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Social relationships in a small habitat-dependent coral reef fish:

an ecological, behavioural and genetic analysis

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Declaration of Ethics

This research presented and reported in this thesis was conducted in compliance with the National Health and Medical Research Council (NHMRC) Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, 7th Edition, 2004 and the Qld Animal Care and Protection Act, 2001. The proposed research study received animal ethics approval from the JCU Animal Ethics Committee Approval Number #A1847.

Signature ___31/3/2016___ Date

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Abstract

Most social animals form prolonged relationships with other individuals, whether as mating partners, parent-offspring bonds or associations with other kin. The processes that determine the strength and longevity of social relationships are often poorly understood. Many animals form monogamous breeding pairs, however, fundamental issues such as why they pair, how they choose partners and whether they mate exclusively with partners remain to be resolved. Many also live in extended family groups, but the advantages of staying or leaving family groups are complex. These issues are especially poorly understood for coral reef fishes, where social monogamy is common but appears to have evolved in species that provide either no parental care, or minimal care provided by the male. It has always been assumed that social groups in reef fish arise through strong site attachment and as a consequence of larval dispersal are not family or kin associations. In this thesis, I combined long-term behavioural observations, field experiments and new molecular tools to address these keys issues related to monogamy and genetic relatedness within social groups of a coral reef cardinalfish – *Sphaeramia nematoptera* (Apogonidae) in Kimbe Bay, Papua New Guinea. This species forms small, highly sedentary social groups, tends to mate in pairs within groups and is a paternal mouthbrooder. The following specific questions were addressed in the four data chapters: 1) What role does site attachment play in the choice of mates? i.e. do they primarily show allegiance to a site or a mate? 2) Are mating pairs assorted by size and which sex chooses mates?; 3) Does the social mating system reflect the genetic composition of offspring or do offspring arise from extrapair or sneak mating?; 4) Are social groups comprised of related individuals and what mechanisms might lead to kin association?

In Chapter 2, I used long term observational data to show that monogamous pairs within social groups were highly site attached compared with unpaired individuals. Following natural losses of partners or experimental mate removals, individuals rapidly formed new partnerships within the same social group rather than moving to other groups. In addition, partners did not follow their mates when they were experimentally relocated to adjacent groups, instead re-pairing with individuals within the same social group. Together, these results indicate that group cohesion is maintained through strong site attachment, and individuals show greater allegiance to sites than to mates. Thus site fidelity plays a big role in influencing pairing and pair longevity in this species.

There was strong size-assortment of males and females in pairs, with larger males pairing with larger females within social groups (Chapter 3). Size-assortative mating was not due to juvenile cardinalfish growing up together, as although individuals pair at a small size, they regularly change partners between spawning cycles until adulthood. Following natural losses, both males and females repaired with partners of more similar size than expected due to chance. Experimental removal of either males or females showed that both sexes actively choose partners, indicating that size-assortative mating was maintained by mutual mate choice.

Although most mature males and females remain paired for at least one and often several breeding cycles, genetic analysis of offspring using 19 highly polymorphic microsatellite markers revealed that both sexes exhibited extrapair mating and sneaking behaviour (Chapter 4). Multiple mothers were found in 11.4% of 105 clutches analysed, indicating that males were mouthbrooding eggs from non-partner females. Multiple paternity was found in 7.6% of clutches. The latter was unexpected, as males were thought to have limited opportunity to fertilize eggs brooded by other males. This suggests that there are males that perform sneak matings, and the high investment paternal care does not guarantee sole paternity for the brooder. Together, these results suggest that while this species is socially monogamous, both sexes will opportunistically mate with other individuals to maximize their reproductive success.

In the final chapter (5), genetic analyses revealed that individuals in social groups exhibited a higher genetic relatedness than expected on the basis of random assortment. Pairwise relatedness was 1.3 times higher within reefs than between reefs and 1.3 times higher within social groups than between social groups on the same reef. There was a negative relationship between relatedness and distance between social groups on a scale of 3.9km2. High genetic relatedness within groups was explained by high levels of self-recruitment to natal reefs and an attraction among sibs, which appear to be able to settle in close proximity. While similar-sized juveniles and sub adults were more closely related when in the same social group, this same trend does not hold true for adults. This suggests an ontogenetic decline in kin attraction, possibly a mechanism to avoid inbreeding.

Overall, the results indicate that social groups of coral reef fishes are likely to be far more complex than previously anticipated. Social groups of *S. nematoptera* appear to arise through strong site attachment and high genetic relatedness, suggesting juveniles have a predisposition to seek out related individuals. Within social groups, strong site attachment facilitates pair formation, with both sexes having a strong preference for partners of similar size. Although pair formation may promote group stability and reproductive success, the unique paternal mouth brooding reproductive system does not preclude cheating by either males or females. Clearly, site attachment, familiarity, relatedness and opportunism all must be considered in any general model of social organization and mating systems of coral reef fishes.

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Chapter 1: Introduction

Most mobile animals spend at least part of their lives associated with others of their kind, and with them they form social relationships. A social relationship or “bonding” may be defined as a close association between two or more individuals in an animal group (Wilson 2000). Social relationships encompass all aspects of animal life, including reproduction, parental care and group living. Sexually reproductive animals form relationships with mating partners that vary from brief encounters to life-long partnerships (Emlen & Oring 1977). Animals that provide parental care form relationships with their offspring, which may last from just a few hours to many decades (Clutton-Brock 1991). A large number of animals form social groups and relationships within groups with unrelated group members, sharing information that may improve foraging success and predator avoidance (Emlen 1995; Krause & Ruxton 2002; Kutsukake 2008; Majolo *et al.* 2008).

