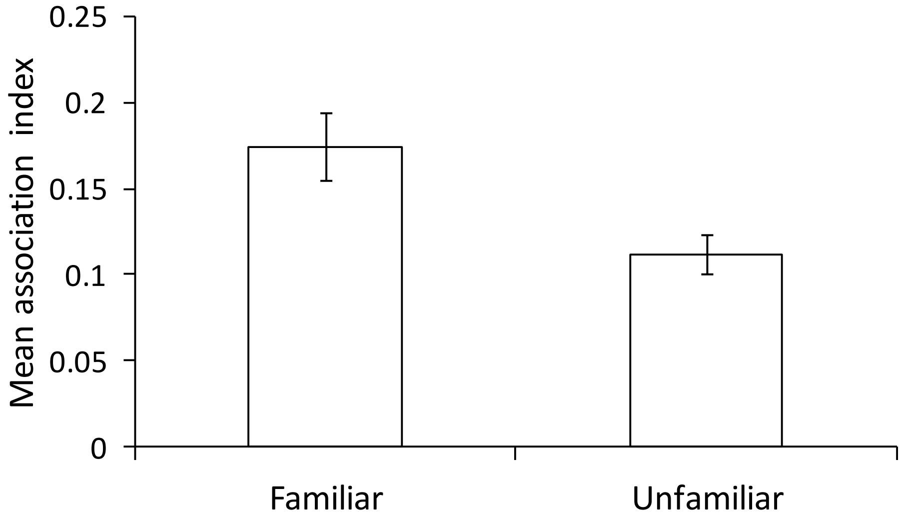

Figure 14. Mean association index between treatments containing familiar and unfamiliar individuals. The index is a measure of overall strength of social attraction between individuals.

Table 3. Summary of the test for preference and avoidance behaviour. The test includes the number of significant pairs (dyads) within each network, between familiar (F) and unfamiliar (UF) treatments. A significant *P* value indicates greater levels of preference and avoidance behaviour compared to 20,000 random iterations of the observed association data.

		Significant Dyads				
Network	Treatment	Preferred	Avoided	Obs. Mean Association	Exp. Mean Association	*P*
1	F	3	2	0.1292	0.1372	0.0007
2	F	2	5	0.2888	0.2990	0.0056
3	F	6	8	0.1512	0.1626	0.0000
4	F	0	4	0.1580	0.1717	0.0001
5	F	2	4	0.1766	0.1904	0.0001
6	F	2	3	0.1645	0.1828	0.0000
7	F	4	2	0.1118	0.1215	0.0002
8	F	7	11	0.2135	0.2268	0.0001
9	UF	1	2	0.1587	0.1721	0.0001
10	UF	5	1	0.1089	0.1153	0.0062
11	UF	1	0	0.0847	0.0930	0.0001
12	UF	4	2	0.0857	0.0907	0.0125
13	UF	7	2	0.1362	0.1464	0.0010
14	UF	3	0	0.1038	0.1090	0.0327
15	UF	3	1	0.1461	0.1568	0.0003
16	UF	3	0	0.0699	0.0760	0.0064

3.5 Discussion

During this study groups of juvenile *S. canicula* were manipulated to quantify the aggregation and social behaviour of the early life-history stages in this benthic, oviparous elasmobranch species. In addition, the study sought to determine the influence of conspecific familiarity on gregariousness. Initial analyses of aggregation, prior to analyses of social associations, revealed that *S. canicula* do indeed aggregate more than would be expected from a random distribution within the arena, but interestingly, only when individuals are familiar with one another. The results provide strong evidence that familiarity, at least in older (> 6 month) juvenile *S. canicula*, is an important mechanistic driver of aggregation behaviour. Both group number and group size in familiar treatments were on average greater than would be expected under a random distribution within the experimental arenas. This result was not reflected in the unfamiliar groups. *Experiment 2* revealed significant non-random social behaviour across all replicated groups, confirming that social preferences appear to play an important role in structuring aggregation behaviour in neonate sharks (< 2 months) through either preference or avoidance between specific individuals. Within these social groups the mean association index (i.e. the strength of social attraction) was also significantly higher in familiar networks than unfamiliar. Whilst some groups did show evidence of assortative association, the overall effect of social preferences between familiars was not significantly greater than between unfamiliar individuals. To my knowledge, this is the first study to explore and experimentally test the influence of conspecific familiarity on shark aggregation.

There are broad accounts of aggregation behaviour across a wide range of elasmobranch species (see *Chapter I* for review) and recently, there has been burgeoning evidence that the challenge has gone beyond simply describing aggregation with a move towards determining the mechanisms and functions of such behaviour in this vertebrate taxa (Guttridge et al. 2011; Mourier et al. 2012). By aggregating with conspecifics on the basis of kin and/or familiarity, teleost fishes have been shown to benefit from reduced levels of competition and predation risk (see Ward and Hart 2003 for review) and it is possible that small elasmobranchs might benefit in similar ways. Given the size of *S. canicula* at birth and tendency for adult sharks of many species to predate upon other elasmobranchs (e.g. Simpfendorfer et al. 2001), predator avoidance is likely to be a significant driver of juvenile behavioural strategy (e.g. Sims et al. 1993 for *S. canicula* hatchlings). Variation in predation risk has been found to significantly influence social network dynamics determining rates of fission-fusion behaviour and the temporal stability of social bonds in a shoaling teleost fish (Kelley et al. 2011). As such, it is possible that juvenile catsharks use aggregation to dilute individual risk of attack and also habitat features in order to hide. The influence of habitat on social behaviour in this species is explored in a subsequent chapter (*Chapter V*) and future research directly assessing habitat and predation risk in a social networks context will no doubt prove insightful.

The results of this study suggest that the tendency for juvenile, benthic sharks to aggregate varies as a function of their immediate social environment, with conspecific familiarity serving to enhance non-random grouping that probably functions as an antipredator strategy (Sih 1987; Rangeley and Kramer 1998). Interestingly however, there is a suggestion that sharks that are unfamiliar with one another, might adopt alternative antipredator strategies, perhaps hiding or camouflaging against the background substrate. Whilst there is no evidence that wild juvenile *S. canicula* occupy nursery habitat until large enough to disperse into deeper water, it seems likely given that large numbers of egg cases are deposited together on and around specific habitat substrate. In this context, familiarity between hatchlings might be expected to develop promoting aggregation in neonates that hatch in close temporal and spatial proximity. Indeed, adult female *S. canicula* on average lay a pair of eggs every two to three weeks and from captive rearing of this species it was found that eggs from the same clutch hatch within a few days of one another provided they are incubated at the same temperature (Griffiths et al. 2012; D.M.P. Jacoby, unpublished data). Thus, by manipulating the social environments of the experimental neonates from hatching, the results of this study appear to support this hypothesis. Further research, however, is clearly required to test whether this occurs under natural conditions.

The merits of being able to individually recognise and repeatedly associate with conspecifics have been explored in great detail using small teleost fishes as a model (Griffiths and Magurran 1999; Krause et al. 2000a; Ward and Hart 2003; Frommen et al. 2007; Jordan et al. 2009). Evidence of non-random social preferences in these neonate *S. canicula* is consistent with temporally stable social bonds in the adults of this species (Jacoby et al. 2010). In the wild, female *S. canicula*, like all other elasmobranch species invest heavily in the growth and cognitive development of offspring prior to birth, but show no evidence of post-natal maternal care. As a result, juvenile sharks are left to fend for themselves from birth, often surviving in nursery groups in shallow protected waters where there is greater opportunity for repeated social interactions (Heupel and Simpfendorfer 2005b; Chapman et al. 2009). Under these circumstances, the reinforcement of individual recognition and stable social bonds between juveniles may prove important during the first years of survival and development. This has been observed in juvenile lemon sharks, which show persistent social preferences, leadership and assortative behaviour for periods of up to eight months (Guttridge et al. 2011). Indeed, social behaviour in these young sharks appears also to be influenced by environmental conditions whereby juvenile *N. brevirostris* utilise warm, shallow mangrove inlets during high tide as a means of avoiding predation from larger adult and subadult conspecific and heterospecific sharks (Guttridge et al. 2012a). Benthic sharks display very different behaviour to many of these well documented carcharhinid species, demonstrating long periods of group refuging/resting behaviour often in association with complex habitat structures (Sims 2003; Sims et al. 2005; Powter and Gladstone 2009; Wearmouth et al. 2012). Juvenile *S. canicula* which hatch in much deeper, coastal waters, however, are likely to be equally susceptible to adult predation and therefore aggregation resulting from non-random social attraction must also confer antipredator benefits. Given that the antipredator benefits of grouping will hold for both familiar and unfamiliar individuals, why then should non-random social grouping be restricted to just familiar sharks? It is probable that like teleost fishes, familiarity confers other additional benefits, such as a reduction in aggression or competition for food between group members (Krause et al. 2000a; Ward and Hart 2003) however, further research would be required to test this idea in sharks.

Conducting experiments on elasmobranchs in captivity poses a number of potential issues including achieving sufficient replication for statistical power and having large enough aquaria to encourage typical behaviour. Some of these issues can be addressed by using small model species such as *S. canicula* and by quantifying behaviour such as group resting. While it is acknowledged that the dimensions of the experimental area could potentially bias active swimming behaviour of these sharks in particular, the potential resting area available in the aquaria and the size of the individuals in each replicate were factors built into the null model. Therefore any potential bias imposed on aggregation behaviour was accounted for by increases in the mean of the distribution in the null aggregation model. Furthermore, it is important to reiterate here that all individuals were free to be solitary during any given sampling period. As such a minimum group number of 'zero' was possible whereas the minimum group size was restricted to 'two' individuals meaning that group size and group number were not expected to negatively co-vary automatically.

Using a replicated experimental design not only allowed general conclusions about behaviour to be drawn but also highlighted the level of individual and group-level heterogeneity in the social behaviour of sharks (Jacoby et al. 2010). This was particularly apparent in the results for social assortment with some networks showing preferential association between familiar individuals and other networks showing avoidance. Within an ecological context it is possible that some individuals, perhaps less social individuals, may gain more information from unfamiliar conspecifics when encountering new habitat surroundings, as familiar individuals have been exposed to similar *a priori* experiences (Brown and Laland 2003; Pinter-Wollman et al. 2009). Tracking the behaviour of sharks of different social connectivity under differing environmental and social contexts will prove an interesting avenue for future studies.

Research on teleost fishes now provides a comprehensive foundation for studying social recognition in aquatic organisms (Ward et al. 2007). Furthermore, significant developments in the application of social network analysis to study animal groups enable us to study these mechanisms at the level of the group and/or the population (Croft et al. 2008). With very different life-history characteristics, elasmobranchs will no doubt provide an interesting group for comparison. The extent to which familiarity influences aggregation and social grouping in sharks is likely to reflect how important individual recognition is to survival during the early stages of growth and development.

This first experimental chapter has established that *S. canicula* form social groups under conditions where conspecifics are familiar with one another. Familiarity has been shown to drive changes in group number and enhance the strength of social association between individuals. It has been discussed how repeated individual interactions are likely to occur in the wild between neonates that hatch in close temporal and spatial proximity, however, it is equally likely under such conditions that hatchlings will also be related to some extent to one another. The next chapter addresses whether kin relatedness is important in structuring juvenile association and discusses the levels of relatedness that might be expected given the female reproductive strategy in this species.

Chapter IV.

Multiple paternity and the opportunity for kin-based associations in the small spotted catshark, *Scyliorhinus canicula*

4.1 Abstract

As with many animals, social grouping between newborn elasmobranchs is potentially important for survival and may be influenced, in part, by both genetic and social drivers such as relatedness and familiarity. Assortment by relatedness depends on two key factors; (i) the opportunity for kin to associate and interact and (ii) the ability of individual's to differentiate among kin and non-kin and for them to actively prefer to associate with kin. The opportunity for associations to occur among kin will in part be determined by the reproductive strategy adopted by the mother. In particular, when there is multiple paternity (MP) – females mating with multiple males – the average degree of relatedness between siblings will be reduced. This chapter investigates these two key factors directly in the small-spotted catshark (*Scyliorhinus canicula*). First, the degree of MP occurring in wild caught individuals is examined. 150 neonates from 13 different mothers were genotyped using 10 microsatellite loci. Descriptive exploration of the reproductive data revealed that larger females laid larger eggs that were more likely to hatch than eggs from smaller females. MP was commonplace, with progeny from 92% of females sired by multiple males. In the second part of this chapter, the role of relatedness in structuring social interactions is explored using the neonate hatchlings from the MP experiment. Analyses of social network structure revealed little evidence for kin assortment or structuring by sex, with the majority of groups showing random associations. High MP is consistent with the reproductive biology of the species, particularly its protracted breeding season and potential for long-term sperm storage. Kin association, perhaps as a result of such high MP, appears less important than familiarity in influencing the behavioural strategies of young *S. canicula*.

4.2 Introduction

Kin recognition has been observed in many different animal species influencing association between siblings in different ways (Fletcher and Michener 1987). There are a number of adaptive benefits to being able to individually recognise kin and these will depend on the species in question and their respective life histories. The ability to distinguish between kin and non-kin for example, allows individuals to preferentially allocate resources to genetically related conspecifics while also enabling them to avoid costly inbreeding (Gerlach et al. 2008). In gregarious mammals and primates in particular, kin association is common, conferring both foraging benefits through reduced competition and social benefits in the form of alloparental care (Greenwood 1980; Silk 2002; Bradley et al. 2007). In freshwater teleost fish that show low dispersal such as the Lake Eacham rainbowfish (*Melanotaenia eachamensis*), female fish are able to balance the benefits of nepotism with the costs of inbreeding through their ability to discriminate and therefore change their degree of association with male kin over male non-kin (Arnold 2000). Other benefits of kin association include a reduction of aggression between group mates which has been shown to lead to increased growth rates in captive salmonids (Brown and Brown 1993) and indirect (genetic) benefits such as increased cooperative behaviours (Ward and Hart 2003).

There appears, however, to be substantial variation between studies and species in the level of kin association observed in fishes (see Ward and Hart 2003 for review). An interesting example is the guppy, *Poecilia reticulata* that are known to be able to recognise kin (Hain and Neff 2007) but show no evidence of relatedness structuring social associations within the wild (Croft et al. 2012). Indeed, there is further evidence that in dynamic, wild fission-fusion social systems, kin assortment does not occur (e.g. Peuhkuri and Seppä 1998; Pouyard et al. 1999) and that even within eusocial insect societies where nepotism is high, explanations other than kin selection might be more appropriate (Nowak et al. 2010). Rapidly developing genetic and behavioural analyses, however, now enable us to detect more subtle effect sizes and allow us to address more questions relating to kin structuring *in situ* (e.g. Croft et al. 2012).

Kin recognition systems vary greatly across species and under different social contexts (Mateo 2004). Differences in mating system and life history are likely to drive the evolution of different mechanisms of social recognition (Ward et al. 2009). Self-referent phenotype matching, for example, allows individuals to discriminate between kin and non-kin by using their own phenotypic or olfactory cues for comparison (Mateo 2010). The concept of 'self' is often controlled by the highly polymorphic genes that make up the major histo-compatibility complex (MHC), the genotype of which has been found to play important roles in mediating kin recognition (see Brown and Eklund 1994 for review). MHC appears to play a crucial role in species where kin recognition is a learned process instead of a self matching mechanism. In a study conducted by Gerlach et al. (2008), zebrafish (*Danio rerio*) learnt kin odour cues through olfactory imprinting only at a specific stage of larval development, a mechanism the authors suggest is controlled by the development of immune system MHC genes (Gerlach et al. 2008).

While genetic control mediates some aspects of kin recognition mechanisms, social factors are also likely to be involved. Social recognition or familiarity of conspecifics can enhance the ability of fish to recognise kin (Ward and Hart 2003) but is often short-lived requiring repeated exposure to familiars (Mateo 2004). This repeated exposure does occur, however, between individuals that are born or hatch within close spatial and temporal proximity to one another, particularly when juveniles remain within their natal area for some time (e.g. Chapman et al. 2009). Consequently the degree of relatedness between offspring within such 'nursery areas' will be dictated by female reproductive strategy. Female promiscuity will no doubt result in a dilution of genetic relatedness between offspring within nursery areas and therefore it is important to understand both female mating system and juvenile association to determine the role of kin in structuring population dynamics. One taxonomic group, that often fulfil important apex predatory roles within their ecosystems and in which questions about kin structuring remain virtually unexplored, is the sharks and rays (although see Guttridge et al. 2011).

In their long evolutionary history elasmobranchs have developed complex reproductive modes expressed by variation in a range of reproductive characters, including the nature of ovarian cycles, gestation periods and mating systems. Nevertheless, descriptions of these characters remain unknown for most sharks (Carrier et al. 2004). Mating systems can fundamentally influence population-level processes, with polyandry (female mating with multiple males) and multiple paternity (MP) directly affecting levels of genetic variability and inbreeding, even altering the potential for adaptation within populations (Frankham 2005). Applying molecular markers to analyse litters of pups or collections of embryos has demonstrated that MP is widespread across shark species. It has been detected in several orders including Squaliformes (Lage et al. 2008; Daly-Engel et al. 2010), Carcharhiniformes (Feldheim et al. 2002; Chapman et al. 2004; Daly-Engel et al. 2006), Hexanchiformes (Larson et al. 2011), Lamniformes (Gubili et al. 2012) and Orectolobiformes (Saville et al. 2002). However, less detail is available on the frequency and extent of MP in sharks, with the data produced suggesting it is highly variable between and within species (Veríssimo et al. 2011; Fitzpatrick et al. 2012). Whilst approximately 40% of sharks are oviparous (Compagno et al. 2005) investigations have generally focused on viviparous sharks, where litters of identical maternal origin can be readily identified often from sharks caught by fishing vessels (e.g. Schmidt et al. 2010). Consequently, the extent of multiple paternity in species of sharks with alternative reproductive modes is largely unstudied, as are the indirect repercussions of female reproductive strategy on offspring behaviour.

It has been demonstrated that polyandry has both benefits and costs, for parents and offspring (e.g. Evans and Magurran 2000; Evans and Kelley 2008). The advantages are perhaps most evident for males, whereby individuals may force females to mate with them in order to skew the paternity of resulting offspring. However, studies of MP in sharks have yet to demonstrate direct benefits for females provided by males, such as gifts or paternal care (Chapman et al. 2004; Portnoy et al. 2007; Dibattista et al. 2008) or indirect benefits, such as increased juvenile survival (Dibattista et al. 2008). Given the high energetic cost associated with mating and the risk of problems arising from bite wounds (Pratt and Carrier 2005), it has been suggested that MP in elasmobranchs may be driven by male benefits and influenced by sexual conflict (Daly-Engel et al. 2007; Portnoy et al. 2007; Dibattista et al. 2008).

There are increasing records that some sharks and rays, for which there is no evidence of parental care or cross-generational social structure, also show high levels of female promiscuity although there is substantial variation between species (Fitzpatrick et al. 2012). Determining levels of female multiple mating in sharks therefore, is likely not only to inform research into the population genetic structure of these species, but also promote greater investigation of potential kin structuring of offspring behaviour within these systems. Amongst predatory elasmobranchs, social preferences and familiarity have been previously demonstrated (Guttridge et al. 2009a; see also *Chapter III*) in addition to female reproductive philopatry (Tillet et al. 2012), all of which represent behavioural mechanisms that directly affect population structure. To the best of my knowledge only one other study, has addressed the extent to which kin relatedness might structure shark groups. Guttridge et al. (2011) obtained mixed results when exploring kin assortment in a wild population of lemon sharks (*Negaprion brevirostris*), with some year groups assorting and others not, a result put down to inconsistencies in sample size and emigration from the study area.

Continuing on from the examination of the structure and mechanisms underpinning aggregation behaviour in *S. canicula* in the previous chapter, this study was designed to determine the level of MP in wild female sharks and explore the extent to which kin relatedness structures associations amongst the subsequent shark pups. Juvenile sharks of this species have been shown to aggregate, demonstrating non-random associative preferences, particularly when grouped with familiar conspecifics (*Chapter III*). However, the behavioural strategies of juvenile *S. canicula* remain relatively unstudied, but are of interest given their small size and the reproductive biology of the adults. MP was expected to be relatively high given the protracted breeding season and long-term sperm storage exhibited by this species (Metten 1939). Should this be the case, relatedness (controlling for the effects of familiarity) was not expected to significantly structure social interactions between juveniles due to increased genetic diversity between offspring. Additionally, as juveniles were sexed upon hatching, effects of sex were also explored but again were not expected to significantly structure shark associations until after maturity (Jacoby et al. 2010). Finally, descriptive analyses of female fecundity were also conducted to evaluate any possible associated fitness benefits.

4.3 Materials and Methods

4.3.1 Study species

S. canicula belong to one of the largest families of sharks, the Scyliorhinidae (Carcharhiniformes). The reproductive strategy in *S. canicula* is one of oviparity, where females lay pairs of eggs which are covered by a protective case and anchored on to macroalgae and other solid surfaces in subtidal habitats (Ellis and Shackley 1997). This species appears to have an unusually protracted breeding season (Ford 1921; Metten 1939; Capapé et al. 2008) with egg-laying in British waters generally occurring between November to July, peaking in June and July (Harris 1952; Craik 1978; Sumpter and Dodd 1979; Ellis and Shackley 1997). Annual fecundity in catsharks from southern England has been estimated at between 29 and 62 eggs. Eggs take five to six months to hatch depending on water temperature, with the average pup measuring 100.5mm total length (L_T) at hatching (Ellis and Shackley 1997). Sperm storage has been well described in this species, with sperm localised to the oviducal gland (Metten 1939). In captivity a group of females continued to lay eggs after 214 days in isolation (Ellis and Shackley 1997), a result that shares much in common with work on *Scyliorhinus retifer* that produced eggs that hatched normally after 843 days in isolation, suggesting long-term sperm storage (Castro et al. 1988). In the Atlantic *S. canicula* is often caught as by-catch in demersal fisheries, but its commercial importance is growing, particularly through its use as whelk bait. Additionally, within the

Mediterranean it is targeted for consumption (Capapé et al. 2008). More information on the general biology of this widely distributed and relatively abundant species is given in *Chapter II*.

4.3.2 Collection and maintenance of females

Female sharks (n = 20) were captured on the 14 January 2010 during a 30 min 'otter' trawl haul at Whitsand Bay, in the English Channel (50.33° N, 4.24° W, see *Chapter II*). Of these 20 sharks 13 produced a sufficient number of eggs to assess MP (minimum = 6, maximum = 32). All sharks were transferred to the Marine Biological Association (MBA) laboratory in Plymouth, UK where they were weighed (mean W; 828.6 g, range 608-1008 g), total length (L_T) was measured (mean; 613.1 mm, range 544-652 mm) and a fin clip was preserved in 100% ethanol. The individuals were tagged with T-bar anchor tags (FD94, Floy Tag, Seattle, WA) to facilitate identification. All sharks were isolated from male contact from the date of capture until their release on 10 May 2010. The four most fecund individuals were held until regular egg-laying appeared exhausted (October 2010). Specific details on the aquaria in which adults were held can be found in *Chapter II*.

4.3.3 Sampling of offspring

Egg-laying females were maintained in single-sex groups for the duration of the experiment. During egg-laying periods, sharks were checked for eggs on a near daily basis with eggs being removed immediately in all individuals where egg tendrils were observed trailing from the cloaca. All eggs were measured (L_T) and weighed (W) before being housed individually in isolated compartments of an egg rack in a separate tank (see *Chapter II* for details). During this time water temperature was subject to seasonal increases between December and June (range: 11.4–18.0^{o}C). Eggs were excluded from subsequent analyses if maternal identity was unclear, that is, the eggs were deposited without physical removal and so they could not be attributed to a specific female. During this study a total of 206 eggs of known maternal heritage were collected, of which 27% were not used due to decay or lack of fertilisation (see *Appendix I* for hatch success and individual laying consistency – Table A.1. and Fig. A.1.). The remaining 150 eggs were all genotyped using 10 hypervariable microsatellite loci (see details below). All healthy eggs were allowed to hatch offering a unique opportunity to assess not only kin interaction within social groups but also incubation time and offspring dimensions. Upon hatching offspring were measured, weighed and a fin clip taken for DNA extraction. For each female the proportion of eggs that hatched successfully was calculated, these values were arcsine transformed in order to standardise values for further analyses.

4.3.4 Genetic analyses

Genomic DNA was isolated from *S. canicula* and 10 microsatellite loci amplified as molecular markers. Reliable estimates of multiple paternity involving unknown parents rely on knowledge of the allele frequencies in population as a whole. Thus, three sample collections from the western Channel were genotyped. The first collection was made at the same time as the gestating females (14th January 2010, *n* = 33), the second collection was made earlier from the same location (3rd April 2008, *n* = 20) and the third originated from the geographically proximate Salcombe Bay (50.21°N 4.76°W; 9th June 2008, *n* = 24). A full description of the techniques adopted for genotyping and for determining differences in allele frequencies to the source population can be found in *Appendix I*.

To test for MP, mother and pup genotypes were arranged into arrays and checked by eye to ensure that each offspring carried a maternal allele. To facilitate comparison with previous studies of MP in sharks, GERUD 2.0 (Jones 2005) and PrDM (Neff and Pitcher 2002) were used to estimate the minimum number of sires and the probability of detecting MP (PrDM) in groups of pups. In order to reduce the computational burden and in accordance with the recommendations of Jones (2005), analyses utilising GERUD (and therefore PrDM) were restricted to the four loci with the highest "exclusion probabilities" (*Scan02*, *Scan06*, *Scan14* and *Scan16*). These loci typically have the greatest polymorphism and thus the best ability to reliably reconstruct parentage. Where the program returned multiple solutions for progeny arrays, the solutions were ranked by likelihood, based on probabilities generated by both Mendelian segregation and allele frequencies derived from all samples included in this study. COLONY 2.0 (Jones and Wang 2010) was then used to estimate the most likely number of sires and reconstruct sib-ship between offspring using 10 loci. COLONY was run using all samples included in this study to estimate the allele frequencies in the source population. The error rate was set to 0.02 and two 'long' runs were completed to ensure convergence of the result. Finally, Genalex (Peakall and Smouse 2006) was used to calculate the Queller and Goodnight (1989) moment estimator of relatedness, giving an indication of full and half sib-ship. This coefficient was chosen due to its widespread use and allowed social behaviour of the offspring to be compared against matrices created from both kin reconstruction and quantitative genetic estimation techniques.

4.3.5 *Behavioural analyses*

4.3.5.1 Kin assortment

Once neonate sharks had been measured, weighed and fin clipped post hatching, individuals were sexed and tagged with visible implant elastomer tags (VIE; Northwest Marine Technology, WA, USA), before being housed in groups of four as part of the experiment described in *Chapter III*. Groups within a holding tank were always familiar with one another, however some were housed with maternal siblings and others with non-maternal siblings. As per *Chapter III*, experimental social groups were constructed in large arenas (1026 l capacity, 1.8 × 1.0 × 0.6 m) by combining 12 individuals comprising four groups of three maternal siblings (i.e. four different maternal groups present during each replicate), each individual taken from a separate holding environment to control for the effects of familiarity. As with previous experiments this allowed the role of kinship in driving social associations to be tested for directly whilst controlling for the known effects of familiarity (see *Chapter III*). Five replicate groups which were kin but unfamiliar with one another, were constructed and their social networks quantified over a period of two days (eight samples). The sampling protocol for this experiment was consistent with the overall methodology used throughout this thesis for defining association (see *Chapter II*). The null simulation model that was outlined in the section on assortment by familiarity (*Chapter III*) was also adopted here to test for statistical significance between kin and non-kin associations. Similarly, the observed test statistic used to test for assortment amongst kin was the value remaining after subtracting mean kin association from mean non-kin association. The null model contained 10,000 randomly generated networks against which the observed level of kin assortment was compared.

4.3.5.2 Kin and sex structuring

Following replicated network treatments, a total of 50 juvenile sharks were entered into the largest aquarium available at the MBA (2321 l capacity, 2.6 × 1.8 × 0.5 m). This allowed the exploration of putative kin structuring on a much larger spatial scale in the event that small effect sizes were not detected in the small network experiments. This large network was restricted to the 50 individuals for which the VIE tag remained clearly distinguishable and estimates of sib-ship had been obtained through both kin reconstruction defined by allele sharing in COLONY and using Genalex to calculate continuous estimates of genetic relatedness. Furthermore, it provided the opportunity to address whether juvenile sex influenced social structure. In addition to the biological insight this provided, it also functioned to determine whether sex of the individuals needed to be controlled for in other laboratory experiments. This network contained the offspring from 12 of the 13 different mothers. The social network was constructed over 12 samples, during a four day period. To determine whether kin relatedness and sex played a role in structuring the social network of juvenile *S. canicula*, the association matrix was correlated against a two different matrices of relatedness using the quadratic assignment correlation procedure (QAP) in UCINET 6 (Borgatti et al. 2002). This statistical test is a modification of the mantel correlation that accounts for non-independence of data within a matrix. QAP analyses were run to determine correlation between matrices of association indices and dissimilarity matrices of estimates of their genetic relatedness calculated in both COLONY (kin reconstruction) and Genalex (quantitative genetic estimates of relatedness). A third and final QAP analysis was run to test for correlation between social partners and sex. A binary dissimilarity matrix was constructed whereby dyads of the same sex were assigned a 1 and those of a different sex a 0. The observed correlation coefficients were tested against 10,000 randomly generated trails to obtain values of significance.

4.4 Results

Neonate hatchlings had a male:female sex ratio of 1.14:1. The mean incubation period was 177.7 days (range 128 - 226 days), which was negatively correlated with seawater temperature (Spearman rank correlation, $n = 150$, $r = -0.547$, $p < 0.001$). The mean L_T of neonates was 103.6 mm (range; 90 - 117 mm) with no significant size differences between the sexes (Student's *t*-test: $t(148) = 0.263$, $p = 0.793$). Mean L_T and mass of neonate individuals were positively correlated with maternal measurements after removing the three females which did not lay any eggs at all (two tailed Pearson correlation; L_T, $n = 17$, $r = 0.507$, $p = 0.038$; W, $n = 17$, $r = 0.565$, $p = 0.018$; Fig. 15). Interestingly, maternal L_T was also positively correlated with arcsine transformed hatch success across the 17 individuals that laid eggs (two tailed Pearson correlation: $n = 17$, $r = 0.573$, $p = 0.016$). There was no influence of the extent of female multiple mating on hatch success (Linear regression, arcsine transformed, $n = 13$, $r^2 = 0.178$, $p = 0.151$).

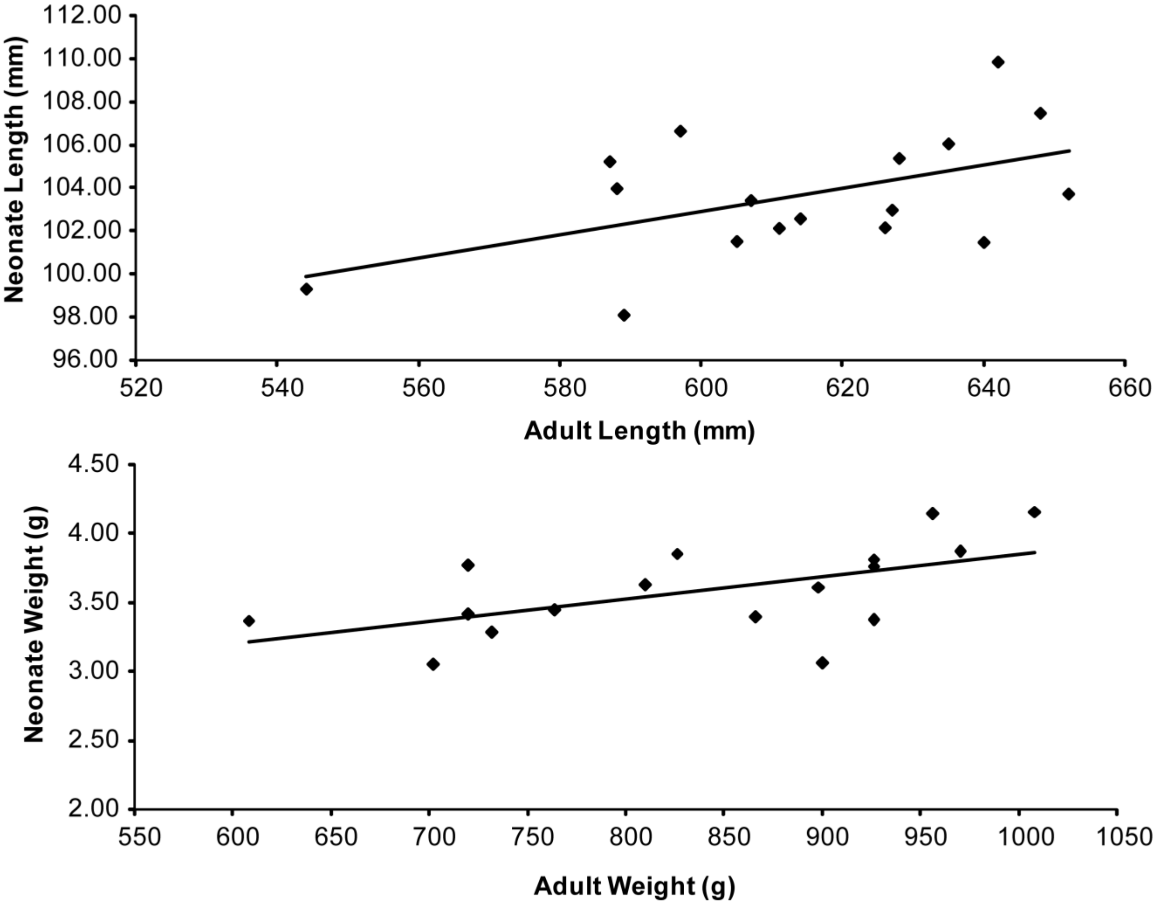

Figure 15. Relationship between female L_T and weight (W) and the mean L_T and W of offspring at hatching.

4.4.1 Genetic analyses

MP was assessed for the 150 offspring of 13 females. The number of eggs analysed from each female ranged between 4 and 28. It was recognised that the number of eggs analysed has a potential influence over the power to detect MP, however, there was no correlation between the most likely number of sires contributing to individual female progeny array and quantity of eggs analysed per individual (Spearman rank correlation, n = 13, r = 0.399, p = 0.177), suggesting that the extent of MP observed was not merely a product of sampling protocol.

Pairwise analysis of sample collections gathered from Salcombe in 2008, and Whitsand Bay in 2008 and 2010 demonstrated no evidence of significant population structure; $p > 0.05$ in tests for genetic differentiation and F_{ST}. Therefore, homogeneity of allele frequencies was assumed and samples were pooled to obtain an estimate of allele frequencies in the source population. No scoring issues or null alleles were detected at the ten remaining markers. There was no evidence of significant linkage disequilibrium or deviation from Hardy-Weinberg equilibrium (see methods and Table A.2. in *Appendix I*), after sequential Bonferroni correction for multiple comparisons (at the $p = 0.05$ significance level the initial alpha value for tests of deviation from Hardy-Weinberg equilibrium =0.005).

Exclusion probabilities from GERUD for each locus (Table A.2. *Appendix I*) identified the four most informative markers (*Scan02*, *Scan06*, *Scan14* and *Scan16*). The total probability using these loci was 0.985, which increased to 0.999 when all 10 loci were used. Using these four most informative loci to estimate the minimum number of sires using GERUD resulted in genetic polyandry being detected in 12 of the progeny arrays (92%). The probability of detection of multiple paternity (PrDM) in the polyandrous progeny arrays was generally high, varying between 78% and 100%, tending to improve as numbers of offspring and sires increased, but fell to approximately 63% in the monogamous progeny array (Table 4). Analysis of the most likely number of sires incorporating all 10 loci using COLONY found evidence of MP in the same 12 progeny arrays. The number of sires estimated in GERUD and COLONY were consistent in 10 of the 13 progeny arrays, while COLONY tended to increase the numbers of sires contributing in 2 of the 13 cases. Sib-ship reconstruction in COLONY showed that patterns of paternal skew were quite variable. Analysis of the 49 sets of paired eggs which were laid at the same time demonstrated that 22 sets were sired by different fathers.

Table 4. Estimated number of sires in 13 progeny arrays of *S. canicula*, inferred using GERUD (3 loci) and COLONY (10 loci) software. Eggs are laid by females in pairs, enabling the number of males contributing to each egg pair to be identified. PrDM is the probability of detecting multiple paternity.

			GERUD		COLONY		
Female ID	No. offspring genotyped	No. egg pairs genotyped	No. sires	PrDM	No. sires	No. egg pairs sired by different males	Paternal skew
G0376	23	5	3	0.999	2	3	17:6
G0377	16	6	3	0.999	3	2	8:7:1
P0551	28	12	3	1.000	3	6	14:10:4
Y0338	21	7	3	1.000	4	4	8:7:5:1
B1801	5	1	1	0.838*	2	0	3:2
B1802	6	2	2	0.938	2	2	4:2
B1803	5	2	2	0.861	2	1	4:1
B1805	12	5	1	0.628*	1	0	-
P0552	8	3	2	0.958	3	3	3:3:2
P0553	5	1	2	0.854	2	0	4:1
R1851	4	1	2	0.776	2	0	2:2
Y0336	6	1	3	0.986	3	0	3:2:1
Y0337	11	3	2	0.952	2	1	8:3

* For the monogamous progeny arrays the PrDM was determined for 2 sires, with one male contributing a single pup.

4.4.2 Behavioural analyses

4.4.2.1 Kin assortment

In four out of the five kin networks there was no evidence of kin assortment ($p < 0.05$, Table 5) with combined p values revealing no significant effects of kinship (Stouffer's test: $n = 5$, $p = 0.244$). One network, however, did show significant assortment ($p = 0.04$, Table 5). Mean associations between kin and those between non-kin were also compared to random and on further investigation this result appeared to be driven by a mean association index (AI) between non-kin that was significantly lower than random, producing a large difference between mean kin and non-kin association (i.e. the test statistic, see Table 5).

Table 5. Statistical comparison of mean kin and non-kin group association index (AI). Levels of kin and non-kin association were compared statistically to random association (P_{rand}) and the difference between these AI values was used as the statistic to test for significant assortment.

	Mean association index					
Network	Kin	P_{rand}	Non-kin	P_{rand}	Difference	P
1	0.171 (0.07)	0.314	0.156 (0.11)	0.716	0.014	0.327
2	0.146 (0.14)	0.055	0.101 (0.11)	**0.955**	0.045	**0.040**
3	0.060 (0.06)	0.913	0.090 (0.09)	0.210	-0.031	0.887
4	0.093 (0.10)	0.338	0.084 (0.13)	0.602	0.008	0.367
5	0.112 (0.13)	0.501	0.102 (0.13)	0.937	0.010	0.412

Standard deviation of kin and non-kin AI is given in parentheses.

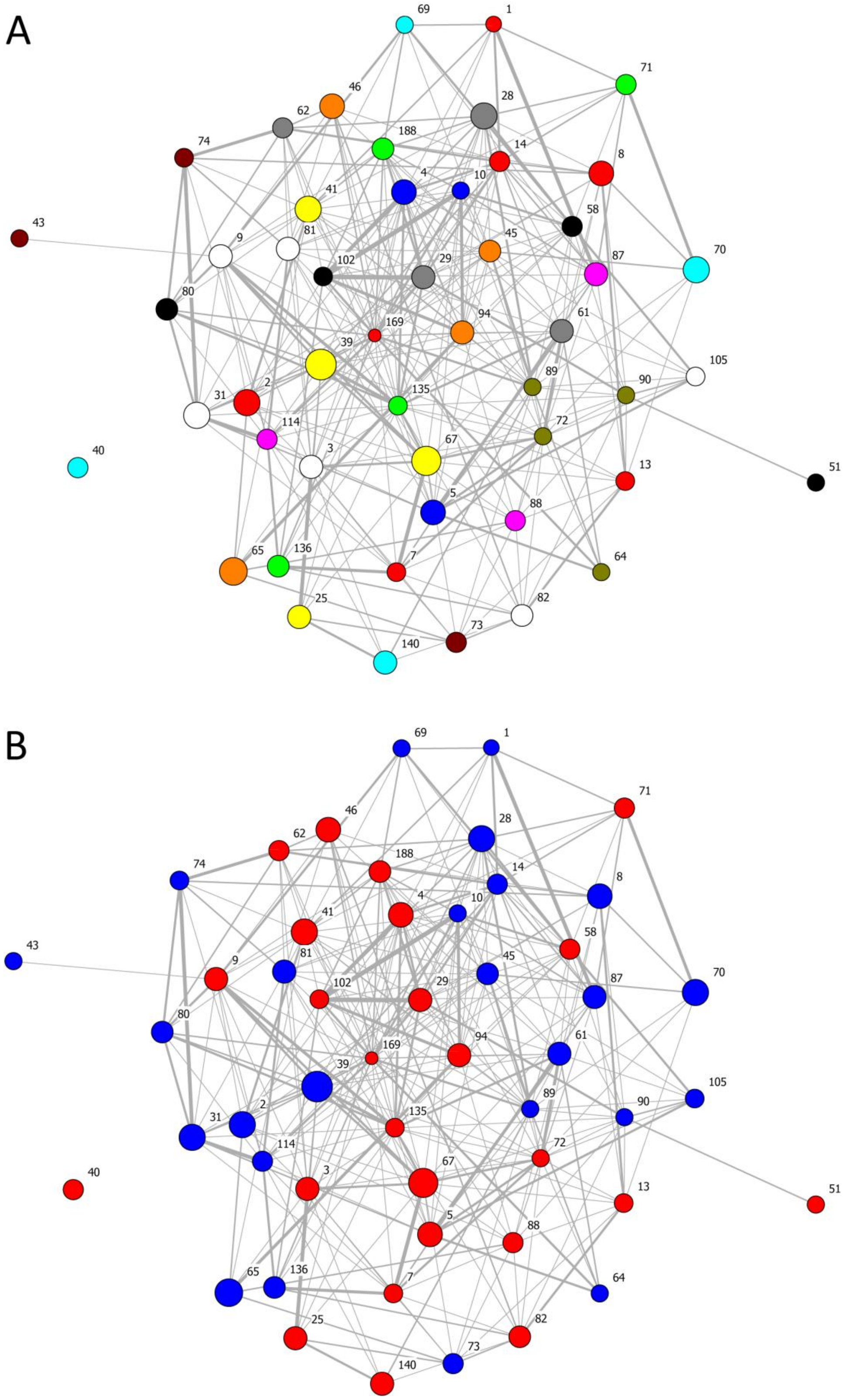

Figure 16. Social network structure of 50 juvenile *S. canicula*. Node colour represents maternal half siblings (A) and sex of individual (B). In both networks node size represents the L_T of the individual and weight of the edge represents strength of association (mean overall network AI = 0.033).

4.4.2.2 Kin and sex structuring

With MP analysis estimates very high for this species (92%), only 15.6% of sibling dyads within the large network of 50 individuals, were likely to be full-sibling related. QAP analyses determined no correlation between social network structure and relatedness based on kin reconstruction estimates (Pearson's $r_{(49)} = 0.012$, $p = 0.278$, Fig. 16A). Using the more accurate measure of relatedness from the quantitative genetics approach, which provided continuous values of relatedness, there remained no correlation with social network structure (Pearson's $r_{(49)} = 0.033$, $p = 0.067$, Fig. 16A). Although the result was nearer to being significant the biological effect size was very small. Finally, the sex of the individual did also not significantly influence social network structure (Pearson's $r_{(49)} = -0.009$, $p = 0.323$, Fig. 16B).

4.5 Discussion

In this chapter the opportunities for kin-based associations and the extent to which kinship was important in structuring social networks, was examined. The first part of this study describes a high frequency of MP (92% of progeny arrays) across a sample of 13 female sharks which suggested that only 15.6% of the subsequent offspring were likely to be full-sibling related. The social preferences amongst related individuals and kin structuring within a large-scale network were then examined using two different estimates of relatedness; kin reconstruction and a quantitative genetics approach. Behavioural experiments using the reared offspring of these females found little evidence for kin assortment or overall structuring of associations by either kin or sex. These results are consistent with the prediction that high MP increases genetic diversity between offspring, reducing the inclusive fitness benefits of preferentially associating with kin. These results are discussed here in two separate sections to reflect the two parts to this study, the first addressing female reproductive strategy and the second, juvenile social behaviour.

4.5.1 MP and female reproductive strategy

S. canicula is the first oviparous shark in which genetic polyandry has been assessed. The results from the two separate analyses (GERUD and COLONY) were relatively consistent (Table 4) and the use of multiple approaches provided a more comprehensive view of MP (Sefc and Koblmüller 2009; Yue and Chang 2010). The only case where a single male sired all the offspring occurred when there was a moderate number of 12 offspring and even the smallest progeny array (*n* = 4) demonstrated evidence of multiple sires. Of the 12 polyandrous progeny arrays half had one male siring at least 60% of the offspring suggesting a degree of male reproductive skew. It is also important to note the number of eggs genotyped per female (4-28) was relatively small compared to the reported fecundity of *S. canicula* (29-62 eggs per year; Ellis and Shackley 1997). This is related to the collection of females prior to the end of the breeding season, suggesting that in a single reproductive year a typical *S. canicula* may produce offspring from far more sires than indicated in this study. However, the results correspond well to previous work on viviparous sharks, where evidence of MP has been widespread (reviewed in Daly-Engel et al. 2010). The high levels of MP observed are comparable to those observed in other shark species, for example the lemon shark *N. brevirostris* (81-87%; Feldheim et al. 2004; Dibattista et al. 2008) and sandbar shark *Carcharhinus plumbeus* (85%; Portnoy et al. 2007). The results are also in accord with investigations of the thornback ray (*Raja clavata*), the only other oviparous elasmobranch in which MP has been assessed, that found evidence of MP in all five groups of eggs analysed (Chevolot et al. 2007).

The predominance of MP in *S. canicula* is consistent with many features of its reproductive biology, particularly its protracted breeding season (Ford 1921) and the potential for long-term sperm storage (Ellis and Shackley 1997). This forms an interesting contrast to recent investigations of MP in *Squalus mitsukurii* (Daly-Engel et al. 2010) and *Squalus acanthias* (Veríssimo et al. 2011), which have documented some of the lowest frequencies of MP in any shark species (11% and 17%, respectively). Both *Squalus* species have a structurally simple oviducal gland that may not allow long-term sperm storage (Hamlett et al. 2005) and a protracted asynchronous reproductive cycle which may have little quiescent period between pregnancies (Daly-Engel et al. 2010). This may limit the potential for multiple matings to occur before fertilization takes place and may also facilitate more effective male avoidance strategies by females (Daly-Engel et al. 2010; Veríssimo et al. 2011).

In larger coastal sharks such as the lemon and sandbar shark, high levels of polyandry have been documented (Feldheim et al. 2004; Portnoy et al. 2007). Since these species have low dispersal potential and a high degree of philopatry, MP may function to increase genetic diversity of broods and decrease sibling competition for resources (Chapman et al. 2004; Daly-Engel et al. 2007). Mark-recapture and acoustic telemetry studies of *S. canicula* suggest limited dispersal and high levels of site philopatry (Sims et al. 2001; Rodríguez-Cabello et al. 2004; Sims et al. 2006; see also *Chapter VII*), which is consistent with this hypothesis. However, broad scale studies of individual movements and assessment of migration rates with molecular markers have yet to be undertaken. Moreover, it is unclear whether genetic variation between siblings has the potential to lead to long-term ecological benefits for offspring.

The failure to identify any indirect benefits to female sharks (Dibattista et al. 2008) has meant that convenience polyandry, where females mate with multiple males as a method of avoiding/reducing harassment from males, may be the most appropriate explanation for MP (Daly-Engel et al. 2007; Portnoy et al. 2007; Dibattista et al. 2008). It is well documented that sexual segregation in sharks is common (Wearmouth and Sims 2008) and this is often attributed to the male mating strategy which is typically aggressive (Carrier et al. 2004; Whitney et al. 2004). A significant literature concerning the behaviour of *S. canicula* has been generated, much of which may be interpreted in light of convenience polyandry as an explanation for MP. The primary requisite for convenience polyandry to develop is that the costs associated with resistance outweigh the costs of mating (Lee and Hays 2004; Dibattista et al. 2008). Direct observation of *S. canicula* has demonstrated that courtship and copulation is extended and may involve harassment and aggression with multiple males in pursuit of a female (Dodd 1983). Furthermore, sexual dimorphism has been shown in mouth and dental morphology, with males developing longer, narrower mouths and longer teeth to aid pectoral biting during copulation (Ellis and Shackley 1995; Crooks 2011). Due to the potentially negative impact of multiple mating on female fitness, unisex refuging behaviour by females has been observed in this species and is likely to serve in part as a male avoidance strategy (Sims 2003; Wearmouth et al. 2012). Aggressive mating in other sharks has already been observed and involves the biting of fins and flank, where serious injury to the females can result (Carrier et al. 2004). The observation that multiple males harass lone female *S. canicula* may suggest cooperative behaviour between males to induce unwilling females to copulate (e.g. mobbing), another behaviour that has previously been noted in sharks (Pratt and Carrier 2001; Whitney et al. 2004). This means that the costs of resisting mating for female catsharks could be high, potentially favouring convenience polyandry. The result of male aggression and female resistance may lead to the development of sexually antagonistic co-evolution (Holland and Rice 1998; Chapman et al. 2003) with sexual segregation (including bathymetric separation, Ford 1921; Steven 1932; Sims et al. 2001) and female refuging in *S. canicula* (Sims 2005; Wearmouth and Sims 2008) reflecting mechanisms to avoid or resist mating. Whilst these inferences remain tentative, the small spotted catshark provides a tractable model in which to begin investigation of shark reproductive systems, particularly as successful observation has been made of the species both in the wild (Sims et al. 2001) and the laboratory (Kimber et al. 2009; Jacoby et al. 2010). The protracted breeding season and potential for long-term sperm storage could also render them particularly useful for studies of sperm precedence and competition, which have yet to be investigated in sharks.

In this study approximately half of the 49 sets of paired eggs analysed were sired by different fathers. This suggests that during each oviposition cycle there is a relatively even chance that eggs in opposing oviducts will be fertilised by the same father. It has been shown that *S. canicula* has a single ovary with ova that are released in pairs, each ova proceeds independently down a separate oviduct, where fertilisation is thought to occur within the oviducal gland (Metten 1939). This biological trait has implications for the degree of half- and full-sibling relatedness between offspring from the same female, which will ultimately influence the level of selection on kin association in this species, as discussed in the next section. In elasmobranch species with paired female reproductive organs, sperm deposition in the oviducts may be independent, meaning that the male's clasper must to be inserted into each oviduct separately (Pratt and Carrier 2005). If a single clasper is inserted during mating then at least two matings would be required to fertilize eggs in both oviducts, suggesting multiple mating may be common. However, accounts suggest *S. canicula* may insert both claspers during copulation (Leigh-Sharpe 1926), which could also explain the occurrence of paired eggs sired by the same father.

4.5.2 Social grouping and genetic relatedness

Kin recognition and discrimination in gregarious fishes appears to be demonstrable under controlled experimental conditions (Olsén et al. 1998; Gerlach and Lysiak 2006) but remains more uncertain under wild conditions with evidence for kin structuring in some species (e.g. Fraser et al. 2005) but not others (e.g. Croft et al. 2012). The costs and benefits of associating with kin are discussed in the introduction to this chapter and are highly variable for different species, age classes and under different ecological and social scenarios (Mateo 2004). Within the elasmobranchs, there is also uncertainty, with the only study considering kin structuring in shark aggregations showing year on year differences (Guttridge et al. 2011). Whilst the results of this current study do show one social group demonstrating significant assortment, overall there is little evidence of kin structuring within *S. canicula* juveniles at either the small or large group scale using either estimates of relatedness. Kin discrimination can be of adaptive importance in both cooperative and non-cooperative animals for avoiding inbreeding within a population (Hatchwell 2010; Mateo 2010). In systems where parental or alloparental care is common or where lek mating systems or mating coalitions occur, kin selection appears relatively widespread amongst vertebrates (see Hatchwell 2010 for review). A component of kin selection theory relies on relatively high frequencies of shared genes within the population through female monogamy and association or cooperation between close relatives (Hamilton 1964). With no parental care in *S. canicula* and the high levels of female multiple mating reported here, genetic diversity is likely to be high between individuals and therefore it is perhaps unsurprising that no evidence of fine-scale genetic structuring was found within these shark groups. It is important to emphasize here that failure to detect any direct link between relatedness and social attraction can't entirely rule out kin selection in *S. canicula*. Indeed, selection may be more subtle than group resting behaviour and thus might go undetected using the current analyses. Equally, sex appeared not to influence grouping behaviour but might also drive behavioural strategy (e.g. dispersal) from an early age perhaps in response to environmental features over longer temporal scales (Litvinov 2006).

Finally, data gathered on the incubation rates and neonate dimensions at hatching revealed relationships between female and neonate body size and female body size and hatch success, with larger females' eggs more likely to survive to hatching and developing into larger offspring. This may serve to counter an overall reduction in egg production during senescence, however further research would be required to test this. There was no relationship found between the extent of multiple mating observed in each female and subsequent standardised hatch success. This result most likely reflects the oviparous mating system in this species in which all maternal investment occurs during egg production, after which other environmental factors and predation probably determine hatch success.

4.5.3 Conclusion

This study combines genetic analyses of multiple paternity and social network analyses of kin association in order to better understand the link between female reproductive strategy in *S. canicula* and the social interactions of her offspring. MP was found to be high in this model species, consistent with many aspects of its reproductive biology, including a protracted breeding season and potential for sperm storage. This result is potentially a consequence of coercive mating by males, a hypothesis that will be discussed again in a subsequent chapter (*Chapter VII*). Neither kin relatedness nor sex were found to influence social network structure, where associations appeared to be all but random suggesting that female multiple mating leads to low levels of offspring relatedness, negating the requirement for strong kin selection within the population.

This and previous chapters in this thesis have examined the influence of social environment and genetic relatedness on grouping behaviour in *S. canicula,* revealing some of the mechanisms that underpin patterns of association in this species. How the ecological environment of these juvenile sharks influences association has yet to be investigated, but will be addressed in the next chapter. Furthermore, social dynamics and individual network position are explored in greater detail with a view to testing individual consistencies in social behaviour across context.

Chapter V.

The effect of habitat complexity on the social behaviour of juvenile sharks

5.1 Abstract

The ecological and social environments into which individuals are born can greatly influence behavioural strategy during the early stages of an animal's life. In particular the degree of complexity of a habitat can alter perceived predation risk by providing a refuge for predators and prey. Work on shoaling teleost fish has demonstrated that the complexity of the habitat can have a major effect on patterns of shoaling behaviour, a body of work which has provided insights into the mechanisms and functions underpinning social behaviour in this taxonomic group. In contrast, no previous work has examined the effect of habitat complexity on the aggregation behaviour of sharks. In the current study the ecological environment experienced by small shark groups was manipulated whilst changes in aggregation behaviour were monitored. Moreover the degree to which individuals show consistent differences in social association under different ecological conditions was investigated. Specifically this experimental study used replicate groups of benthic, small spotted catsharks (*Scyliorhinus canicula*) to test for consistent individual differences in social network position across three different habitat types that varied in their structural complexity. Juvenile *S. canicula* showed consistency in their ranked social network position despite evidence that more complex structures induced the formation of a greater number of groups. To my knowledge, this is the first study to suggest that sharks possess consistent 'personality' traits through time and across contexts. Furthermore, it also contributes to the current discussion surrounding the use of social network position as a meaningful descriptor of animal personality.

5.2 Introduction

Animals obtain information from a wide variety of sources which feedback to influence subsequent behavioural decisions (Cahan et al. 2002). In many species, environmental features can be a strong determinant of whether or not an animal chooses to associate with conspecifics (Krause and Ruxton 2002; Orpwood et al. 2008; Edenbrow et al. 2011). For example, when living under the risk of predation, fish may associate with other group mates as a shoal and/or occupying areas containing complex habitat structures such as rock, coral or algal structures. Associating with group mates can reduce an individuals' perception of risk from predation (Pitcher et al. 1988; Brown et al. 2006) due to mechanisms associated with the selfish herd hypothesis (Hamilton 1971) and confusion/dilution effects (reviewed in Krause and Ruxton 2002). Alternatively, complex habitats may simply provide cover under which both predators and prey can hide. The complex relationship between social behaviour and habitat can thus have profound influence at the individual level, for example, through the ways in which animals acclimate to novel environments (Pinter-Wollman et al. 2009) or make decisions as to whether to join or leave a group (Krause and Ruxton 2002; see also *Chapter I* for more detail). These behavioural strategies can also determine gene flow within a population (Natoli et al. 2005; Andrews et al. 2010), information transfer between conspecifics (Darden et al. 2008) or dictate the growth and development of individuals, ultimately influencing the structure of fish communities (Allouche and Gaudin 2001). Understanding how the ecological environment shapes patterns of social interactions and the mechanisms underpinning population and individual differences in these responses is thus of great importance.

The structural complexity of habitat has been shown to influence in profound ways the grouping behaviour of teleost fishes, particularly of those under threat from a predator (Orpwood et al. 2008) or those engaging in courtship or sexual behaviour (Hibler and Houde 2006). Orpwood et al. (2008) for example, demonstrated that European minnows (*Phoxinus phoxinus*), in the presence of a predator, increased their shoal size in structurally simple habitats only, choosing instead to reduce their rate of movement in more structurally complex environments. These strategies may be intuitive for prey species attempting to avoid active predators, as predator foraging success is reduced in complex habitat but may make them more susceptible to ambush predators also using these complex environments to remain hidden (Warfe and Barmuta 2004). The influence of habitat complexity on the individual social interactions in fish, whether under risk of predation or not, has received considerably less attention in the literature. Recent work by Edenbrow et al. (2011), however, suggests that individual social interactions might actually be fairly resilient to changes in habitat complexity.

Juvenile sharks of small benthic species such as *S. canicula*, used throughout this thesis, are also likely to be susceptible to predation by larger conspecific and heterospecific predators. Consequently, individuals are often born or hatch in areas of structural complexity such as mangrove habitat or rocky substrate (Simpfendorfer and Milward 1993; Carraro and Gladstone 2006). They can remain in and around these areas for a considerable time (Chapman et al. 2009); a strategy adopted to counter their slow growth rates. Within nursery areas, by definition, individual juvenile sharks repeatedly use the same locations across years (Heupel et al. 2007) and as a result aggregation and social grouping can occur (e.g. Guttridge et al. 2011). Despite burgeoning evidence that sharks are capable of 'socialising' in a non-random manner (see *Chapter I* for review, see also *Chapter III*), there remains little information on the behavioural strategies adopted by juvenile elasmobranchs for surviving the early stages of development (see Guttridge et al. 2012 a for an exception). Shark social networks have been described in both laboratory and wild studies (e.g. Jacoby et al. 2010; Guttridge et al. 2011; Mourier et al. 2012), yet the degree to which social behaviour in sharks might be repeatable across different ecological contexts remains unexplored.

It is now well documented that across taxonomic groups individuals can show consistent differences in specific behaviours such as aggressiveness, boldness and general activity (Dall et al. 2004; Sih et al. 2004; Bell et al. 2009). Such behavioural differences are often referred to as animal personalities which can be broadly defined as a suite of correlated behaviours displayed within or across contexts (Sih et al. 2004) that are characterised by consistent individual differences through time (Dall et al. 2004; Cote and Clobert 2007). Personality traits such as boldness, aggression and activity can have substantial effects on both an individuals' gregariousness and how individuals make social decisions under different environmental conditions. For example, in three-spined sticklebacks (*Gasterosteus aculeatus*) and guppies (*Poecilia reticulata*) the boldness of an individual is known to be an important determinant of its position within a social network (Pike et al. 2008; Croft et al. 2009). Animals demonstrating different personality traits are also likely to mediate and maintain the structure of a social network. Under patchy food distributions, for instance, highly exploratory individuals in populations of European shore crabs (*Carcinus maenas*) were found to play an important role in connecting otherwise poorly connected individuals, whilst crabs exhibiting less exploration (low exploratory) formed stable subgroups (Tanner and Jackson 2012). There is now a considerable body of research exploring the role of behavioural syndromes in teleost fishes, with notable progress made towards linking individual differences in gene expression pathways to consistent behavioural variation (see Conrad et al. 2011 for review). Despite this progress, there remains very little work considering individual consistency in social network position under different ecological environments.

Manipulation of animal groups under controlled conditions allows repeated exposure of social groups to different ecological environments and the subsequent network structures can then be compared. Consistency of social network position might then be tested for across context indicating inter-individual differences in sociality. To date, considerable research into the mechanisms underpinning the fine-scale social structure and individual social network position has been conducted using model, freshwater teleost fishes (e.g. Croft et al. 2004; Croft et al. 2005; Pike et al. 2008; Kelley et al. 2011) or global network structure in marine mammals (e.g. Lusseau 2003; Lusseau et al. 2006; Frére et al. 2010). It is only recently that chondrichthyans, with their very different, *K* selected life-history traits compared to teleost fishes, have begun to attract the attention of network practitioners wanting to study their grouping behaviour (Jacoby et al. 2010; Guttridge et al. 2011; Mourier et al. 2012).

This chapter addresses how a changing environment influences aggregation behaviour and social network structure in a model shark species. *S. canicula* are oviparous, benthic elasmobranchs found in abundance in UK and Irish coastal waters, where this species has been extensively studied (Sims et al. 2001, 2006; *Chapter VI* ; Wearmouth et al. 2012). Neonate *S. canicula* hatch from egg cases that are laid on macroalgae, rocky substrata and other structurally complex marine features. The behavioural strategies these juvenile sharks subsequently exhibit to survive, as with most benthic elasmobranch species, remains a mystery, although non-random social interaction between individuals familiar to one another appears to drive aggregation behaviour (see *Chapter III*). Building upon previous experimental chapters that discuss the social and genetic mechanisms dictating aggregation behaviour in juvenile *S. canicula*, this study tests specifically for individual personality defined by consistency in social network position across habitats of increasing structural complexity. Habitat was manipulated experimentally to reflect more natural surroundings likely to be experienced by these juvenile sharks whilst replicated social groups were monitored for fine-scale changes in social structure. In accordance with widespread research into animal personalities, it was expected that individual sharks would show some degree of consistency in their social behaviour (i.e. their relative network positions). It was also predicted however, that within more complex environments individuals would be drawn to occupy areas that might offer protection instead of remaining out in the open, thus increasing the level of aggregation at these locations. Such refuging behaviour is commonly observed in teleost fishes as an antipredator strategy (Krause et al. 2000b). To my knowledge, this is the first study to test directly for individual consistency of behavioural traits in elasmobranchs.

5.3 Methods

5.3.1 *Experimental sharks*

The group resting behaviour of juvenile (< 1 yr) *S. canicula* was used to assess the influence of habitat complexity on the aggregation and social behaviour of young sharks. Juvenile sharks were reared in the Marine Biological Association Laboratory, Plymouth, U.K. from eggs laid by wild females caught locally at Whitsand Bay, Cornwall, UK (see *Chapters II* and *IV*). The experiment was conducted between August and September 2011 on juveniles which were approximately 8 – 10 months old (*n* = 100) with a mean total length (L_T ± SD) of 179.7 ± 27.4 mm and a mean weight of 17.98 ± 7.6 g. Size-matched individuals were selected from large holding aquaria (858 l capacity, 1.65 x 0.80 x 0.65 m) before being tagged for individual identification using visible implant elastomer tags (VIE; Northwest Marine Technology, WA, USA) as per previous chapters. Once tagged, sharks were distributed across five smaller holding aquaria (149 l capacity, 1.22 x 0.61 x 0.20 m, temperature: 17.0 ^{o}C), with 20 individuals per aquaria where they were allowed to recover for > 10 days. As the sex of the individual did not appear to influence association between juveniles (*Chapter IV*) sex was chosen randomly from a stock sex ratio of ~1:1. The husbandry of experimental sharks, including feeding schedule and light cycle, can be found in *Chapter II*.

5.3.2 *Experimental design*

A repeated measures design was used to assess the effects of habitat on gregariousness and social interactions between juvenile sharks. Each group of 10 individuals, of which there were 10 replicates, were transferred from the recovery aquaria where they would have familiarised with one another (see *Chapter III*) to the large experimental arenas (858 l capacity, 1.65 x 0.80 x 0.65 m). Four experimental groups at a time were run concordantly for two days during which aggregation behaviour and activity rates were examined periodically. Social networks were constructed from scan samples of associations taken at two hourly intervals between 08:00 and 18:00 h (6 samples per day). Individuals were deemed to be associating if they were within one body length of one another (see *Chapter II* and *III* for more detail). During the 12 samples, data were gathered on group size, number of groups, the proportion of individuals active and the participants of each group for social network measures. Following data collection all individuals were returned to their specific holding aquaria.

To determine whether environment influenced how the juvenile sharks associated, all 10 groups were subject to three habitat treatments which differed in their level of structural complexity:

1) Gravel – each experimental aquaria was given a natural, medium gravel substrate (size range diameter: 8-16 mm) spread evenly throughout the area. This was defined as a *simple* habitat.
2) Stones – each experimental aquaria contained three discrete clusters of large stones (~18 x 9 x 10 cm) always in the same location and orientation constituting sites 1, 2 and 3. (NB. Stone 'structures' were sufficiently large for several groups of individuals to form independently of one another at each cluster). This was defined as a *complex* habitat.

3) Mixed – each experimental aquaria contained both of the above habitat types. This was defined as a combination of *simple* and *complex* habitats. More information is given in figure 17.

Little is known about the type of habitat favoured by juvenile *S. canicula* in the wild, however, based on knowledge of the structures upon which egg cases are deposited, these treatments were designed to reproduce some of the habitats which are likely to be experienced by young sharks of this species. The subsequent ordering of these treatments was randomised for each group in this repeated measures design to control for any potential order effects. Each trial was then conducted as described above. During data collection, the structures around which individuals were aggregating (in the Stones and Mixed treatments only) were also recorded to enable preferences for specific locations to be assessed.

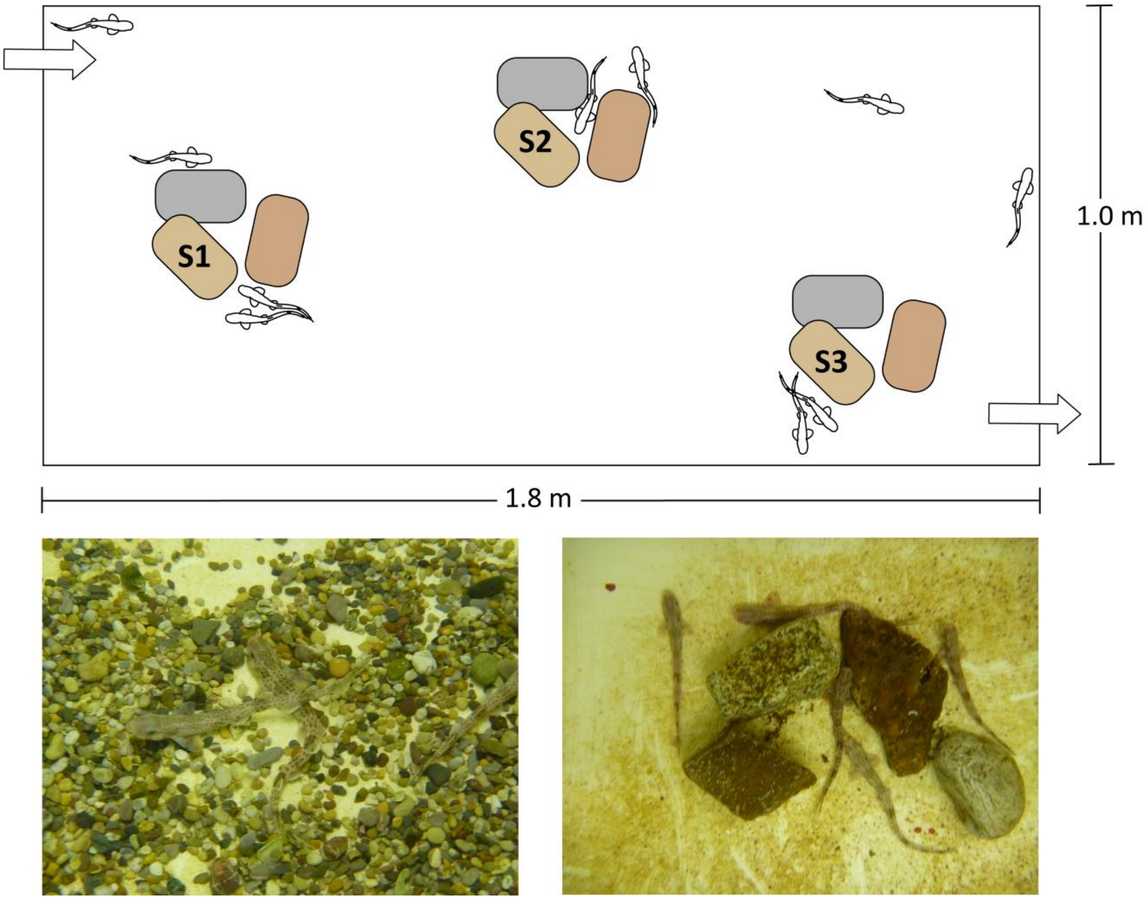

Figure 17. Schematic diagram illustrating the three experimental stone structures (S1, S2 and S3). Photographs show juvenile sharks aggregating during the simple gravel treatment (bottom left) and the complex stones treatment (bottom right). Arrows indicate the flow circulation of the water.

5.3.3 Statistical analysis

To test for effects of environment on aggregation behaviour across habitats of increasing complexity a multivariate, repeated measures general linear model (GLM) was used. The dependent variables of group size, group number and activity were entered into the model, with an independent variable of treatment. Repeated, within-subject contrasts, applying the Bonferroni correction for pairwise comparisons, were used to determine the relative effects of treatment on behaviour. As per Briffa et al. (2008), effect size estimates (η^2) were also calculated to determine the proportion of variation in the dependent variables that were explained by the independent variable. This estimate was then compared to effect size estimates from analyses of association data (see below). Finally, in treatments that contained complex structures (Stones and Mixed) a Pearson's chi-squared analysis was conducted to test for significant preferences for a particular site location.

5.3.3.1 Network analysis

Group membership of individuals marked with VIE tags was recorded during each scan sample. Weighted social networks were then constructed for the duration of each experiment by accumulating data on partner associations during resting behaviour for each of the 12 sampling periods. Using the Simple Ratio Index (SRI; Cairns and Schwager 1987), all dyadic pairings (two associating individuals) were assigned a weighted value between 0 and 1 representing the strength of association between these individuals. A SRI closer to 0 indicates that individuals were rarely/never seen associating, where as a SRI of 1 means individuals were never seen apart. A matrix of association from the SRI was then constructed for each of the 10 groups under each habitat treatment. Individual node-based metrics, derived from matrices of association, were calculated and included *strength*, the sum of an individual's association indices with all other individuals in the group; *reach*, an indirect measure of connectedness that gauges the proportion of individuals that are connected to the node of interest via one, two, three links etc, and *clustering coefficient*, which is an indication of the role an individual plays in interconnecting groups and communities based on neighbour connectivity. To test for consistency in social behaviour, the *strength* of each individual's network position was first ranked before being tested for concordance across treatments using Kendall's coefficient of concordance (*W*). For each of the 10 groups *W* was calculated, before a permutation test was used to determine significance as individual ranks were non-independent of one another (Croft et al. 2011). The permutation test, conducted in Poptools (Hood 2010), reshuffled individual ranks 20,000 times providing a distribution against which observed values for concordance in strength were compared. The 10 individual significance values were then combined using Stouffer's method as per *Chapter III*. These analyses were adopted to explore the level of behavioural consistency in the social interactions of juvenile *S. canicula*. Briffa et al. (2008) discuss how a value for *W* also represents a scaled effect size estimate and therefore a mean value for *W* was then directly compared to η^2 from previous analyses to determine the relative strength of behavioural plasticity and consistency in this species. Unless otherwise stated, all statistical analyses were conducted in PASW Statistics 18 (IBM Corp., Somers, NY, USA) and network analyses in SOCPROG 2.4 (Whitehead 2009).

5.4 Results

5.4.1 Aggregation behaviour

With the assumption of sphericity met for all three treatments ($p > 0.05$), the multivariate, repeated measures GLM revealed that there was a significant main effect of habitat type on aggregation behaviour ($F_{(6,32)} = 3.239$, $p = 0.013$). Further exploration showed that there were significant effects of habitat on the number of groups ($F_{(2,18)} = 10.939$, $p < 0.001$) but not on the group size ($F_{(2,18)} = 1.089$, $p = 0.358$) or proportion of active individuals ($F_{(2,18)} = 1.150$, $p = 0.339$). Interestingly, group sizes remained virtually constant across the three treatments (Fig. 18). Analysis of contrasts revealed a significant increase in mean group number between the Gravel (simple) and Stones (complex) habitat treatments ($p = 0.005$,

Fig. 18) and a subsequent significant decrease between the Stones and the Mixed (simple/complex combination) treatments (*p* = 0.023, Fig. 18). As such the three dimensionally 'complex' stone structures appeared to encourage aggregation tendencies in these juvenile sharks whilst the gravel substrate appeared to have a dispersive influence.

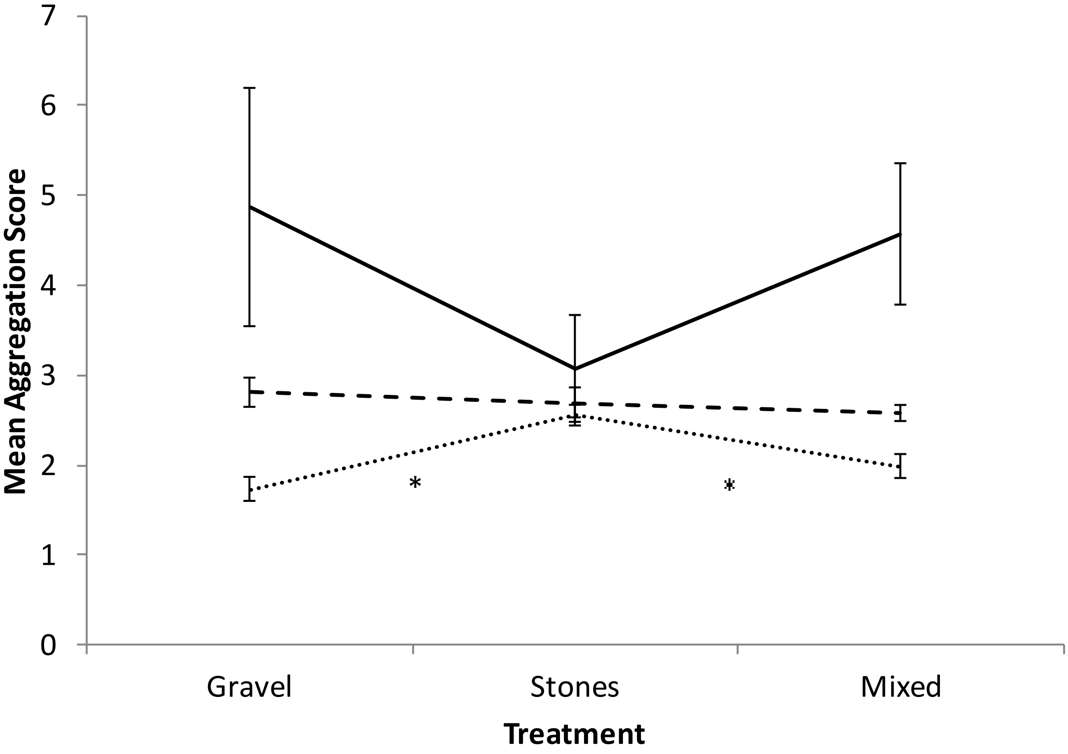

Figure 18. Interaction graph (± SE) of the number of active individuals (solid line), the group size (dashed line) and the number of groups (dotted line) during the three habitat manipulation experiments. * Denotes significant differences at the *p* < 0.05 level.

Should aggregation in and around stone structures occur due to a shared requirement for a resource, an even distribution of groups across the three discrete clusters of stones would be expected. A Pearson's chi-squared analysis was used to test for deviation from this expected distribution. During both the Stones and the Mixed habitat treatments there was clear deviation from the expected distribution with site 3 being favoured under both conditions (Stones: $\chi^2 = 140.621$, df = 18, $p < 0.001$; Mixed: $\chi^2 = 117.030$, df = 18, $p < 0.001$, Fig. 19).

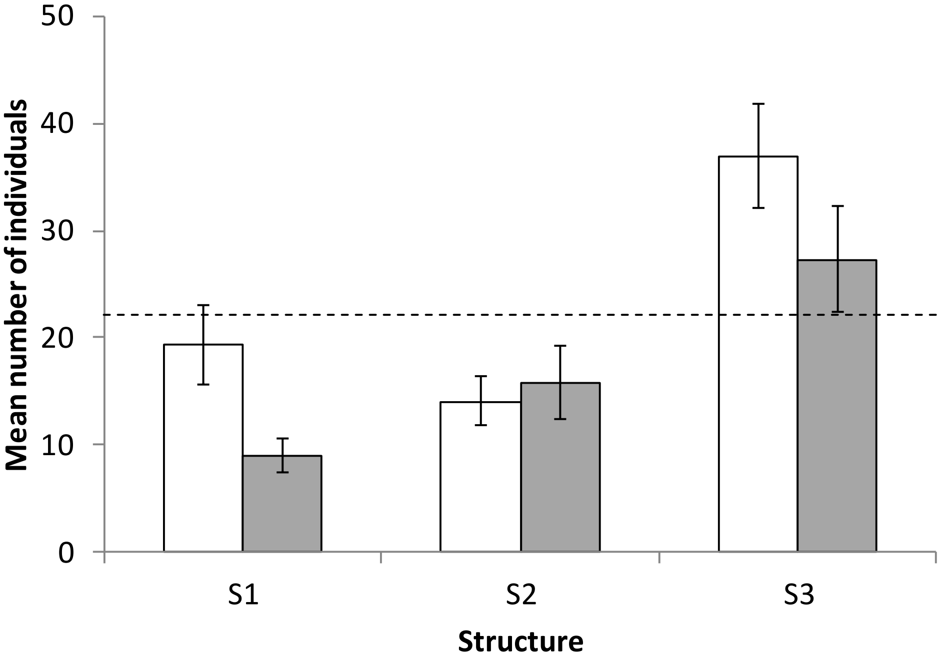

Figure 19. Mean number of individuals (± SE) associated with each of the three discrete stone clusters. Both the Stones (white bars) and Mixed (grey bars) treatments differed significantly from the expected distribution (dashed line).

5.4.2 Social behaviour

Descriptive analyses of the social network data revealed little variation between the mean network metrics across the three different habitats (Fig. 20). Significant concordance was found in strength of individual social network position across the three treatments (Stouffer's test: $n = 10$, $P < 0.001$) with a mean concordance of $W = 0.464$, although there was relatively high variation between groups (range: 0.300 – 0.731). This variation is likely due to the small number of individuals in each experimental group (n = 10) resulting in a fairly high random mean value for W within the null model. Overall however, this result indicated that individuals of high social connectivity in one type of habitat remained highly connected in subsequent habitat treatments despite the randomisation of the order in which treatments were presented.

5.4.3 Plasticity and consistency in behaviour

Scaled effect size estimates were calculated for the plasticity observed in juvenile aggregation behaviour ($\eta^2 = 0.158$), which proved lower than the effect size for social concordance across habitats ($W = 0.464$) as per the methods outlined in Briffa et al. (2008). This result indicated that whilst juvenile sharks appear to significantly adapt their aggregation tendencies depending upon the type of habitat available, there is a stronger effect dictating the relative levels of social connectivity each individual commands, which appear highly consistent across these different habitats.