Social relationships are almost ubiquitous in the animal kingdom, and understanding them is of utmost importance to grasp the ecology of many taxa, as has been shown for example in long term studies of cooperatively breeding seabirds, mammals, insects and freshwater fish (Crook *et al.* 1976; Perrone 1978; Kellogg *et al.* 1995; Arnqvist *et al.* 1996; Cézilly *et al.* 2000). Some relationships may arise through mutual attraction between individuals, while others are a fortuitous consequence of individuals attracted to the same resources (Emlen & Oring 1977). One relationship of particular interest is that of socially monogamous mating partners, among whom interactions can be particularly complex. For most pair-forming species there is considerable doubt as to whether the mating systems represent true genetic monogamy, which may be the exception rather than the rule (Griffith *et al.* 2002; Dolan *et al.* 2007). For some group living species in the marine environment the extent to which groups are composed of kin can be completely unknown. Resolving the nature of these relationships requires a fundamental understanding of proximate and ultimate causes of social behaviour. This thesis takes a holistic approach, using long-term observational, experimental and genetic approaches, to unravel the basis of individual relationships in a group-living coral reef fish.

The social and mating systems of animals are closely linked to patterns of parental care with the prevalence of monogamy closely associated with extensive biparental care in terrestrial vertebrates (Ar & Yom-Tov 1978; Kleiman & Malcom 1981). However, in teleost fish, monogamy is often present in species with minimal or no parental care (Whiteman & Côte 2004; Reavis & Copus 2011). Parental care involving a brief period of tending developing embryo's occurs in 30% of fish families, and mostly this takes the form of paternal brood care (50-84%) (Gross & Sargent 1985). The differing parental care strategies of males and females lead to differing mating strategies (Kokko *et al.* 2006). Paternal brood care in fish can potentially lead to reversed gender roles, where males invest more into offspring and thus become the choosy sex, and females compete with each other (Jones *et al.* 1999b; Sogabe & Yanagisawa 2007). Here, I consider mate selection, fidelity and parental care strategies together, to gain a full understanding of bonding among individuals in a group-living reef fish species.

1. Mating relationships: mate versus site fidelity

Arguably the most important social relationship in an animal's life is the association with reproductive partner(s). Most aspects of a species' ecology, behaviour and life history are impossible to understand without taking into account the evolution of its mating strategy (Ar & Yom-Tov 1978; Arnqvist & Nilsson 2000; Kokko *et al.* 2003; Lukas & Clutton-Brock 2012). A prolonged relationship between mating partners can be strongly associated with site fidelity and homing. For example, in some colonially nesting sea birds, individuals return to the same cliff site every year to find the same partner again (Danchin & Wagner 1997; Brown *et al.* 2000). Salmon and other anadromous fish may live many years at sea, but home to natal streams where they take long, strenuous journeys upstream in order to find mating partners and breed (Dittman & Quinn

1996). Despite the obvious links with site attachment and homing, the relative importance of attraction between individuals or association with places in determining mating partnerships is poorly understood.

Mating with the same partner over long periods of time has known advantages, with familiarity contributing to an increased reproductive output (Thibault 1994; Naves *et al.* 2006). Similarly, familiarity with breeding sites can significantly decrease the likelihood of predation and contribute to foraging efficiency (Shields 1984; Wakefield *et al.* 2015). In storks, order Ciconiiforms, those species that have the highest rate of site attachment are also the most likely to be monogamous (Cézilly *et al.* 2000). And even within one species, those individuals loyal to their partner are also the ones most likely to repeatedly return to the same breeding site (Naves *et al.* 2006). However, while animals appear to be closely attached to both mates and sites, it is not known which factor is of ultimate importance. Animals may primarily return to the same site to mate with their preferred partner or may be returning to their preferred breeding site and mating with their previous partner out of convenience. In Chapter 2, I use observations and experiments to establish whether either site or mate fidelity takes precedence in the mating system of a coral reef fish species.

2. Mate choice: size assortative mating

The formation of pair bonds within social groups can have important implications for patterns of sexual selection and the evolution of reproductive systems. For instance, assortative mating, where individuals with similar phenotypes or genotypes choose one another more often than would be expected under random mating, can lead to the preservation of phenotypic variation and, in extreme cases, to sympatric speciation (Cézilly 2004). Such patterns can arise because of direct or indirect selection (Jiang *et al*. 2013), and can be a consequence of mutual mate choice or exclusive male or female choice (McNamara & Collins 1990). Who chooses whom and which is the “choosy” sex often depends on reproductive investment. Typically, females have higher investment into offspring, due to the greater cost of producing eggs rather than sperm and the often associated pregnancy or brood care costs (Kokko *et al.* 2003). There are examples of animals however, where gender roles are reversed and males are the primary choosers (Jones *et al.* 2000). This is usually related to paternal brood care (Vincent *et al.* 1994). In monogamous animals, which often have mutual offspring care or similar gender investments, mutual mate choice is expected. In these circumstances assortative mating by body size is common (Kuwamura *et al.* 1993; Harari *et al.* 1999a). Size is a likely characteristic to play an important role in mate choice and pairing patterns, since for many animals it is directly correlated with reproductive output and offspring fitness (Davies & Halliday 1978; Partridge *et al.* 1987; Olsson 1993). Matching theory predicts that when both sexes are choosy and select on body size, both should choose the biggest partner possible which then leads to assortative pairs that are stable (Puebla *et al.* 2011). The behavioural mechanisms leading to size-assortative pairing have received little attention in many taxa. Here (in Chapter 3) assortative mating and the interactions leading to this mating pattern are studied for the first time in a reef fish with paternal brood care.

3. Social versus genetic monogamy

While social monogamy is ubiquitous in the animal kingdom, molecular tools are beginning to establish that this is not necessarily reflected in the parentage of offspring. Extra-pair mating has been found in 90% of socially monogamous bird species studied thus far, with an average of 15% of offspring with extra-pair paternity (Griffith *et al.* 2002; Dolan *et al.* 2007). In some cases, the advantages of having extra partners are material. For example, in the ground cricket, *Allonemobius socius*, the female receives a nuptial gift in the form of nutrition (Fedorka & Mousseau 2002). In most cases however, having offspring outside of the mated pair leads to increased genetic diversity in the offspring and may be a form of genetic bet hedging (Fedorka & Mousseau 2002). Cheating on one’s partner can bring fitness gains without brood care investments, for example when males fertilize offspring with the mated female of another male (Gross 1996). In the fish literature such behaviour is called “sneaking”, whereas in birds it is often described as cuckoldry.

Male parental care that involves great investment, such as brooding in internal or external brood pouches, is thought to evolve where there is a high confidence of paternity (Kahn *et al.* 2013). Confidence in paternity may be high in fishes where there is external fertilization and males can monopolize the eggs at the time of fertilization. Some seahorses and pipefish, where males brood embryos in pouches, have been extensively studied with molecular tools and there has not been any evidence of multiple paternity in brooded offspring (Jones & Avise 1997b; Jones *et al.* 1999b; McCoy *et al.* 2001). Therefore, it is thought that male parental care strategies of this magnitude evolve in a context where sole paternity is guaranteed.

Females in monogamous relationships are considered less likely to participate in extra-pair mating as this will not increase their potential reproductive output. However, there may be some advantages in terms of the genetic diversity of the offspring. Sneak matings by females in monogamous species with paternal care are rarely observed. However, among some species of freshwater cichlids with maternal mouthbrooding, brood mixing is common. For example, in *Protomelas spilopterus* 5-65% of broods were sired by the brooding mother, while the remaining offspring had different mothers (Kellogg *et al.* 1998). The advantage of looking after the offspring of other females is unknown, and it is not clear if they are being duped into providing parental care. In goldeneye ducks, *Bucephala islandica*, older females lay eggs in younger females' nests without the latter being aware of the process (Eadie & Fryxell 1992). In monogamous species with paternal care, a female may opportunistically mate with more than one male if they can produce more eggs than one male can care for. In chapter 4 I examine for the first time in a coral reef fish the link between social and genetic monogamy, and study alternative mating tactics in males and females.

4. Relatedness in animal groups

Often animals live in groups in which they mate and provide parental care. Group living has many known advantages for unrelated individuals such as increased foraging efficiency and predator avoidance (Inman & Krebs 1987; Majolo *et al.* 2008). It may be even more beneficial to live in groups of kin: more stable social systems and decreased aggressiveness (Hamilton 1964; Ward & Hart 2003; Frommen & Bakker 2004). Many animal societies are thought to have evolved on the basis of co-operation among kin in producing the next generation. For example, eusociality in insects has evolved in the context of kin selection, where the fitness of an individual can be greater by helping a close relative reproduce, rather than having its own offspring (Hamilton 1964; Hughes *et al.* 2008). In terrestrial animals, kin groups are often established through pedigree analysis and many examples exist of the advantages arising from living in close proximity to relatives. Mammals, for example, often use group structures to effectively raise and protect young (Clutton-Brock 2009b; Clutton-Brock & Lukas 2011). However, when living with kin, the potential for inbreeding becomes an issue, and most species have mechanisms to avoid inbreeding through kin recognition and directed mate choice (Waldman 1987; Frommen & Bakker 2006; Harrison *et al.* 2011). In marine fishes, the potential for individuals to live in kin groups has received almost no attention due to long-held assumptions about larval dispersal. The final aim of this thesis (Chapter 5) was to examine the potential for self-recruitment and kin group structures in a group-living coral reef fish and study possible mechanisms of outbreeding.

Tools: Integrating behavioural studies and molecular methods

Understanding reproduction, dispersal and group structures in detail requires integrating molecular methods and long-term behavioural studies. It is known from birds that reproductive systems are often misinterpreted when our understanding of them is solely based on observational studies. For example, recent research has revealed that most bird species deemed monogamous actually perform extrapair matings, or have mating systems that should be called polygamous or polyandrous (Griffith *et al.* 2002). Genetic studies have revealed dispersal patterns and pedigrees within populations that were otherwise almost impossible to see for many species, especially in the marine realm (Avise *et al.* 2002; Fraser *et al.* 2005; Piyapong *et al.* 2011). Here I integrate molecular tools with behavioural observations and experiments to study the reproductive system of a coral reef cardinalfish as well as research group structures and dispersal.

Social relationships in fishes

Coral reef fish offer an ideal context to study social relationships as many species are habitat dependent and form small sedentary social groups (Barlow 1981). Many such species are socially monogamous, with apparently strong pair bonds (Whiteman & Côte 2004). They also exhibit strong site fidelity and strong homing abilities (e.g. Marnane 2000, Kaunda-Arara & Rose 2004, Kolm et al. 2005). However, the extent to which mate choice is direct, or mediated through site attachment is unknown for almost all groups of coral reef fish. Mate choice and fidelity in monogamous reef fishes are poorly understood, and have generally not been investigated with long term behavioural or molecular methods. Notable exceptions include work done on anemonefish and coral gobies, which revealed aspects of social dynamics within groups (Kuwamura *et al.* 1993; Wong *et al.* 2005; Buston *et al.* 2009) and questioned established concepts of the evolution of mating systems (Wong *et al.* 2007), emphasizing the importance of such studies. Almost all reef fish have a dispersive pelagic larval phase, but in the last two decades many examples of self-recruitment, in which a large proportion of juveniles return to their natal reef, have been detected (Jones *et al.* 1999a; Swearer *et al.* 1999; Jones 2015). High levels of local-scale self-recruitment leave open the possibility of interactions among kin in the formation of social relationships.

Cardinalfish

Coral reef cardinalfish (f. Apogonidae) offer a unique opportunity to investigate questions about social relationships, such as the evolution of monogamy, mate choice and parental care, and the structure of social groups. Many species of cardinalfish are group-living and extremely site attached to their diurnal resting sites (Marnane 2000). Some form pairs within their social groups, which can be short lived or in some cases appear to be long lasting, socially monogamous partnerships (Kuwamura 1985). All species studied so far have been found to be paternal mouthbrooders

(Breder & Rosen 1966; Thresher 1984), although the extent of paternity has not yet been studied. Almost all cardinalfish have a dispersive, pelagic larval stage, but self-recruitment and population genetic structures have scarcely been investigated.