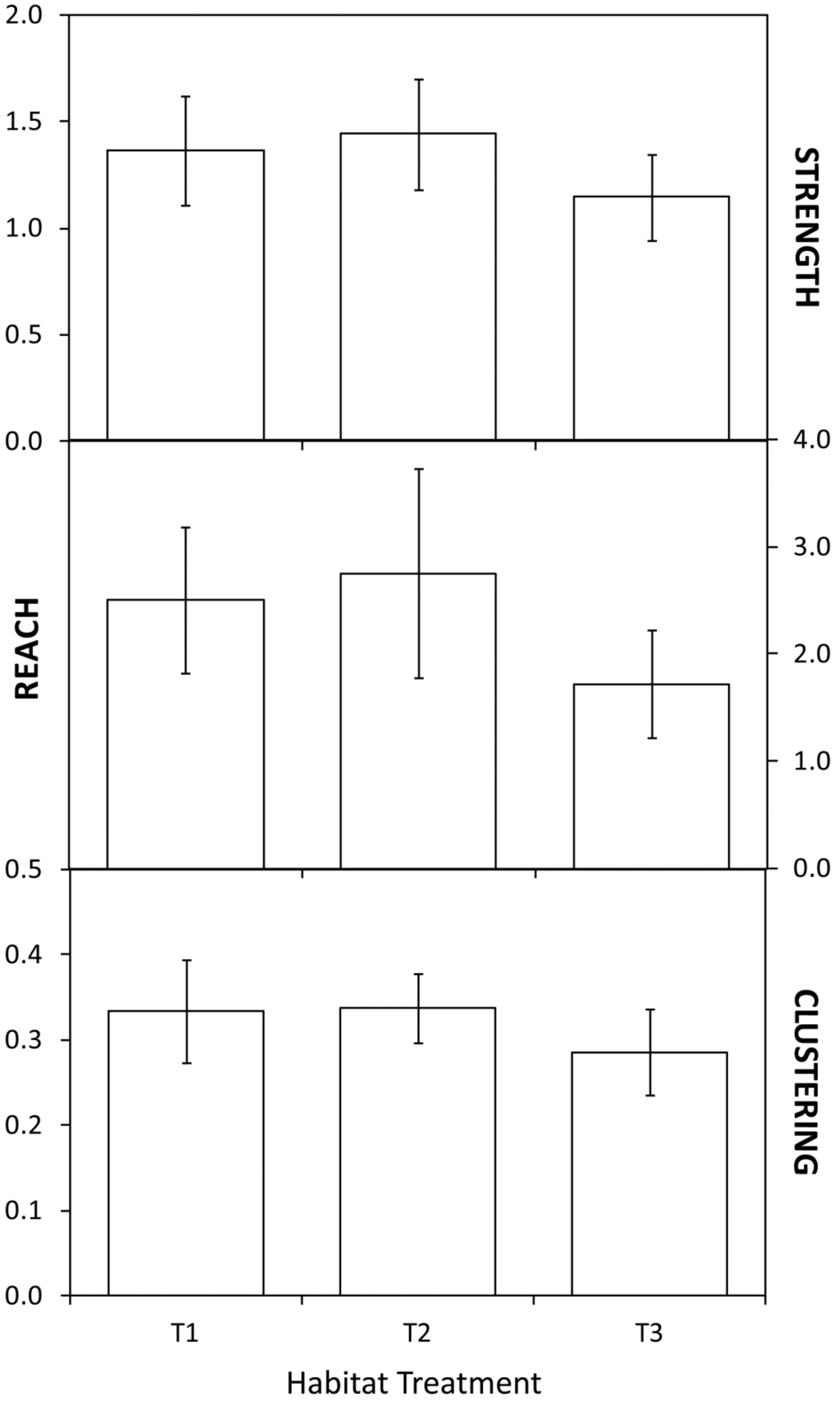

Figure 20. Mean social network metrics across habitat treatments. Mean strength, reach and clustering coefficient were calculated for the Gravel (T1), Stones (T2) and Mixed (T3) treatments.

5.5 Discussion

It has been shown through experimentation using teleost fishes that the ecological environment experienced by individuals influences the social interactions of gregarious species through the direct (Croft et al. 2006a; Orpwood et al. 2008) and indirect effects of predation (Croft et al. 2003). While predation risk was not tested for specifically in this study, gregarious behaviour that likely serves as an antipredator strategy in juvenile *S. canicula* was explored under different habitat environments, to determine the level of individual consistency in social behaviour across context (otherwise known as a personality trait). Groups of sharks were manipulated experimentally to experience multiple habitat types of varying structural complexity. As predicted aggregation of these sharks was significantly influenced by habitat whereby the number of groups significantly increased during the treatment containing the stone structures (Stones), suggesting at least some evidence of behavioural plasticity. Furthermore, of the three discrete stone clusters, one (S3) was always preferentially sought by individuals attempting to aggregate. Despite relatively high variation between replicates, significant consistency in the strength of individual social network position was found across the three habitat treatments. This is the first indication, of animal personality traits being found within predatory elasmobranchs. In addition, the effect size estimates showed that consistency in sociality was a stronger driver of behavioural strategy than plasticity in aggregation behaviour.

By exposing replicated experimental groups of sharks to multiple environments, the complex interaction between habitat and social environment can begin to be explored. The presence of a uniform layer of gravel in both the Gravel and Mixed treatments, for example, appeared to reduce aggregation tendency perhaps as individuals were better colour/contrast matched with the substrate. The skin colouration of *S. canicula* was observed to adopt a lighter or darker shade dependent upon the colour of the aquaria background (DMPJ *Pers. obs.*). There is a suggestion from the results of the aggregation analyses that the presence of the stone structures in the arena served, to some extent, to drive aggregation behaviour in these juveniles. However, most individuals preferentially sought the same structure around which to aggregate and indeed maintained relative social network positions whilst doing so. This indicates that given the choice, juvenile *S. canicula* opt to utilise refuging habitat but would rather do so with other conspecifics. Indeed, by aggregating at three-dimensionally complex habitat features individuals are likely to benefit from both the dilution of risk offered by grouping with phenotypically similar conspecifics (Krause and Godin 1994) and a reduction in predator success in structurally complex environments (Warfe and Barmuta 2004). This appeared to be borne out in this study where the Stones treatment induced the highest number of groups. Interestingly, group size was not affected and this was potentially due to the way in which aggregation was quantified. For simplicity, individuals were deemed to be in association if they were situated within one body length of the centre point of another individual (Franks et al. 2010). Using this type of sampling it was assumed that individual sharks use visual cues to determine where and with whom to associate. Given the important role of olfaction in social recognition in teleost fishes (Ward et al. 2007), it is probable that odour cues also play a role in structuring elasmobranch groups. This could prove an interesting subject for future research but in the context of the current study might explain the preferential attachment of small isolated groups to the same site across both treatments (Stones and Mixed). It is possible that a strong olfactory signal was drawing these groups to aggregate at the same location but that the large stone clusters created a physical and visual barrier between groups potentially explaining why group size was not influenced by habitat.

The discovery of highly repeatable personality traits in animals is currently receiving considerable attention in the behavioural ecology literature (Sih et al. 2004; Bell et al. 2009; Krause et al. 2010; Webster and Ward 2010; Briffa and Greenaway 2011; Conrad et al. 2011). As discussed in previous chapters (*Chapter III* specifically) there is now burgeoning evidence to suggest that some elasmobranch species demonstrate non-random social interactions in both the laboratory (Jacoby et al. 2010; *Chapter III*) and the field (Guttridge et al. 2011; Mourier et al. 2012). However, individual consistency in shark behaviour has never been addressed, despite the need to better understand and predict the response of species at risk from habitat disturbance and other anthropogenic influences (Robbins et al. 2006). Perhaps predictably, given the mounting evidence for repeatable personality traits in other taxa (Dall et al. 2004; Sih et al. 2004; Dingemanse et al. 2010), this study revealed consistent individual differences in social network position through time and across habitats of differing structural complexity. Social and non-social measures of behavioural consistency have been widely recorded in teleost fishes (see Conrad et al. 2011 for review) but relatively few of these studies have specifically tested for consistency under differing levels of habitat complexity. Recently however, Edenbrow et al. (2011) found that the social network structure of two isolated guppy populations was not significantly influenced by habitat complexity, despite being significantly different from one another due to the variation in predation risk associated with their source geographic locations. The results from Edenbrow et al. (2011) are therefore consistent with the current study suggesting that individuals possess consistently different levels of sociality under changing environmental contexts. In the present study these individual network positions (ranked by strength of association) are actually consistent relative to others in the group.

Non-random social preferences influence the social structure and aggregation tendencies of a wide variety of animals across a range of taxa (Krause and Ruxton 2002; Croft et al. 2008). Until recently, social behaviour in sharks was almost exclusively anecdotal, but as discussed in *Chapters I* and *III* is now beginning to be quantified in a variety of species. Numerous environmental features, such as tidal cycle, depth, water temperature and light level are often cited as significant drivers of aggregation behaviour in sharks (Economakis and Lobel 1998; Hight and Lowe 2007; Powter and Gladstone 2009) and it is probable that these will all vary considerably in nearshore environments where juvenile sharks are normally studied. Indeed, slow growing elasmobranchs often require complex habitat structures such as mangroves and rocky openings (crevices, caves) which provide protection in the form of cover from larger marine predators (Yokota and Lessa 2006). As shown here, permanent environmental features appear to be important influences on the aggregation behaviour of juvenile sharks suggesting antipredator motivation in this species as well, although further research would be required to test this specifically.

By occupying coastal nursery areas juvenile sharks are more likely to be influenced by human activity, so despite strong evidence that inshore environments offer ideal habitat for small and juvenile shark species, there comes a need to better manage these areas from inshore fishing activities and development for an ever growing global population (Pauly et al. 2002; Knip et al. 2010). Thus, developing a clear understanding of the ways in which habitat influences the tendency for juvenile sharks to aggregate and indeed interact socially, is of great importance to the implementation of future conservation initiatives and this study serves to highlight the need for further research in this direction.

In conclusion, inshore habitats play a particularly important role in the survival and development of many species of shark (Yokota and Lessa 2006; Heupel et al. 2007; Knip et al. 2010). Although considerable attention has been paid to exploring home ranging behaviour and space use within these nursery habitats (e.g. Morrissey and Gruber 1993; Heupel et al. 2004; Heupel and Simpfendorfer 2005 a,b), it is important to address, as here, the influence of changing environmental features on the social associations of aggregations of juvenile sharks. Consistency in social preference, coupled with a degree of plasticity in aggregation tendency under different habitat types, perhaps paints a more complex ecological picture than originally thought. However, greater knowledge of the behavioural strategies adopted by juvenile sharks for surviving the early stages of development will surely contribute positively towards improved conservation efforts, particularly for threatened or endangered species.

This chapter, along with the two previous chapters, addresses the mechanisms and functions behind grouping behaviour in *S. canicula* using controlled and replicated captive experimentation. This concludes the laboratory component of the thesis with the next two chapters focusing on wild catshark behaviour from data gathered by passive telemetry techniques. As such, the laboratory experiments provide context for some of the patterns of behaviour observed in the wild population.

Chapter VI.

Developing a deeper understanding of animal movements and spatial dynamics through novel application of network analyses

6.1 Abstract

Determining how animals move within their environment is fundamental knowledge that contributes to effective management and conservation. Continuous 'round-the-clock' animal movement data is frequently gathered using biotelemetry technology, providing discrete data packages on the presence-absence of animals at known locations through time. Current analyses of such data do not generally account for the interconnectivity of locations as animals move between them and consequently do not integrate graphically or statistically a temporal component to spatial changes. This study describes the novel application of network analyses to electronic tag data whereby nodes represent locations, and edges the movements of individuals between locations. The aim of this chapter is to demonstrate some of the descriptive and quantitative approaches for determining how an animal's movement interconnects home range habitats. Telemetry data from arrays of recorders provide movement data of individual animals, and as examples of the method proposed the movements of two shark species, the small spotted catshark (*Scyliorhinus canicula*) and the Caribbean reef shark (*Carcharhinus perezi*) are examined. In doing so, both local and global network properties are considered from an animal movement perspective as are the simulated effects of node removal as a proxy for habitat disturbance. Comparative visual representations of two *S. canicula* movement networks suggest, for example, potential differences in space use. Multiple regression quadratic assignment procedure (MRQAP) showed that habitat was a significant predictor of movement behaviour. Null modelling of *C. perezi* movement data, corrected for the spatial restriction of static nodes, demonstrated a significant, non-random distribution of directed move-

ments among sites. Additionally, the connectivity of this animal's movement network was significantly reduced through targeted disruption of a site of high centrality but not through disruption of a randomly selected site. Network theory is a well established theoretical framework and its integration into the fast developing field of animal movement and telemetry has the potential to yield significant advances to how we interpret animal space use from electronically recorded data. This technique has potentially wide application in animal behaviour and ecology but may also inform the management of habitat harbouring threatened or endangered species via the simulation, modelling and intuitive visualisation of animal movement.

6.2 Introduction

Understanding how and why animals move and migrate is fundamental to the effective management and conservation of wild animal populations. The spatial and temporal structure of movement cycles are based on evolutionarily successful behavioural decisions in response to numerous physical, biological and environmental stimuli (Patterson *et al.* 2008; Sims 2010). As human impacts on natural habitats become more widespread, understanding the cyclical trends involved in movement and the driving forces behind them are vital to the identification and potential mitigation of anthropogenic disruption (Southwood and Henderson 2000).

There are acute difficulties associated with observing and recording data for highly mobile and wide-ranging species in their natural habitats. As such, ecologists are increasingly relying on animal-borne electronic tags designed to store and in some cases transmit data relating to an animal's movement, speed, direction or environment (Rutz and Hays 2009). In recent years, advances in biologging and telemetry techniques have enhanced considerably our understanding of space use in a variety of animals from a broad range of environments (Cooke 2008). Ecologists now rely on such technology to estimate population density, home ranges and identify localised movement patterns. Radio telemetry, for example, has been used to track terrestrial animals including sugar gliders (*Petaurus breviceps*: Quin et al. 1992), jaguars (*Panthera onca*: Soisalo and Cavalcanti 2006) and mice (*Apodemus flavicollis*: Perkins et al. 2009), but also freshwater fish (McCleave et al. 1978). However, the frequencies used in radio telemetry are insufficient to track fish within a marine environment (Sims 2010). Consequently static spatial arrays of acoustic recorders are frequently used to monitor the short and long-term movements of a wide variety of marine taxa including elasmobranchs (Heupel and Simpfendorfer 2005 a,b), crustaceans (Freire and González-Gurriarán 1998) and teleost fishes (Anras et al. 1997). Electronic data-logging (archival) tags, satellite-linked archival tags and Global Positioning System (GPS) tags are also used regularly to track large predatory fish that roam across vast oceanic distances, experiencing considerable changes in both temperature and depth (Brunnschweiler et al. 2009; Queiroz et al. 2010). Equally, large terrestrial predators are tracked using motion-sensitive, camera trapping techniques that are being employed to estimate population density and study the visitation patterns on regularly traversed routes in remote places, such as the Brazilian Pantanal (Soisalo and Cavalcanti 2006). Such advances in technology can provide 'round-the-clock' monitoring of individuals and it is not unusual for this type of dataset to contain tens of thousands of individual data points (Fancy et al. 1989; Heupel et al. 2010). These data however, often share a generic feature, namely a time series of presence-absence data with each individual's time stamp and location creating a single data point.

The size and complexity of telemetry datasets can be challenging to visualise, analyse and interpret. In virtually all contemporary analyses (for review see Sims 2010), presence-absence data at known locations are viewed as separate entities, the abundance and frequency of which can be correlated to biotic or abiotic variables. Descriptive analyses such as frequency distributions of detections and multiple tag presence-absence graphs offer a useful approach for exploring common patterns within a population. The most frequent examples of these types of analyses are found in the marine environment where static array telemetry is widely used to track coastal marine predators (Dawson and Starr 2009; Speed et al. 2011). Basic inferential analyses include the generation of temporally and spatially structured residency indices which can be empirically compared (Abecasis and Erzini 2008; O'Toole et al. 2011). A more complex method for defining space utilisation and home ranges of animals is kernel utilisation distribution (KUD), widely used in both marine and terrestrial ecology. For example, home range estimates for white-nosed coatis (*Nasua narica*) in Arizona, US, were calculated for both individual solitary males and whole troops using a fixed-kernel density estimator with a smoothing factor which was then compared to the minimum convex polygons (MCPs) from previous studies (Hass 2002). The result of such analyses is a graphical depiction of the likelihood of an individual being found in a given area at a given time period, thus providing information on 'core areas' (see Dawson and Starr 2009; Speed et al. 2011 for further examples). These types of analyses offer powerful insights into the movements and habitat use of animals; however there is still a wealth of information held within these large datasets which, to the best of my knowledge, is not yet being fully exploited. Further development and refinement of analysis techniques is required to not only identify core areas using presence and absence data, but also to identify, quantify and compare empirically linkages and movements between core areas, for example.

For some time, ecologists have advocated the use of graph theory for explaining metapopulation structure and the impact of landscape connectivity on conservation (Urban and Keitt 2001). Graph theory considers the local and global structure of networks constructed from pairwise interactions of connected elements in a graphic format using nodes linked by one or a series of edges (see Materials and Methods for more detail). More recently, a desire to better understand how the internet or transport routes are connected has given rise to the exploration of anthropogenic spatial network structures (Gastner and Newman 2006; Kaluza et al. 2010). There has been some exploration of biological spatial networks, for example the spatial network topography of both freshwater ponds and roosting trees interconnected by amphibians and bats has been examined (Fortuna et al. 2006, 2009). However, these studies do not look at individual animal movement from automated electronic equipment. They do however, provide new insight into the importance and connectivity of specific habitat features on the aggregation and disease transfer of the animals moving between them. Individual-based telemetry data presents an opportunity to utilise graph theory and enhance the potential for hypothesis-driven, telemetry-based field studies. This is particularly important because the frequency at which an animal or a group of animals occur at a given location does not necessarily underlie the ways in which animals move between locations. Here, an integrative and holistic approach that accounts for the connectivity of locations is proposed that has the potential to significantly improve our understanding and interpretation of animal movement and habitat use. As such we might begin to use electronic tag data to address a number of important hypotheses regarding the movement of an individual or group of animals (see Table 6 for examples).

Table 6. Example hypotheses (*H*) that could be addressed using network analysis of movement data obtained from animal biotelemetry data.

H_1	*Animals demonstrate repeatable movement patterns/show site fidelity*
H_2	*Movements and space use differ significantly between time of day/year or between sex/age class*
H_3	*Environmental variables can be used to predict movement between areas or general movement patterns*
H_4	*Habitat disturbance at key locations will impact animal movement*

From its early development in human sociology, social network analysis (SNA) has now become a fast growing ecological discipline adapted to study animal interactions and the formation of group related behaviours (Croft et al. 2008). Broadly applicable to any complex, interconnected system, network analysis has been used to study hyperlinkage on the World Wide Web (Park 2003), scientific collaboration networks (Barrat et al. 2004), power distribution within the national grid (Watts and Strogatz 1998) and a host of biological systems from protein interactions in yeast cells (Rives and Galitski 2003) to shoaling behaviour in fish (Croft et al. 2005). What appears most striking about this broad analytical approach to date is that unlike in human sociology which has employed SNA to study a variety of indirect human interactions (e.g. Song et al. 2010), network analyses of non-human vertebrates remains almost exclusively grounded in the assessment of social connections, whether it be grooming (Lehmann and Dunbar 2009), shoaling (Krause et al. 2000a) or vocal communication networks (Fichtel and Manser 2010). There seems great potential then, to amalgamate these two apparently disparate fields of animal movement analysis and network analysis, in order to explore the interconnectivity of habitat or resources via the animals which move freely between them (Urban and Keitt 2001; Fortuna et al. 2006).

The aim of this chapter is to encourage an interdisciplinary approach to the analysis of animal movement and tracking data. The potential application of network analyses to improving the visualisation and statistical analyses of presence-absence data gathered by the wide variety of telemetry devices currently in use is demonstrated. This accessible approach is then demonstrated using two distinct telemetry datasets of shark movements within two separate static acoustic arrays in temperate and tropical ecosystems. The movement tracks within and between individuals are then compared before applying null modelling and node disruption simulation techniques to determine space use and vulnerability of the network to the loss of nodes (habitat patches). Furthermore, the application of this technique is discussed in the context of hypothesis-driven animal telemetry and the future management and conservation strategies of a wide variety of terrestrial and aquatic taxa.

6.3 Materials and methods

In an attempt to illustrate the potential benefits of using network analyses to analyse, visualise and interpret these vast and often disparate packets of data, two distinct datasets are interrogated from the marine environment where static array, acoustic telemetry is widely used (Sims 2010). The movements of two predatory shark species are investigated: *S. canicula* tracked within a small, deep-water acoustic array at Whitsand Bay, Cornwall, UK (Fig. 21) and *C. perezi* tracked using a large acoustic array (32 receivers) in the shallow waters off the coast of Cape Eleuthera, Bahamas (Fig. 22).

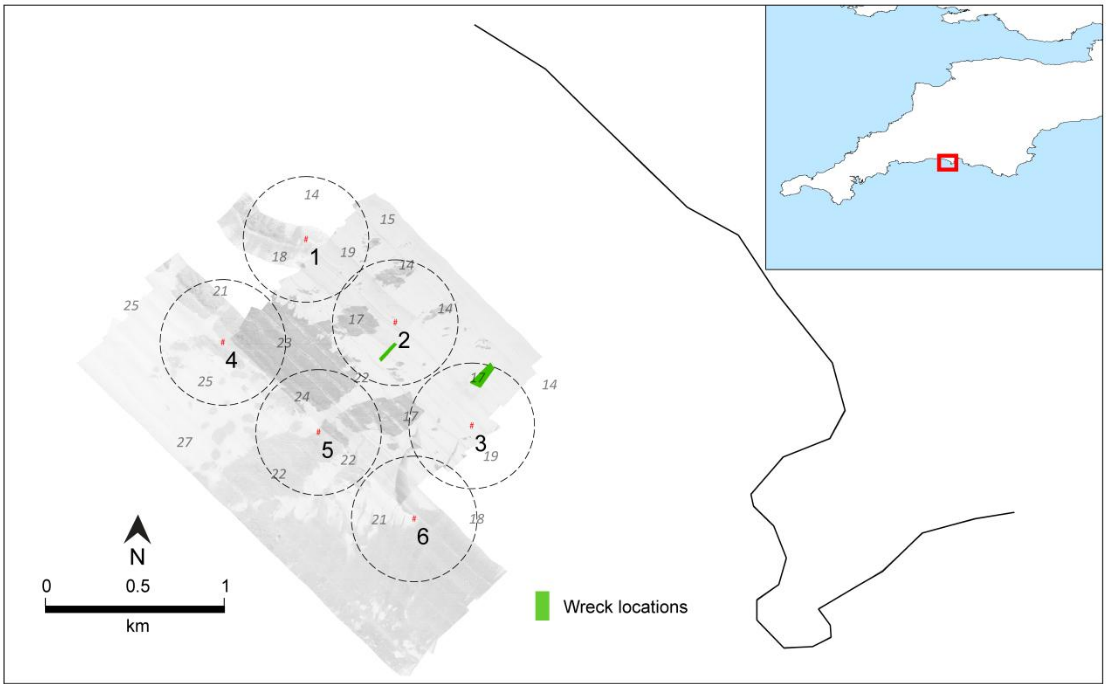

Figure 21. Spatial layout and topography of an acoustic receiver array at Whitsand Bay, Cornwall, UK. The array contains six data-logging receivers where the dotted circles around each receiver indicate the approximate detection range. Topography is represented by multibeam sonar swaths with darker colours indicating rocky habitat. The continuous black line denotes the coastline.

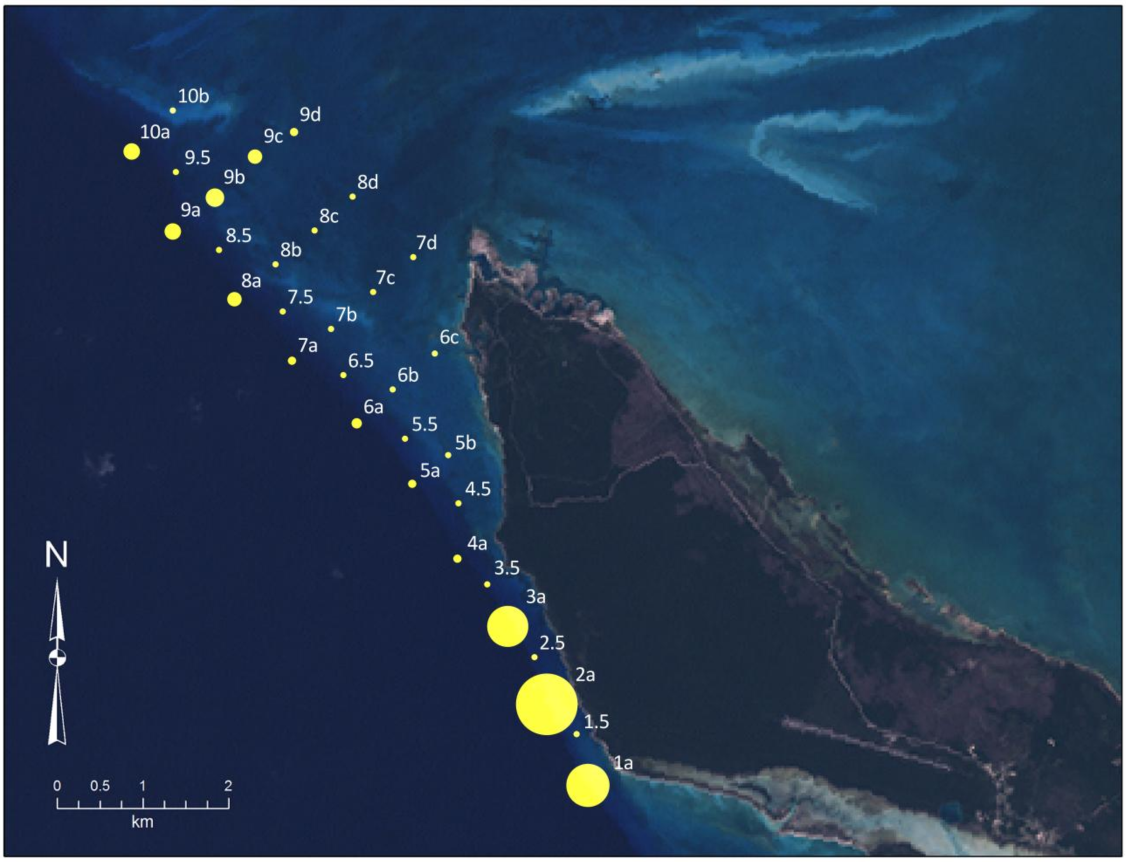

Figure 22. Spatial layout of acoustic receivers within an array situated off the coast of Cape Eleuthera, Bahamas. Circles represent receiver location and the size of the circle represents the number of detections logged during the movements of a male *C. perezi* tracked for 14 months.

Methodological details of the capture, tagging and acoustic tracking of *S. canicula* is provided in the next chapter (*Chapter VII*) and in *Appendix II: A.2* for the Caribbean reef shark dataset. The data here represent individual, unreplicated animal tracks which are used to demonstrate the specific application of this approach. These data have been selected to illustrate how network analyses account for the interconnectivity synonymous with telemetry data and furthermore how statistical analyses of these animal tracks can be explored in their entirety or in a restricted format enabling specific hypothesis testing. For ecological insight into *S. canicula* movements using this methodological approach see *Chapter VII*.

6.3.1 Working with network data

Network theory relies on the notion that complex interconnected systems are made up of *nodes* connected by *edges*. Nodes may represent anything from individual organisms, physical locations or centres of information and may be dynamic or static within their environment. Edges, whether binary (0 or 1) or weighted relative to an index (continuous values between 0-1, see *Chapter II*), are equally variable and might encapsulate physical or emotional interactions or associations between network nodes (social networks), directional flow of information or disease, or the mobility of organisms between fixed locations. Nodes and edges are assimilated into a network of interconnected nodes (e.g. Fig. 4) from which a number of quantitative metrics can be calculated that can describe both local and global network structure. In particular, node-based metrics (local properties) can be used to describe the influence individual nodes (*i*) have on the overall network structure and are determined from the level of interaction one node has with any other node, either directly or via intermediaries. Unlike in previous chapters, in the present study an interaction (or edge) between two nodes constitutes the movement of an individual or group between two areas with the weighting of this movement edge relative to the frequency with which this directed movement is made by individuals. Common node and edge-based metrics are summarised below:

Centrality – Measures of local centrality indicate a nodes' importance directly via its level of connectedness. *Degree* (k_i) for instance, is a local measure of the number of direct links or edges attached to a node and therefore *degree centrality* gives an indication of the reachability of a node or location. Degree may be further refined to account for *In-degree* (k_i^{in}) and *Out-degree* (k_i^{out}) in order to explain entry and exit points or gateways to an area of interest or conservation concern. An area with a high degree centrality for example, would suggest strong site fidelity by wide ranging animals. In this instance animals may return from many different areas but always back to the same location, as described for the home ranges of Galápagos sealions (*Zalophus wollebaeki*; Wolf et al. 2007) for example.

Betweenness – *Betweenness* (B_i) offers a property that can be measured for individual nodes or as a global mean and is based on the number of paths that pass through a specific node, from one node to another via the shortest path length. Transient or migratory species for example may divert to a specific habitat, such as a watering hole, *en route* to another location (Wolanski and Gereta 2001). These areas of high betweenness might provide access to a limited resource or be important for the social exchange of information and therefore are likely to promote aggregation (Krause and Ruxton 2002; *Chapter I*). Such localised node metrics may prove beneficial in assessing the relative importance of specific locations to the core movements of site faithful or territorial animals.

Degree Distribution – Equally there are global properties of these movement networks, such as the degree distribution *P(K)* across all habitat locations, which reveal important structural components within the network. These can be used to model the possible effects of disturbance on the current space use of an animal (see Rhodes et al. 2006 for example).

Average Path Length – Another global property worth considering is the average path length (*L*) between all nodes. This metric provides a measure of how easily, or indeed likely, an animal moves between locations on average and is useful for comparing networks between individuals or age classes.

Density – Finally, *Edge density* (E) represents the proportion (or percentage) of actual edges present, out of the total number of edges possible in a given network ($E = 1$). This measure is likely to inform analyses of random and non-random space use in animals.

6.3.2 *Data processing*

The large amounts of data produced from biotelemetry techniques can today be explored in spreadsheet or database programs for a variety of statistical analyses. Modern programs offer a range of functionality and algorithms for sorting, sampling and randomising data and it is relatively straight forward to automate searches or run queries within the data to pick out interactions or movements of different animals between receivers or locations. Spreadsheets or simple text files of interaction data can then be fed directly into one of several programs developed specifically for analysing interaction or association data. In this study SOCPROG, a MATLAB based program designed for analysing social structures in animal groups (Whitehead 2009) and UCINET (Borgatti et al. 2002) are used, both of which offer a comprehensive set of network analysis tools (see *Appendix II: A.2.2* for links to programs and manuals). These programs were used to manipulate the data into full matrices of directed shark movement interactions between receivers within their respective arrays, upon which all further analyses were conducted (see *Appendix II: A.2.3* for example matrices). Once the data is entered into a program, there are a number of considerations to bear in mind when creating a movement interaction matrix:

Absolute or Relative Edge Weighting – The matrix can either comprise absolute values, where weighted edges represent a total count of movements between two nodes or a relative proportion of the number of times a movement is made between node A and node B, divided by the total number of movement edges in the network (see *A.2.3* for further information). Absolute interaction data (hereafter AID) and relative interaction data (RID) are likely to produce very different movement networks as RID controls for the time spent within the monitored area. However, AID can provide a useful insight into temporal space use in animals. Both RID and AID, each containing directed movements, are used in the following analyses in order to illustrate the potential uses of count and proportional data for biologically meaningful exploration.

Sampling rate – When an interaction measure relates to a physical movement, we are primarily working with dyadic pairings (two locations and an edge between them), in the form of directed movements between one node and another. Therefore, each sample will simply relate to each movement step made between a pair of locations. Weighted and directed movements are considered in this paper.

Filtering/restriction – It is at this stage in the data preparation that a network/matrix can be easily filtered or restricted using various column headings in the raw data file in order to consider only certain aspects of the dataset. This might include restricting a network to purely immediate movements between locations, by filtering at an edge duration (E_t) of ≤ 1 hr or 30 min for example. Additionally, temporal restrictions can be placed on the data to consider specific seasonal movements or alternatively tagged animals might be categorised for quantitative comparison of movement networks between classes.

6.3.3 Network visualisation

Suppose the data now comprise a complete or filtered matrix format, visual exploration of the data is highly recommended. This can be achieved in both SOCPROG and NETDRAW, the latter being a visualisation component of UCINET, by simply loading the file containing the interaction matrix. Furthermore, a list of node-based properties, often referred to as 'attributes', can be entered parallel to the interaction matrix. Node attributes within a movement network, for example, might include habitat type, canopy cover, water depth or number of animals present. Node size and colour can be altered in accordance with these attribute data to illustrate detection frequency, sex or specific geographic variables. Additionally, visual filtering of edge weight in some movement networks (e.g. NETDRAW allows the switching on and off of nodes and edges) might reveal clear distinction between home ranges and exploratory movement behaviour. Network visualisations can then be laid out in a number of different ways based on the structural properties of the network in question (see Fig. 22). Equally useful however is the ease with which nodes in these programs can be moved around or referenced to specific locations or environmental features, facilitating the overlay of a network on a map using mapping tools such as GIS. Alternatively, movement networks might not require spatial reference, in which case there are a number of functions such as multidimensional scaling or spring embedding that will aid visual representation of networks based on edge weights and measures of centrality. As such, network analyses offer a vast and intuitive array of visual manipulation techniques which are extremely useful for informing further quantitative analyses.

Model visualisation 1. The AID for the annual movement of two example *S. canicula* individuals (9114, male) and (9127, female) were drawn up into comparative network visualisations using NETDRAW. Node size was manipulated to reflect the residency times throughout the year based on the frequency of detections at each acoustic monitor (Fig. 23).

Model visualisation 2. The Cape Eleuthera array represents a dynamic system of 32 receivers. The annual space use of a juvenile male reef shark (0.89 m L_T) represented as RID is given in figure 24 and includes a spring embedded visualisation of the network (Fig. 24A). Node colour was then manipulated to indicate the core areas or home ranges of this animal during the first and last 7 months of its total track (Fig. 24B and 24C) using the centrality metric described above (K_i).

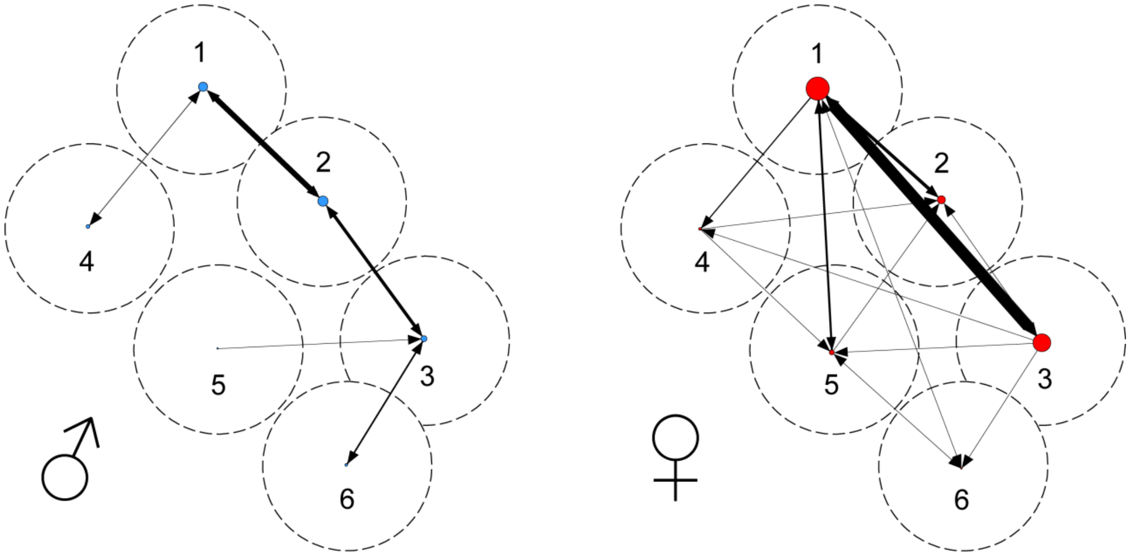

Figure 23. Movement networks of a male and a female *S. canicula* tracked in Whitsand Bay during 2010. Networks represent absolute interaction data (total counts) of movements between receiver locations (i.e. greater edge weight shows more frequent interactions). Node size indicates detection frequency. Circles around receivers indicate approximate detection range.

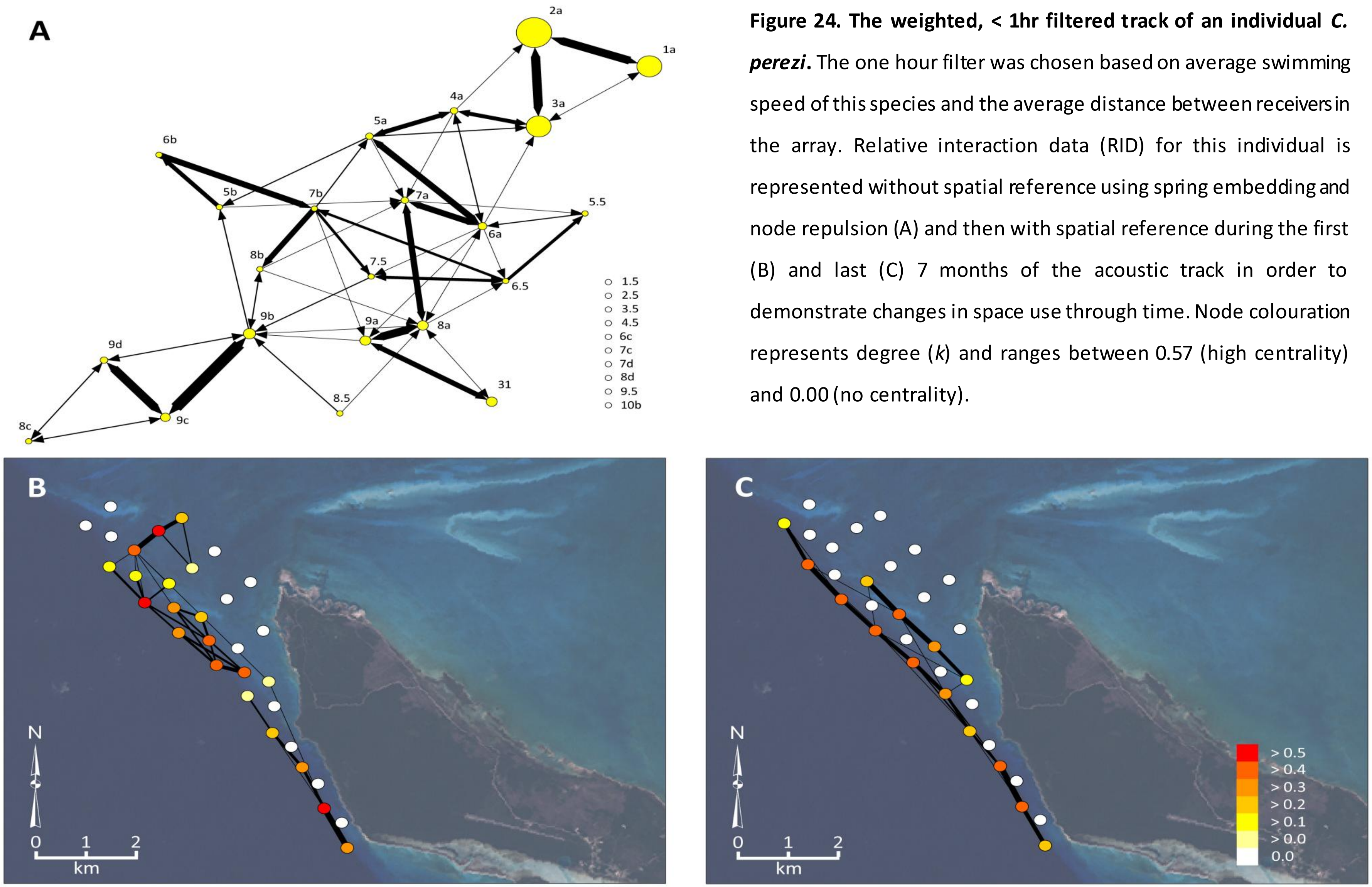

Figure 24. The weighted, < 1hr filtered track of an individual *C. perezi*. The one hour filter was chosen based on average swimming speed of this species and the average distance between receivers in the array. Relative interaction data (RID) for this individual is represented without spatial reference using spring embedding and node repulsion (A) and then with spatial reference during the first (B) and last (C) 7 months of the acoustic track in order to demonstrate changes in space use through time. Node colouration represents degree (*k*) and ranges between 0.57 (high centrality) and 0.00 (no centrality).

6.3.4 Statistical analyses and null modelling

Model analysis 1. Calculation of node-based metrics were carried out in UCINET using the 'Network > Centrality > Degree/Betweenness' functions, which are a small subset of an extensive range of analyses designed to calculate different structural properties within network data. Some of these analyses, not presented here, are also likely to prove suitable for analysing interaction data on animal movements.