Study species

Throughout this thesis, I use the pajama cardinalfish, *Sphaeramia nematoptera,* as a focal species to answer questions about social relationships in coral reef fishes. *Sphaeramia nematoptera* lives in small groups of 5-40 individuals in branching corals or caves on tropical coral reefs of the Indo-Pacific (Randall *et al.* 1997). The small groups and possible pair formation, as well as the unusual reproductive strategy of paternal mouthbrooding and high site fidelity they share with all other species of the family, make them a good focal species for studies of pair formation, mate choice and habitat dependence. Similar to *Pterapogon kauderni* it has a long brooding period of about 8 days, but in contrast to the well-studied banggai cardinalfish, the pajama cardinalfish has a pelagic larval phase, which makes it more representative of small habitat dependent coral reef fish for questions of dispersal and fine scale genetic structures. In the laboratory it was found to have a larval duration of 24-26 days (Fisher & Bellwood 2003).

Aims & Outline

The overall aim of this thesis is to understand the basis of prolonged social relationships in small, site attached coral reef fish, focusing on the structure of social groups, the mating system and the potential for kin associations. I use the pajama cardinalfish, *Sphaeramia nematoptera*, as a focal system to understand why groups form and why individuals form relationships within groups. The specific aims for each chapter were as follows: 1) Determine whether there is a connection between site fidelity and pair formation, and experimentally investigate the underlying basis of the relationship; 2) investigate size- assortative mating and possible role of mutual mate choice in pair bonding; 3) use genetic tools to establish the extent to which offspring are derived from socially monogamous pairs or extra-pair mating and sneak-mating by either males or females; and finally 4) investigate the pairwise relatedness of animals within and between social groups, determining whether recruits settle close to their parents, whether siblings settle together, and whether there is a fine scale genetic structure within the population.

These aims are addressed in four separate chapters. **Chapter 1** provides insight into the relationship between site attachment and mate attachment in *Sphaeramia nematoptera*. Using long term observational studies and behavioural experiments, I investigate whether fish in this species first choose a site and then choose their mate from possible partners on this same site, or whether they directly choose mates and return to the same sites to keep the pair bond, in **Chapter 2** I study pairing and mate choice within social groups of the study species. In this chapter I establish the nature of assortative mating and how such mating patterns came to be through mate choice or incidental pairing as juveniles by employing behavioural data gathered for many mating pairs and a mate removal experiment. **Chapter 3** compares the observational findings of mating and parentage patterns with molecular findings from the same population. Additionally, alternative reproductive tactics in both sexes are investigated. Finally, in **Chapter 4**, the social relationships of *S. nematoptera* within the social group and with offspring is investigated. Ecological data assembled in previous chapters is used and pairwise relatedness is calculated for all sampled members of the population. This allows the investigation of whether fish live in groups of kin and whether relatedness is different among and between reefs. The mechanisms behind levels of relatedness are also studied, including self-recruitment and associations among siblings at settlement.

Chapter 2 : Love the one you’re with: Site fidelity facilitates pair formation in aggregations of coral reef cardinalfish

Abstract

Colonial animals often form stable pair bonds, returning to the same site to breed with the same partner every year. Familiarity with both the partner and the site can enhance reproductive success. However, it is often unknown whether the mating system arises either as a result of site fidelity, mate fidelity or both. Here, observational and experimental studies are used to identify causal links between site fidelity and pair formation in a group-living coral reef cardinalfish, *Sphaeramia nematoptera*. A long-term field tagging study was undertaken to quantify site- and mate-attachment. This was followed by mate removal and mate transplant experiments to test whether the prolonged association with home sites was primarily because of mate or site fidelity. Adult *S. nematoptera* exhibited a prolonged association with home sites and partners, with some pairs lasting more than four months at the same site. Generalized linear models showed that individuals in pairs were more likely to remain site attached and males were more site attached than females. Following mate removal, 78% of *S. nematoptera* found a new partner within two weeks on the same site, supporting the hypothesis that individuals are primarily exhibiting site fidelity. This was confirmed by the partner translocation experiment, with only 1 of 24 fish following their translocated partner to a new site. In these cardinalfish, strong site fidelity facilitates long lasting pair bonds as well as new pair formation when necessary.

Introduction

Mate and site fidelity are two important characteristics of individuals that define the social and mating systems of animals (Cézilly *et al.* 2000; Naves *et al.* 2007; Bai & Severinghaus 2012). Many animals exhibit prolonged association with the same mates and breeding sites over multiple breeding seasons. For example, among colonially breeding sea birds that return to the same location to reproduce every year, monogamy is associated with high nest site fidelity (Danchin & Wagner 1997; Brown *et al.* 2000). The choice of preferred mates and sites is often inextricably linked. Hence, it is difficult to discern whether individuals are primarily attracted to sites where they choose from available mating partners (site fidelity) or whether they are primarily attracted to particular mates that continue to use familiar breeding sites (mate fidelity). Both site and mate fidelity are likely to enhance breeding success through familiarity. Whether one or both have primacy is an important aspect of the evolution of mating systems, in that it indicates whether site or mate fidelity came first in the history of a species. This information is also crucial in understanding a species dependency on the, often threatened, habitat.

Few published studies have demonstrated whether species primarily exhibit mate or site fidelity. Aebisher et al (1995) found that female shags, *Phalacrocorax aristotelis*, show greater fidelity to mates than sites. Individuals tend to leave their former nest site if their partner is removed and many follow their partners to new sites. On the other hand, the Lanyu scops owl, *Otus elegans botelensis*, has been found to exhibit both breeding site and partner fidelity, but path analysis suggests site fidelity is of primary importance (Bai & Severinghaus 2012). Similarly, Morse & Kress (1984) showed experimentally that site fidelity is of primary importance to Leach's storm-petrels. A phylogenetic analysis by Cézilly et al. (2000) showed that in the order Ciconiiformes, including 29 families, nest site fidelity evolved before mate fidelity, suggesting that the former has primacy in this group. Overall, the potential links between mate and site fidelity are poorly understood for most taxa. Both long-term observations on pair formation and longevity, combined with removal and/or displacement experiments, provide ways to explore whether pairing is facilitated by site fidelity or vice versa .

Among fishes, the relationship between site and mate attachment is best known for species with polygynous mating systems. Mate and site fidelity by females have been experimentally tested by male removal and replacement experiments (Jones 1981; Warner 1987, 1990). Females continue to spawn at the same sites, regardless of the male occupying them, suggesting that mate choice is mediated through site use by males and not vice versa. For paternal caring and polygynous fishes there is mixed evidence for both strong mate and site choice by females. In the freshwater cottid *Cottus bairdi*, females choose quality males over the breeding site (Downhower & Brown 1980). However, in the triplefin blenny *Forsterygion varium*, site quality is more important than mate quality, as females switched mates when nest site quality was manipulated (Thompson 1986). The relative roles of mate and site fidelity are less understood for monogamous fishes, where individuals often remain associated with the same site and mate throughout their lives.

Many coral reef fish species appear to be both socially monogamous (Barlow 1984) and characterised by strong site attachment (Sale 1971; Fricke 1986), but the links between pairing and site attachment and the consequences on social system dynamics are often unclear. Barlow (1984, 1986) suggested a close connection between the formation of lasting pair bonds and a species' site attachment. However, this has not been widely tested. Most monogamous fish families are closely associated with discrete benthic habitats such as coral colonies, rocky reefs or rubble patches (Whiteman & Côte 2004). However, their degree of site attachment can vary among individuals by reproductive status and sex. For instance, in the goby *Gobiodon histrio*, breeding pairs and juveniles move much less than solitary individuals (Wall & Herler 2008), while in the cardinalfish *Ostorhinchus cyanosoma*, females have a much higher movement rate than males (Rueger *et al.* 2014). Many coral reef fish form large social groups that are associated with discrete patches of habitat (Sale 1972; Booth & Wellington 1998; Fishelson 1998). Within these groups, many individuals may also form pairs or display strong social hierarchies. The relationships of individuals in these groups are poorly understood as there have been very few long- term studies on the social system of aggregating reef fish species. Detailed studies on the degree of mate and site fidelity are necessary to understand the evolution of monogamy in group-living reef fish.

Coral reef cardinalfish (family Apogonidae) typically live in large social groups and appear to be highly attached to their diurnal refuges (Gardiner & Jones, 2005; Marnane, 2000), many with strong homing to specific resting sites after their nocturnal foraging period (Marnane, 2000). A likely consequence of this site attachment and group formation is that individuals form mating partnerships within their groups. Cardinalfish are paternal mouthbrooders in which the male is the primary caregiver. There is increasing evidence that some species form monogamous pair bonds for at least one breeding cycle (e.g. *Ostorhinchus cyanosoma*, (Rueger *et al.* 2014), during which the male carries eggs from one female and the female gives the male all eggs from one clutch (Fishelson, 1970; Kuwamura, 1983; Kuwamura, 1985, 1986). However, the duration of the pair bond appears to vary, with some species forming pairs for mating once (e.g. *Ostorhinchus doerderleini* (Kuwamura, 1985), *Apogon niger* (Kuwamura, 1985; Okuda, 1999; Okuda & Ohnishi, 2001), while other pair bonds continue over multiple cycles (e.g. *Apogon notatus* (Kuwamura, 1983), *Pterapogon kaunderni* (Kolm & Berglund 2004). The degree to which the longevity of pair bonds is associated with the strength of site fidelity has not been investigated, and it is clear that the high level of site attachment and variability in social structure and pair duration, makes cardinalfish an ideal model to address questions on the nature of mate and site attachment.

In this study I investigated whether individuals in social groups of the Pajama cardinalfish *Sphaeramia nematoptera* exhibit a prolonged association with particular sites and mates, and whether this is explained by site or mate fidelity. Firstly, a 10-month long tagging and observational study was undertaken in Kimbe Bay (Papua New Guinea) to quantify site associations of individuals within social groups and whether individuals form stable pair bonds over multiple breeding cycles. Specifically, it was tested; (1) whether *S. nematoptera* exhibits long-lasting site attachment, (2) whether pairs are more site attached than single fish, and (3) whether site attachment differs among sexes and reproductive stages. Following this, a mate removal experiment was carried out. I predicted that if site attachment was more important than mate attachment, when partners were removed, individuals would continue to use the same sites and re-mate with other members of the social group. I then undertook a mate translocation experiment in which partners were moved to adjacent sites and social groups. Here I predicted that if mate attachment is stronger than site attachment then significantly more newly unmated individuals would follow their mates to the new sites than staying at their home site.

Methods

Study location and species

The study was carried out on cardinalfishes inhabiting inshore reefs in Kimbe Bay, Papua New Guinea (5°30'S, 150°05'E) near the vicinity of Mahonia Na Dari Research & Conservation Centre. The inshore reefs of Kimbe Bay host an abundant and diverse assemblage of cardinalfishes (Gardiner & Jones 2005). Breeding and recruitment of cardinalfish takes place year round at this low latitude location where the range in water temperature is minimal (29-32°C). Behavioural data were collected across multiple breeding cycles from several survey trips (October-November 2012, February - March 2013 and July - August 2013) and experiments conducted in April 2014 and May 2015.

The study focussed on one species of cardinalfish in Kimbe Bay: *Sphaeramia nematoptera*, which rests diurnally in large branching colonies of *Porites cylindrica*, on which they are habitat specialists (Gardiner & Jones 2005). In Kimbe Bay, *S. nematoptera* occurs in small groups of 5-20 individuals and appear to form pairs within those groups (pers. obs.). *Sphaeramia nematoptera* is a paternal mouthbrooder and the brooding phase typically takes 6-8 days, with several days of feeding required between broods, while the interval between spawning of females is 10-19 days (Kuwamura 1983; Kume *et al.* 2000a). In low latitudes these cardinalfish are presumed to be relatively short lived (<2 years) (Klein, 2007; Kume, Yamaguchi, & Aoki, 2003; Mees, Mwamasojo, & Wakwabi, 1999). The social system of this species has not been assessed before.