Node-based metrics, of betweenness (B_i) and directed In- and Out-degree (k_i) were calculated and compared between the male and female (RID) catshark movements (Fig. 23) to illustrate how replication of movement tracks between different sexes might yield insight into behavioural strategies. Network measures are summarised in Table 7.

Table 7. Comparative summary of the node-based metrics betweenness, in degree and outdegree for the male and female *S. canicula* throughout 2010. Male and female sharks made intermittent use of the habitats covered by the acoustic array for 277 and 317 days respectively. Values represent network analyses of RID with AID given in parentheses.

	Male (9114)			**Female (9127)**		
Receiver	B_i	k_i^{in}	k_i^{out}	B_i	k_i^{in}	k_i^{out}
1	10	0.28 (17)	0.30 (18)	5.5	0.45 (84)	0.47 (86)
2	10	0.40 (24)	0.40 (24)	2	0.10 (19)	0.09 (17)
3	7	0.23 (14)	0.18 (11)	1.5	0.32 (59)	0.35 (65)
4	0	0.02 (1)	0.05 (3)	0	0.03 (6)	0.01 (2)
5	0	0.00 (0)	0.017 (1)	2	0.07 (13)	0.07 (13)
6	0	0.067 (4)	0.05 (3)	0	0.02 (4)	0.01 (2)
Mean	**4.50**	**0.17 (10)**	**0.17 (10)**	**1.83**	**0.17 (31)**	**0.17 (31)**
Network Centralisation (%)		**19.77**			**22.40**	

Model analysis 2. The inherent nature of network data rules out the use of many traditional statistical analyses due to the violation of the common assumption of independent data points. As such, current statistical approaches largely rely on generating null network populations by randomly permuting various aspects of the dataset (Croft et al. 2011). The observed network structure is then compared to the distribution of a large number of randomly permuted networks generating a *P* value of significance (see previous chapters for examples). Clearly, general biological conclusions about species specific movements also require multiple individual tracks. Incorporated within the network programs discussed above are a number of other statistical analyses designed to handle non-independent, matrix data. Multiple regression quadratic assignment procedures (MRQAP) are designed for permuting multiple linear regression coefficients of data in square matrix format (Dekker et al. 2007). These techniques have been successfully employed in studies of non-human (yellow-bellied marmots, *Marmota flaviventris*; Wey and Blumstein 2010) and human social networks (Harrison et al. 2011) for regressing age, relatedness and cooperative input against social network structure.

Using the double Dekker semi-partialling procedure built in to UCINET, MRQAP was used to determine the environmental variables linked to individual directed catshark movements within the acoustic array. Environmental data attributed to each of the six acoustic receivers were converted into similarity matrices using the 'Data > Attribute to matrix' function whereby absolute differences between receivers yielded positive values of the distance between the attribute scores of a pair of nodes (or locations). The attributes included were (1) inshore or offshore receiver location, (2) mean depth of the area within detection range of each receiver, and (3) a habitat complexity score based upon averaging arbitrary, but consistent values of rugosity and habitat substrate. These similarity matrices were then regressed against the RID on the annual, direct movements (< 1 hr) and space use of the two catsharks throughout 2010.

Model analysis 3. There are several methods for carrying out statistical analyses of network data using null modelling and randomisation of real movement data that can be interpreted intuitively from a movement analysis perspective. Comparing some structural property of the movement network, such as mean density (*D*), centrality (*C*) or path length (*L*) to a suite of random networks is a useful method for determining whether an animal is using an area in a random manner. This is likely to prove useful when combined with the above regression analyses, for testing specific hypotheses (see Table 6). There are however, a number of ways in which interaction matrices can be permuted to create random networks and these might differ from how some networks of social interaction are randomised (Whitehead et al. 2005; Croft et al. 2011). Given that the network nodes are spatially restricted in telemetric movement networks (i.e. direct movements between one pair of nodes is more likely than another because they are nearer one another) it would be unwise to permute either nodes or edges in the random networks without accounting for this spatial bias. Therefore, the random networks need to preserve aspects of the most likely spatial arrangement of the network structure.

To determine whether the model Caribbean reef shark showed random use of the acoustic array, the variance in degree (k_i) for the <1 hr filtered AID was calculated for the observed track disregarding any nodes which were not visited by this individual (i.e. those nodes not connected in Fig. 24). This observed value was compared to the frequency distribution of the same test statistic calculated on a series of permuted (randomised networks). In order to preserve spatial structure in the data, observed individual movement events (n = 624), which contributed to overall edge weighting, were permuted to create replicated (weighted) movements from the raw data. Reassignment of these individual directed movements during each permutation, were constrained to only directional edges between pairs of locations which occurred in the empirical movement network (see Fig. 24). The permutation was done using a Monte Carlo simulation in POPTOOLS (Hood 2010) creating a distribution of 10,000 random networks against which variance in degree (k_i) of the empirical data was compared to generate a P value. This provided a conservative test, based only on the known movements from the observed data, with which to determine whether the frequencies of movement between specific receivers were likely to have occurred under random movement.

Model analysis 4. It is probable that if an animal is demonstrating non-random space use of an area over extended periods of time, then there are attributes associated with those areas that are favourable to that animal during its current phase of development, whether it is protection, food availability or appropriate temperature ranges. It is useful then to attempt to predict how an animals' movement might be impacted by disturbances in habitat at different locations. The disruption of nodes in a movement network (i.e. removal of edges connected to a node) might serve to simulate the impact that habitat destruction is likely to have on an animal's space use. Thus this can be used to model the effects of natural and anthropogenic disturbance on animal movements, particularly among animals with high levels of site attached behaviour. Consequently, this technique has the potential to be used for impact assessment and strategic conservation measures by determining how likely an animal or group of animals is to disperse from an area if one or several nodes become unavailable (Rhodes et al. 2006). However, it does not account for the potential for animals to adapt to such disturbances.

The ecological basis for this type of 'knock out' analysis is perhaps most suitable for movement between more isolated and diverse habitat locations and as such the relatively small-scale *C. perezi* movement track presented here, does not necessarily offer a real world example where a knock out is expected to have population consequences. It does, however, serve an illustrative purpose, indicating the potential use of this approach to conservation measures whilst also highlighting the importance of considering interconnectivity in telemetry data as compared to analyses of static individual locations. Using the full, < 1hr filtered, RID interaction network, edge betweenness (B_e) was calculated between nodes in a matrix format and compared before and after the targeted disruption of a node with a characteristically high centrality (9b) and that of a randomly generated node (9a). Matrices of B_e for the targeted and random node-disrupted networks were compared to B_e from the full movement network to determine whether network connectedness was significantly impacted. This was achieved using the 'Network>Compare densities' function in UCINET. Statistical tests were one-tailed as we would expect connectedness to reduce to some extent with the removal of any movement edges. Random sub-sampling (20,000 bootstrap samples) of the network provided a way of estimating variance within these tests.

6.4 Results

6.4.1 Small spotted catshark dataset

Model visualisation and model analysis 1. Comparative descriptive analyses of the example, unreplicated male and female *S. canicula* movement data reveal distinct differences in habitat use and residency times within the area under acoustic observation (Fig. 23). Node size and edge weight, representative of detection frequency and frequency of movement interactions (AID) respectively, suggest that this particular female is utilising the area to a greater extent than the male. Individual node-based metrics support this idea (Table 7), revealing greater overall network centralisation or density (*E*) in the female movements (female: 22.40%, male: 19.77%), but interestingly, a higher mean betweenness centrality in the male network (female: 1.83, male: 4.50). This value of *B* for the male shark track appears to be heavily influenced by exclusive and persistent movements along the inshore half of the array. Even at this scale, using model data, there is apparent segregation in space use and movement between locations as evidenced by the stark differences in degree (*k*) at node 2 (Table 7).

Model analysis 2. Using more quantitative analyses, relative interaction data (RID) on the directed movements of the male and female catsharks were regressed against the environmental attributes of each of the six receiver stations in order to address the potential influence of habitat type on shark behaviour. Attribute similarity matrices were entered into a multiple regression quadratic assignment procedure (MRQAP) which was run against individual movement networks. The regression coefficients individually were non-significant predictors of either movement track. As a model however, the difference between the edges in terms of habitat similarity, can be used to predict the strength of the movements made by both the example female ($R^2 = 0.181$, $P = 0.018$) and the male shark ($R^2 = 0.166$, $P = 0.036$), perhaps with a suggestion that in these cases habitat type is a stronger determinant of the female's behavioural strategy.

6.4.2 Caribbean reef shark dataset

Model visualisation 2. Using a single shark track from a much larger, more dynamic telemetric dataset, a number of other animal movement and space-use analyses are explored from a network perspective. The RID for a Caribbean reef shark (*C. perezi*) track has been visually represented in several different ways (Fig. 24) which can be used interchangeably depending upon the type of data exploration required. Figure 22, shows the spatial arrangement of the array with the detection frequencies for each of the receivers overlaid on top. Spring embedding of the network (Fig. 24A) disregards the spatial arrangement of the receivers, but demonstrates the centrality of some locations over others based upon individual node-based metrics. These visualisations are easily manipulated to mirror the exact spatial arrangement of the array (Fig. 24B, 24C) and further insight is gained by representing receiver attributes across nodes. Visual analyses of the movement patterns of this juvenile shark, between the first and last seven months of tracking, illustrate interesting changes in space use and home range of this animal throughout time and ontogeny.

Model analysis 3. Null modelling, which preserved the spatial network structure, was used to create a frequency distribution of the variance of k_i across 10,000 random networks. Significance values were produced from direct comparison of the observed data to this frequency distribution. The variance distribution of edge weighting amongst the observed shark movement network was significantly higher than would be expected from random space use ($P < 0.001$).

Model analysis 4. The targeted node disruption (i = 9b) had the effect of significantly reducing network connectedness (Paired sample t test; $t(31) = 1.904$, $p = 0.044$), whereas assigning a random node for disruption (i = 9a), despite being adjacent to the targeted node, appeared not to impact connectedness significantly and, thus, the animal's movement pattern (Paired sample t test; $t(31) = 0.675$, $p = 0.210$). Interestingly, both nodes had a similar number of detections suggesting detection frequencies, often relied upon quite heavily in more traditional analyses of telemetry data, are not necessarily indicative of the underlying movement network of an individual. Standard error for this analysis was calculated using 20,000 bootstrap samples and the results are summarised in Table 8.

Table 8. Statistical comparison of edge betweenness (B_e) for targeted and random node removal. This was calculated by comparing a measure of connectedness between the full Caribbean reef shark RID movement track (14 months) and the same track with the simulated random and targeted disruption of one node. Standard error (SE) estimates were produced from 20000 bootstrap samples.

Network	Node Removed	E	$E\text{-}E_{sim}$	Detections (n)	p*
Full	**-**	**1.207**	**0.000**	**-**	**-**
Random	9a	1.129	0.079	462	0.210
Targeted	9b	0.754	0.454	550	0.044

* Values indicate one tailed significance tests as mean B_e is expected to reduce with the removal of edges.

6.5 Discussion

This study examines the novel use of network analyses to understand and interpret animal movement and space use gathered via passive electronic tagging equipment. Traditional analyses of animal biotelemetry data appear largely to ignore the connectivity between areas preferring instead to evaluate presence-absence data in a non-dynamic, static format. Given that biologging and biotelemetry techniques are currently undergoing rapid development (Rutz and Hays 2009) there is a need to address this connectivity in relation to how tagged animals are moving within and between these data collection points (Urban and Keitt 2001). The conversion of data into a movement network/matrix format provides edge-based data to complement static node telemetry time stamps. This allows researchers to capture both the spatial and temporal dynamics associated with animal movement in a holistic analysis of what are often very large, complex datasets.

Static array acoustic telemetry data were used to illustrate some of the many approaches offered by network analyses to this type of data. The handling and sorting of raw data, followed by the visual exploration of animal tracks is discussed and examples given (Figs. 23 and 24). A range of statistical analyses which can be conducted on matrix data are also described allowing comparison of individual tracks and environmental variables or null modelling and simulated predictions of movement under different scenarios. These tools offer greater flexibility to identify movement patterns associated with habitat use and furthermore, can help to model the space use shifts in the event of critical habitat loss. It is important to emphasise here that the use of these analyses for studying and comparing behaviours across multiple individuals (i.e. replicating movement tracks) is certainly encouraged in order to draw general conclusions about how species are using an area.

6.5.1 Summary of visual and statistical approaches

The unreplicated movements and space use of a benthic shark *S. canicula*, a species for which the biology, physiology and ecology have been the subject of wide research (Ellis and Shackley 1997; Sims 2003; Jacoby et al. 2010), were assessed within a deep-water acoustic array. The movement tracks of the two example *S. canicula* reveal obvious differences in the temporal space use within a relatively small area of an open coastal bay. MRQAP analyses, at least in the case of these individuals, suggest that habitat features are significant predictors of an individual's movement, a finding which is supported by short-term continuous tracking and underwater surveys of this species (Sims et al. 2001). Further exploration of individual node-based metrics of each of the six receivers (Table 7) reveals high male betweenness, suggesting a roaming strategy in comparison to what is likely a refuging (philopatric) strategy in the female; such behaviour has also been documented from observational studies (Sims 2003). These node-based metrics on directed movements might also be used to explore the entry and exit points of an open water array such as this. Replication of male and female movement tracks and the implications for differential behavioural strategies between the sexes in this species is addressed in *Chapter VII*.

By contrast, data collected on the comparatively wide ranging behaviour of a larger species of coastal shark, *C. perezi* revealed that detection frequency can be a deceptive and inaccurate predictor of an animal's underlying movement strategy (i.e. differences in edge betweenness impact between random and targeted node disruption despite similar detection rate, Table 8). This is an important result given the largely static nature of current analyses of electronic tag data. Spring embedding and other network visualisation is explored (Fig. 24) and node manipulation is used to demonstrate differences in home range between the first and second half of the movement track, as this male shark grew and its movement became more orientated towards the coastal shelf. On land, radio telemetry has also been used to explain sex differences and seasonal changes in home ranging behaviour in terrestrial mammals such as the Eurasian lynx (*Lynx lynx*; Herfindal et al. 2005) or the Sichuan snub-nosed monkey (*Rhinopithecus roxellana*; Li et al. 2000); as such, there is now the potential to analyse such data with some of the statistical approaches outlined in this study.

Null modelling of *C. perezi* movements demonstrated that this animal was not using the area in a random manner favouring instead to move between several well connected locations (receivers). Furthermore the likelihood for dispersal is significantly greater if one of these few highly interconnected nodes becomes impacted beyond use. This result does not account for adaptation to disturbance and perhaps needs to be explored in greater detail given the nature of this particular acoustic array and the likelihood of individual node disturbance at such a small scale. It does however, provide support for the differential influence of specific areas within an animal's home range movements. The techniques applied here on movement data could be extended to include assessments of disturbance in other systems. For example, Amstrup and Gardner (1994) use radio tracking of individuals to assess the effects of industrial development and increased hunting on the temporal and spatial distribution of polar bear (*Ursus maritimus*) denning sites. In a more recent study, Rhodes et al. (2006) explored the robustness and fragility of white-striped freetail bat (*Tadarida australis*) roosting networks from targeted and random node removal. These types of simulations, as shown here, can be used to predict the impact of habitat disturbance on individual behaviour and overall community space use by anticipating displacement and susceptibility to dispersal, thus indicating priority habitat for conservation (Rhodes et al. 2006).

6.5.2 Analysis accessibility

Currently there are a variety of programs which can be used to determine the movement of animals from static telemetry data. Descriptive and quantitative analyses for these data were carried out across different programs using different platforms, which highlights a need to consolidate ideas and analyses for this type of data into a single more holistic program. Current developers of network programs make it particularly easy to download and use their software and manuals (Borgatti et al. 2002; Whitehead 2009; *A.2.2*) providing all the necessary tools needed for the non-specialist to conduct these types of analyses. An appreciation of these types of analytical approaches might, in addition, be useful during the planning stages of an experiment and could perhaps help to refine the hypotheses leading a telemetry-based study of animal movement.

6.5.3 Future developments

The field of movement ecology has seen the development of rapid, convergent approaches to the study of animal movement. Using a wide range of animal-borne electronic tags is one way in which to condense these species-specific movement differences into simplified presence-absence data, GPS locations and the environmental variables associated with these tracks. Radio tracks of mountain lions (*Felis concolor*), for example were analysed to determine movement 'signatures' revealing specific types of behaviour when hunting, feeding or mating (Beier et al. 1995). Equally, within the marine environment, smart positioning and temperature logging tags (SPOT tags) are used to track large marine predators that surface relatively frequently (Sims 2010) and have been used to explore niche expansion in salmon sharks (*Lamna ditropis*; Weng et al. 2005). Alternatively, small injectable radio frequency ID tags or passive integrated transponder (PIT) tags, can be used to track the movements of very small birds, mammals and amphibians. Pyrenean brook salamanders (*Calotriton asper*), for instance, have been successfully tagged with PIT tags in order to enhance the efficiency of determining individuals at different habitat locations (Cucherousset et al. 2008). How animal tracks are interpreted remains open to debate (Cooke 2008), however the need to visualise better and statistically analyse the spatial and temporal relationship inherent in animal movement remains a key barrier in movement ecology. Incorporating the interconnectivity of habitat locations into such analyses using node and edge-based metrics as here is likely to prove beneficial to the study of movement of many terrestrial and marine organisms. Furthermore, with the burgeoning development of animal social network analysis (Croft et al. 2008) there seems great potential to explore the role of biological spatial networks and movement analyses, in the context of animal social networks.

6.6 Conclusion

Exploring the spatial and temporal dynamics of the ways in which animals move is a particular challenge. With availability of 'round-the-clock' tracking of animals using sophisticated biotelemetry technology, we now have the capacity to gain a real insight into the behaviour and stimuli influencing an animal's movement patterns and habitat use. It is suggested here that the analysis of this data currently requires greater consideration of how temporal dynamics interact with spatial parameters. The use of visually intuitive network representations and statistical analyses which account for the interconnectivity of habitat locations is one way to achieve this goal. This study demonstrates how well established network analysis techniques can be manipulated to explore electronic tag movement data. Actual acoustic data from shark movements are used as a test bed along with suggestions of how best to clean and filter the data into a biologically meaningful format. Specific analyses were chosen to indicate the possible ecological, conservation and management benefits of this approach, whilst attempting to highlight the potentially broad appeal of these techniques to different species and types of study. These techniques are now used in greater detail to address specific ecological questions in the next chapter.

Chapter VII.

Revealing sexual relationships: predicting spatio-temporal dynamics in a behaviourally cryptic species

7.1 Abstract

For a variety of behaviourally cryptic species there remains little information about the timing and location of key life-history stages and yet this has important implications for their conservation. For sexually segregated species in particular, the ability to predict the temporal and spatial patterning of these species, *in situ*, is likely to be of significant ecological importance. This chapter tests an approach based on network theory, in conjunction with long-term monitoring of individual movements, to identify previously unknown aspects of the spatial dynamics of a sexually segregating shark species, the small spotted catshark (*Scyliorhinus canicula*). High levels of site fidelity to an inshore, coastal location monitored revealed diel and seasonal trends as well as periods of synchronicity in the presence of both sexes. Dynamic network analyses, adopted from the social sciences and used here to explore movement, demonstrate that male behaviour significantly predicts female use of complex habitat. Furthermore, comparison of movement networks between the sexes reveal that during the springtime months of March – May, the different sexes show statistically similar patterns of movement and co-occur in space and time more than at any other periods of the year. Interpreting movement patterns and co-occurrences from long-term, continuous telemetry data in threatened species could provide potentially valuable information towards the implementation of 'closed' areas designed to protect species during periods of reproductive importance.

7.2 Introduction

Sharks, like many other marine predators, often exhibit complex population dynamics that are difficult to study due to the inaccessible environments in which they inhabit and the spatial scales over which they occur. Importantly, for many species of shark, there remains a significant scarcity of data on the occurrence of important life history stages, although many studies speculate (e.g. Jorgensen et al. 2009), for example where and when mating occurs and the location and timing of parturition. Furthermore, slow growth rates and low fecundity combine with species-specific behavioural traits, such as aggregation, site philopatry and behavioural thermoregulation, making sharks particularly vulnerable to overfishing (Speed et al. 2010; Barnett et al. 2011; Queiroz et al. 2012; see also *Chapter I*). One particular behavioural trait which can often further obscure this picture is the spatial, temporal and/or social separation of the two sexes, otherwise known as sexual segregation.

A widespread characteristic of many elasmobranchs (Wearmouth and Sims 2008), sexual segregation is common in species that show sexual dimorphism with respect to body size such as the scalloped hammerhead (*Sphyrna lewini*; Klimley 1987), shortfin mako (*Isurus oxyrinchus*; Mucientes et al. 2009) and blue sharks (*Prionace glauca*; Litvinov 2006), but is also found in species that are sexually monomorphic in body size such as the small spotted catshark (*S. canicula*; Sims et al. 2001). The different mechanisms likely to influence sexual segregation in elasmobranchs are similar to those established for terrestrial vertebrates, such as ungulates, upon which much of the early mechanistic research was first conducted (Ruckstuhl and Neuhaus 2002; Ruskstuhl and Clutton-Brock 2005). For marine species, the *predation risk hypothesis* (1), the *forage selection hypothesis* (2), the *activity* *budget hypothesis* (3) and the *thermal niche-fecundity hypothesis* (4) are all reviewed extensively by Wearmouth and Sims (2008), with (5) the *social factors hypothesis* thoroughly explored and empirically tested in a species-specific study by Wearmouth et al. (2012). To briefly summarise, these hypotheses can be used to explain how (1) asymmetry in reproductive investment can enhance the predation risk of the sexes disproportionately leading to sex differences in habitat use; (2) sex differences in body size can drive sexual segregation due to differential dietary requirements needed for somatic growth; (3) activity budgets between sexes are likely to vary as a function of body size potentially leading to fission of sexes moving at different speeds driving social sexual segregation and (4) in order to maximise life-time reproductive success, female ectotherms (such as many shark species) will occupy thermal environments sub-optimal to males, again leading to spatial separation. Finally, social factors (5) such as sexual harassment of one sex by the other is likely also to contribute to sexual segregation (Darden and Croft 2008) due to the differential costs and reproductive pay-off associated with multiple mating events that ultimately promote different behavioural strategies (see Wearmouth and Sims 2008; Wearmouth et al. 2012 for a review of these hypotheses). With sexual segregation common in elasmobranchs (Wearmouth and Sims 2008) there is increasing need to understand the temporal and spatial dynamics of the two sexes, especially periods when synchronicity is more pronounced than segregation and hence of putative reproductive importance for example. Yet there remains multiple logistic and analytical challenges to achieving this information.

Determining periods of temporal synchronicity is notoriously difficult in wide ranging marine species, even more so in species that demonstrate sexual segregation. This very trait, in fact, renders sharks even more susceptible to anthropogenic drivers of population decline putting the onus on researchers to better understand the spatial and temporal elements associated with reproductive behaviour in elasmobranchs. Differential exploitation of the sexes is particularly detrimental to elasmobranchs that are characterised by *K* selected life-history traits such as low fecundity and slow growth to maturity (Sims 2008: Mucientes et al. 2009). Dwindling shark stocks are a global concern with management efforts to reduce overexploitation often lagging considerably behind the measures that are required (Godin and Worm 2010). The implementation of marine protected areas (MPAs) have been shown to increase the abundance and activity, through reduced anthropogenic disturbance, of some shark species that show high levels of site fidelity to coastal locations (Garla et al. 2006). Furthermore, there are now calls to implement high seas MPAs around areas of high productivity, such as oceanographic fronts, known to attract large pelagic sharks and other ecologically and economically valuable predators (Malakoff 2004; Queiroz et al. 2012). These MPAs, however, need not necessarily be a permanent fixture but might rather operate during periods known for frequent visitation of these species (Heupel and Simpfendorfer 2005b). Consequently, estimating the timing and location of periods where behaviour becomes synchronised, particularly in sexually segregated or endangered shark populations, becomes of great importance to conservation. It is also probable that environmental variables are, to some extent, likely to influence how and when sexual segregation occurs. Currently, electronic tagging of coastal and pelagic shark species is beginning to facilitate significant progress towards this endeavour (Sims 2010).

The rapid development of electronic tags and biotelemetry techniques are continuing to enhance our understanding of the movements, residency patterns and social interactions of wide ranging animals (Rutz and Hayes 2009; Krause et al. 2011) and this progress has always been widely embraced by the elasmobranch research community since these species are very difficult to study in their natural environment (Sims 2010). Furthermore, new, interdisciplinary analyses are now available that simplify the notoriously large telemetry data sets, enabling general principles to be explored across multiple species and spatial scales (Sims et al. 2008) or that account for the interconnectivity of habitats as individuals move between them (*Chapter VI*). The acoustic tracking of coastal sharks is now commonplace, providing important data on the round-the-clock movements and home ranging behaviour of site-attached sharks (Heupel et al. 2006; Andrews et al. 2009; Barnett et al. 2011; Knip et al. 2011). As discussed in *Chapter VI*, acoustic receivers record the presence of individually tagged sharks in both space and time. This apparently simple presence/absence data, however, can harbour a wealth of information about the behavioural strategies adopted by the tagged sharks, particularly when those data are considered in a dynamic format to account for movement behaviour and individual co-occurrences. Using a hypothesis driven approach to the analyses of such data is likely to reveal important behavioural signatures within the acoustic data (Dowd and Joy 2011) that might be useful for informing management and policy decisions.

Using data collected by acoustic telemetry techniques the aim of this study was to explore the influence of environmental and social factors on movement and define periods of temporally and spatially synchronised behaviour in a sexually segregated species, *S. canicula*. A common resident of shallow coastal habitat around the UK, this species is a small oviparous elasmobranch and a model species for physiological, ecological and behavioural research (e.g. Ballard et al. 1993; Sims 2003; Kimber et al. 2009). Female *S. canicula* refuge in single sex communities in shallow water openings in rocky substrata (e.g.

caves or large crevices) as a result of harassment by males seeking multiple mating opportunities (Jacoby et al. 2010; Wearmouth et al. 2012). As discussed at length in *Chapter IV*, these females can mate year round, are capable of storing sperm for long periods (see Griffiths et al. 2012) and as a result have a protracted egg-laying season. In this chapter analyses of static acoustic monitoring of detection frequencies of individually-tagged *S. canicula* were combined with dynamic analyses of movement interactions and individual co-occurrences (a weighted proxy for social interactions) in an attempt to predict patterns of synchronicity in behaviour. Predicting the timing and location of these patterns, in concert with the ecological variables driving them, may add new insight to the population dynamics of sexually segregated and other behaviourally cryptic elasmobranch species. This information might also prove useful for informing conservation strategies through the use of seasonally implemented MPAs.

7.3 Materials and methods

7.3.1 Study area

Whitsand Bay is an open embayment area in Cornwall, on the South West coast of the UK (50^{o} 19.549 N, 4^{o} 15.246 W, Fig. 21, *Chapter VI*). The bay is approximately 5.5 km wide containing two large shipwrecks, the *James Eagan Lane* and HMS *Scylla*, both of which provide complex habitat for fish to live in and around. The substrate consists of large areas of sand and gravel interspersed with rocky habitat which provides ideal refuging habitat for *S. canicula*. Whilst low intensity, commercial trawling does occur sporadically, fishing activity is mainly restricted to close inshore (< 15 m deep, where sharks to be tagged were captured) or considerably further offshore due to the unsuitable substrate and diving activities operating on and around the wrecks. Advertised, monetary rewards were offered to local fisherman that catch fish tagged with Marine Biological Association tags, acoustic or otherwise, ensuring relatively high return rates for recaptured fish.

7.3.2 Acoustic equipment and tagging procedure

Sharks were tracked using six acoustic receivers mounted at the top of 2-m-high seabed 'landers' (Fig. 25) in an acoustic array situated approximately 1.5 km offshore and covering an area of 1.5 km^2 (Fig. 21). The landers were deployed at depths ranging from 14 – 25 m including all of the above described habitat types. A total of 47 *S. canicula* (31 ♀, 16 ♂) were tagged with V9 coded transmitters (VEMCO, Halifax, Nova Scotia) and released into the non-overlapping, acoustic array consisting of six VR3-UWM (underwater modem) receivers (VEMCO, Halifax, Nova Scotia). Sharks were tagged and released in stages in an attempt to produce year-on-year replication. In January 2010, 15 female and four male sharks were released followed by a further 16 females and four males in January 2011. *A priori* hypotheses about how the different sexes were likely to use this area led to a strong female bias, given their propensity to spend time refuging in shallow water, rocky substrata to avoid male conspecifics (Sims et al. 2001; Sims 2003). In June 2011, however, the decision was taken to increase the number of male sharks by adding a further eight to the acoustic array based on prolonged residency patterns in some of the previously released males. It was also expected that refuging behaviour, unless out in the open, was likely to significantly reduce the ability of the receivers to detect the transmitters. Therefore, for the females particularly, detections were more likely to be an indication of the sharks moving or at least being out in the open away from openings in rocky substrata. Sharks trawled from inside the acoustic array were transferred to the MBA laboratory for surgical implantation of the V9 transmitters (see details of tagging and tracking below).

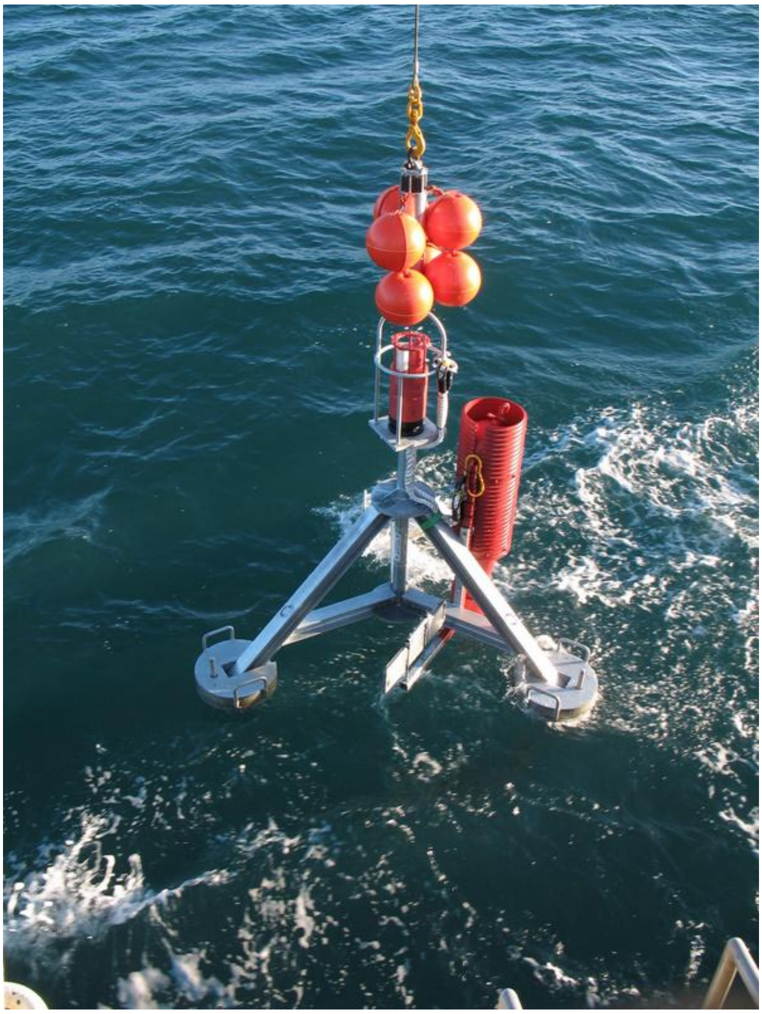

Figure 25. Seabed landers housing acoustic tracking equipment. One of six landers deployed in a relatively deep water acoustic array. This image shows the acoustic receiver (in red below the buoys) and the acoustic release device (also red, to the right side of the receiver). Photograph courtesy of the MBA.

Individual sharks were anaesthetised prior to surgery using benzocaine solution (1g of ethyl p-aminobenzoate in 100 ml of 70% ethanol) before a small incision (<15 mm) was made on the ventral surface (offset from the ventral midline) midway along the trunk, between the base of the pectoral and pelvic fins. The transmitter (28 mm) was then inserted into the peritoneum and the incision was closed with one silk braided, non-absorbable and two coated vicryl, absorbable sutures (MidMeds, Waltham Abbey, UK). Each shark was also tagged externally with a Floy T-bar anchor tag for identification and recapture. All fish were recovered from the anaesthesia in clean, aerated water where they were held into a current to irrigate the gills. Individuals were monitored for several days before being returned to the wild at the location of capture. Furthermore, tagged sharks that were subsequently caught and returned to the MBA, of which there were four, indicated that the incision healed well leaving nothing but a small pink line indicating where the acoustic tag was inserted. This procedure, as with all other procedures conducted during this project, were approved by the MBA Ethical Review Committee, licensed by the UK Home Office under the Animals (Scientific Procedures) Act 1986 and carried out by licensed personnel.

Acoustic transmitters implanted in the sharks were set to emit randomly a coded pulse (at 69 kHz) at a nominal delay of anytime between 180 and 360 seconds (to reduce the chance of any two tag's signals 'colliding' at a detector), giving a minimum battery life of over 400 days. Sharks occurring anywhere (in the open) within approximately 300 m of any one of the six receivers when this pulse was emitted, would then be logged with an individual identification, a time and a date stamp. These presence/absence data comprised the raw data logs which were periodically downloaded, every four to six months, via a surface modem aboard the MBA research vessel, *MBA Sepia*.

7.3.3 Tag detection range testing

The radius at which VR3 receivers are able to detect a pulse from a transmitter is dependent on a number of factors including environmental features and output strength of the tag. As such, the range of detection for each receiver was tested using a test tag suspended 2 metres below the hull of the research vessel; this tag was preset to emit a pulse every 10 s. Range testing occurred during a flood tide along a predetermined path whereby the vessel was allowed to drift from 500 m on one side of the receiver to 500 m on the opposite side, as close to the GPS location for each receiver as possible. GPS locations of the boat position were taken concurrently with transmitter pulses which were recorded by the receiver in question and the data subsequently downloaded and compared. Detection frequencies were then calculated based on the number of minutes the boat spent within a specified range bin and the number of test tag detections recorded by the receiver of interest. This was replicated for each of the six receivers and a mean detection frequency calculated. A nonlinear regression was used to determine that a three parameter model (Gompertz model) which predicts a sigmoidal trend in the detection range, was the model which best fit the data (R^2 = 0.861 Fig. 26).

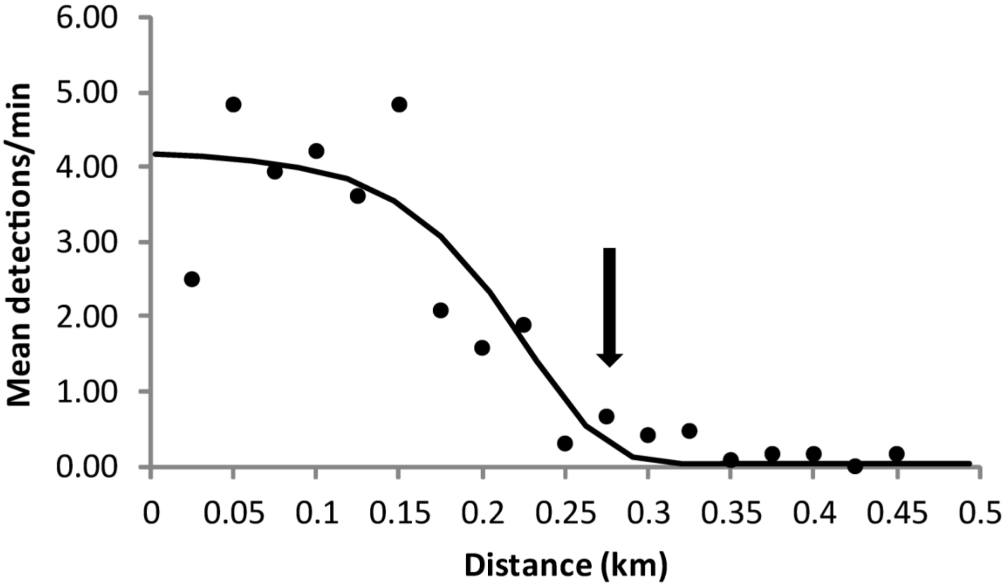

Figure 26. Range testing of acoustic receivers. A three parameter model [b1*exp(–b2*exp(-b3*x))] best explained the sigmoidal relationship between the mean number of detections per minute and distance. In the model, b represents three different parameter estimates and x is the independent variable of distance. This relationship predicts a value of approximately 275 m as the upper distance estimate within which acoustic pings are reliably detected (arrow).

7.3.4 Data management

Following a download, the raw telemetry data were imported into a Microsoft Access database. During the import, data were adjusted to correct for clock drift between the PC clock used to download the data and the receiver clock, which varied between each receiver from a minimum of 0.34 (receiver 6) to a maximum of 1.35 (Receiver 4) seconds per day (mean = 0.78 s/d). In addition, the import program also highlighted the false positive detections which can result from two or more tags emitting pulses at exactly the same time at the same receiver or from background acoustic 'pollution' which occurs at the same frequency as the transmitters (69 kHz). False positive detections are likely to produce a tag 'ping' number outside of those which have been deployed on actual fish and are thus often easy to isolate. The chance of a spurious 'ping' number actually replicating a number of a tagged fish is very remote even with relatively high numbers of tags within the area. A total of 51 false positive detections were isolated in a multi-species dataset containing 170,951 detections, less than 0.03%. Finally, Whitsand Bay has been extensively surveyed using multibeam and side-scan sonar (see Fig. 21, *Chapter VI*) providing topographic data which was not only used to plan the positioning of the receivers but also provided data for the analysis of the animal tracks themselves (see *Data analysis*). Year round, environmental data, including sea surface temperature (SST), wave height, wind speed and direction were also obtained from an environmental monitoring station situated on a buoy in a neighbouring bay (Looe Bay Channel Coastal Observatory).

7.3.5 Data analysis

Both static and dynamic information held within acoustic telemetry data was explored here using a combination of more traditional analyses of presence/absence data and also the application of network analyses, outlined in *Chapter VI*, to consider movements made by these tracked sharks between receiver stations. In addition, co-occurrences of individuals in space and time were analysed using social network analysis (SNA) in an attempt to address the influence of social strategy on space use. This amalgamation of descriptive and statistical approaches is introduced conceptually in *Chapter I* (Fig. 5) and is then explored in greater detail in *Chapter VI*.

7.3.5.1 Static analyses – presence/absence

The frequency and abundance of acoustic detections were assessed for diel and seasonal trends using an actogram to determine the degree and longevity of site fidelity within the population. Temporal trends were then considered in relation to SST data obtained from the Looe Bay Channel Coastal Observatory buoy. Furthermore, data on male and female detections were pooled across multiple individuals allowing general hypotheses about differential sexual strategies to be explored. Home ranging behaviour was plotted using kernel density estimates in ArcGIS 10 and these were subsequently compared to analyses of movement interaction networks to assess the reliability of static analyses for understanding shark movements (see *Dynamic analyses*).

7.3.5.2 Dynamic analyses – movement behaviour

Static analyses of detection frequencies do not necessarily capture dynamic behaviours such as short-term, repeated movements. Therefore, to understand the degree of site fidelity in males and females, dynamic network analyses of movement interactions were undertaken using the methodology described in *Chapter VI*, whereby nodes in the network represent receivers and edges the movement of individuals between receivers. Matrices containing values of relative interaction data (RID) – a relative proportion of the number of times a movement is made between locations divided by the total number of movement edges – were constructed from pooled male ($n = 16$) and female ($n = 31$) shark tracks. The weighted networks described only the direct (< 1 hr) movements of individuals between receiver locations.

In an attempt to understand the mechanisms driving male and female strategies in the wild, the pooled movement matrices were regressed against a number of biotic and abiotic predictor variables using a multiple regression quadratic assignment procedure (MRQAP). This permutation procedure, similar to node-label permutation conducted in previous chapters, was used to test whether differences in similarity of environmental variables at each of the six locations, were significant predictors of movement behaviour. The fixed environmental variables were outlined in *Chapter VI* and included mean depth of the receivers, distance from shore and a habitat complexity score derived from knowledge of the substrate and available refuging habitat around each receiver. These variables were then converted into matrices of similarity before they were regressed against the male and female movement networks. The models were then rerun to include the movement matrices for the opposite sex as a biotic or social predictor variable.