Observational studies on site attachment and pairing behaviour

Site attachment, pairing behaviour and group structure were examined by a 10-month tagging study, and given that many species live less than a year (Marnane 2000; Kume *et al.* 2003), may encompass the lifespan of many individuals. Fish were monitored for at least two breeding cycles over this period. This was repeated three and eight months later to assess the longevity of pair bonds. All handling and manipulating of fish was done in accordance with JCU Ethics Committee, approval number A1847.

Fish were tagged in nine groups from five reefs (N=102), based on their natural occurrences in the study area. Fish were caught using diluted clove oil (Munday & Wilson 1997) and hand nets and their standard length was measured to the nearest millimetre. Visible Implant Elastomer (VIE) tags were used to tag individual fish uniquely, using six colours and five positions on the fish. VIE has been shown to last for at least several months and does not impact fish behaviour, nor affect susceptibility to predation (Marnane, 2000). During each of the three observational periods (October - November 2012, February - March 2013 and July - August 2013) all individuals were re-located every two to three days via visual census, and information recorded on their location (to assess site fidelity, i.e. whether they were present on the same coral colony), their nearest neighbour (most likely mate), and whether they were breeding. Brooding males and gravid females were clearly identified by a distended buccal cavity and bulging abdomen respectively.

Preliminary observations showed that most adult individuals in social groups were more closely associated with one other individual and the overall sex ratio was slightly male biased. To quantify pairing, fish were categorized as “paired” if they were observed in close proximity (≤ 20cm) consistently over at least one breeding cycle (appr.14 days) (cf. Tetsuo Kuwamura, 1985; Pratchett, Pradjakusuma, & Jones, 2006). Fish that were not observed close to a particular fish, over multiple days were categorized as “solitary”.

To verify the method of classifying pairs, a short pilot survey was undertaken to test the hypothesis that the distance between apparent partners was significantly smaller than that to the next nearest individual in the group. Three minute focal views were conducted on 20 tagged individuals from four sites, their classified partner (usually a fish within the same coral branches, and who was occasionally in non-aggressive physical contact such as brushing of fins) and the nearest "non-partner" neighbour within the group. The distances between the focal individual and its partner, and between the focal individual and the next nearest neighbour, were estimated by trained observers on SCUBA every 20 seconds over three minutes. Mean distances between the paired fish and the neighbouring fish were compared using a paired t-test. Test assumptions were checked by visual assessment of the residuals. Partners were also assumed if only two of the group members were adults and one was brooding. Sex was determined by distended buccal cavity during brooding (male) and bulging abdomen shortly before brooding (female). All pairs were heterosexual and courtship behaviour was only observed between partners defined on this basis.

Site attachment was quantified by monitoring the seven sites of tagged groups and an area of approximately 30m around each specific home site every two to three days. This was done for three to six weeks, on each of the three survey trips (October-November 2012, February-March 2013 and July-August 2013). For each survey, every tagged fish was categorized as present on their original tagging site, moved to another known site, or absent.

In order to examine the effect of reproductive status on site attachment, it was necessary to determine the size at maturity. To do this, 34 individuals from groups not part of the observational and experimental study were collected, sized (SL), and their gonads preserved in 4% Formaldehyde solution. These fish encompassed the naturally occurring size range seen on Kimbe Bay reefs (10 – 54mm SL). Fish were captured with clove oil, and while still under anaesthesia, put into a 50% sea water–ice bath for an hour until dissections were performed (a standard method of euthanizing marine fish (Barnett & Bellwood, 2005; Marnane & Bellwood, 2002)). Samples were cast in paraphin wax, cut into 5µm sections and stained using Mayer's Haematoxylin and Young's Eosin Erythrosine. Sections were assessed for sex and maturity ("adult" with fully developed gonads, "subadult" with distinguishable oocytes or spermatocytes not fully developed, and "juvenile" with undifferentiated gonads). Results were compiled for each individual and size at maturity subsequently estimated. Each tagged individual was categorized as either adult, subadult or juvenile, based on their standard length at time of tagging.

Generalized linear models (GLM) were applied to assess which factor best explained the fishes' level of site attachment. Factors tested were sex, maturity, pairing and home site and all interactions between them. The response variable for site attachment was the number of times a fish was present out of the total number of possible observations within the initial one-month observation period. The first month was used because the rate of resighting was highest in this period such that a high number of individuals could be included in the analysis. To account for proportional data, a binomial error term was used. In case of overdispersion this was changed to quasibinomial. Dredge analysis was applied to assess the best approximating model according to Akaike Information Criterion with correction for finite sample sizes (AICc) (Burnham & Anderson 2002). All models with Δi values of up to 6 were considered (Richards 2005). Models were validated with diagnostic plots; (1) qqnorm of residuals for testing the assumption of normality, (2) residuals vs fit for testing the assumption of homogeneity of variance. All analyses were done in R statistical package R 3.0.0 (R Core Team 2015). To minimize observer bias, whenever possible, blinded methods were used when behavioural data were analysed. In some cases, however, it was not possible to record data blind because the study involved focal animals in the field.

Mate removal experiment

A mate removal experiment was undertaken to test the hypothesis that if site attachment drove pairing associations, the newly unmated individuals would remain on the same site and re-mate within the same social group, rather than leave and find another group. Thirteen paired females and 10 paired males were removed from their home sites using diluted clove oil and hand nets, while their partners remained at the home sites. The now unmated partners and home sites were surveyed every day for 15 days before and 15 days after the removal. The number of times an individual was observed to be on their home site or on a different site or lost was compared between the time before and after the experiment, using Fisher's exact test.