Given the time period over which data was collected and the degree of site fidelity exhibited by this species, the analysis of shark movement data were restricted to specific seasonal movements (*Spring*: Mar-May, *Summer*: Jun-Aug, *Autumn*: Sep-Nov and *Winter*: Dec-Feb) based on sea surface temperatures. The edge density (*E*) of the movement networks were then compared between male and female sharks for each season using a paired samples *t*-test on bootstrapped estimates of standard error. The null hypothesis for this test, which was conducted in UCINET (Borgatti et al. 1999), was that the mean tie strength between receiver locations was the same for both sexes.

7.3.5.3 Co-occurrence networks

The acoustic equipment used in this study was not designed to measure direct social interactions or association between individual sharks due to the broad field of detection. However, by tagging a relatively high number of sharks that are known to show site attached behaviour (Wearmouth and Sims 2008), co-occurrences in space and time could still be explored as proxies for potential interactions or associations. A co-occurrence reflected an instance where two or more individuals were detected within one hour of one another at the same location and was deemed an estimation of the opportunity for social association to take place, rather than direct measures. These individuals might have occurred anywhere within a 550 m diameter of the acoustic receiver and in an attempt to overcome this, dyadic pairs co-occurring at the same location and the same time, were assigned an index weighting (I_w) based on the number of times the pairing occurred together in total and over a period of how long (Equation 3).

Equation 3:
$$I_w = \sqrt{\left(\frac{x}{Ty_x - T1_x}\right)x}$$

where x is the number of detections in which a pair of sharks were recorded together in the same group, *T* represents the time of co-occurrence on the receiver and *y* is a constant for the time of the last co-occurrence between the pair.

This weighting was then implemented in the calculation of the half weight index (HWI, *Chapter II*) using SOCPROG 2.4 (Whitehead 2009). Pairs of individuals were assigned a HWI value creating a matrix of co-occurrence. In the calculation of HWI, dyads or groups that co-occurred most frequently and over the longest periods of time were given the most (or strongest) weighting. It was presumed that these pairs of individuals were most likely to be in actual close proximity to one another. It is recognised that this simple weighting might require some modification to ensure that identical weights are not obtained for different ratios of association to duration data (see chapter discussion for more on this). It is included here, however, in an attempt to control the potential bias associated with the spatial scale over which co-occurrences might occur. SNA were used to construct co-occurrence networks excluding any dyads that co-occurred on the day of release. By restricting the matrices, season trends in co-occurrences were explored for an indication of putative mating behaviour.

7.4 Results

7.4.1 Static analyses

Of the 47 sharks released into the acoustic array in Whitsand Bay (Fig. 21) and tracked during this two year study, the individual detection frequencies ranged from zero to 8221 in the females (Mean ± SE: 736.87 ± 335.88) and zero to 17,133 in the males (Mean ± SE: 2043.00 ± 1074.51). Summary details of the sharks tracked during a two year period are provided in Table 9. Despite nearly twice as many female *S. canicula* released, male sharks recorded approximately three times as many detections across the six receivers indicating males spent more time in open habitat where successful detections were more likely. On average males also showed a greater number of movements between receiver locations ($U_{(45)} = 154.50$, $z = -2.12$, $p = 0.035$, Fig. 27) suggesting more of a roaming strategy this sex. There were similarities, however, in the maximum period of residency between the sexes with a female shark still being detected after 757 days (Mean ± SE: 188.35 ± 43.02) and a male after 745 days (Mean ± SE: 225.75 ± 54.90). These detection frequencies indicate that both sexes show high site fidelity to this area and, as predicted, results here suggest that females were probably not detected because they refuge in rocky substrata, which masks acoustic transmissions leading to a considerably lower relative number of detections (Fig. 27).

Table 9. Summary of the sharks tagged and tracked at Whitsand Bay, Cornwall, UK.

Shark	Individual	Sex	Release date	Last detection	Days at liberty	No. of detections	Movements between receivers
1	9126	M	27-Jan-2010	27-Jun-2010	77	72	10
2	9115	F	27-Jan-2010	08-Mar-2011	405	85	5
3	9118	F	27-Jan-2010	23-Feb-2010	27	153	10
4	9119	F	27-Jan-2010	09-Sep-2010	225	513	25
5	9121	F	27-Jan-2010	27-Jan-2010	0	4	1
6	9122	F	27-Jan-2010	27-Jan-2010	0	5	1
7	9123	F	27-Jan-2010	16-Oct-2010	262	8221	101
8	9125	F	27-Jan-2010	11-Aug-2010	196	29	7
9	9129	F	27-Jan-2010	23-Feb-2012	757	2769	184
10	9131	F	27-Jan-2010	09-Jan-2012	712	2133	165
11	9132	F	27-Jan-2010	24-Dec-2011	696	241	27
12	9133	F	27-Jan-2010	27-Jan-2010	0	3	0
13	9136	F	27-Jan-2010	21-Mar-2010	53	82	3
14	9113	M	10-Feb-2010	16-May-2010	95	358	17
15	9114	M	10-Feb-2010	06-Nov-2011	605	2609	179
16	9117	M	10-Feb-2010	25-Feb-2012	745	567	62
17	9127	F	10-Feb-2010	12-Jan-2012	701	1849	143
18	9128	F	10-Feb-2010	-	0	0	0
19	9130	F	10-Feb-2010	20-Nov-2010	271	90	16
20	9391	M	03-Feb-2011	29-Feb-2012	391	17133	940
21	9394	M	03-Feb-2011	31-Aug-2011	184	1077	39
22	9396	M	03-Feb-2011	28-Feb-2012	390	28	3
23	9397	M	03-Feb-2011	-	0	0	0
24	9398	F	03-Feb-2011	03-Feb-2011	0	4	0
25	9399	F	03-Feb-2011	29-Mar-2011	0	1	0
26	9400	F	03-Feb-2011	04-Mar-2011	29	35	3
27	9401	F	03-Feb-2011	07-Nov-2011	277	6175	325

Table 9 cont.

28	9402	F	03-Feb-2011	17-Apr-2011	73	45	16
29	9403	F	03-Feb-2011	29-Mar-2011	54	47	8
30	9404	F	03-Feb-2011	13-Feb-2012	375	62	15
31	9405	F	03-Feb-2011	03-Feb-2011	0	14	0
32	9406	F	03-Feb-2011	09-Apr-2011	65	64	5
33	9407	F	03-Feb-2011	02-Apr-2011	58	110	20
34	9169	F	03-Feb-2011	05-Aug-2011	183	6	0
35	9170	F	03-Feb-2011	-	0	0	0
36	9171	F	03-Feb-2011	14-Nov-2011	284	38	11
37	9172	F	03-Feb-2011	19-Jun-2011	136	59	9
38	9173	F	03-Feb-2011	03-Feb-2011	0	3	0
39	9174	F	03-Feb-2011	03-Feb-2011	0	3	0
40	9175	M	07-Jun-2011	13-Dec-2011	189	85	8
41	9176	M	07-Jun-2011	29-Nov-2011	3	27	2
42	9177	M	07-Jun-2011	28-Feb-2012	266	5269	327
43	9179	M	07-Jun-2011	19-Oct-2011	134	3518	332
44	9180	M	07-Jun-2011	07-Jun-2011	0	3	0
45	9182	M	07-Jun-2011	28-Feb-2012	266	1066	115
46	9183	M	07-Jun-2011	12-Jun-2011	5	85	25
47	9187	M	07-Jun-2011	24-Feb-2012	262	791	63

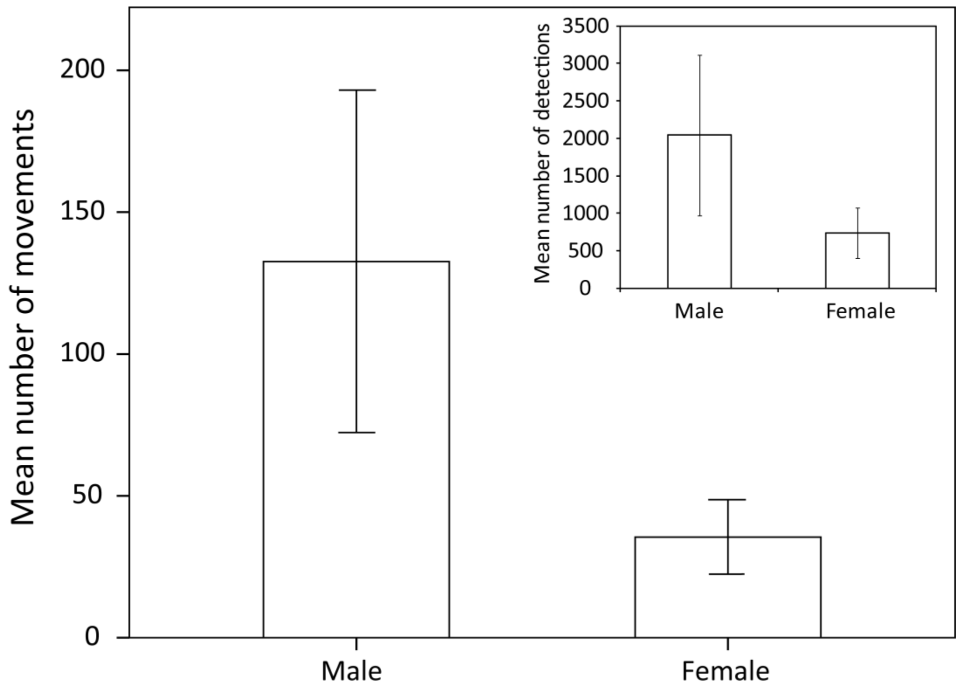

Figure 27. Mean number of movements (± SE) made between receivers for male and female sharks. These movements influence the mean number of detections between sexes (inset) as females were not likely detected when in their refuges.

To investigate the diel and seasonal trends between the sexes all detections from January 2010 to March 2012 were plotted as an actogram for both male (*n* = 32 688) and female (*n* = 22 843) sharks in relation to SST data (Fig. 28). Strong signals within the detection plots for the females reveal apparent crepuscular movements to and from refuges which consistently track the increasing day length and SST during the summer months (Fig. 28A). This is evident by the upper and lower curves which form 'circles' of detections that correspond to the increase and decrease of light levels and temperature (albeit lagged slightly) during the course of the year (Fig. 28A). Males, however, were detected regularly throughout daylight and night time hours (Fig. 28B). Interestingly both female and male sharks were detected relatively infrequently during the winter months (Nov – Feb) when SSTs were at their lowest. Once these temperatures began to rise (approximately March), detection frequencies became abundant with surprising synchronicity between the sexes

(Fig. 28). This is an indication that both SST and intersexual behaviour impose substantial influence on movement and residency in this species, something that was explored in greater detail in subsequent sections (see *Dynamic analyses*).

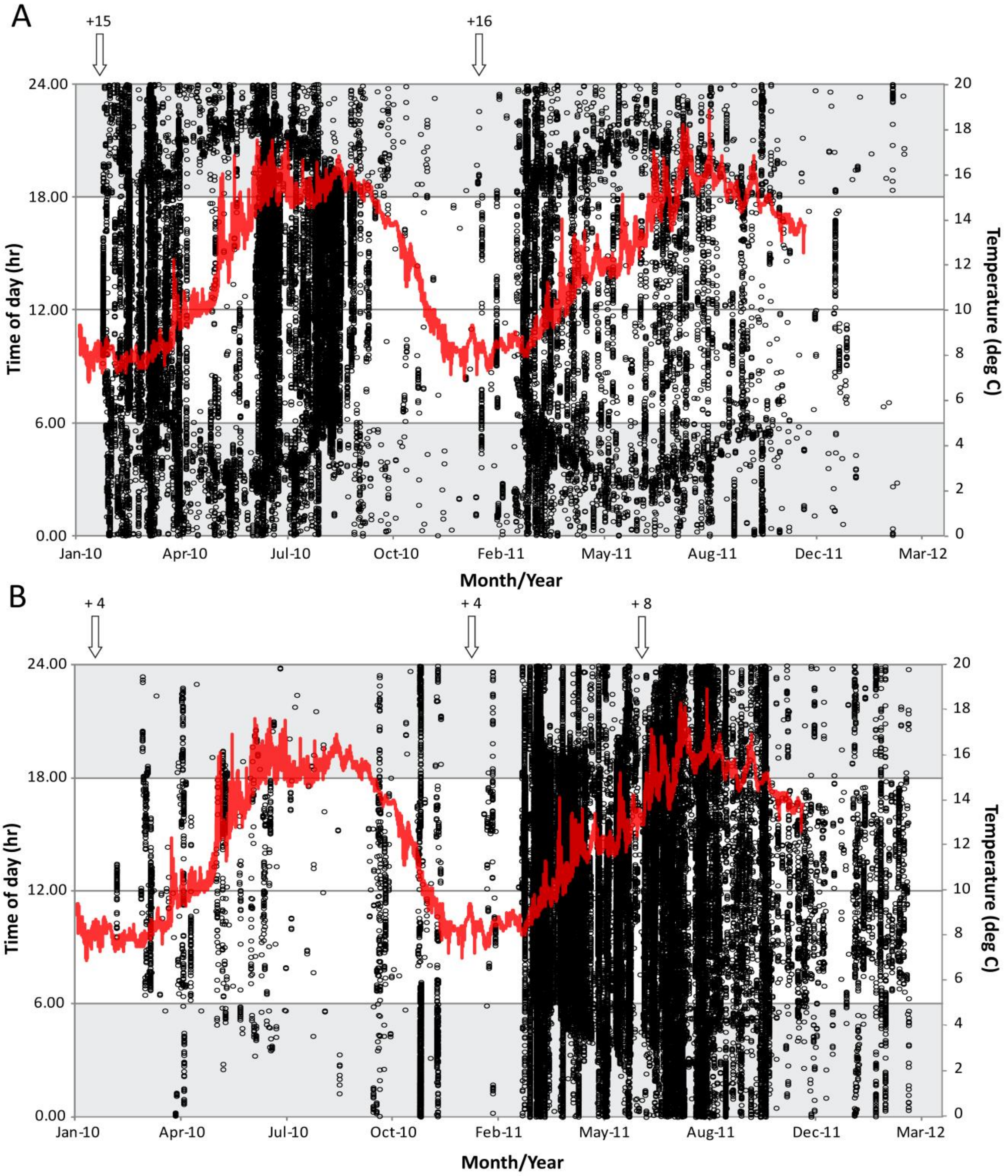

Figure 28. Actogram of female (A) and male (B) diel and seasonal detections in relation to sea surface temperatures (SST). Black circles represent individual detections and the red line, values of SST. Grey areas approximate hours of darkness with arrows above the plots informing when tagged sharks were released into the array.

7.4.2 Dynamic analyses

Movement networks, overlaid on kernel density (KD) plots, revealed interesting insight into the differential strategies adopted by female and male *S. canicula* (Fig. 29). Female detections and thus KD home range estimates were most abundant at receiver 1, followed by receiver 2. Female movements were strongly biased to the inshore locations (receivers 1, 2 and 3), however, they appeared to move between all receivers suggesting greater residency to the area as a whole (Fig. 29A). Conversely, the detection frequencies of the male sharks suggest residency that was restricted to receiver 2. The male movement network, however, describes very high levels of patrolling behaviour between receivers 1 and 2, the area in which the females occur most frequently, a behaviour that is not identified by the kernel density plot (Fig. 29B). This demonstrates the differential information captured by static and dynamic analyses of acoustic telemetry data.

The results of the MRQAP analyses are explained here in the text and summarised in Table 10. As a model, the environmental data which included depth, inshore/offshore location and a measure of habitat complexity, proved a significant predictor of both female and male movement, despite relatively low r^2 values (Female & Male: $p < 0.05$). Individually, however, none of these environment parameters were significant predictors of movement. Results from previous research (Sims et al. 2001) in conjunction with the static and descriptive analyses from this data suggested that male behaviour might strongly influence female behaviour and *vice versa*. As such, the model was re-run to include the movement interaction matrix of the opposite sex (see Table 10).

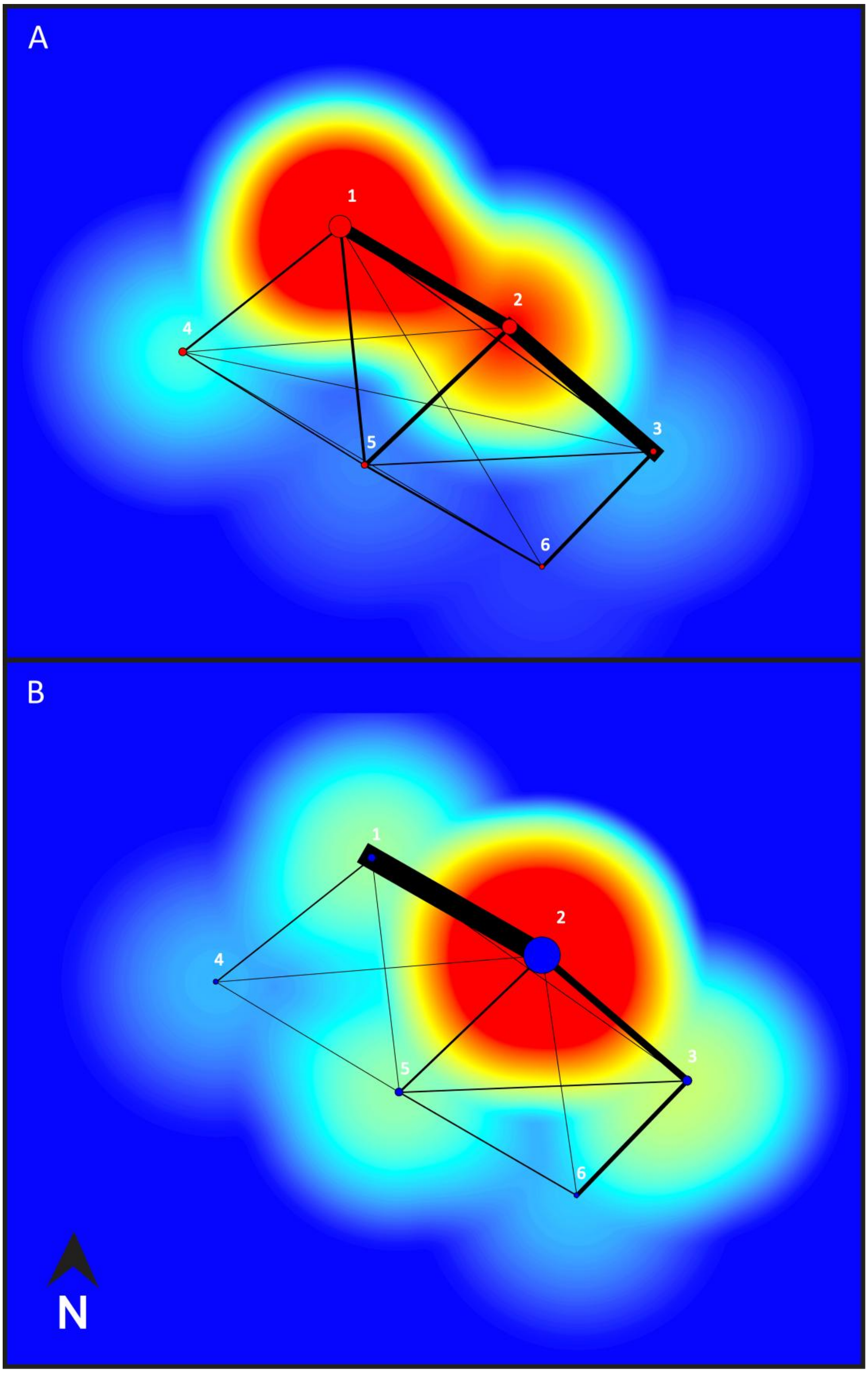

Figure 29. Kernel density (KD) plots overlaid with movement interaction networks. Red KD areas indicate periods of prolonged residency whilst the size of the network nodes illustrates the detection frequencies for female (A) and male (B) sharks. Network edges show the abundance of movements between locations.

As predicted, these social explanatory variables substantially increased the amount of variation accounted for by the model, indicated by increased r^2 values (Female & Male: p <0.01). Female and male movements individually were significant predictors of intersexual movement behaviour, however, when male movements were included in the female model, habitat complexity also became a significant predictor, a result not reciprocated for the male model (Table 10). This result is consistent with the literature describing the female necessity to refuge away from male sharks which are likely to harass them for mating opportunities (Wearmouth and Sims 2008; Jacoby et al. 2010).

Table 10. Summary of MRQAP analyses predicting shark movements from environmental and social data. Overall fit of the model and the significance of individual explanatory variables are given.

	R^2	R^2_{adj}	P	Parameters	P
Model 1 - F movements	0.197	0.138	**0.045**	*Habitat complexity*	0.409
				Distance from shore	0.949
				Mean depth	0.389
Model 2 - M movements	0.187	0.127	**0.044**	*Habitat complexity*	0.260
				Distance from shore	0.316
				Mean depth	0.269
Model 3 - F movements (inc. M)	0.693	0.658	**0.007**	*Habitat complexity*	**0.039**
				Distance from shore	0.077
				Mean depth	0.088
				Male movement	**0.011**
Model 4 - M movements (inc. F)	0.689	0.653	**0.002**	*Habitat complexity*	0.062
				Distance from shore	0.188
				Mean depth	0.103
				Female movement	**0.009**

The temporal influence of sexual segregation was then explored by comparing the network density (*E*) of the seasonal movements of these sharks between the sexes. A bootstrap approach to the paired sample *t*-test was run using 10,000 sub-samples to estimate standard error. This two-tailed test revealed significant differences in male and female movements across all seasons except spring (*t*-test: Spring – $t_{(14)} = -0.851$, $p = 0.326$; Summer – $t_{(14)} = 2.637$, $p = 0.013$; Autumn – $t_{(14)} = 2.014$, $p = 0.042$; Winter – $t_{(14)} = -3.326$, $p = 0.002$). Thus, the null hypothesis for the same mean tie strength between the sexes was accepted for the *Spring* months only (Mar – May), where movements made by female and male *S. canicula* appear more synchronised.

7.4.3 Co-occurrence networks

To allow comparison to the previous analyses, weighted networks of co-occurrences were divided into seasons to investigate the influence of time of year on putative social interactions. In accordance with the movement data, female sharks appeared more integrated in the co-occurrence network during the spring months and seem to become increasingly less so towards the end of the year (Fig. 30). During this time, females co-occurred in greater numbers with both male and female conspecifics highlighting potential periods when mating behaviour, perhaps as a result of convenience polyandry or increased female receptivity, are more likely to occur. Acoustic co-occurrences must be interpreted with caution for the reasons already stated, however, with evidence from both spatial and temporal analyses of movement and social data, there is a strong case implicating this seasonal window as important in the life-history of this sexually segregated species.

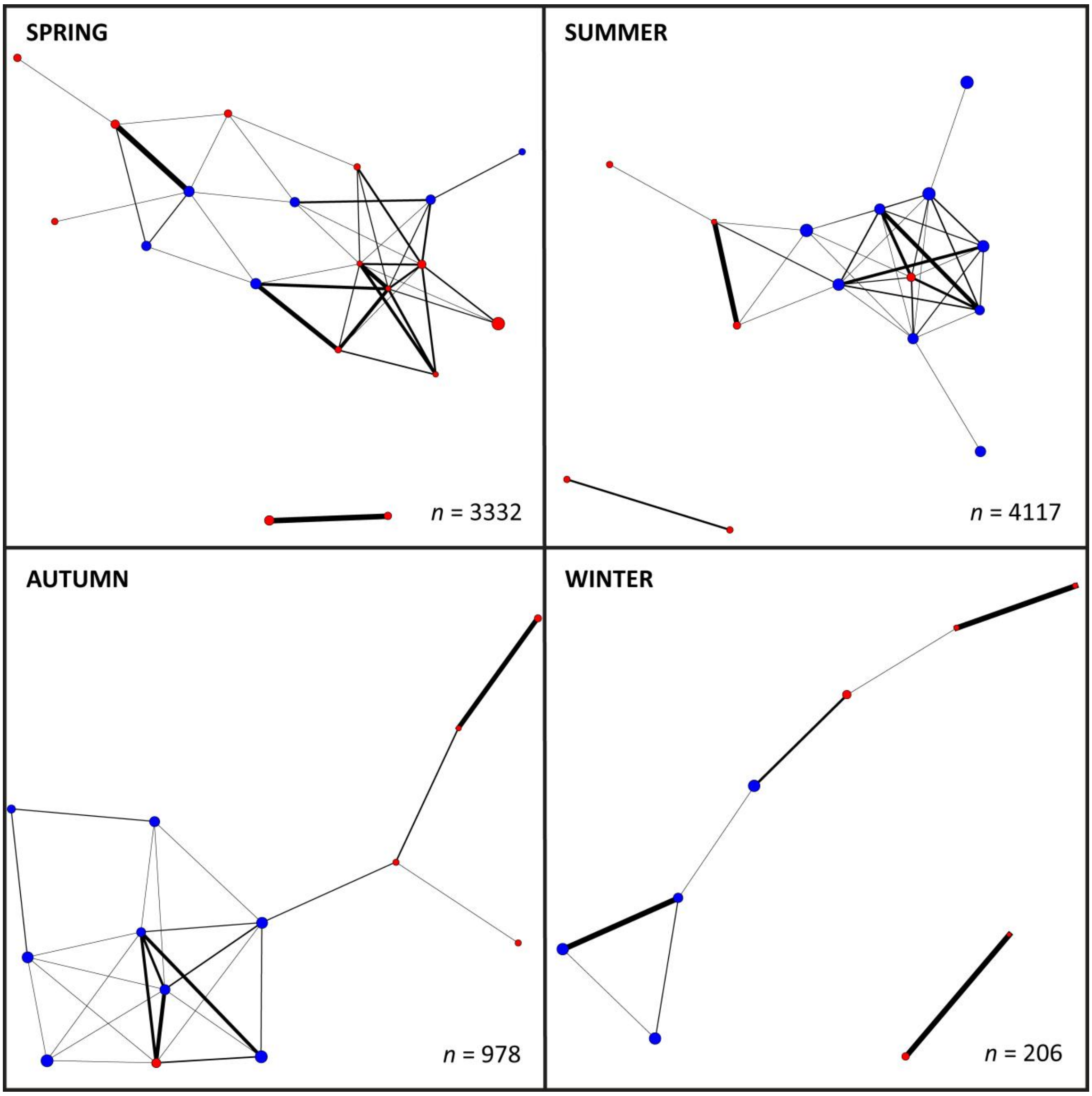

Figure 30. Seasonal networks of co-occurrences in wild *S. canicula*. Here nodes represent individual sharks and edges their associations inferred from co-occurrences at the receivers. Female (red) and male (blue) co-occurrences were derived from weighting the half-weight index (edge weight) based on the frequency and duration of co-occurrences at specific locations. Node size represents the size of the individual when it was released. *n* values represent the number of co-occurences within season.

7.5 Discussion

Determining periods of synchronicity and the environmental drivers of these patterns in behaviourally cryptic, sexually segregating species is of significant ecological importance. For many species of elasmobranch sexual segregation is common (Wearmouth and Sims 2008) and yet knowledge of when the sexes are most likely to encounter one another often remains elusive even for species that are of global conservation concern (Martin 2007; Mucientes et al. 2009). Here, a tractable, model species of elasmobranch, *S. canicula* was tracked within an array of acoustic receivers in an open embayment off the South West coast of the UK. Differential use of habitat by the two sexes was considered in relation to localised environmental data in order to explore for the first time in an open coast environment, the mechanisms driving segregation in a species known to sexually segregate from observations made in an enclosed marine lough (Sims et al. 2001; Wearmouth et al. 2012). Analyses of static detection frequencies revealed strong crepuscular movements in female sharks and site philopatric behaviour in both sexes that appeared strongly coupled with sea surface temperatures. Network analyses of movement and individual co-occurrences defined clear synchronicity in the behaviour of both sexes during the months of March, April and May in stark contrast to marked segregation during the rest of the year. These types of analyses attempt to encapsulate both the temporal and spatial information contained within acoustic telemetry data that can often be overlooked (see *Chapter VI*).

The relatively small acoustic array used in this study was limited in spatial range, covering an area of just 1.5 km^2. Situated within an open embayment there were large amounts of habitat deemed suitable for this species found outside the area under acoustic surveillance. Despite this both male and female *S. canicula* showed remarkably high levels of site attached behaviour consistent with other demersal elasmobranchs that share similar life history traits (Hight and Lowe 2007; Powter and Gladstone 2009). The present study showed spatial and temporal trends in detection frequency suggesting preferential locations or home ranges, particularly in the inshore areas, with an apparent influence of sea surface temperature (SST) and light levels on the presence and absence of these sharks. Light level and daily fluctuations in temperature have also been linked to aggregation behaviour in other larger species of elasmobranch, such as the grey reef shark (*Carcharhinus amblyrhynchos*; Economakis and Lobel 1998). Additionally, the longevity of the acoustic tracks in this study confirm some of the behavioural trends discussed in previous acoustic research on this species conducted over shorter time periods (Sims et al. 2001). Female *S. canicula*, for example, are most likely to leave their refuges at dawn and dusk (Wearmouth et al. 2012) and strong crepuscular signals, year-on-year as here, appear to support this (Fig. 28).

By employing a networks approach to analyse movement behaviour in this study (as described in *Chapter VI*), the interconnectivity of areas containing different habitat were considered complimentary to static analyses of detection frequencies. Movement networks overlaid on kernel density plots revealed that directed movements of males, which are substantially more numerous than female movements, were largely restricted to patrolling the inshore locations around receivers 1 and 2. These areas were where most of the female detections occurred (i.e. when they were entering and leaving their refuges), thus supporting recent research validating male harassment as the main driver of sexual segregation in this species (Jacoby et al. 2010; Wearmouth et al. 2012).

In species demonstrating site fidelity it is expected that certain habitat features, such as bottom substrate (Powter and Gladstone 2009) or abundance of temporally distributed prey (Barnett et al. 2011), play an important role in mediating a continued return to specific habitat. It is less obvious, however, how habitat then influences the movement of individuals once they return. MRQAP analyses, run on the pooled movement networks for both female and male sharks, showed that together the environmental parameters were significant predictors of movement in both sexes. The amount of variance explained increased substantially, however, when the movement networks of the opposite sex were included in each of the respective models. Furthermore, habitat complexity alone was a significant predictor of female but not male movement networks, confirming previous suggestions that harassment by male sharks is persistent enough to drive females to use refuges as an avoidance strategy (Sims et al. 2001; Sims 2003). This finding is also consistent with some freshwater systems where female Trinidadian guppies (*Poecilia reticulata*) alter their habitat use by occupying deeper, more risky habitat due to very high levels of male sexual harassment (Darden and Croft 2008).

Despite marked sexual segregation reinforced by intersexual harassment, there are likely to be occasions where female sharks are more receptive to male mating attempts, even if this behaviour is driven by convenience polyandry as is suggested in *Chapter IV*. Although it is not possible to determine whether breeding events have taken place in the wild, periods of temporal and spatial synchronicity are perhaps indicative of when breeding behaviour is likely to occur. It is important, therefore, to be able to predict this synchronisation, especially for species under significant anthropogenic pressure (Mucientes et al. 2009; Sims 2008). Partitioning the data into seasons showed that all networks were significantly different from one another apart from the movements made during the months March, April and May. This finding was the first indication advocating springtime as a period when the two sexes demonstrate statistically similar behavioural patterns. In other, more wide-ranging elasmobranch species such as the white shark (*Carcharodon carcharias*) spring and summer months are proposed to be times when sharks move offshore to mate, although no firm evidence of mating has yet been obtained (Weng et al. 2007).

In an attempt to substantiate this time period as being important in the reproductive strategy of *S. canicula*, the weighted co-occurrences of individuals were calculated for sharks that occurred at the same location within ≤1 hr of each other. These co-occurrences did not necessarily denote associative behaviour, but rather the potential for association to take place and hence the weighting of the data to account for the duration and the frequency of these co-occurrences through time. Social network analyses of these co-occurrences reiterated the spring months as a period when female and male sharks co-occurred much more frequently together in a network that was well mixed (Fig. 28). More single- and mixed-sex ties were apparent in spring in comparison to all the other seasons where female occurrences barely register (i.e. there are very few red nodes in summer, autumn and winter). This implies that during the months of Mar – May, not only were the sexes moving in similar ways but that they were also co-occurring in space and time. In more northern latitudes, aggregations of salmon sharks (*Lamna ditropis*) and blue sharks (*Prionace glauca*) occur later in the year during summer months (Williams et al. 2010) suggesting that, as in this study, temperature might indeed be a driver of reproductive behaviour. It is well known, in fact, that temperature cues regulate reproductive behaviour in many species of fish through its influence on the endocrine system and thus egg/sperm production (Van Der Kraak and Pankhurst 1997). Interestingly, Garnier et al. (1999) found that sperm reserves in male *S. canicula* peaked from March – May, the period in which synchronised behaviour was found in this study. Behaviourally, it is possible that this period marks a 'season' when male sharks are more aggressive or persistent in their harassment and are either more successful in their mating attempts or the females allow more mating due to the energetic expense of avoidance/resistance (Dodd 1983).

Finally, as suggested during the methods section of this chapter, there remains room for improving the calculation of the index weighting and perhaps incorporating this temporal component directly into the HWI. Lagged association rates (Whitehead 2008) were calculated for all inter- and intrasexual co-occurrences in this study but due to high variation in dyadic longevity, this did not provide a meaningful estimate for weighting the inferred associations. Model data with known association parameters would be required to validate the index and work on this is already underway but was not complete at the time of thesis completion.

7.6 Conclusion

Knowledge of how habitat features influence the movements of site faithful elasmobranchs and how this impacts sexual segregation is extremely important, particularly for threatened or endangered species. Management strategies, designed to reduce localised fishing mortality and enhance population recovery must also consider, where possible, the timing and location of important life-history stages of populations, such as nursery area and mating or pupping grounds, particularly if such behaviour is restricted to specific seasons. This study employed long-term acoustic telemetry of an elasmobranch species common to UK coastal waters, to answer these more broadly applicable questions. Static and dynamic analyses showed that abiotic features of these animals' environments were significant predictors of their movement, particularly in the females that are driven to use refuges in order to avoid male harassment. Furthermore, a period of synchronised behaviour between the sexes was highlighted between the months of March through to May in which females were more likely to appear outside of these refuges, co-occurring with male sharks during a period that coincided with seasonal increases in sea surface temperatures. These types of hypothesis-driven analyses could potentially prove extremely useful in the implementation of seasonal marine protected areas to protect more threatened species during periods most pertinent to their reproductive behavioural strategies.

Chapter VIII.

General discussion and future directions

Chondrichthyans are an extremely successful, highly diverse group of marine vertebrate taxa. Due to the inaccessible nature of much of our oceans and the vast ranging behaviour of many species belonging to this class, they are often extremely difficult to study. Consequently there remain many unanswered questions regarding much of their natural behaviour, home ranges, mating and feeding grounds and life-history traits. Many elasmobranchs for example, are known to aggregate in small and large groups, a behaviour that can often exacerbate their vulnerability to fishing pressure (Roberts and Sargant 2008; Mucientes et al. 2009). The aim of this thesis was twofold. Firstly, I was interested in exploring how and why sharks aggregate, looking specifically at the structure, mechanisms and functions of group living in sharks and a comprehensive review of the research already conducted on this is provided in *Chapter I*. With this as a foundation, I then wanted to understand how these mechanistic drivers of aggregation might influence the localised movements of multiple sharks tracked in the wild using electronic tags. As a broad analytical framework that is both rapidly developing and widely used, social network analysis (SNA) provided a vehicle for me to address both aspects of this thesis. Network theory facilitates the analysis of large, complex, interrelational systems allowing us to determine groups and subgroups within our data (Newman 2006) and it is now widely used to explore animal social networks (Croft et al. 2008; Wey et al. 2008; Krause et al. 2009). Throughout this thesis network analysis is used to explore shark social preferences under different social and ecological contexts. This approach is also adopted to try and better understand how habitat features are connected by wild sharks that swim between them in an attempt to encourage more of a predictive approach to analysis of shark tracking data. The key findings from applying this approach are listed and discussed in more detail in the next section.

8.1 Major findings

Throughout this thesis I have alluded to the complexity surrounding the social and reproductive biology of the small spotted catshark (*Scyliorhinus canicula*) as a model elasmobranch. This species is oviporous, occurring across a range of depths (5 – 400 m); it shows distinct sexual segregation at a diel scale due to strong social factors (male harassment) and is reproductively active for a large majority of the year (Ellis and Shackley 1997; Wearmouth et al. 2012). In addition, *S. canicula* are behaviourally cryptic with females refuging in rocky substrata (i.e. crevices, caves) and both sexes (and juveniles) demonstrating long periods of inactivity on a daily basis (Sims et al. 1993, 2001, 2006). These aspects of their behavioural ecology not only made them a challenging species to study but also provided many interesting questions regarding the mechanisms and functions of their behaviour, some of which are addressed in this thesis.

8.1.1 Social preferences

One of main results in this thesis is that juvenile, benthic *S. canicula* are capable of showing associative, non-random preferences when aggregating with conspecifics and that this pattern is driven by familiarity of group mates but not relatedness. Social recognition has been shown to confer numerous benefits in teleost fishes (Ward et al. 2007; Ward et al. 2009) and indeed in terrestrial animals (Mateo 2004). It was demonstrated in *Chapter III* that juvenile *S. canicula* were capable of recognising familiar conspecifics and that non-random social structuring developed where individuals were familiar with one another. Interestingly, these results were detectable at the level of the group whereby the number of groups increased along with the mean association index of social attraction, however, it was not detectable at the level of the individual with mixed results for the analysis of assortment by familiarity. Population level effects of familiarity have been documented in guppies (*Poecilia reticulata*) that use social facilitation to enhance foraging ability (Swaney et al. 2001), although this species are known to be able to distinguish conspecifics individually and in the case of the females use this knowledge to influence male harassment behaviour (e.g. Brask et al. 2012). Knowledge of how familiarity influences social behaviour in *S. canicula*, however, is interesting in the context of a previous study. Jacoby et al. (2010) demonstrate that socially peripheral adult female *S. canicula* are more susceptible to social disruption by both male and novel female conspecifics. This suggests perhaps that familiarity might also play a part in structuring adult aggregations and further research would be useful to confirm this, particularly as determining familiarity effects on adult sharks of many other species, would be logistically very difficult.

8.1.2 Reproductive strategy, relatedness and assortment

Evidence that social recognition exists in this species demonstrates that active partner preferences help to structure social networks with interesting implications for the exploration of other mechanisms. No evidence of kin structuring was found in juvenile sharks that are known to hatch in close physical and temporal proximity to one another, often from the same mother. These results, outlined in *Chapter IV*, imply that perhaps there is low selection acting upon kin association in this species possibly in response to the high levels of female polyandry (in 92% of females) resulting in increased genetic diversity between offspring sired by multiple males. Tracking of adult male and female sharks in the wild (*Chapter VII*) provides context to these results, offering interesting insight into long-term, intersexual behavioural patterns. Male harassment as a driver of sexual segregation has been demonstrated previously in this species (Jacoby et al. 2010; Wearmouth et al. 2012) as has segregation in movement patterns between the sexes (Sims et al. 2001, 2006). Until now, however, it was not known fully, the extent to which this impacted female reproductive strategy and the subsequent strategy of her offspring. Mobbing behaviour of females by males has certainly been hypothesised in this species (Dodd 1983) and witnessed in others (Pratt and Carrier 2001; Whitney et al. 2004) and co-occurrences in the wild might support this theory. During summer and autumn particularly, there was evidence of a single female that was well contacted to multiple males (Fig. 30), whereas all other strong co-occurrence ties are within sex (i.e. consistent with segregation during those periods). These tagged sharks are, of course, just a subsample of the population and it is likely that tagged individuals are interacting with non-tagged individuals both inside and outside the range of the acoustic receivers. Given the high residency patterns of both male and female sharks though, this does appear to support this hypothesis, especially given the extremely small number of male – female co-occurrences observed during at least three quarters of the year.