Mate translocation experiment

To establish whether mate attachment plays a greater role in determining the position of a fish, a mate translocation experiment was conducted. I hypothesized that individuals would follow their partner to a different site and thus display stronger mate fidelity than site fidelity. 12 males and 12 females from known pairs were captured as described above and relocated onto a nearby colony of *Porites cylindrica*. Translocation sites were located 4-10m away from the home site. *Sphaeramia nematoptera* travel at least 20m each night to their feeding grounds (pers. obs.), so this is well within the range of their normal diurnal movements. To avoid homing of the translocated partner, it was placed in a 15x15x15cm mesh cube (i.e. cage), which still allowed the other fish to see and smell it. Fish could have been influenced by the presence of conspecifics due to social hierarchies and aggression and the quality of the coral colony itself. To avoid any deterring effect of the coral colony or conspecifics, half the translocation sites had other adult *S. nematoptera* present (N_{males}=6, $N_{females}$=6) and half did not (N_{males}=6, $N_{females}$=6). To check for an effect of brooding status on mate and site attachment, 10 pairs were separated during a breeding cycle, when the male was brooding. The partner left behind was monitored and its position recorded 20-30min, 2-3hours and 20-24hours after the translocation of its mate. No more than two fish of the same sex were translocated from each group, in order to avoid effects of changing group structures. A cage control was employed at the same time on every site (N=20), with the ratios of males and females and brooding/non-brooding mirroring the treatment group. For the controls a fish was placed into a mesh cube on the same site and the site attachment of the partner was observed. A control for catching and handling involved catching one partner of a pair and releasing it back onto their home site and then recording the mate's behaviour (N=20). The number of fish moved were compared between controls and treatments.

Results

Site and mate attachment

Sphaeramia nematoptera showed high site attachment over a four-week period, with the majority of tagged fish (90.9%) still present at the same location on their original colony (Figure 2-1). After four months most tagged fish (80.8%) were still present and even after nine months half of the tagged fish remained (Figure 2-1). Within the eight-month period six fish (5.8%) were recorded to move between observed colonies.

There was strong evidence of stable pair bonds in *S. nematoptera*. Three quarters of tagged adults were paired, each pair always consisting of a male and a female (N=24 pairs). Pair quantification showed that partners were significantly closer to one another than to other members of the group (Figure 2-2, t = -4.8468, df = 22, p<0.001). These pairs stayed together for at least one breeding cycle (i.e. 4 weeks). After four months, 46.5% of pairs were still found together (N=10 pairs) (Figure 2-1). The rest had either formed new pairs (9.3%) or were solitary (44.2%). Social groups varied in size, with a mode of seven fish and ranging between four and 26 individuals.

Histological examination showed mature oocytes present in females above 38mm standard length (SL) and mature sperm in males above 39mm. All subadults were measured to be between 33 and37mm SL and all individuals smaller than that were juveniles. Based on these maturation sizes, at the time of tagging two-thirds of members in these groups were adults (N=65), a quarter were juveniles (N=25) and an eighth were sub-adults (N=12).

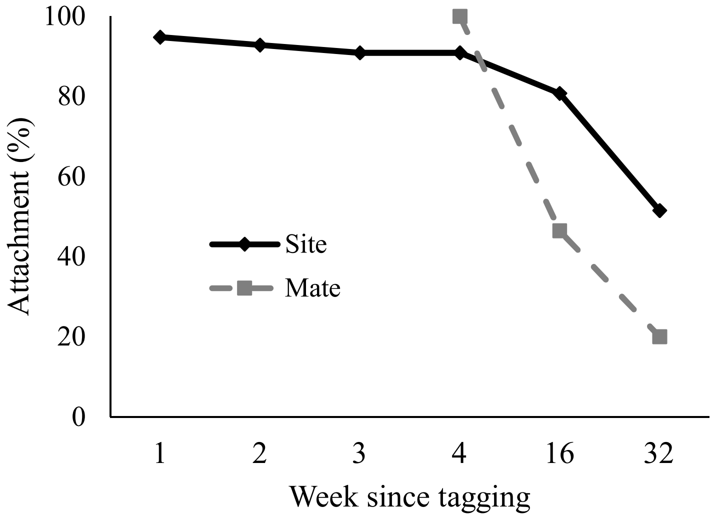

Figure 2-1 Site and mate attachment of *Sphaeramia nematoptera* to home coral colonies and mates. Site attachment is: the total frequency (%) of tagged fish present at the capture location after one, two, three, four, 13 (3 months) and 33 weeks (8 months). After four weeks observed individuals in pairs were designated as mates. Mate attachment is: the frequency (%) of pairs where both partners were still present initially (4 weeks), after 13 weeks and after 33 weeks.

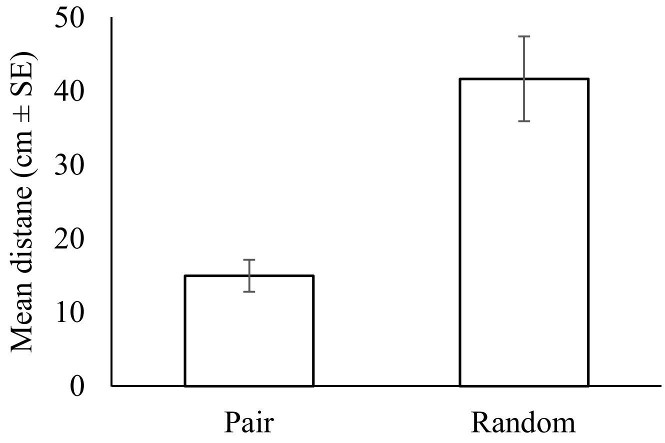

Figure 2-2 Mean distance (cm ±SE) of 23 focal *Sphaeramia nematoptera* to their partner (Pair) and a random other fish within their social group (Random) within a three-minute focal period

Determinants of site attachment

Variation in site attachment in *S. nematoptera* was best described by the sex and paired/solitary status of individuals (Figure 2-2a). This is backed by the strong support for the two highest ranked models (models 1 and 2, Table 2-1), which together make up an accumulative Akaike weight of almost 0.75, and thus the probability that these models describe the variability in site attachment is high. Paired individuals had greater site attachment than solitary individuals (Figure 2-2a), with paired fish on average 12.4% more likely to be at the original home site than solitary individuals. The effect of sex on site attachment was strong, with males 1.15 times more site attached than females in groups of *S. nematoptera* (Figure 2-2a, Table 2-1). Solitary females also show the highest variability in site attachment (Figure 2-2a). The GLM analysis showed that reproductive maturity (Figure 2-2b) and location (i.e. home site identity) of individuals only played a minor role (Table 2-1).