8.1.3 Environmental influence on social behaviour

The social environment is just one factor that influences the ways in which animals group. The ecological environment and the degree of structural complexity of the surrounding habitat are also important factors influencing grouping behaviour particularly in the context of perceived predation risk in small or vulnerable fish (Orpwood et al. 2008). When considered in the context of male behaviour, habitat complexity alone actually proved to be a significant predictor of adult female movement behaviour of those individuals tracked in the wild (*Chapter VII*). However, the influence of habitat on behaviour was addressed at a much finer scale in the laboratory using juveniles (*Chapter V*). The number of individuals forming groups increased within more complex environments and decreased when a gravel substrate provided what appeared to be an opportunity to camouflage. This plasticity to environmental conditions appears consistent with some teleost fish, such as brown trout (*Salmo trutta*), that change behavioural strategy under different ecological conditions and during different seasons (Heggenes et al. 1993). Despite this degree of plasticity in overall grouping behaviour, at the individual level, juvenile *S. canicula* were able to maintain consistent social network positions across each of the three habitat treatments.

This expression of consistent individual differences through time and across context, often referred to as a personality trait, is the first indication of personality in elasmobranchs. It is perhaps not a surprising result given the taxonomic breadth of organisms for which repeatable behaviours have already been quantified (e.g. Rhesus macaques, *Macaca mulatta*: Stevenson-Hinde et al. 1980; domestic dogs, *Canis familiaris*: Svartberg and Forkman 2002; beadlet anemones, *Actinia equine*: Briffa and Greenaway 2011; teleost fishes: Conrad et al. 2011). It is hypothesised throughout that grouping behaviour in these juvenile sharks is, in part, driven by the antipredator benefits of associating with phenotypically similar conspecifics. Unfortunately, due to ethical licensing constraints the influence of predation risk on grouping could not be directly tested during this thesis, but is rather inferred from other experimentation. These experiments, particularly the study manipulating habitat (*Chapter V*), suggests an antipredator strategy and under such conditions consistency in network position, particularly for those well connected to one another, might enable individuals to be more resilient to perturbation or attack (Chivers et al. 1995; see also Jacoby et al. 2010, for example, which addresses harassment not predation). Furthermore, behavioural consistency is likely to be adaptive for survival in a highly dynamic coastal environment where juvenile *S. canicula* hatch from their egg cases.

8.1.4 Network analyses of movement

This thesis demonstrates that network analysis can enhance our understanding of animal biotelemetry data by incorporating the interconnectivity of habitats that the animals move between, allowing specific hypotheses to be tested about how these animals are utilising their environment. The methodology developed in *Chapter VI* and applied in *Chapter VII* attempts to demonstrate the different aspects of wild shark behaviour that can be illuminated through dynamic analyses of passive tracking data. Indeed these analyses helped to reveal that wild *S. canicula*, known to be highly sexually segregated, show periods of behavioural synchronicity during the springtime months of March, April and May, conducting statistically similar movement behaviours and showing greater levels of mixed-sex co-occurrences than at any other time of year. Male patrolling of female refuging locations, supportive of male harassment behaviour (Wearmouth et al. 2012), is apparent in the wild movement networks but not when analysing detection frequencies in a static format. Complex networks of movement are relatively common in research on human mobility (e.g. Song et al. 2010) and in some instances might have important consequences for the global economy (Porter 2000) or the spread of invasive species (Kaluza et al. 2010).

Likewise, knowledge of animal movement can be an important prerequisite for understanding and predicting population dynamics (Morales et al. 2010). As demonstrated in *Chapter VII*, network analysis of animal movement between fixed locations can offer a powerful predictive tool in determining, for example, periods of synchronicity and possibly reproduction in behaviourally cryptic species or the extent to which environmental features influence habitat use.

First and foremost, these findings advance our understanding of the life-history of *S. canicula* by determining some of the mechanisms and/or functions driving their aggregation behaviour and movement ecology. Beyond this, this well studied species offers a tractable model with which to address questions relating to other elasmobranchs of a similar life-history (Sims 2003), many of which have received considerable less attention in the literature than the larger carcharhinid species. Studies linking reproductive mode and behavioural strategy, as here, are directly comparable to other species of benthic elasmobranch that show similar intersexual behaviours and spatial segregation (Pratt and Carrier 2001). Furthermore there are hypotheses tested in this thesis that regard the importance of both individual and group-level behaviours, explored in a social network context, that make for an interesting comparison with similar studies on teleost fishes (e.g. the development and maintenance of familiarity); although many of these hypotheses require further experimentation to fully determine the mechanisms behind the patterns of social interaction (see *Future research directions*). Finally, in the last few years, several studies have quantified elasmobranch social networks (Jacoby et al. 2010; Guttridge et al. 2011; Mourier et al. 2012) contributing to the vast array of organisms for which social networks have been described (Croft et al. 2008). Further investigation of predatory shark social networks, particularly in the wild, will no doubt reveal important ecological consequences, not least for the trophic ecology of their respective food webs (Heithaus et al. 2008, 2009).

8.2 Conservation implications

Throughout this thesis reference is made to the conservation implications of the work being conducted. It is important to reiterate, however, that *Scyliorhinus canicula*, as a model shark species is widely distributed and relatively abundant in UK waters. The International Union for Conservation of Nature (IUCN) declare that population trends are stable and list this species as *least concern* on their Red List of Threatened Species. During the course of this research I have therefore been fortunate enough to be able to obtain and rear sufficient numbers of individuals (all of which were released post-experiment) to allow rigorous hypothesis testing with statistically powerful replication. Logistically, this would not have been possible of course using an endangered or threatened species of elasmobranch. Increasing our understanding of the biology of relatively abundant species will likely prove informative in the management and conservation of those species under much greater ecological threat. Throughout this thesis the individual ecological benefits that elasmobranchs might gain by aggregating or forming social groups are discussed at length and in doing so, I have attempted to provide an overview of how and where social network analyses are likely to contribute to the field of shark behavioural ecology. It is important here to also address the anthropogenic-driven costs associated with grouping behaviour in elasmobranchs and explore how some of the implications of this body of work might be applicable in a wider conservation context.

Anthropogenic influences impose costs at the population level (i.e. fishing pressure and habitat destruction) that are likely to be exacerbated by behavioural mechanisms such as grouping/schooling behaviour, sexual segregation and site philopatry (Wearmouth and Sims 2008; Mucientes et al. 2009). Sharks pose a particularly interesting and indeed worrying model for examining these costs, especially given their *K* selected life-history traits and the fact that many species occupy an apex position within their ecological niche and, thus, their ability to influence processes at lower trophic levels (Heithaus et al. 2008). By repeatedly aggregating *en masse* at specific locations, at specific times, slow-growing elasmobranchs make themselves particularly vulnerable to overfishing (Mucientes et al. 2009). Now, with modern and efficient fishing equipment, countries where shark landings represent a major economic advantage can substantially increase their catch per unit effort through simple targeted fishing practices and knowledge of these ecological events. One example of this is the basking shark (*Cetorhinus maximus*) fishery at Achill Island, Co. Mayo, Republic of Ireland where 12,360 sharks were landed between 1947 and 1975 (Sims 2008). Of these total reported landings, 75% were caught between 1950 and 1956 with mean catch per year being reduced from 1323 individuals to just 60 by 1962 – 1975. Basking sharks have long gestation periods (between 1 and 2 years) and show particularly low fecundity even among elasmobranchs. It is proposed that one explanation for this dramatic reduction in this north-eastern Atlantic population is that a large majority of the sharks taken were probably mature adult females engaging in seasonal coastal aggregation behaviour (Sims et al. 2000; Sims 2008). This putative differential exploitation of the sexes is pertinent when we consider that females of some pelagic shark species seek shallow inshore waters during parturition (Feldheim et al. 2002; Hueter et al. 2005), potentially enhancing encounter rates with fishing vessels. Furthermore, it highlights the urge for more detailed research on the composition of shark aggregations and for evaluation of minimum group size thresholds at which reproduction will remain viable within a population, particularly for short-term mating aggregations in more solitary species.

In fisheries where sharks are apparently not the principal target species, such as pelagic longline fisheries (for tunas and billfish), the tendency for pelagic shark species to school can increase elasmobranch bycatch, further inflating mortality rates (Gilman et al. 2008). Worldwide declines in shark populations and the ecological consequences of these declines have been reported widely (Myers and Worm 2003; Worm et al. 2006; Myers et al. 2007; Heithaus et al. 2008; Ferretti et al. 2010). One consideration to help stem these trends must be conservation and management strategies that address these behaviourally mediated increases in fisheries mortality and as discussed in *Chapter VII*, consideration of seasonal or temporary marine protected areas designed to safeguard habitat locations during periods of reproductive importance. With philopatric behaviour being such a prevalent characteristic among elasmobranch populations, there are now calls for shark fisheries to incorporate spatially structured management strategies which account for the degree of site philopatry of the respective target species (Hueter et al. 2005; Robbins et al. 2006; Knip et al. 2010). Furthermore, detailed analyses of aggregation events and individual and collective movement networks that aid the prediction of diel and seasonal space use should also be considered in future management policies (Mucientes et al. 2009; *Chapters VI* and *VII*).

A second implication for elasmobranch grouping behaviour is an increase in popularity among people wishing to dive with sharks and rays with a concomitant surge in ecotourism operations around the world. In parts of the world where elasmobranchs were once prized for their meat and fins, communities are now adjusting their focus to conserve these species and their aggregation sites. Ecotourist ventures seek to utilise local knowledge of ecological events, such as periodic grouping, in offering experiences interacting with animals that would otherwise be targeted for food or fins. Whether tourism offers a sufficient economic alternative to fishing for some communities is still open to debate (Topelko and Dearden 2005), and is to a large extent dictated by culture-driven consumer demand, particularly within the far eastern markets where shark products are prized as delicacies and for medicinal purposes. From a conservation perspective, ecotourist ventures appear important in the publicity of threatened elasmobranch species at a global level. Furthermore, under the correct management, ecotourist operators may also provide an invaluable source of temporal data on the nature of shark aggregations that may inform future conservation initiatives (Theberge and Dearden 2006) and scientific research.

A wide variety of elasmobranch species utilise coastal, nearshore environments either periodically to feed or mate or year-round as a nursery habitat (Heupel et al. 2007; Chapman et al. 2009; Knip et al. 2010). These environments not only experience major fluctuations in temperature, salinity, depth, turbidity and flow rate (Mann 2000) but are also highly susceptible to anthropogenic perturbation such as intense fishing activities and habitat alteration (Castilla 1999). The ecological benefits (and costs) individual sharks incur from recognising and associating with familiar group mates are frequently discussed throughout this thesis. In the wild, it seems plausible that within such a dynamic and often heavily impacted environment, behavioural consistency, shown to be a strong driver of shark behavioural strategy in *Chapter V*, provides a mechanism for small and developing sharks to withstand considerable changes to their habitat which might otherwise negatively impact their reproductive success. It would be extremely interesting to test for repeatability of behaviour under natural, more fluctuating environments. Examination of plasticity and consistency in shark behaviour however, even under laboratory conditions, is likely to become an important aspect of elasmobranch conservation by determining the extent to which sharks can adapt or are resilient to disturbance. Indeed in *Chapter VI*, node removal analyses attempt to address this by quantifying the resilience of movement networks to simulated disturbance.

8.3 Future research directions

Reviewing the literature on shark aggregation, which contributed towards the general introduction of this thesis (*Chapter I*), highlighted for me the need to include more rigorous hypothesis testing of how and why sharks group in the ways they do. A strong descriptive component to studies of grouping prevails (see *Chapter I*) although there are recent notable exceptions suggesting a change in this trend (e.g. Guttridge et al. 2011; Mourier et al. 2012; Jirik and Lowe 2012). The thesis attempts to address this change, indicating where possible, further research that might be conducted to enhance our understanding of the motivation and mechanisms behind grouping behaviour in elasmobranchs. Fortunately, technological advances of tags and tracking equipment provide increasing opportunities in this endeavour (Holland et al. 2009; Guttridge et al. 2010; Krause et al. 2011, Rutz et al. 2012). This section will discuss ideas and thoughts that I would like to have expanded on during this research had it not been for time or logistical constraints.

8.3.1 Experimentation

Laboratory experimentation comprises a large component of this thesis and allowed hypotheses to be tested using small, replicated groups of *S. canicula*. During each of the three separate experiments (*Chapters III*, *IV* and *V*) it was apparent that there were high levels of behavioural variation at both the group level and between individuals within groups. This, to some extent, reduced the effect size of the results and therefore it would have been interesting to consider the effects of experimental group size on non-random social preferences and the ability to detect these. This, of course, would have required larger experimental arenas, more juvenile sharks or lower numbers of replication so the decision was taken to concentrate efforts elsewhere. Equally, quantifying association based on group resting behaviour made it particularly difficult to design experiments that specifically tested the mechanisms behind social recognition in *S. canicula*. Binary choice experiments were initially conducted to determine the time taken for familiarity to develop, adopting methods frequently used in studies on teleost fishes (e.g. Griffiths and Magurran 1997; Darden et al. 2009). Due to the length of time devoted to resting behaviour in this species, however, this methodology proved impractical. Other, more mobile elasmobranch species such as the lemon shark (*Negaprion brevirostris*) are more amenable to this type of experimentation (see Guttridge et al. 2009a) and would likely prove interesting candidates for investigating the role of vision and olfaction on social recognition in sharks (Gardiner et al. 2012).

The finding of repeatable social network positions across context (*Chapter V*) really opens up a wealth of opportunity for further experimental investigation. How the balance between consistent and plastic phenotypes is expressed throughout ontogeny in elasmobranchs would be a logical progression for this work. The intriguing dynamics between individual behavioural consistency and age, for example, were recently explored by Edenbrow and Croft (2011) who used the mangrove killifish (*Kryptolebias marmoratus*), a model clonal vertebrate, to show that the expression of behavioural type became more pronounced towards sexual maturity, after which it was expressed less. The ease with which *S. canicula* can be hatched and reared in the laboratory, make this shark an ideal candidate to address this question in future studies. It is also noteworthy to mention here that across studies of animal personality there appears to be significant variation in the analyses adopted to test statistically for consistency and repeatability in behaviour (Lessells and Boag 1987; Cote and Clobert 2007; Stamps et al. 2012) with many adopting a form of linear mixed-effects model (e.g. Wilson et al . 2011). Within these studies behaviours contributing to personality types are generally behaviours that can be quantified in isolation for each individual (e.g. testing for boldness in teleost fishes by dropping a weight into a tank to simulate a predatory bird attack) thus providing independent data points to test for repeatability. Quantifying personality based on consistent individual social behaviour, however, is a new endeavour. As such, the statistical analysis for determining repeatability in social network position will likely be open to debate due to the non-independent nature of network metrics. As with other analyses of network data, permutation tests and null models will no doubt prove useful (Croft et al. 2011). Analyses of interrelational, social network data continue to develop in an attempt to both reduce subjectivity in sampling protocol (Haddadi et al. 2011) and fully account for the non-orthogonal nature of network data (Croft et al. 2011) and some of these developments will now be discussed.

8.3.2 *Analytical developments*

There is currently a variety of network software available reflecting the broad application of these types of analyses and it is hoped that this thesis goes someway to demonstrating the versatility of this analytical approach. A small number of statistical analyses developed for analysing social networks were adapted to study animal movement in *Chapter VI*, however, this is just a small subset of the techniques that are available. Clearly further research is required to fully determine the extent to which social network analyses might be incorporated into studies of movement behaviour particularly in light of the fact that network edges can not only be weighted based on the frequency of movement between locations, but also can contain temporal information (i.e. it takes an animal a certain amount of time to travel from one node to another). For the purposes of the present study this temporal component was simply used to filter out indirect movements but it is expected that the durations associated with individual edges might perhaps become incorporated into the statistical analyses as discussed below.

Up until now, the temporal dynamics of animal social networks, that is the time scale or number of samples over which a network is condensed, have been largely overlooked and yet the topology of a network can change over multiple temporal scales (Blonder et al. 2012). The result is a static network which suggests constancy in network structure throughout the period of sampling, whereas in reality this represents a relative equilibrium around which fluctuations in the network structure occur. Recent work, suggests that these fluctuations might harbour interesting temporal information about the nature of associations within the population (Blonder and Dornhaus 2011; Blonder et al. 2012) and might also prove useful for determining a statistically-derived sampling protocol (Haddadi et al. 2011). Thus the integration of time-ordered networks into behavioural ecology will surely enhance our understanding of the duration and ordering of network connectivity that can often be critical in directed networks or networks tracking the flow of resources or information (Sumpter 2006; Sih et al. 2009). Such analytical developments offer an exciting prospect for the future of animal social network research and will no doubt facilitate progress towards answering some of the questions outlined in this section.

8.4 Conclusion

This thesis advocates the use of network analyses to test specific hypotheses about how and why social grouping occurs within a model species of elasmobranch, *Scyliorhinus canicula*. Familiarity appeared to structure social associations between individuals that were able to maintain these network positions under different ecological contexts. Kin relatedness did not influence network structure likely due to increased genetic diversity between offspring caused by the highest level of female polyandry yet observed in a shark. Despite strong sexual segregation, due to male sexual harassment and a protracted breeding season in this species, periods of behavioural synchronicity in the wild implicate the spring months of March, April and May as a period that is of potential reproductive importance.

Widespread legal and illegal fishing practices and ever expanding anthropogenic use of the marine environment have fuelled growing concern over the conservation status of many shark species globally (Baum et al. 2003; Ferretti et al. 2010). Consequently, there is an increasingly urgent need to better understand both the movements and space use and also the behavioural strategies adopted by these predators to survive. The mechanisms underpinning one such strategy, namely the aggregation and social grouping of individuals, are receiving burgeoning attention in the literature. Coupled with current rapid development of electronic tagging technology and advances in broad analytical frameworks such as social network analyses (SNA), we are beginning to address the predictability of these behaviours which is likely to prove critical for the conservation of these animals in the future.

References.

Abecasis, D. & Erzini, K. (2008) Site fidelity and movements of gilthead sea bream (*Sparus aurata*) in a coastal lagoon (Ria Formosa, Portugal). *Estuarine, Coastal and Shelf Science,* **79,** 758-763.

Allee, W. C. & Dickinson, J. C. J. (1954) Dominance and subordination in the smooth dogfish *Mustelus canis* (Mitchill). *Physiological Zoology,* **27,** 356-364.

Allouche, S. & Gaudin, P. (2001) Effects of avian predation threat, water flow and cover on growth and habitat use by chub, *Leuciscus cephalus*, in an experimental stream. *Oikos,* **94,** 481-492.

Amstrup, S. C. & Gardner, C. (1994) Polar bear maternity denning in the Beaufort Sea. *The Journal of Wildlife Management,* **58,** 1-10.

Andrews, K. R., Karczmarski, L., Au, W. W. L., Rickards, S. H., Vanderlip, C. A., Bowen, B. W., Gordon Grau, E. & Toonen, R. J. (2010) Rolling stones and stable homes: social structure, habitat diversity and population genetics of the Hawaiian spinner dolphin (*Stenella longirostris*). *Molecular Ecology,* **19,** 732-748.

Andrews, K. S., Williams, G. D., Farrer, D., Tolimieri, N., Harvey, C. J., Bargmann, G. & Levin, P. S. (2009) Diel activity patterns of sixgill sharks, *Hexanchus griseus*: the ups and downs of an apex predator. *Animal Behaviour,* **78,** 525-536.

Anras, M. L. B., Lagardére, J. P. & Lafaye, J. Y. (1997) Diel activity rhythm of seabass tracked in a natural environment: group effects on swimming patterns and amplitudes. *Canadian Journal of Fisheries and Aquatic Sciences,* **54,** 162-168.

Arnold, K. E. (2000) Kin recognition in rainbowfish (*Melanotaenia eachamensis*): sex, sibs and shoaling. *Behavioral Ecology and Sociobiology,* **48,** 385-391.

Ballard, W. W., Mellinger, J. & Lechenault, H. (1993) A series of normal stages for development of '*Scyliorhinus canicula*', the lesser spotted dogfish (Chondrichthyes: Scyliorhinidae). *Journal of Experimental Zoology,* **267,** 318-336.

Barber, I. & Wright, H. A. (2001) How strong are familiarity preferences in shoaling fish? *Animal Behaviour,* **61,** 975-979.

Barnett, A., Abrantes, K. G., Stevens, J. D. & Semmens, J. M. (2011) Site fidelity and sex-specific migration in a mobile apex predator: implications for conservation and ecosystem dynamics. *Animal Behaviour,* **81,** 1039-1048.

Barrat, A., Barthélemy, M., Pastor-Satorras, R. & Vespignani, A. (2004) The architecture of complex weighted networks. *Proceedings of the National Academy of Sciences of the United States of America,* **101,** 3747-3752.

Baum, J. K., Myers, R. A., Kehler, D. G., Worm, B., Harley, S. J. & Doherty, P. A. (2003) Collapse and conservation of shark populations in the Northwest Atlantic. *Science,* **299,** 389-392.

Beauchamp, G. & Fernández-Juricic, E. (2005) The group-size paradox: effects of learning and patch departure rules. *Behavioral Ecology,* **16,** 352-357.

Beier, P., Choate, D. & Barrett, R. H. (1995) Movement patterns of mountain lions during different behaviors. *Journal of Mammalogy,* **76,** 1056-1070.

Bejder, L., Fletcher, D. & Bräger, S. (1998) A method for testing association patterns of social animals. *Animal Behaviour,* **56,** 719-725.

Bell, A. M., Hankison, S. J. & Laskowski, K. L. (2009) The repeatability of behaviour: a meta-analysis. *Animal Behaviour,* **77,** 771-783.

Blonder, B. & Dornhaus, A. (2011) Time-ordered networks reveal limitations to information flow in ant colonies. *PLoS ONE,* **6,** e20298.

Blonder, B., Wey, T. W., Dornhaus, A., James, R. & Sih, A. (2012) Temporal dynamics and network analysis. *Methods in Ecology and Evolution*, In press.

Borgatti, S. P. (2002) NetDraw: graph visualization software. Analytic Technologies, Harvard.

Borgatti, S. P., Everett, M. G. & Freeman, L. C. (1999) *UCINET 6.0 Version 1.00,* Analytic Technologies, Natick.

Borgatti, S. P., Everett, M. G. & Freeman, L. C. (2002) UCINET 6 For Windows: software for social network analysis. Analytic Technologies, Harvard.

Borgatti, S. P., Mehra, A., Brass, D. J. & Labianca, G. (2009) Network analysis in the social sciences. *Science,* **323,** 892-895.

Bozzanao, A., Murgia, R., Vallerga, S., Hirano, J. & Archer, S. (2001) The photoreceptor system in the retinae of two dogfishes, *Scyliorhinus canicula* and *Galeus melastomus*: possible relationship with depth distribution and predatory lifestyle. *Journal of Fish Biology,* **59,** 1258-1278.

Bradley, B. J., Doran-Sheehy, D. M. & Vigilant, L. (2007) Potential for female kin associations in wild western gorillas despite female dispersal. *Proceedings of the Royal Society B: Biological Sciences,* **274,** 2179-2185.

Brask, J. B., Croft, D. P., Thompson, K., Dabelsteen, T. & Darden, S. K. (2012) Social preferences based on sexual attractiveness: a female strategy to reduce male sexual attention. *Proceedings of the Royal Society B: Biological Sciences,* **279,** 1748-1753.

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

Briffa, M., Rundle, S. D. & Fryer, A. (2008) Comparing the strength of behavioural plasticity and consistency across situations: animal personalities in the hermit crab *Pagurus bernhardus*. *Proceedings of the Royal Society B: Biological Sciences,* **275,** 1305-1311.

Brown, C. & Laland, K. N. (2003) Social learning in fishes: a review. *Fish and Fisheries,* **4,** 280-288.

Brown, C. & Warburton, K. (1999) Social mechanisms enhance escape responses in shoals of rainbowfish, *Melanotaenia duboulayi*. *Environmental Biology of Fishes,* **56,** 455-459.

Brown, G., Rive, A., Ferrari, M. & Chivers, D. (2006) The dynamic nature of antipredator behavior: prey fish integrate threat-sensitive antipredator responses within background levels of predation risk. *Behavioral Ecology and Sociobiology,* **61,** 9-16.

Brown, G. E. (2003) Learning about danger: chemical alarm cues and local risk assessment in prey fishes. *Fish and Fisheries,* **4,** 227-234.

Brown, G. E. & Brown, J. A. (1993) Social dynamics in salmonid fishes: do kin make better neighbours? *Animal Behaviour,* **45,** 863-871.

Brown, J. L. & Eklund, A. (1994) Kin recognition and the major histocompatibility complex: an integrative review. *American Naturalist,* **143,** 435-461.

Brunnschweiler, J. M., Baensch, H., Pierce, S. J. & Sims, D. W. (2009) Deep-diving behaviour of a whale shark *Rhincodon typus* during long-distance movement in the western Indian Ocean. *Journal of Fish Biology,* **74,** 706-714.

Cahan, S. H., Blumstein, D. T., Sundström, L., Liebig, J. & Griffin, A. (2002) Social trajectories and the evolution of social behavior. *Oikos,* **96,** 206-216.

Cairns, S. J. & Schwager, S. J. (1987) A comparison of association indices. *Animal Behaviour,* **35,** 1454-1469.

Capapé, C., Vergne, Y., Reynaud, C., Guélorget, O. & Quignard, J. P. (2008) Maturity, fecundity and occurrence of the small spotted catshark *Scyliorhinus canicula* (Chondrichthyes: Scyliorhinidae) off the Languedocian coast (southern France, north-western Mediterranean). *Vie et Milieu,* **58,** 47-55.

Caraco, T. (1979) Time budgeting and group size: a test of theory. *Ecology,* **60,** 618-627.

Carey, F. G., Scharold, J. V. & Kalmijn, A. J. (1990) Movements of blue sharks (*Prionace glauca*) in depth and course. *Marine Biology,* **106,** 329-342.

Carraro, R. & Gladstone, W. (2006) Habitat preferences and site fidelity of the ornate wobbegong shark (*Orectolobus ornatus*) on rocky reefs of New South Wales. *Pacific Science,* **60,** 207-223.

Carrier, J. C., Pratt, H. L. & Castro, J. L. (2004) Elasmobranch reproduction. *Biology of Sharks and Their Relatives* (eds J. C. Carrier, J. Musick & M. R. Heithaus), pp. 269-286. CRC Press, Boca Raton, FL.

Carrier, J. C., Pratt, H. L., Jr. & Martin, L. K. (1994) Group reproductive behaviors in free-living nurse sharks, *Ginglymostoma cirratum*. *Copeia,* **1994,** 646-656.

Castilla, J. C. (1999) Coastal marine communities: trends and perspectives from human-exclusion experiments. *Trends in Ecology & Evolution,* **14,** 280-283.

Castro, J. L., Bubucis, P. M. & Overstrom, N. A. (1988) The reproductive biology of the chain dogfish, *Scyliorhinus retifer*. *Copeia,* **3,** 740-746.

Chapman, D. D., Babcock, E. A., Gruber, S. H., Dibattista, J. D., Franks, B. R., Kessel, S. A., Guttridge, T., Pikitch, E. K. & Feldheim, K. A. (2009) Long-term natal site-fidelity by immature lemon sharks (*Negaprion brevirostris*) at a subtropical island. *Molecular Ecology,* **18,** 3500-3507.

Chapman, D. D., Prodöhl, P. A., Gelsleichter, J., Manire, C. A. & Shivji, M. S. (2004) Predominance of genetic monogamy by females in a hammerhead shark, *Sphyrna tiburo*: implications for shark conservation. *Molecular Ecology,* **13,** 1965-1974.

Chapman, T., Arnqvist, G., Bangham, J. & Rowe, L. (2003) Sexual conflict. *Trends in Ecology & Evolution,* **18,** 41-47.

Chevolot, M., Ellis, J. R., Rijnsdorp, A. D., Stam, W. T. & Olsen, J. L. (2007) Multiple paternity analysis in the thornback ray *Raja clavata* L. *Journal of Heredity,* **98,** 712-715.

Chivers, D, P., Brown, G, E., Smith & R. J, F. (1995) Familiarity and shoal cohesion in fathead minnows (*Pimephales promelas*): implications for antipredator behaviour. *Canadian Journal of Zoology,* **73,** 955-960.

Clarke, T. A. (1971) The ecology of the scalloped hammerhead shark, *Sphyrna lewini*, in Hawaii. *Pacific Science,* **25,** 133-144.

Compagno, L., Dando, M. & Fowler, S. (2005) *Sharks of the World,* Princeton University Press, Princeton, NJ.

Compagno, L. J. V. (1990) Alternative life-history styles of cartilaginous fishes in time and space. *Environmental Biology of Fishes,* **28,** 33-75.

Connor, R. C., Heithaus, M. R. & Barre, L. M. (2001) Complex social structure, alliance stability and mating access in a bottlenose dolphin 'super-alliance'. *Proceedings of the Royal Society of London. Series B: Biological Sciences,* **268,** 263-267.

Conrad, J. L., Weinersmith, K. L., Brodin, T., Saltz, J. B. & Sih, A. (2011) Behavioural syndromes in fishes: a review with implications for ecology and fisheries management. *Journal of Fish Biology,* **78,** 395-435.

Cooke, S. J. (2008) Biotelemetry and biologging in endangered species research and animal conservation: relevance to regional, national, and IUCN Red List threat assessments. *Endangered Species Research,* **4,** 165-185.

Côté, I. M. & Poulin, R. B. (1995) Parasitism and group size in social animals: a meta-analysis. *Behavioral Ecology,* **6,** 159-165.

Cote, J. & Clobert, J. (2007) Social personalities influence natal dispersal in a lizard. *Proceedings of the Royal Society B: Biological Sciences,* **274,** 383-390.

Couzin, I. D., Krause, J., Franks, N. R. & Levin, S. A. (2005) Effective leadership and decision-making in animal groups on the move. *Nature,* **433,** 513-516.

Couzin, I. D., Krause, J., James, R., Ruxton, G. D. & Franks, N. R. (2002) Collective memory and spatial sorting in animal groups. *Journal of Theoretical Biology,* **218,** 1-11.

Craik, J. C. A. (1978) An annual cycle of vitellogenesis in the elasmobranch *Scyliorhinus canicula*. *Journal of the Marine Biological Association of the United Kingdom,* **58,** 719-726.

Croft, D. P., Morrell, L. J., Wade, A. S., Piyapong, C., Ioannou, C. C., Dyer, J. R. G., Chapman, B. B., Yan, W. & Krause, J. (2006a) Predation risk as a driving force for sexual segregation : a cross-population comparison. *American Naturalist,* **167,** 867-878.

Croft, D. P., James, R., Thomas, P., Hathaway, C., Mawdsley, D., Laland, K. & Krause, J. (2006b) Social structure and co-operative interactions in a wild population of guppies (*Poecilia reticulata*). *Behavioral Ecology and Sociobiology,* **59,** 644-650.

Croft, D. P., Arrowsmith, B. J., Bielby, J., Skinner, K., White, E., Couzin, I. D., Magurran, A. E., Ramnarine, I. & Krause, J. (2003) Mechanisms underlying shoal composition in the Trinidadian guppy, *Poecilia reticulata*. *Oikos,* **100,** 429-438.

Croft, D. P., Hamilton, P., Darden, S. K., Jacoby, D. M. P., James, R., Bettaney, E. & Tyler, C. (2012) The role of relatedness in structuring the social network of a wild guppy population. *Oecologia***,** 1-9.

Croft, D. P., James, R. & Krause, J. (2008) *Exploring Animal Social Networks,* Princeton University Press, Princeton, NJ.

Croft, D. P., James, R., Ward, A. J. W., Botham, M. S., Mawdsley, D. & Krause, J. (2005) Assortative interactions and social networks in fish. *Oecologia,* **143,** 211-219.

Croft, D. P., Krause, J., Darden, S., Ramnarine, I., Faria, J. & James, R. (2009) Behavioural trait assortment in a social network: patterns and implications. *Behavioral Ecology and Sociobiology,* **63,** 1495-1503.

Croft, D. P., Krause, J. & James, R. (2004) Social networks in the guppy (*Poecilia reticulata*). *Proceedings of the Royal Society B: Biological Sciences,* **271,** 516-519.

Croft, D. P., Madden, J. R., Franks, D. W. & James, R. (2011) Hypothesis testing in animal social networks. *Trends in Ecology & Evolution,* **26,** 502-507.

Crooks, N. (2011) Sexual and seasonal dimorphisms in the dermal, dental and ampullary structures of the lesser-spotted catshark, *Scyliorhinus canicula*. PhD Thesis, University of Portsmouth, UK.

Cucherousset, J., Marty, P., Pelozuelo, L. & Roussel, J.-M. (2008) Portable PIT detector as a new tool for non-disruptively locating individually tagged amphibians in the field: a case study with Pyrenean brook salamanders (*Calotriton asper*). *Wildlife Research,* **35,** 780-787.

Cutts, C. J. & Speakman, J. R. (1994) Energy savings in formation flight of pink-footed geese. *Journal of Experimental Biology,* **189,** 251-261.

Dall, S. R. X., Houston, A. I. & McNamara, J. M. (2004) The behavioural ecology of personality: consistent individual differences from an adaptive perspective. *Ecology Letters,* **7,** 734-739.

Daly-Engel, T. S., Grubbs, R. D., Bowen, B. W. & Toonen, R. J. (2007) Frequency of multiple paternity in an unexploited tropical population of sandbar sharks (*Carcharhinus plumbeus*). *Canadian Journal of Fisheries and Aquatic Sciences,* **64,** 198-204.

Daly-Engel, T. S., Grubbs, R. D., Feldheim, K. A., Bowen, B. W. & Toonen, R. J. (2010) Is multiple mating beneficial or unavoidable? Low multiple paternity and genetic diversity in the shortspine spurdog *Squalus mitsukurii*. *Marine Ecology Progress Series,* **403,** 255-267.

Daly-Engel, T. S., Grubbs, R. D., Holland, K. N., Toonen, R. J. & Bowen, B. W. (2006) Assessment of multiple paternity in single litters from three species of carcharhinid sharks in Hawaii. *Environmental Biology of Fishes,* **76,** 419-424.

Darden, S. K. & Croft, D. P. (2008) Male harassment drives females to alter habitat use and leads to segregation of the sexes. *Biology Letters,* **4,** 449-451.

Darden, S. K., James, R., Ramnarine, I. W. & Croft, D. P. (2009) Social implications of the battle of the sexes: sexual harassment disrupts female sociality and social recognition. *Proceedings of the Royal Society B: Biological Sciences,* **276,**2651-2656.

Darden, S. K., Steffensen, L. K. & Dabelsteen, T. (2008) Information transfer among widely spaced individuals: latrines as a basis for communication networks in the swift fox? *Animal Behaviour,* **75,** 425-432.

Dawson, C. L. & Starr, R. M. (2009) Movements of subadult prickly sharks *Echinorhinus cookei* in the Monterey Canyon. *Marine Ecology Progress Series,* **386,** 253-262.

Dekker, D., Krackhardt, D. & Snijders, T. (2007) Sensitivity of MRQAP tests to collinearity and autocorrelation conditions. *Psychometrika,* **72,** 563-581.

Dewar, H., Mous, P., Domeier, M., Muljadi, A., Pet, J. & Whitty, J. (2008) Movements and site fidelity of the giant manta ray, *Manta birostris*, in the Komodo Marine Park, Indonesia. *Marine Biology,* **155,** 121-133.

DiBattista, J. D., Feldheim, K. A., Gruber, S. H. & Hendry, A. P. (2008) Are indirect genetic benefits associated with polyandry? Testing predictions in a natural population of lemon sharks. *Molecular Ecology,* **17,** 783-795.

Dingemanse, N. J., Kazem, A. J. N., Réale, D. & Wright, J. (2010) Behavioural reaction norms: animal personality meets individual plasticity. *Trends in Ecology & Evolution,* **25,** 81-89.

Dodd, J. M. (1983) Reproduction in cartilaginous fishes (Chondrichthyes). *Fish Physiology* (eds W. S. Hoar, D. J. Randall & E. M. Donaldson), pp. 31-95. Academic Press, San Diego, CA.

Domeier, M. & Nasby-Lucas, N. (2007) Annual re-sightings of photographically identified white sharks (*Carcharodon carcharias*) at an eastern Pacific aggregation site (Guadalupe Island, Mexico). *Marine Biology,* **150,** 977-984.

Dowd, M. & Joy, R. (2011) Estimating behavioral parameters in animal movement models using a state-augmented particle filter. *Ecology,* **92,** 568-575.

Dudgeon, C. L., Noad, M. J. & Lanyon, J. M. (2008) Abundance and demography of a seasonal aggregation of zebra sharks *Stegostoma fasciatum*. *Marine Ecology Progress Series,* **368,** 269-281.

Dunbar, R. I. M. & Shultz, S. (2007) Evolution in the social brain. *Science,* **317,** 1344-1347.

Ebert, D. A. (1991) Observations on the predatory behaviour of the sevengill shark *Notorynchus cepedianus*. *South African Journal of Marine Science,* **11,** 455 - 465.

Eckert, S. A. & Stewart, B. S. (2001) Telemetry and satellite tracking of whale sharks, *Rhincodon Typus*, in the Sea of Cortez, Mexico, and the North Pacific Ocean. *Environmental Biology of Fishes,* **60,** 299-308.

Economakis, A. & Lobel, P. (1998) Aggregation behavior of the grey reef shark, *Carcharhinus amblyrhynchos*, at Johnston Atoll, Central Pacific Ocean. *Environmental Biology of Fishes,* **51,** 129-139.

Edenbrow, M. & Croft, D. P. (2011) Behavioural types and life history strategies during ontogeny in the mangrove killifish, *Kryptolebias marmoratus*. *Animal Behaviour,* **82,** 731-741.

Edenbrow, M., Darden, S. K., Ramnarine, I. W., Evans, J. P., James, R. & Croft, D. P. (2011) Environmental effects on social interaction networks and male reproductive behaviour in guppies, *Poecilia reticulata*. *Animal Behaviour,* **81,** 551-558.

Ellis, J. R. & Shackley, S. E. (1995) Ontogenic changes and sexual dimorphism in the head, mouth and teeth of the lesser spotted dogfish. *Journal of Fish Biology,* **47,** 155-164.

Ellis, J. R. & Shackley, S. E. (1997) The reproductive biology of *Scyliorhinus canicula* in the Bristol Channel, U.K. *Journal of Fish Biology,* **51,** 361-372.

Evans, J. P. & Kelley, J. L. (2008) Implications of multiple mating for offspring relatedness and shoaling behaviour in juvenile guppies. *Biology Letters,* **4,** 623-626.

Evans, J. P. & Magurran, A. E. (2000) Multiple benefits of multiple mating in guppies. *Proceedings of the National Academy of Sciences,* **97,** 10074-10076.

Excoffier, L., Laval, G. & Schneider, S. (2005) Arlequin (version 3.0): an integrated software package for population genetics data analysis. Libertas Academica.

Fancy, S. G., Pank, L. F., Whitten, K. R. & Regelin, W. L. (1989) Seasonal movements of caribou in arctic Alaska as determined by satellite. *Canadian Journal of Zoology,* **67,** 644-650.

Feldheim, K. A., Gruber, S. H. & Ashley, M. V. (2002) The breeding biology of lemon sharks at a tropical nursery lagoon. *Proceedings of the Royal Society B: Biological Sciences,* **269,** 1655-1661.

Feldheim, K. A., Gruber, S. H. & Ashley, M. V. (2004) Reconstruction of parental microsatellite genotypes reveals female polyandry and philopatry in the lemon shark, *Negaprion brevirostris*. *Evolution,* **58,** 2332-2342.

Ferretti, F., Worm, B., Britten, G. L., Heithaus, M. R. & Lotze, H. K. (2010) Patterns and ecosystem consequences of shark declines in the ocean. *Ecology Letters,* **13,** 1055-1071.

Fichtel, C. & Manser, M. (2010) Vocal communication in social groups. *Animal Behaviour: Evolution and Mechanisms* (ed P. Kappeler), pp. 29-54. Springer Berlin Heidelberg.

Fitzpatrick, J. L., Kempster, R. M., Daly-Engel, T. S., Collin, S. P. & Evans, J. P. (2012) Assessing the potential for post-copulatory sexual selection in elasmobranchs. *Journal of Fish Biology,* **80,** 1141-1158.

Fletcher, D. J. C. & Michener, C. D. (1987) *Kin recognition in animals,* Wiley, New York.

Ford, E. (1921) A contribution to our knowledge of the life-histories of the dogfishes landed at Plymouth. *Journal of the Marine Biological Association of the United Kingdom* **12,** 468-505.

Fortuna, M. A., Gómez-Rodríguez, C. & Bascompte, J. (2006) Spatial network structure and amphibian persistence in stochastic environments. *Proceedings of the Royal Society B: Biological Sciences,* **273,** 1429-1434.

Fortuna, M. A., Popa-Lisseanu, A. G., Ibáñez, C. & Bascompte, J. (2009) The roosting spatial network of a bird-predator bat. *Ecology,* **90,** 934-944.

Frankham, R. (2005) Genetics and extinction. *Biological Conservation,* **126,** 131-140.

Franks, D., Ruxton, G. & James, R. (2010) Sampling animal association networks with the gambit of the group. *Behavioral Ecology and Sociobiology,* **64,** 493-503.

Fraser, D. J., Duchesne, P. & Bernatchez, L. (2005) Migratory charr schools exhibit population and kin associations beyond juvenile stages. *Molecular Ecology,* **14,** 3133-3146.

Freire, J. & González-Gurriarán, E. (1998) New approaches to the behavioural ecology of decapod crustaceans using telemetry and electronic tags. *Hydrobiologia,* **371-372,** 123-132.

Frére, C. H., Krützen, M., Mann, J., Connor, R. C., Bejder, L. & Sherwin, W. B. (2010) Social and genetic interactions drive fitness variation in a free-living dolphin population. *Proceedings of the National Academy of Sciences,* **107,** 19949-19954.

Frommen, J., Mehlis, M., Brendler, C. & Bakker, T. (2007) Shoaling decisions in three-spined sticklebacks (*Gasterosteus aculeatus*)—familiarity, kinship and inbreeding. *Behavioral Ecology and Sociobiology,* **61,** 533-539.

Gardiner, J., Hueter, R., Maruska, K., Sisneros, J., Casper, B., Mann, D. & Demski, L. (2012) Sensory physiology and behavior of elasmobranchs. *Biology of Sharks and Their Relatives, Second Edition* (eds J. C. Carrier, J. Musick, A. & M. R. Heithaus), pp. 349-401. CRC Press.

Garla, R., Chapman, D., Wetherbee, B. & Shivji, M. (2006) Movement patterns of young Caribbean reef sharks, *Carcharhinus perezi*, at Fernando de Noronha Archipelago, Brazil: the potential of marine protected areas for conservation of a nursery ground. *Marine Biology,* **149,** 189-199.

Garnier, D. H., Sourdaine, P. & Jégou, B. (1999) Seasonal variations in sex steroids and male sexual characteristics in *Scyliorhinus canicula*. *General and Comparative Endocrinology,* **116,** 281-290.

Gastner, M. T. & Newman, M. E. J. (2006) The spatial structure of networks. *The European Physical Journal B - Condensed Matter and Complex Systems,* **49,** 247-252.

Gerlach, G., Hodgins-Davis, A., Avolio, C. & Schunter, C. (2008) Kin recognition in zebrafish: a 24-hour window for olfactory imprinting. *Proceedings of the Royal Society B: Biological Sciences,* **275,** 2165-2170.

Gerlach, G. & Lysiak, N. (2006) Kin recognition and inbreeding avoidance in zebrafish, *Danio rerio*, is based on phenotype matching. *Animal Behaviour,* **71,** 1371-1377.

Gilman, E., Clarke, S., Brothers, N., Alfaro-Shigueto, J., Mandelman, J., Mangel, J., Petersen, S., Piovano, S., Thomson, N., Dalzell, P., Donoso, M., Goren, M. & Werner, T. (2008) Shark interactions in pelagic longline fisheries. *Marine Policy,* **32,** 1-18.

Ginsberg, J. R. & Young, T. P. (1992) Measuring association between individuals or groups in behavioural studies. *Animal Behaviour,* **44,** 377-379.

Girard, M. & Du Buit, M.-H. (1999) Reproductive biology of two deep-water sharks from the British Isles, *Centroscymnus coelolepis* and *Centrophorus squamosus* (Chondrichthyes: Squalidae). *Journal of the Marine Biological Association of the United Kingdom,* **79,** 923-931.

Godin, A. C. & Worm, B. (2010) Keeping the lead: how to strengthen shark conservation and management policies in Canada. *Marine Policy,* **34,** 995-1001.

Goodale, E., Beauchamp, G., Magrath, R. D., Nieh, J. C. & Ruxton, G. D. (2010) Interspecific information transfer influences animal community structure. *Trends in ecology & evolution,* **25,** 354-361.

Gowans, S., Whitehead, H. & Hooker, S. K. (2001) Social organization in northern bottlenose whales, *Hyperoodon ampullatus*: not driven by deep-water foraging? *Animal Behaviour,* **62,** 369-377.

Greenwood, P. J. (1980) Mating systems, philopatry and dispersal in birds and mammals. *Animal Behaviour,* **28,** 1140-1162.

Griffiths, A., Casane, D., McHugh, M., Wearmouth, V., Sims, D. & Genner, M. (2011) Characterisation of polymorphic microsatellite loci in the small-spotted catshark (*Scyliorhinus canicula* L.). *Conservation Genetics Resources,* **3,** 705-709.

Griffiths, A. M., Jacoby, D. M. P., Casane, D., McHugh, M., Croft, D. P., Genner, M. J. & Sims, D. W. (2012) First analysis of multiple paternity in an oviparous shark, the small-spotted catshark (*Scyliorhinus canicula* L.). *Journal of Heredity,* **103,** 166-173.

Griffiths, S. W. & Magurran, A. E. (1997) Familiarity in schooling fish: how long does it take to acquire? *Animal Behaviour,* **53,** 945-949.

Griffiths, S. W. & Magurran, A. E. (1999) Schooling decisions in guppies (*Poecilia reticulata*) are based on familiarity rather than kin recognition by phenotype matching. *Behavioral Ecology and Sociobiology,* **45,** 437-443.

Griffiths, S. W., Ojanguren, A. F., Orpwood, J. E., Magurran, A. E. & Armstrong, J. D. (2007) Familiarity-biased patterns of association shift with time among European minnows. *Journal of Fish Biology,* **71,** 1602-1612.

Gruber, S. H., Nelson, D. R. & Morrissey, J. F. (1988) Patterns of activity and space utilization of lemon sharks, *Negaprion brevirostris*, in a shallow Bahamian lagoon. *Bulletin of Marine Science,* **43,** 61-76.

Gubili, C., Duffy, C., Cliff, G., Wintner, S., Shivji, M., Chapman, D., Bruce, B., Martin, A. P., Sims, D. W., Jones, C. S. & Noble, L. R. (2012) Application of molecular genetics for conservation of the great white shark, *Carcharodon carcharias*, L. 1758. *Global perspectives on the biology and life history of the great white shark.* (ed M. Domeier). CRC Press, Boca Raton, FL.

Guttal, V. & Couzin, I. D. (2010) Social interactions, information use, and the evolution of collective migration. *Proceedings of the National Academy of Sciences,* **107,** 16172-16177.

Guttridge, T., van Dijk, S., Stamhuis, E., Krause, J., Gruber, S. & Brown, C. (2012b) Social learning in juvenile lemon sharks, *Negaprion brevirostris*. *Animal Cognition***,** 1-10.

Guttridge, T. L., Gruber, S. H., DiBattista, J. D., Feldheim, K. A., Croft, D. P., Krause, S. & Krause, J. (2011) Assortative interactions and leadership in a free-ranging population of juvenile lemon shark *Negaprion brevirostris*. *Marine Ecology Progress Series,* **423,** 235-245.

Guttridge, T. L., Gruber, S. H., Franks, B. R., Kessel, S. T., Gledhill, K. S., Uphill, J., Krause, J. & Sims, D. W. (2012a) Deep danger: intra-specific predation risk influences habitat use and aggregation formation of juvenile lemon sharks *Negaprion brevirostris*. *Marine Ecology Progress Series,* **445,** 279-291.

Guttridge, T. L., Gruber, S. H., Gledhill, K. S., Croft, D. P., Sims, D. W. & Krause, J. (2009a) Social preferences of juvenile lemon sharks, *Negaprion brevirostris*. *Animal Behaviour,* **78,** 543-548.

Guttridge, T. L., Gruber, S. H., Krause, J. & Sims, D. W. (2010) Novel acoustic technology for studying free-ranging shark social behaviour by recording individuals' interactions. *PLoS ONE,* **5,** e9324.

Guttridge, T. L., Myrberg, A. A., Porcher, I. F., Sims, D. W. & Krause, J. (2009b) The role of learning in shark behaviour. *Fish and Fisheries,* **10,** 450-469.

Haddadi, H., King, A., Wills, A., Fay, D., Lowe, J., Morton, A., Hailes, S. & Wilson, A. (2011) Determining association networks in social animals: choosing spatial–temporal criteria and sampling rates. *Behavioral Ecology and Sociobiology,* **65,** 1659-1668.

Hain, T. J. A. & Neff, B. D. (2007) Multiple paternity and kin recognition mechanisms in a guppy population. *Molecular Ecology,* **16,** 3938-3946.

Hall, A., J. , McConnell, B. J. & Barker, R. J. (2001) Factors affecting first-year survival in grey seals and their implications for life history strategy. *Journal of Animal Ecology,* **70,** 138-149.

Hamilton, W. D. (1964) The genetical evolution of social behaviour. II. *Journal of Theoretical Biology,* **7,** 17-52.

Hamilton, W. D. (1971) Geometry for the selfish herd. *Journal of Theoretical Biology,* **31,** 295-311.

Hamlett, W. C., Knight, D. P., Pereira, F. T. V., Steele, J. & Sever, D. M. (2005) Oviducal glands in chondrichthyans. *Reproductive biology and phylogeny of chondrichthyes* (ed W. C. Hamlett), pp. 301-336. Science Publishers Inc, Enfield, NH.

Harris, J. E. (1952) A note on the breeding season, sex ratio and embryonic development of the dogfish *Scyliorhinus canicula* (L.). *Journal of the Marine Biological Association of the United Kingdom,* **31,** 269-275.

Harrison, F., Sciberras, J. & James, R. (2011) Strength of social tie predicts cooperative investment in a human social network. *PLoS ONE,* **6,** e18338.

Hass, C. C. (2002) Home-range dynamics of white-nosed coatis in Southeastern Arizona. *Journal of Mammalogy,* **83,** 934-946.

Hatchwell, B. J. (2010) Cryptic kin selection: kin structure in vertebrate populations and opportunities for kin-directed cooperation. *Ethology,* **116,** 203-216.

Hearn, A., Ketchum, J., Klimley, A., Espinoza, E. & Peñaherrera, C. (2010) Hotspots within hotspots? Hammerhead shark movements around Wolf Island, Galapagos Marine Reserve. *Marine Biology,* **157,** 1899-1915.

Heggenes, J., Krog, O. M. W., Lindas, O. R., Dokk, J. G. & Bremnes, T. (1993) Homeostatic behavioural responses in a changing environment: brown trout (*Salmo trutta*) become nocturnal during winter. *Journal of Animal Ecology,* **62,** 295-308.

Heithaus, M. R., Frid, A., Wirsing, A. J. & Worm, B. (2008) Predicting ecological consequences of marine top predator declines. *Trends in Ecology & Evolution,* **23,** 202-210.

Heithaus, M. R., Wirsing, A. J., Burkholder, D., Thomson, J. & Dill, L. M. (2009) Towards a predictive framework for predator risk effects: the interaction of landscape features and prey escape tactics. *Journal of Animal Ecology,* **78,** 556-562.

Helfman, G. S. & Schultz, E. T. (1984) Social transmission of behavioural traditions in a coral reef fish. *Animal Behaviour,* **32,** 379-384.

Henningsen, A. D. (1994) Tonic immobility in 12 elasmobranchs: use as an aid in captive husbandry. *Zoo Biology,* **13,** 325-332.

Herfindal, I., Linnell, J. D. C., Odden, J., Nilsen, E. B. & Andersen, R. (2005) Prey density, environmental productivity and home-range size in the Eurasian lynx (*Lynx lynx*). *Journal of Zoology,* **265,** 63-71.

Herskin, J. & Steffensen, J. F. (1998) Energy savings in sea bass swimming in a school: measurements of tail beat frequency and oxygen consumption at different swimming speeds. *Journal of Fish Biology,* **53,** 366-376.

Heupel, M. R., Carlson, J. K. & Simpfendorfer, C., A. (2007) Shark nursery areas: concepts, definition, characterization and assumptions. *Marine Ecology Progress Series,* **337,** 287-297.

Heupel, M. R. & Simpfendorfer, C. (2005a) Quantitative analysis of aggregation behavior in juvenile blacktip sharks. *Marine Biology,* **147,** 1239-1249.

Heupel, M. R. & Simpfendorfer, C. A. (2005b) Using acoustic monitoring to evaluate MPAs for shark nursery areas: the importance of long-term data. *Marine Technology Society Journal,* **39,** 10-18.

Heupel, M. R., Semmens, J. M. & Hobday, A. J. (2006) Automated acoustic tracking of aquatic animals: scales, design and deployment of listening station arrays. *Marine and Freshwater Research,* **57,** 1-13.

Heupel, M. R., Simpfendorfer, C. A. & Hueter, R. E. (2004) Estimation of shark home ranges using passive monitoring techniques. *Environmental Biology of Fishes,* **71,** 135-142.

Heupel, M. R., Yeiser, B. G., Collins, A. B., Ortega, L. & Simpfendorfer, C. A. (2010) Long-term presence and movement patterns of juvenile bull sharks, *Carcharhinus leucas*, in an estuarine river system. *Marine and Freshwater Research,* **61,** 1-10.

Hibler, T. L. & Houde, A. E. (2006) The effect of visual obstructions on the sexual behaviour of guppies: the importance of privacy. *Animal Behaviour,* **72,** 959-964.

Hight, B. V. & Lowe, C. G. (2007) Elevated body temperatures of adult female leopard sharks, *Triakis semifasciata*, while aggregating in shallow nearshore embayments: evidence for behavioral thermoregulation? *Journal of Experimental Marine Biology and Ecology,* **352,** 114-128.

Hoare, D. J., Couzin, I. D., Godin, J. G. J. & Krause, J. (2004) Context-dependent group size choice in fish. *Animal Behaviour,* **67,** 155-164.

Hoare, D. J., Krause, J., Peuhkuri, N. & Godin, J. G. J. (2000) Body size and shoaling in fish. *Journal of Fish Biology,* **57,** 1351-1366.

Hoelzel, A. R. (1991) Killer whale predation on marine mammals at Punta Norte, Argentina; food sharing, provisioning and foraging strategy. *Behavioral Ecology and Sociobiology,* **29,** 197-204.

Höjesjö, J., Johnsson, J. I., Petersson, E. & Jarvi, T. (1998) The importance of being familiar: individual recognition and social behavior in sea trout (Salmo trutta). *Behavioral Ecology,* **9,** 445-451.

Holland, B. & Rice, W. R. (1998) Chase-away sexual selection: antagonistic seduction versus resistance. *Evolution,* **52,** 1-7.

Holland, K. N., Meyer, C. G. & Dagorn, L. C. (2009) Inter-animal telemetry: results from first deployment of acoustic 'business card' tags. *Endangered Species Research,* **10,** 287-293.

Holland, K. N., Wetherbee, B. M., Peterson, J. D. & Lowe, C. G. (1993) Movements and distribution of hammerhead shark pups on their natal grounds. *Copeia,* **1993,** 495-502.

Hood, G. M. (2010) PopTools version 3.2.5.

Hueter, R. E., Heupel, M. R., Heist, E. J. & Keeney, D. B. (2005) Evidence of philopatry in sharks and implications for the management of shark fisheries. *Journal of Northwest Atlantic Fishery Science,* **35,** 239-247.

Hulbert, L. B., Aires-da-Silva, A. M., Gallucci, V. F. & Rice, J. S. (2005) Seasonal foraging movements and migratory patterns of female *Lamna ditropis* tagged in Prince William Sound, Alaska. *Journal of Fish Biology,* **67,** 490-509.

Jacoby, D. M. P., Busawon, D. S. & Sims, D. W. (2010) Sex and social networking: the influence of male presence on social structure of female shark groups. *Behavioral Ecology,* **21,** 808-818.

Jirik, K. E. & Lowe, C. G. (2012) An elasmobranch maternity ward: female round stingrays *Urobatis halleri* use warm, restored estuarine habitat during gestation. *Journal of Fish Biology,* **80,** 1227-1245.

Johnson, D. D. P., Kays, R., Blackwell, P. G. & Macdonald, D. W. (2002) Does the resource dispersion hypothesis explain group living? *Trends in Ecology & Evolution,* **17,** 563-570.

Jones, A. G. (2005) GERUD 2.0: a computer program for the reconstruction of parental genotypes from half-sib progeny arrays with known or unknown parents. *Molecular Ecology Notes,* **5,** 708-711.

Jones, O. R. & Wang, J. (2010) COLONY: a program for parentage and sibship inference from multilocus genotype data. *Molecular Ecology Resources,* **10,** 551-555.

Jordan, L. A., Wong, M. Y. L. & Balshine, S. S. (2009) The effects of familiarity and social hierarchy on group membership decisions in a social fish. *Biology Letters,* **6,** 301-303.

Jorgensen, S. J., Reeb, C. A., Chapple, T. K., Anderson, S., Perle, C., Van Sommeran, S. R., Fritz-Cope, C., Brown, A. C., Klimley, A. P. & Block, B. A. (2009) Philopatry and migration of Pacific white sharks. *Proceedings of the Royal Society B: Biological Sciences,* **277,** 679-688.

Kaluza, P., Kölzsch, A., Gastner, M. T. & Blasius, B. (2010) The complex network of global cargo ship movements. *Journal of The Royal Society Interface,* **7,** 1093 - 1103.

Kelley, J. L., Morrell, L. J., Inskip, C., Krause, J. & Croft, D. P. (2011) Predation risk shapes social networks in fission-fusion populations. *PLoS ONE,* **6,** e24280.

Kimber, J. A., Sims, D. W., Bellamy, P. H. & Gill, A. B. (2009) Male-female interactions affect foraging behaviour within groups of small-spotted catshark, *Scyliorhinus canicula*. *Animal Behaviour,* **77,** 1435-1440.

Klimley, A. P. (1985) Schooling in *Sphyrna lewini*, a species with low-risk of predation - a non-egalitarian state. *Zeitschrift Fur Tierpsychologie-Journal of Comparative Ethology,* **70,** 297-319.

Klimley, A. P. (1987) The determinants of sexual segregation in the scalloped hammerhead shark, *Sphyrna lewini*. *Environmental Biology of Fishes,* **18,** 27-40.

Klimley, A. P. & Nelson, D. R. (1984) Diel movement patterns of the scalloped hammerhead shark (*Sphyrna lewini*) in relation to El Bajo Espiritu Santo: a refuging central-position social system. *Behavioral Ecology and Sociobiology,* **15,** 45-54.

Knip, D. M., Heupal, M. R. & Simpfendorfer, C. (2010) Sharks in nearshore environments: models, importance and consequences. *Marine Ecology Progress Series,* **402,** 1-11.

Knip, D. M., Heupel, M. R., Simpfendorfer, C. A., Tobin, A. J. & Moloney, J. (2011) Ontogenetic shifts in movement and habitat use of juvenile pigeye sharks

Carcharhinus amboinensis in a tropical nearshore region. *Marine Ecology Progress Series,* **425,** 233-246.

Krause, J., Butlin, R. K., Peuhkuri, N. & Pritchard, V. L. (2000a) The social organization of fish shoals: a test of the predictive power of laboratory experiments for the field. *Biological Reviews,* **75,** 477-501.

Krause, J., Cheng, D. J. S., Kirkman, E. & Ruxton, G. D. (2000b) Species-specific patterns of refuge use in fish: the role of metabolic expenditure and body length. *Behaviour,* **137,** 1113-1127.

Krause, J., Croft, D. & James, R. (2007) Social network theory in the behavioural sciences: potential applications. *Behavioral Ecology and Sociobiology,* **62,** 15-27.

Krause, J. & Godin, J.-G. J. (1994) Shoal choice in the banded killifish (*Fundulus diaphanus*, Teleostei, Cyprinodontidae): effects of predation risk, fish size, species composition and size of shoals. *Ethology,* **98,** 128-136.

Krause, J., James, R. & Croft, D. P. (2010) Personality in the context of social networks. *Philosophical Transactions of the Royal Society B: Biological Sciences,* **365,** 4099-4106.

Krause, J., Lusseau, D. & James, R. (2009) Animal social networks: an introduction. *Behavioral Ecology and Sociobiology,* **63,** 967-973.

Krause, J. & Ruxton, G. D. (2002) *Living in Groups,* Oxford University Press, Oxford.

Krause, J., Wilson, A. D. M. & Croft, D. P. (2011) New technology facilitates the study of social networks. *Trends in Ecology & Evolution,* **26,** 5-6.

Lachlan, R. F., Crooks, L. & Laland, K. N. (1998) Who follows whom? Shoaling preferences and social learning of foraging information in guppies. *Animal Behaviour,* **56,** 181-190.

Lage, C. R., Petersen, C. W., Forest, D., Barnes, D., Kornfield, I. & Wray, C. (2008) Evidence of multiple paternity in spiny dogfish (*Squalus acanthias*) broods based on microsatellite analysis. *Journal of Fish Biology,* **73,** 2068-2074.

Larson, S., Christiansen, J., Griffing, D., Ashe, J., Lowry, D. & Andrews, K. (2011) Relatedness and polyandry of sixgill sharks, *Hexanchus griseus*, in an urban estuary. *Conservation Genetics,* **12,** 679-690.

Lee, P. L. M. & Hays, G. C. (2004) Polyandry in a marine turtle: females make the best of a bad job. *Proceedings of the National Academy of Sciences of the United States of America,* **101,** 6530-6535.

Lehmann, J. & Dunbar, R. I. M. (2009) Network cohesion, group size and neocortex size in female-bonded Old World primates. *Proceedings of the Royal Society B: Biological Sciences,* **276,** 4417-4422.

Leigh-Sharpe, W. H. (1926) The comparative morphology of the secondary sexual characters of elasmobranch fishes. The claspers, clasper siphons, and clasper glands. Memoir X. *Journal of Morphology,* **42,** 335-348.

Lessells, C. M. & Boag, P. T. (1987) Unrepeatable repeatabilities: a common mistake. *The Auk,* **104,** 116-121.

Li, B., Chen, C., Ji, W. & Ren, B. (2000) Seasonal home range changes of the Sichuan snub-nosed monkey (*Rhinopithecus roxellana*) in the Qinling Mountains of China. *Folia Primatologica,* **71,** 375-386.

Litvinov, F. (2006) On the role of dense aggregations of males and juveniles in the functional structure of the range of the blue shark *Prionace glauca*. *Journal of Ichthyology,* **46,** 613-624.

Liu, K. & Muse, S. V. (2005) PowerMarker: an integrated analysis environment for genetic marker analysis. *Bioinformatics,* **21,** 2128-2129.

Lürling, M. & Scheffer, M. (2007) Info-disruption: pollution and the transfer of chemical information between organisms. *Trends in Ecology and Evolution,* **22,** 374-379.

Lusseau, D. (2003) The emergent properties of a dolphin social network. *Proceedings of the Royal Society of London. Series B: Biological Sciences,* **270,** S186-S188.

Lusseau, D., Whitehead, H. & Gero, S. (2008) Incorporating uncertainty into the study of animal social networks. *Animal Behaviour,* **75,** 1809-1815.

Lusseau, D., Wilson, B., Hammond, P. S., Grellier, K., Durban, J. W., Parsons, K. M., Barton, T. R. & Thompson, P. M. (2006) Quantifying the influence of sociality on population structure in bottlenose dolphins. *Journal of Animal Ecology,* **75,** 14-24.

Magurran, A. E. (1990) The adaptive significance of schooling as an anti-predator defence in fish. *Annales Zoologici Fennici,* **27,** 51-66.

Magurran, A. E., Seghers, B. H., Shaw, P. W. & Carvalho, G. R. (1994) Schooling preferences for familiar fish in the guppy, *Poecilia reticulata. Journal of Fish Biology,* **45,** 401-406.

Malakoff, D. (2004) New tools reveal treasures at ocean hot spots. *Science,* **304,** 1104-1105.

Manly, B. F. J. (1995) A note on the analysis of species co-occurrences. *Ecology,* **76,** 1109-1115.

Manly, B. F. J. (2006) *Randomization, Bootstrap and Monte Carlo Methods in Biology,* Chapman & Hall/CRC, Boca Raton, FL.

Mann, K. H. (2000) *Ecology of coastal waters with implications for management,* Blackwell Science, Malden, MA.

Martin, R. A. (2007) A review of behavioural ecology of whale sharks (*Rhincodon typus*). *Fisheries Research,* **84,** 10-16.

Martin, R. A., Rossmo, D. K. & Hammerschlag, N. (2009) Hunting patterns and geographic profiling of white shark predation. *Journal of Zoology,* **279,** 111-118.

Mateo, J. M. (2004) Recognition systems and biological organization: the perception component of social recognition. *Annales Zoologici Fennici,* **41,** 729-745.

Mateo, J. M. (2010) Self-referent phenotype matching and long-term maintenance of kin recognition. *Animal Behaviour,* **80,** 929-935.

McCleave, J. D., Power, J. H. & Rommel, S. A. (1978) Use of radio telemetry for studying upriver migration of adult Atlantic salmon (*Salmo salar*). *Journal of Fish Biology,* **12,** 549-558.

McHugh, M., Sims, D. W., Partridge, J. C. & Genner, M. J. (2011) A century later: long-term change of an inshore temperate marine fish assemblage. *Journal of Sea Research,* **65,** 187-194.

McKibben, J. N. & Nelson, D. R. (1986) Patterns of movement and grouping of gray reef sharks, *Carcharhinus amblyrhynchos*, at Enewetak, Marshall Islands. *Bulletin of Marine Science,* **38,** 89-110.

McLaughlin, R. H. & O'Gower, A. K. (1971) Life history and underwater studies of a Heterodont shark. *Ecological Monographs,* **41,** 271-289.

Metten, H. (1939) Studies on the reproduction of the dogfish. *Philosophical Transactions of the Royal Society of London,* **230,** 217-238.

Morales, J. M., Moorcroft, P. R., Matthiopoulos, J., Frair, J. L., Kie, J. G., Powell, R. A., Merrill, E. H. & Haydon, D. T. (2010) Building the bridge between animal movement and population dynamics. *Philosophical Transactions of the Royal Society B: Biological Sciences,* **365,** 2289-2301.

Morrell, L. J., Croft, D. P., Dyer, J. R. G., Chapman, B. B., Kelley, J. L., Laland, K. N. & Krause, J. (2008) Association patterns and foraging behaviour in natural and artificial guppy shoals. *Animal Behaviour,* **76,** 855-864.

Morrissey, J. F. & Gruber, S. H. (1993) Habitat selection by juvenile lemon sharks, *Negaprion brevirostris*. *Environmental Biology of Fishes,* **38,** 311-319.

Mourier, J., Vercelloni, J. & Planes, S. (2012) Evidence of social communities in a spatially structured network of a free-ranging shark species. *Animal Behaviour,* **83,** 389-401.

Mucientes, G. R., Queiroz, N., Sousa, L. L., Tarroso, P. & Sims, D. W. (2009) Sexual segregation of pelagic sharks and the potential threat from fisheries. *Biology Letters,* **5,** 156-159.

Myers, R. A., Baum, J. K., Shepherd, T. D., Powers, S. P. & Peterson, C. H. (2007) Cascading effects of the loss of apex predatory sharks from a coastal ocean. *Science,* **315,** 1846-1850.

Myers, R. A. & Worm, B. (2003) Rapid worldwide depletion of predatory fish communities. *Nature,* **423,** 280-283.

Myrberg, A. A., Jr. & Gruber, S. H. (1974) The behavior of the bonnethead shark, *Sphyrna tiburo*. *Copeia,* **1974,** 358-374.

Natoli, A., Birkun, A., Aguilar, A., Lopez, A. & Hoelzel, A. R. (2005) Habitat structure and the dispersal of male and female bottlenose dolphins (*Tursiops truncatus*). *Proceedings of the Royal Society B: Biological Sciences,* **272,** 1217-1226.

Neff, B. D. & Pitcher, T. E. (2002) Assessing the statistical power of genetic analyses to detect multiple mating in fishes. *Journal of Fish Biology,* **61,** 739-750.

Newman, M. E. J. (2006) Modularity and community structure in networks. *Proceedings of the National Academy of Sciences,* **103,** 8577-8582.

Niwa, H.-S. (2003) Power-law versus exponential distributions of animal group sizes. *Journal of Theoretical Biology,* **224,** 451-457.

Northcutt, R. G. (1977) Elasmobranch central nervous system organization and its possible evolutionary significance. *American Zoologist,* **17,** 411-429.

Nowak, M. A., Tarnita, C. E. & Wilson, E. O. (2010) The evolution of eusociality. *Nature,* **466,** 1057-1062.

Olsén, K. H., Grahn, M., Lohm, J. & Langefors, Å. (1998) MHC and kin discrimination in juvenile Arctic charr, *Salvelinus alpines* (L.). *Animal Behaviour,* **56,** 319-327.

Orpwood, J. E., Magurran, A. E., Armstrong, J. D. & Griffiths, S. W. (2008) Minnows and the selfish herd: effects of predation risk on shoaling behaviour are dependent on habitat complexity. *Animal Behaviour,* **76,** 143-152.

O'Toole, A., Danylchuk, A., Goldberg, T., Suski, C., Philipp, D., Brooks, E. & Cooke, S. (2011) Spatial ecology and residency patterns of adult great barracuda (*Sphyraena barracuda*) in coastal waters of The Bahamas. *Marine Biology,* **158,** 2227-2237.

Park, H. W. (2003) Hyperlink network analysis: a new method for the study of social structure on the web. *Connections,* **25,** 49-61.

Patterson, T. A., Thomas, L., Wilcox, C., Ovaskainen, O. & Matthiopoulos, J. (2008) State-space models of individual animal movement. *Trends in Ecology & Evolution,* **23,** 87-94.

Pauly, D., Christensen, V., Guenette, S., Pitcher, T. J., Sumaila, U. R., Walters, C. J., Watson, R. & Zeller, D. (2002) Towards sustainability in world fisheries. *Nature,* **418,** 689-695.

Peakall, R. O. D. & Smouse, P. E. (2006) genalex 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes,* **6,** 288-295.

Perkins, S. E., Cagnacci, F., Stradiotto, A., Arnoldi, D. & Hudson, P. J. (2009) Comparison of social networks derived from ecological data: implications for inferring infectious disease dynamics. *Journal of Animal Ecology,* **78,** 1015-1022.

Peuhkuri, N. & Seppä, P. (1998) Do three-spined sticklebacks group with kin? *Annales Zoologici Fennici,* **35,** 21-27.

Piegorsch, W. W. & Bailer, A. J. (2005) *Analyzing Environmental Data,* Wiley.

Pike, T. W., Samanta, M., Lindström, J. & Royle, N. J. (2008) Behavioural phenotype affects social interactions in an animal network. *Proceedings of the Royal Society B: Biological Sciences,* **275,** 2515-2520.

Pinter-Wollman, N., Isbell, L. A. & Hart, L. A. (2009) The relationship between social behaviour and habitat familiarity in African elephants (*Loxodonta africana*). *Proceedings of the Royal Society B: Biological Sciences,* **276,** 1009-1014.

Pitcher, T. J. (1983) Heuristic definitions of fish shoaling behaviour. *Animal Behaviour,* **31,** 611-613.

Pitcher, T. J., Lang, S. H. & Turner, J. A. (1988) A risk-balancing trade off between foraging rewards and predation hazard in a shoaling fish. *Behavioral Ecology and Sociobiology,* **22,** 225-228.

Pitcher, T. J. & Parrish, J. K. (1993) Functions of shoaling behaviour in teleosts. *Behaviour of teleost fishes* (ed T. J. Pitcher), pp. 363-469. Chapman & Hall, London.

Porter, M. E. (2000) Location, competition, and economic development: local clusters in a global economy. *Economic Development Quarterly,* **14,** 15-34.

Portnoy, D., S., Piercy, A., N., Musick, J., A., Burgess, G., H. & Graves, J., E. (2007) Genetic polyandry and sexual conflict in the sandbar shark, *Carcharhinus plumbeus*, in the western North Atlantic and Gulf of Mexico. *Molecular Ecology,* **16,** 187-197.

Pouyaud, L., Desmarais, E., Chenuil, A., Agnese, J. F. & Bonhomme, F. (1999) Kin cohesiveness and possible inbreeding in the mouthbrooding tilapia *Sarotherodon melanotheron* (Pisces Cichlidae). *Molecular Ecology,* **8,** 803-812.

Powter, D. M. & Gladstone, W. (2009) Habitat-mediated use of space by juvenile and mating adult Port Jackson sharks, *Heterodontus portusjacksoni*, in Eastern Australia. *Pacific Science,* **63,** 1-14.

Pratt, H. L. & Carrier, J. C. (2001) A review of elasmobranch reproductive behavior with a case study on the nurse shark, *Ginglymostoma Cirratum*. *Environmental Biology of Fishes,* **60,** 157-188.

Pratt, H. L. & Carrier, J. C. (2005) Elasmobranch courtship and mating behaviour. *Reproductive biology and phylogeny of chondrichthyes* (ed W. C. Hamlett), pp. 129-170. Science Publishers Inc, Enfield, NH.

Queiroz, N., Humphries, N. E., Noble, L. R., Santos, A. M. & Sims, D. W. (2010) Short-term movements and diving behaviour of satellite-tracked blue sharks *Prionace glauca* in the northeastern Atlantic Ocean. *Marine Ecology Progress Series,* **406,** 265-279.

Queiroz, N., Humphries, N. E., Noble, L. R., Santos, A. n. M. & Sims, D. W. (2012) Spatial dynamics and expanded vertical niche of blue sharks in oceanographic fronts reveal habitat targets for conservation. *PLoS ONE,* **7,** e32374.

Queller, D. C. & Goodnight, K. F. (1989) Estimating relatedness using genetic markers. *Evolution,* **43,** 258-275.

Quin, D. G., Smith, A. P., Green, S. W. & Hines, H. B. (1992) Estimating the home ranges of sugar gliders (*Petaurus breviceps*) (Marsupialia: Petauridae), from grid-trapping and radiotelemetry. *Wildlife Research,* **19,** 471-487.

Rangeley, R. W. & Kramer, D. L. (1998) Density-dependent antipredator tactics and habitat selection in juvenile pollock. *Ecology,* **79,** 943-952.

Revill, A. S., Dulvy, N. K. & Holst, R. (2005) The survival of discarded lesser-spotted dogfish (*Scyliorhinus canicula*) in the Western English Channel beam trawl fishery. *Fisheries Research,* **71,** 121-124.

Rhodes, M., Wardell-Johnson, G. W., Rhodes, M. P. & Raymond, B. (2006) Applying network analysis to the conservation of habitat trees in urban environments: a case study from Brisbane, Australia. *Conservation Biology,* **20,** 861-870.

Rice, W. R. (1989) Analyzing tables of statistical tests. *Evolution,* **43,** 223-225.

Riley, M. J., Hale, M. S., Harman, A. & Rees, R. G. (2010) Analysis of whale shark *Rhincodon typus* aggregations near South Ari Atoll, Maldives Archipelago. *Aquatic Biology,* **8,** 145-150.

Rives, A. W. & Galitski, T. (2003) Modular organization of cellular networks. *Proceedings of the National Academy of Sciences,* **100,** 1128-1133.

Robbins, R. L. (2007) Environmental variables affecting the sexual segregation of great white sharks *Carcharodon carcharias* at the Neptune Islands South Australia. *Journal of Fish Biology,* **70,** 1350-1364.

Robbins, W. D., Hisano, M., Connolly, S. R. & Choat, J. H. (2006) Ongoing collapse of coral-reef shark populations. *Current Biology,* **16,** 2314-2319.

Roberts, C. M. & Sargant, H. (2002) Fishery benefits of fully protected marine reserves: why habitat and behavior are important. *Natural Resource Modelling,* **15,** 487-507.

Rodríguez-Cabello, C., Sánchez, F., Fernández, A. & Olaso, I. (2004) Is the lesser spotted dogfish (*Scyliorhinus canicula*) population from the Cantabrian Sea a unique stock? *Fisheries Research,* **69,** 57-71.

Rodríguez-Cabello, C., Sánchez, F. & Olaso, I. (2007) Distribution patterns and sexual segregations of *Scyliorhinus canicula* (L.) in the Cantabrian Sea. *Journal of Fish Biology,* **70,** 1568-1586.

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

Rousset, F. (2008) Genepop’007: a complete re-implementation of the genepop software for Windows and Linux. *Molecular Ecology Resources,* **8,** 103-106.

Rowat, D., Meekan, M., Engelhardt, U., Pardigon, B. & Vely, M. (2007) Aggregations of juvenile whale sharks (*Rhincodon typus*) in the Gulf of Tadjoura, Djibouti. *Environmental Biology of Fishes,* **80,** 465-472.

Ruckstuhl, K. E. & Clutton-Brock, T. H. (2005) Sexual segregation and the ecology of the two sexes. *Sexual segregation in vertebrates: ecology of the two sexes* (eds K. E. Ruckstuhl & P. Neuhaus). Cambridge Univeristy Press, Cambridge, UK.

Ruckstuhl, K. E. & Neuhaus, P. (2002) Sexual segregation in ungulates: a comparative test of three hypotheses. *Biological Reviews,* **77,** 77-96.

Rutz, C., Burns, Z. T., James, R., Ismar, S. M. H., Burt, J., Otis, B., Bowen, J. & St Clair, J. J. H. (2012) Automated mapping of social networks in wild birds. *Current Biology,* **22,** R669-R671.

Rutz, C. & Hays, G. C. (2009) New frontiers in biologging science. *Biology Letters,* **5,** 289-292.

Saville, K. J., Lindley, A. M., Maries, E. G., Carrier, J. C. & Pratt, H. L. (2002) Multiple paternity in the nurse shark, *Ginglymostoma Cirratum*. *Environmental Biology of Fishes,* **63,** 347-351.

Schmidt, J. V., Chen, C. C., Sheikh, S. I., Meekan, M. G., Norman, B. M. & Joung, S. J. (2010) Paternity analysis in a litter of whale shark embryos. *Endangered Species Research,* **12,** 117-124.

Scott, G. W., Gibbs, K. & Holding, J. (1997) Group 'resting' behaviour in a population of captive bull huss (*Scyliorhinus stellaris*). *Aquarium Sciences and Conservation,* **1,** 251-254.

Sefc, K. M. & Koblmüller, S. (2009) Assessing parent numbers from offspring genotypes: the importance of marker polymorphism. *Journal of Heredity,* **100,** 197-205.

Semeniuk, C. A. D. & Dill, L. M. (2006) Anti-predator benefits of mixed-species groups of cowtail stingrays (*Pastinachus sephen*) and whiprays (*Himantura uarnak*) at rest. *Ethology,* **112,** 33-43.

Semeniuk, C. A. D. & Rothley, K. D. (2008) Costs of group-living for a normally solitary forager: effects of provisioning tourism on southern stingrays *Dasyatis americana*. *Marine Ecology Progress Series,* **357,** 271-282.

Sih, A. (1987) Prey refuges and predator-prey stability. *Theoretical Population Biology,* **31,** 1-12.

Sih, A., Bell, A. M., Johnson, J. C. & Ziemba, R. E. (2004) Behavioral syndromes: an integrative overview. *The Quarterly Review of Biology,* **79,** 241-277.

Sih, A., Hanser, S. & McHugh, K. (2009) Social network theory: new insights and issues for behavioral ecologists. *Behavioral Ecology and Sociobiology,* **63,** 975-988.

Silk, J. B. (2002) Kin selection in primate groups. *International Journal of Primatology,* **23,** 849-875.

Silliman, W. R. & Gruber, S. H. (1999) Behavioral biology of the spotted eagle ray, *Aetobatus narinari* (Euphrasen, 1790), in Bimini, Bahamas; an interim report. *Bahamas Journal of Science,* **7,** 13-20.

Simpfendorfer, C. A., Goodreid, A. B. & McAuley, R. B. (2001) Size, sex and geographic variation in the diet of the tiger shark, *Galeocerdo Cuvier*, from Western Australian waters. *Environmental Biology of Fishes,* **61,** 37-46.

Simpfendorfer, C. A. & Milward, N. E. (1993) Utilisation of a tropical bay as a nursery area by sharks of the families Carcharhinidae and Sphyrnidae. *Environmental Biology of Fishes,* **37,** 337-345.

Sims, D. W. (2003) Tractable models for testing theories about natural strategies: foraging behaviour and habitat selection of free-ranging sharks. *Journal of Fish Biology,* **63,** 53-73.

Sims, D. W. (2005) Differences in habitat selection and reproductive strategies of male and female sharks. *Sexual segregation in vertebrates: ecology of the two sexes* (eds K. E. Ruckstuhl & P. Neuhaus). Cambridge University Press, Cambridge, UK.

Sims, D. W. (2008) Chapter 3 Sieving a living: a review of the biology, ecology and conservation status of the plankton-feeding basking shark *Cetorhinus Maximus*. *Advances in Marine Biology*, pp. 171-220. Academic Press.

Sims, D. W. (2010) Tracking and analysis techniques for understanding free-ranging shark movements and behavior. *Sharks and their relatives II. Biodiversity, adaptive physiology, and conservation* (eds J. C. Carrier, J. Musick, A. & M. R. Heithaus), pp. 351-392. CRC Press Taylor & Francis Group, Boca Raton, FL.

Sims, D. W. & Davies, S. J. (1994) Does specific dynamic action (SDA) regulate return of appetite in the lesser spotted dogfish, *Scyliorhinus canicula*? *Journal of Fish Biology,* **45,** 341-348.

Sims, D. W., Davies, S. J. & Bone, Q. (1993) On the diel rhythms in metabolism and activity of post-hatching lesser spotted dogfish, *Scyliorhinus canicula*. *Journal of Fish Biology,* **43,** 749-754.

Sims, D., Nash, J. & Morritt, D. (2001) Movements and activity of male and female dogfish in a tidal sea lough: alternative behavioural strategies and apparent sexual segregation. *Marine Biology,* **139,** 1165-1175.

Sims, D. W., Southall, E. J., Humphries, N. E., Hays, G. C., Bradshaw, C. J. A., Pitchford, J. W., James, A., Ahmed, M. Z., Brierley, A. S., Hindell, M. A., Morritt, D., Musyl, M. K., Righton, D., Shepard, E. L. C., Wearmouth, V. J., Wilson, R. P., Witt, M. J. & Metcalfe, J. D. (2008) Scaling laws of marine predator search behaviour. *Nature,* **451,** 1098-1102.

Sims, D. W., Southall, E. J., Quayle, V. A. & Fox, A. M. (2000) Annual social behaviour of basking sharks associated with coastal front areas. *Proceedings of the Royal Society B: Biological Sciences,* **267,** 1897-1904.

Sims, D. W., Southall, E. J., Wearmouth, V. J., Hutchinson, N., Budd, G. C. & Morritt, D. (2005) Refuging behaviour in the nursehound *Scyliorhinus stellaris* (Chondrichthyes: Elasmobranchii): preliminary evidence from acoustic telemetry. *Journal of the Marine Biological Association of the UK,* **85,** 1137-1140.

Sims, D. W., Wearmouth, V. J., Southall, E. J., Hill, J. M., Moore, P., Rawlinson, K., Hutchinson, N., Budd, G. C., Righton, D., Metcalfe, J. D., Nash, J. P. & Morritt, D.

(2006) Hunt warm, rest cool: bioenergetic strategy underlying diel vertical migration of a benthic shark. *Journal of Animal Ecology,* **75,** 176-190.

Soisalo, M. K. & Cavalcanti, S. M. C. (2006) Estimating the density of a jaguar population in the Brazilian Pantanal using camera-traps and capture-recapture sampling in combination with GPS radio-telemetry. *Biological Conservation,* **129,** 487-496.

Song, C., Qu, Z., Blumm, N. & Barabasi, A.-L. (2010) Limits of predictability in human mobility. *Science,* **327,** 1018-1021.

Southwood, T. R. E. & Henderson, P. A. (2000) *Ecological Methods,* Blackwell Science Ltd, Oxford.

Speed, C. W., Field, I. C., Meekan, M. G. & Bradshaw, C. J. A. (2010) Complexities of coastal shark movements and their implications for management. *Marine Ecology Progress Series,* **408,** 275-293.

Speed, C. W., Meekan, M. G., Field, I. C., McMahon, C. R., Stevens, J. D., McGregor, F., Huveneers, C., Berger, Y. & Bradshaw, C. J. A. (2011) Spatial and temporal movement patterns of a multi-species coastal reef shark aggregation. *Marine Ecology Progress Series,* **429,** 261-275.

Sperone, E., Micarelli, P., Andreotti, S., Spinetti, S., Andreani, A., Serena, F., Brunelli, E. & Tripepi, S. (2010) Social interactions among bait-attracted white sharks at Dyer Island (South Africa). *Marine Biology Research,* **6,** 408 - 414.

Springer, S. (1967) Social organization of shark populations. *Sharks, Skates and Rays* (eds P. W. Gilbert, R. F. Mathewson & D. P. Rall), pp. 149 - 174. John Hopkins Press, Baltimore.

Stamps, J. A., Briffa, M. & Biro, P. A. (2012) Unpredictable animals: individual differences in intraindividual variability (IIV). *Animal Behaviour,* **83,** 1325-1334.

Steven, G. A. (1932) Rays and skates of Devon and Cornwall. II. A study of the fishery; with notes on the occurrence, migrations and habits of the species. *Journal of the Marine Biological Association of the United Kingdom,* **18,** 1-33.

Stevenson-Hinde, J., Stillwell-Barnes, R. & Zunz, M. (1980) Individual differences in young rhesus monkeys: consistency and change. *Primates,* **21,** 498-509.

Sumpter, D. J. T. (2006) The principles of collective animal behaviour. *Philosophical Transactions of the Royal Society B: Biological Sciences,* **361,** 5-22.

Sumpter, D. J. T. (2009) *Collective Animal Behavior,* Princeton University Press, Princeton.

Sumpter, J. P. & Dodd, J. M. (1979) The annual reproductive cycle of the female lesser spotted dogfish, *Scyliorhinus canicula* L., and its endocrine control. *Journal of Fish Biology,* **15,** 687-695.

Svartberg, K. & Forkman, B. (2002) Personality traits in the domestic dog (*Canis familiaris*). *Applied Animal Behaviour Science,* **79,** 133-155.

Swaney, W., Kendal, J., Capon, H., Brown, C. & Laland, K. N. (2001) Familiarity facilitates social learning of foraging behaviour in the guppy. *Animal Behaviour,* **62,** 591-598.

Tanner, C. J. & Jackson, A. L. (2012) Social structure emerges via the interaction between local ecology and individual behaviour. *Journal of Animal Ecology,* **81,** 260-267.

Theberge, M. M. & Dearden, P. (2006) Detecting a decline in whale shark *Rhincodon typus* sightings in the Andaman Sea, Thailand, using ecotourist operator-collected data. *Oryx,* **40,** 337-342.

Tibbetts, E. A. & Dale, J. (2007) Individual recognition: it is good to be different. *Trends in Ecology & Evolution,* **22,** 529-537.

Tillett, B. J., Meekan, M. G., Field, I. C., Thorburn, D. C. & Ovenden, J. R. (2012) Evidence for reproductive philopatry in the bull shark *Carcharhinus leucas*. *Journal of Fish Biology,* **80,** 2140-2158.

Topelko, K. N. & Dearden, P. (2005) The shark watching industry and its potential contribution to shark conservation. *Journal of Ecotourism,* **4,** 108 - 128.

Urban, D. & Keitt, T. (2001) Landscape connectivity: a graph-theoretic perspective. *Ecology,* **82,** 1205-1218.

Van der Kraak, G. & Pankhurst, N. W. (1997) Temperature effects on the reproductive performance of fish. *Global warming: Implications for freshwater and marine fish* (eds C. M. Wood & D. G. McDonald), pp. 159-176. Cambridge University Press, Cambridge.

Van Oosterhout, C., Hutchinson, W. F., Wills, D. P. M. & Shipley, P. (2004) Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes,* **4,** 535-538.

Veríssimo, A., Grubbs, D., McDowell, J., Musick, J. & Portnoy, D. (2011) Frequency of multiple paternity in the spiny dogfish *Squalus acanthias* in the Western North Atlantic. *Journal of Heredity,* **102,** 88-93.

Ward, A., J. W. & Hart, P., J. B. (2003) The effects of kin and familiarity on interactions between fish. *Fish and Fisheries,* **4,** 348-358.

Ward, A. J. W., Botham, M. S., Hoare, D. J., James, R., Broom, M., Godin, J.-G. J. & Krause, J. (2002) Association patterns and shoal fidelity in the three-spined stickleback. *Proceedings of the Royal Society of London. Series B: Biological Sciences,* **269,** 2451-2455.

Ward, A. J. W., Sumpter, D. J. T., Couzin, I. D., Hart, P. J. B. & Krause, J. (2008) Quorum decision-making facilitates information transfer in fish shoals. *Proceedings of the National Academy of Sciences,* **105,** 6948-6953.

Ward, A. J. W., Webster, M. M. & Hart, P. J. B. (2007) Social recognition in wild fish populations. *Proceedings of the Royal Society B: Biological Sciences,* **274,** 1071-1077.

Ward, A. J. W., Webster, M. M., Magurran, A. E., Currie, S. & Krause, J. (2009) Species and population differences in social recognition between fishes: a role for ecology? *Behavioral Ecology,* **20,** 511-516.

Warfe, D. M. & Barmuta, L. A. (2004) Habitat structural complexity mediates the foraging success of multiple predator species. *Oecologia,* **141,** 171-178.

Watts, D. J. & Strogatz, S. H. (1998) Collective dynamics of `small-world' networks. *Nature,* **393,** 440-442.

Wearmouth, V., Southall, E., Morritt, D., Thompson, R. C., Cuthill, I., Partridge, J. & Sims, D. W. (2012) Year-round sexual harassment as a behavioral mediator of vertebrate population dynamics. *Ecological Monographs*.

Wearmouth, V. J. & Sims, D. W. (2008) Sexual segregation in marine fish, reptiles, birds and mammals: behaviour patterns, mechanisms and conservation implications. *Advances in Marine Biology, Vol 54*, pp. 107-170. Elsevier Academic Press Inc, San Diego.

Webster, M. M. & Ward, A. J. W. (2010) Personality and social context. *Biological Reviews,* **86,** 759-773.

Weng, K., Boustany, A., Pyle, P., Anderson, S., Brown, A. & Block, B. (2007) Migration and habitat of white sharks (*Carcharodon carcharias*) in the eastern Pacific Ocean. *Marine Biology,* **152,** 877-894.

Weng, K. C., Castilho, P. C., Morrissette, J. M., Landeira-Fernandez, A. M., Holts, D. B., Schallert, R. J., Goldman, K. J. & Block, B. A. (2005) Satellite tagging and cardiac physiology reveal niche expansion in salmon sharks. *Science,* **310,** 104-106.

Wetherbee, B. M., Gruber, S. H. & Rosa, R. S. (2007) Movement patterns of juvenile lemon sharks *Negaprion brevirostris* within Atol das Rocas, Brazil: a nursery characterized by tidal extremes. *Marine Ecology Progress Series,* **343,** 283-293.

Wey, T., Blumstein, D. T., Shen, W. & Jordán, F. (2008) Social network analysis of animal behaviour: a promising tool for the study of sociality. *Animal Behaviour,* **75,** 333-344.

Wey, T. W. & Blumstein, D. T. (2010) Social cohesion in yellow-bellied marmots is established through age and kin structuring. *Animal Behaviour,* **79,** 1343-1352.

Whitehead, H. (1997) Analysing animal social structure. *Animal Behaviour,* **53,** 1053-1067.

Whitehead, H. (2008) *Analyzing animal societies: quantitative methods for vertebrate social analysis.,* The University of Chicago Press, Chicago, IL.

Whitehead, H. (2009) SOCPROG programs: analysing animal social structures. *Behavioral Ecology and Sociobiology,* **63,** 765-778.

Whitehead, H., Bejder, L. & Andrea Ottensmeyer, C. (2005) Testing association patterns: issues arising and extensions. *Animal Behaviour,* **69,** e1-e1.

Whitney, N. M., Pratt, J. H. L. & Carrier, J. C. (2004) Group courtship, mating behaviour and siphon sac function in the whitetip reef shark, *Triaenodon obesus*. *Animal Behaviour,* **68,** 1435-1442.

Williams, R., Okey, T. A., Wallace, S. S. & Gallucci, V. F. (2010) Shark aggregation in coastal waters of British Columbia. *Marine Ecology Progress Series,* **414,** 249-256.

Wilson, A. J., de Boer, M., Arnott, G. & Grimmer, A. (2011) Integrating personality research and animal contest theory: aggressiveness in the green swordtail *Xiphophorus helleri*. *PLoS ONE,* **6,** e28024.

Wilson, S. G. (2004) Basking sharks (*Cetorhinus maximus*) schooling in the southern Gulf of Maine. *Fisheries Oceanography,* **13,** 283-286.

Wolanski, E. & Gereta, E. (2001) Water quantity and quality as the factors driving the Serengeti ecosystem, Tanzania. *Hydrobiologia,* **458,** 169-180.

Wolf, J. B. W., Mawdsley, D., Trillmich, F. & James, R. (2007) Social structure in a colonial mammal: unravelling hidden structural layers and their foundations by network analysis. *Animal Behaviour,* **74,** 1293-1302.

Worm, B., Barbier, E. B., Beaumont, N., Duffy, J. E., Folke, C., Halpern, B. S., Jackson, J. B. C., Lotze, H. K., Micheli, F., Palumbi, S. R., Sala, E., Selkoe, K. A., Stachowicz, J. J. & Watson, R. (2006) Impacts of biodiversity loss on ocean ecosystem services. *Science,* **314,** 787-790.

Yokota, L. & Lessa, R. (2006) A nursery Area for Sharks and Rays in Northeastern Brazil. *Environmental Biology of Fishes,* **75,** 349-360.

Yopak, K. E., Lisney, T. J., Darlington, R. B., Collin, S. P., Montgomery, J. C. & Finlay, B. L. (2010) A conserved pattern of brain scaling from sharks to primates. *Proceedings of the National Academy of Sciences,* **107,** 12946-12951.

Yue, G. H. & Chang, A. (2010) Molecular evidence for high frequency of multiple paternity in a freshwater shrimp species *Caridina ensifera*. *PLoS ONE,* **5,** e12721.

Appendices.

Appendix I

This appendix contains additional information relating to female reproductive success, descriptive detail about offspring and complimentary explanation of genetic analyses of the study outlined in *Chapter IV*.

Table A.1. Gestation and egg hatch success sorted by maternal clutch.

Egg	Maternity	Date (laid)	Date Hatch	Gestation Period (days)	Sex	Length (mm)	Weight (g)	Successful DNA Extraction
57	B1801	01/02/2010	-					N
59	B1801	02/02/2010	-					N
87	B1801	15/02/2010	16/08/2010	182	M	107	3.49	Y
88	B1801	15/02/2010	16/08/2010	182	F	103	3.24	Y
113	B1801	01/03/2010	-					N
114	B1801	01/03/2010	25/08/2010	177	M	103	3.39	Y
170	B1801	05/04/2010	13/09/2010	161	F	99	2.99	Y
192	B1801	05/05/2010	24/09/2010	142	M	101	3.32	Y
45	B1802	28/01/2010	12/08/2010	196	M	105	3.55	Y
46	B1802	28/01/2010	18/08/2010	202	F	108	3.69	Y
65	B1802	06/02/2010	21/08/2010	196	M	112	4.03	Y
66	B1802	06/02/2010	-					N
94	B1802	18/02/2010	26/08/2010	189	F	107	3.77	Y
95	B1802	19/02/2010	24/08/2010	186	M	106	3.97	Y
183	B1802	28/04/2010	27/09/2010	152	M	102	3.62	Y
71	B1803	06/02/2010	19/08/2010	194	F	103	3.57	Y
135	B1803	11/03/2010	31/08/2010	173	F	102	3.64	Y
136	B1803	11/03/2010	03/09/2010	176	M	105	3.67	Y
188	B1803	04/05/2010	27/09/2010	146	F	105	3.60	Y
189	B1803	04/05/2010	23/09/2010	142	M	105	3.66	Y
203	B1803	10/05/2010	-					N
83	B1805	15/02/2010	19/08/2010	185	F	98	3.53	Y

84	B1805	15/02/2010	19/08/2010	185	M	100	3.43	Y
109	B1805	25/02/2010	26/08/2010	182	M	101	3.76	Y
110	B1805	25/02/2010	26/08/2010	182	F	101	3.83	Y
127	B1805	10/03/2010	01/09/2010	175	M	102	3.92	Y
128	B1805	10/03/2010	31/08/2010	174	M	103	3.94	Y
153	B1805	22/03/2010	07/09/2010	169	M	102	4.01	N
154	B1805	22/03/2010	-					N
166	B1805	02/04/2010	10/09/2010	161	F	103	3.93	Y
167	B1805	02/04/2010	08/09/2010	159	M	103	3.88	Y
173	B1805	13/04/2010	15/09/2010	155	M	103	3.97	Y
185	B1805	30/04/2010	-					N
186	B1805	30/04/2010	23/09/2010	146	F	103	4.07	Y
200	B1805	10/05/2010	28/09/2010	141	F	102	3.91	Y
201	B1805	10/05/2010	03/10/2010	146	M	107	3.95	Y

51	G0376	29/01/2010	20/08/2010	203	F	100	3.83	Y
58	G0376	01/02/2010	17/08/2010	197	F	103	4.09	Y
77	G0376	09/02/2010	-					N
80	G0376	11/02/2010	22/08/2010	192	M	105	3.65	Y
97	G0376	19/02/2010	26/08/2010	188	F	100	3.93	Y
102	G0376	23/02/2010	26/08/2010	184	F	101	3.87	Y
123	G0376	03/03/2010	31/08/2010	181	F	105	4.28	Y
124	G0376	05/03/2010	31/08/2010	179	M	105	3.72	N
138	G0376	15/03/2010	30/08/2010	168	M	99	3.78	Y
139	G0376	15/03/2010	03/09/2010	172	F	102	3.77	Y
157	G0376	23/03/2010	06/09/2010	167	F	105	3.84	Y
160	G0376	25/03/2010	07/09/2010	166	M	100	3.65	Y
174	G0376	13/04/2010	13/09/2010	153	M	102	3.88	Y
179	G0376	20/04/2010	14/09/2010	147	F	102	3.72	Y
180	G0376	20/04/2010	21/09/2010	154	F	107	3.94	Y
194	G0376	05/05/2010	-					N
195	G0376	05/05/2010	24/09/2010	142	M	104	3.83	Y
210	G0376	15/05/2010	29/09/2010	137	M	101	3.88	Y
211	G0376	15/05/2010	04/10/2010	142	F	97	3.59	Y
212	G0376	17/05/2010	01/10/2010	137	M	99	3.88	Y
213	G0376	17/05/2010	29/09/2010	135	M	102	3.86	Y
216	G0376	24/05/2010	05/10/2010	134	M	105	3.78	Y
217	G0376	24/05/2010	06/10/2010	135	F	103	3.67	Y
222	G0376	01/06/2010	11/10/2010	132	F	101	3.50	Y
223	G0376	01/06/2010	13/10/2010	134	M	100	3.79	Y
230	G0376	09/06/2010	-					N

231	G0376	09/06/2010	18/10/2010	131	F	99	3.60	Y
44	G0377	28/01/2010	-					N
52	G0377	29/01/2010	-					N
64	G0377	06/02/2010	17/08/2010	192	M	100	3.13	Y
72	G0377	08/02/2010	18/08/2010	191	F	99	3.16	Y
89	G0377	16/02/2010	18/08/2010	183	M	100	2.98	Y
90	G0377	16/02/2010	19/08/2010	184	M	100	3.22	Y
115	G0377	01/03/2010	-					N
116	G0377	01/03/2010	23/08/2010	175	F	98	3.06	Y
129	G0377	10/03/2010	30/08/2010	173	M	100	3.13	Y
130	G0377	10/03/2010	30/08/2010	173	M	100	3.09	Y
155	G0377	22/03/2010	03/09/2010	165	M	99	3.08	Y
156	G0377	22/03/2010	03/09/2010	165	F	100	3.10	Y
163	G0377	30/03/2010	06/09/2010	160	F	96	3.02	Y
164	G0377	30/03/2010	06/09/2010	160	M	100	3.12	Y
198	G0377	05/05/2010	-					N
199	G0377	05/05/2010	-					N
206	G0377	13/05/2010	24/09/2010	134	M	94	3.01	Y
207	G0377	13/05/2010	29/09/2010	139	M	96	2.96	Y
218	G0377	24/05/2010	29/09/2010	128	M	96	2.91	Y
219	G0377	24/05/2010	30/09/2010	129	F	96	2.97	Y
224	G0377	01/06/2010	11/10/2010	132	M	96	2.90	N
225	G0377	01/06/2010	-					N
232	G0377	15/06/2010	-					N
233	G0377	15/06/2010	25/10/2010	132	F	100	3.02	Y
25	P0551	21/01/2010	19/08/2010	210	F	107	4.30	Y
26	P0551	21/01/2010	-					Y
39	P0551	27/01/2010	19/08/2010	204	M	116	4.28	Y
41	P0551	27/01/2010	20/08/2010	205	F	110	4.27	Y
67	P0551	06/02/2010	19/08/2010	194	F	115	4.04	Y
68	P0551	06/02/2010	24/08/2010	199	F	105	4.27	Y
85	P0551	15/02/2010	20/08/2010	186	F			Y
86	P0551	15/02/2010	-					N
100	P0551	23/02/2010	29/08/2010	187	F	117	4.16	Y
101	P0551	23/02/2010	29/08/2010	187	M	112	4.32	Y
119	P0551	02/03/2010	29/08/2010	180	F	110	4.41	Y
120	P0551	02/03/2010	01/09/2010	183	F	108	4.15	Y
133	P0551	11/03/2010	07/09/2010	180	F	109	4.12	Y
134	P0551	11/03/2010	31/08/2010	173	M	110	4.19	Y

149	P0551	20/03/2010	08/09/2010	172	M	110	4.25	Y
150	P0551	20/03/2010	10/09/2010	174	M	115	4.22	Y
161	P0551	29/03/2010	09/09/2010	164	F	110	4.18	Y
162	P0551	29/03/2010	09/09/2010	164	M	111	4.12	Y
171	P0551	13/04/2010	17/09/2010	157	M	107	4.10	Y
172	P0551	13/04/2010	17/09/2010	157	M	111	4.17	Y
181	P0551	25/04/2010	24/09/2010	152	F	113	4.31	Y
182	P0551	25/04/2010	29/09/2010	157	M	106	4.08	Y
196	P0551	05/05/2010	28/09/2010	146	M	106	4.11	Y
197	P0551	05/05/2010	29/09/2010	147	F	106	4.17	Y
204	P0551	10/05/2010	01/10/2010	144	M	110	3.93	Y
205	P0551	10/05/2010	30/09/2010	143	F	108	3.91	Y
214	P0551	18/05/2010	-					N
215	P0551	18/05/2010	06/10/2010	141	F	105	3.83	Y
220	P0551	28/05/2010	-					N
221	P0551	28/05/2010	-					N
228	P0551	07/06/2010	20/10/2010	135	M	102	3.54	Y
229	P0551	07/06/2010	18/10/2010	133	F	107	3.77	Y
28	P0552	23/01/2010	16/08/2010	205	M	110	3.67	Y
29	P0552	23/01/2010	16/08/2010	205	F	106	3.66	Y
61	P0552	04/02/2010	18/08/2010	195	M	106	3.78	Y
62	P0552	04/02/2010	17/08/2010	194	F	103	3.56	Y
91	P0552	18/02/2010	22/08/2010	185	M	103	3.26	N
92	P0552	18/02/2010	-					N
158	P0552	23/03/2010	07/09/2010	168	M	100	3.47	Y
159	P0552	23/03/2010	06/09/2010	167	F	104	3.33	Y
177	P0552	14/04/2010	14/09/2010	153	M	99	3.23	Y
190	P0552	04/05/2010	-					N
191	P0552	04/05/2010	21/09/2010	140	F	100	3.07	Y
40	P0553	27/01/2010	20/08/2010	205	F	104	3.77	Y
69	P0553	06/02/2010	16/08/2010	191	M	100	3.89	Y
70	P0553	06/02/2010	22/08/2010	197	M	110	3.87	Y
140	P0553	15/03/2010	06/09/2010	175	F	107	4.09	Y
141	P0553	15/03/2010	-					N
193	P0553	05/05/2010	28/09/2010	146	M	106	3.75	Y
42	R1851	28/01/2010	13/08/2010	197	M	100	3.34	Y
43	R1851	28/01/2010	13/08/2010	197	M	99	3.37	Y
73	R1851	08/02/2010	18/08/2010	191	M	104	3.27	Y

74	R1851	08/02/2010	19/08/2010	192	M	102	3.53	Y
96	R1851	19/02/2010	23/08/2010	185	F	102	3.38	N
99	R1851	23/02/2010	24/08/2010	182	M	102	3.38	N
4	Y0336	15/12/2009	29/07/2010	226	F	108	3.3705	N
5	Y0336	18/12/2009	26/07/2010	220	F	109	3.2849	N
10	Y0336	04/01/2010	04/08/2010	212	M	100	3.1672	Y
16	Y0336	14/01/2010	-					N
17	Y0336	14/01/2010	-					N
49	Y0336	29/01/2010	-					N
50	Y0336	29/01/2010	-					N
53	Y0336	31/01/2010	-					N
54	Y0336	31/01/2010	-					N
60	Y0336	02/02/2010	23/08/2010	202	F	106	3.63	Y
93	Y0336	18/02/2010	-					N
98	Y0336	23/02/2010	-					N
103	Y0336	23/02/2010	25/08/2010	183	F	102	3.32	Y
104	Y0336	23/02/2010	22/08/2010	180	M	106	3.49	Y
131	Y0336	10/03/2010	-					N
132	Y0336	10/03/2010	30/08/2010	173	F	101	3.39	Y
148	Y0336	20/03/2010	31/08/2010	164	M	110	3.66	Y
3	Y0337	15/12/2009	26/07/2010	223	F	106	3.1993	Y
9	Y0337	02/01/2010	04/08/2010	214	F	107	3.4775	Y
11	Y0337	08/01/2010	-					N
12	Y0337	08/01/2010	-					N
18	Y0337	18/01/2010	-					N
30	Y0337	25/01/2010	24/08/2010	211	M	106	3.35	Y
31	Y0337	25/01/2010	21/08/2010	208	M	110	3.79	Y
63	Y0337	04/02/2010	20/08/2010	197	F	106	3.71	Y
81	Y0337	12/02/2010	26/08/2010	195	M	107	3.58	Y
82	Y0337	12/02/2010	24/08/2010	193	F	105	3.58	N
105	Y0337	23/02/2010	24/08/2010	182	M	101	3.65	N
106	Y0337	23/02/2010	27/08/2010	185	M	106	3.84	Y
121	Y0337	03/03/2010	-					N
122	Y0337	03/03/2010	03/09/2010	184	F	105	3.73	Y
142	Y0337	15/03/2010	06/09/2010	175	M	106	3.54	Y
143	Y0337	15/03/2010	03/09/2010	172	M	108	3.85	Y
151	Y0337	22/03/2010	10/09/2010	172	F	106	3.67	Y
1	Y0338	15/12/2009	20/07/2010	217	M	98	3.7985	Y

2	Y0338	15/12/2009	29/07/2010	226	M	111	3.6808	Y
6	Y0338	18/12/2009	29/07/2010	223	M	107	3.4882	Y
7	Y0338	02/01/2010	03/08/2010	213	F	101	3.531	Y
8	Y0338	02/01/2010	02/08/2010	212	M	108	3.7343	Y
13	Y0338	11/01/2010	04/08/2010	205	F	101	3.4026	N
14	Y0338	11/01/2010	09/08/2010	210	M	103	3.317	N
15	Y0338	11/01/2010	10/08/2010	211	M	105	3.3063	N
19	Y0338	20/01/2010	12/08/2010	204	F	103	3.31	Y
20	Y0338	20/01/2010	11/08/2010	203	M	100	3.43	Y
55	Y0338	31/01/2010	12/08/2010	193	F	98	3.43	Y
56	Y0338	31/01/2010	15/08/2010	196	M	103	3.28	Y
75	Y0338	09/02/2010	24/08/2010	196	M	102	3.49	Y
76	Y0338	09/02/2010	23/08/2010	195	M	111	3.42	Y
107	Y0338	23/02/2010	-					N
108	Y0338	23/02/2010	26/08/2010	184	M	97	3.39	Y
125	Y0338	05/03/2010	-					N
126	Y0338	05/03/2010	02/09/2010	181	M	100	3.35	Y
144	Y0338	15/03/2010	02/09/2010	171	F	97	3.27	Y
145	Y0338	15/03/2010	-					N
168	Y0338	02/04/2010	11/09/2010	162	F	101	3.21	Y
169	Y0338	02/04/2010	10/09/2010	161	F	94	3.16	Y
175	Y0338	13/04/2010	13/09/2010	153	M	99	3.35	Y
176	Y0338	13/04/2010	13/09/2010	153	F	95	3.11	Y
208	Y0338	15/05/2010	-					N
209	Y0338	15/05/2010	-					N
226	Y0338	01/06/2010	11/10/2010	132	M	100	3.23	Y
227	Y0338	01/06/2010	-					N
234	Y0338	18/06/2010	15/10/2010	119	F	92	2.80	Y
235	Y0338	18/06/2010	18/10/2010	122	M	98	3.07	Y

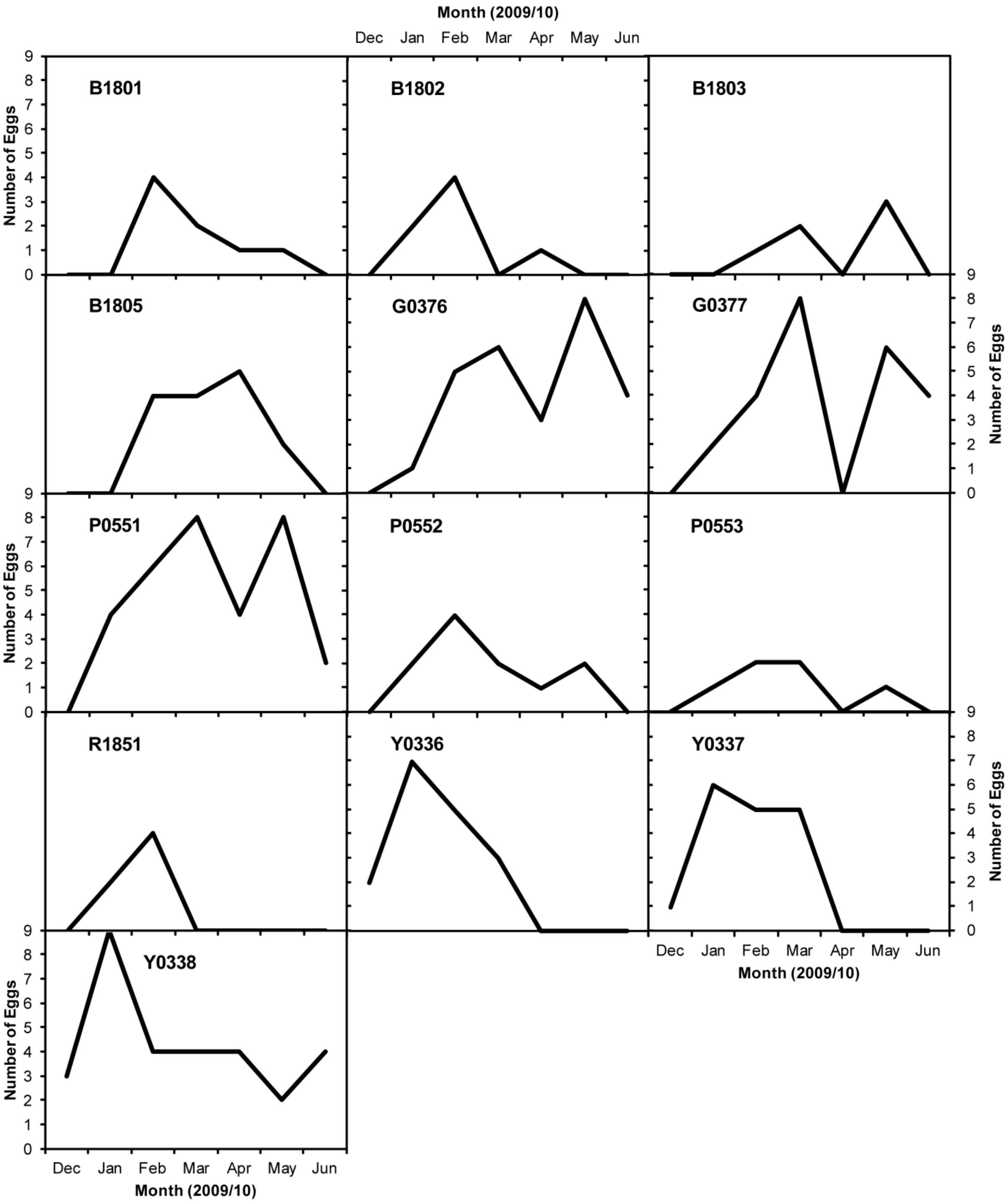

Figure A.1. Summary of the egg laying consistency of 13 female *S. canicula*.

A1.1 Genotyping techniques

Genomic DNA was isolated from *S. canicula* using the Wizard technique (Promega Madison, WI). Ten microsatellite loci; Scan02, Scan03, Scan05, Scan06, Scan09, Scan12, Scan13, Scan14, Scan15 and Scan16 (Griffiths et al. 2011), were amplified according to the manufacturer's instructions with the QUIAGEN multiplex polymerase chain reaction (PCR) kit (QIAGEN, Valencia, CA). Briefly, PCRs were carried out in 10µl reaction volumes containing: 1 µl 1/50 diluted genomic DNA, 1 µl of a primer mix containing all primers at an equal 2 µM concentration, 5 µl PCR master mix and 3 µl RNase-free water. The reactions were performed in a PTC-200 Peltier thermocycler with an initial denaturation for 15 min at 95°C, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 3 min, and extension at 72°C for 60 s, before a final extension step of 72°C for 10 min. Products were labelled with fluorescent HEX, TET or FAM primers following Griffiths et al. (2011). Allele sizes were determined using a MegaBace 1000 DNA sequencer, a 550 bp size standard and Fragment Profiler v1.2 (GEHealthcare).

A1.2 Source population sampling

To check for significant differences in allele frequencies between these sample collections, pairwise F_{ST} values were generated in Arlequin (Excoffier et al. 2005) and genic tests of differentiation were carried out in Genepop (Rousset 2008). Powermarker (Lui and Muse 2005) was used to calculate observed and expected heterozygosity and Genepop was used to test for linkage disequilibrium (LD) and conformity to expectations of Hardy-Weinberg equilibrium (HWE). Microchecker (Van Oosterhout et al. 2004) was also used to check for scoring issues and the presence of null alleles. Sequential Bonferroni corrections (Rice 1989) were applied when appropriate.

Table A.2. Characteristics of *S. canicula* microsatellite loci in the source population. Ni = number of individuals scored, Na = numbers of alleles, H_E = expected heterozygosity, H_O = observed heterozygosity, P_{HW} = p-value of the test for HWE and EP = exclusion probability.

Locus	**Label**	**Ni**	**Na**	**H_E**	**H_O**	**P_{HW}**	**EP**
Scan02	TET	76	9	0.786	0.790	0.685	0.609
Scan03	FAM	74	8	0.594	0.541	0.492	0.390
Scan05	TET	75	7	0.613	0.653	0.463	0.374
Scan06	FAM	75	9	0.799	0.827	0.737	0.621
Scan09	HEX	76	3	0.088	0.092	1.000	0.047
Scan12	FAM	77	6	0.613	0.653	0.224	0.441
Scan13	HEX	75	8	0.555	0.573	0.202	0.328
Scan14	TET	71	11	0.839	0.831	0.015	0.690
Scan15	FAM	74	9	0.772	0.716	0.242	0.573
Scan16	HEX	73	7	0.821	0.808	0.884	0.664

Appendix II

A.2 Caribbean reef shark array, Cape Eleuthera, Bahamas

A.2.1 Design and data collection

Data on the movements of Caribbean reef sharks (*Carcharhinus perezi*), which comprise part of the collaborative study conducted in *Chapter VI,* were collected by Edd Brooks of the Cape Eleuthera Institute (CEI), The Bahamas and the University of Plymouth, UK. The methods for the acoustic tracking of these sharks are outlined below.

This study was conducted between 18th June 2009 and 05th August 2010, off the coast of Eleuthera, The Bahamas (24.54° N 76.12° W, Fig. 20). *C. perezi* were captured using midwater longlines consisting of a 500 m mainline with approximately 35-45 baited gangions approximately 3.5 m long, ending in a 16/0 circle hook. Gangions were equally spaced 8-10 m along the mainline and supported by buoys every six hooks. Each longline was soaked for approximately 90 minutes. Only sharks that were hooked cleanly in the corner of the jaw, that have no obvious external trauma, and that are not visibly exhausted, were selected for transmitter deployment. Candidate sharks were inverted alongside the boat until the onset of tonic immobility (Henningsen 1994). A small 4 cm incision was made ~8 cm anterior to the origin of the pelvic fins and offset from the ventral midline. Prior to inserting the transmitter, all surgery and suture material was bathed in 5% iodine solution. The transmitters were inserted into the peritoneal cavity and the incision closed with 3-6 interrupted dissolving nylon monofilament sutures (Ethicon PDS II, Sommerville, NJ). All research was carried out under research permits MAF/FIS/17 and MAF/FIS/34 issued by the Bahamian Department of Marine Resources and in accordance with CEI animal care protocols developed within the guidelines of the Association for the Study of Animal Behaviour and the Animal Behaviour Society (Rollin & Kessel 1998).

Acoustic transmitters of three different types (8 x V16 Location Only Tags, 3 x V16TP Depth and Temperature Tags, 15 x V9 Accelerometer Tags) have been deployed on Caribbean reef sharks off Cape Eleuthera and have been tracked using an array of acoustic hydrophones for over eighteen months. Eleven of the 27 transmitters are VEMCO V16 (VEMCO, Halifax, Nova Scotia) transmitters, which will still be active for another seven years. The array of 32 VEMCO VR2 acoustic hydrophones was deployed in a non-overlapping grid on 6th June 2009 (Fig. 20). Preliminary analysis of the data has revealed that this more structured array has proven more effective for analysis of temporal patterns in activity space, fine scale movements, and habitat association.

A.2.2 Useful links to social network analysis software

Programs for the analysis of animal social structure (**SOCPROG**) – Developed by Hal Whitehead

- For programme download and manual see http://whitelab.biology.dal.ca/
- Citation: Whitehead, H. (2009) SOCPROG programs: analysing animal social structures. *Behavioral Ecology and Sociobiology,* **63,** 765-778.

UCINET/NETDRAW – Developed by Steve Borgatti, Martin Everett and Lin Freeman and distributed by Analytic Technologies

- Programme download at http://www.analytictech.com/ucinet/download.htm
- Citation UCINET: Borgatti, S. P., Everett, M. G. & Freeman, L. C. (2002) UCINET 6 For Windows: Software for Social Network Analysis. Analytic Technologies, Harvard.
- Citation NETDRAW: Borgatti, S. P. (2002) NetDraw: Graph Visualization Software. Analytic Technologies, Harvard.
- Online textbook based on UCINET and written by Robert Hanneman and Mark Riddle can be found at http://www.faculty.ucr.edu/~hanneman/nettext/

For further information on preparing and using data in these and other network-related programs refer to Croft *et al.* (2008), Whitehead (2008), Whitehead (2009) and Borgatti *et al.* (2009).

A.2.3 Movement interaction matrices

AID	**1**	**2**	**3**	**4**	**5**	**6**
1	0	14	57	4	10	1
2	15	0	2	0	0	0
3	58	2	0	2	1	2
4	0	1	0	0	1	0
5	10	2	0	0	0	1
6	1	0	0	0	1	0

Absolute interaction data (AID) matrix for the asymmetric movement of female catshark 9127 between six locations/acoustic receivers in the UK. Data has been filtered to only include direct movement edges of ≤ 1 hr.

RID	**1**	**2**	**3**	**4**	**5**	**6**
1	0.000	0.076	0.308	0.022	0.054	0.005
2	0.081	0.000	0.011	0.000	0.000	0.000
3	0.314	0.011	0.000	0.011	0.005	0.011
4	0.000	0.005	0.000	0.000	0.005	0.000
5	0.054	0.011	0.000	0.000	0.000	0.005
6	0.005	0.000	0.000	0.000	0.005	0.000

Proportional, relative interaction data (RID) matrix converted from the above count data by simply dividing each value by the total number of movement interactions across the entire network (*n* = 185). Again data was filtered to only include direct movement edges of ≤ 1 hr.