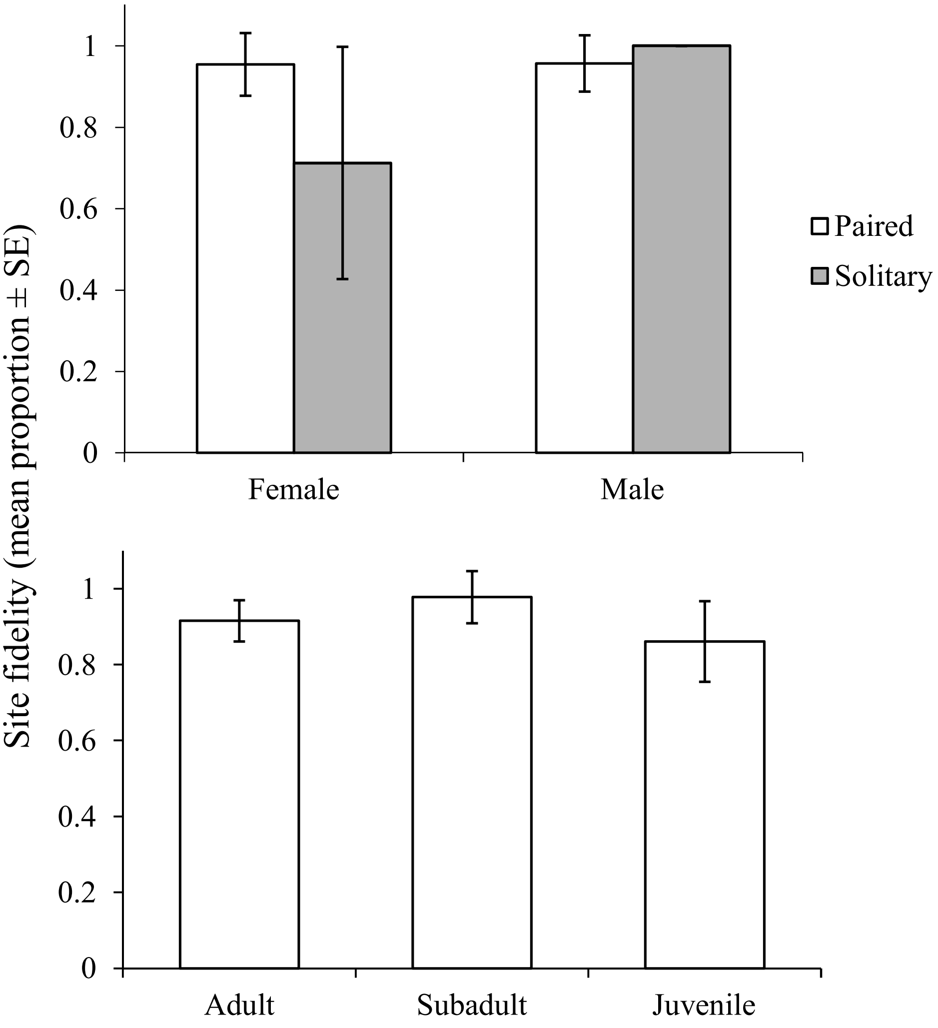

Figure 2-3 Influence of maturity, sex and paired/non-paired status on site attachment for *Sphaeramia nematoptera*. Site attachment is represented by the mean proportion (+/- SE) of number of observations on home sites; a) by sexual maturity (Adult, Subadult, Juvenile), b) by sex and paired/solitary status.

Table 2-1 Best approximating models (delta ≤ 6) for the influence of social system variables on site attachment, for *Sphaeramia nematoptera*. Displayed is: degrees of freedom, AICc, difference in AICc to first model (delta) and Akaike weight, which represent the relative likelihood of the model (weight). Variables included paired versus solitary status, sex, reproductive maturity, total length and home site. Accumulative Akaike weight of first five models= 0.9581.

Model			**AICc**	**delta**	**weight**
	Site attachment~				
1	Sex	2	15.0842	0	0.4118
2	Sex + Paired/Solitary	3	15.5113	0.4271	0.3326
3	Sex + Paired/Solitary + Sex : Paired/Solitary	4	17.2010	2.1168	0.1429
4	Sex + Maturity	4	19.9499	4.8657	0.0362
5	Sex + Maturity + Paired/Solitary	5	20.3689	5.2847	0.0293

Mate removal experiment

Following the mate removal, only two individuals, one male and one female, disappeared from the colony and were not seen again. There was no significant difference in the degree of site attachment fish displayed before and after their partners were removed (Fisher's p= 0.236), with fish using the same sites before and after mate removal. Most fish whose mate was removed (N= 18 ,78%) remained in the colony and had remated by the end of the 15-day observation period, with another fish from the same social group, which was significantly more than those that remained unmated (N=3, 13%, Fisher's p=0.998). Over half of these were actually observed reproducing, either brooding themselves or, in the case of females, with a brooding mate. Only three of the 23 unmated fish (13%) remained solitary on their home site, all of them males.

Mate translocation experiment

Only one out of 24 partners (4.2%) followed their translocated partner to the new refuge site. There was no difference in movement between males and females. It did not matter if there were other fish present on the new site or whether one of the pairs was in brooding phase or not. Treatment fish did not follow their mates. All controls stayed on their home site and in vicinity to their partner during the same time frame and there was no difference between treatment and control (Fisher's exact test, P=0.999). The control fish showed behaviours to indicate they recognized their partner fish inside the cage. They stayed close to the cage and in return the partner within the cage stayed close to the partner on the outside. The only *S. nematoptera* which followed their partner was a small, adult male (SL=45mm) paired, uncharacteristically, with a large female (SL=54mm). After the experiment the caged female was returned to its' home site and set free, the male followed within a few hours. The treatment fish whose partner was translocated also stayed within the same area of the coral colony they had previously occupied.

Figure 2-4 Number of *Sphaeramia nematoptera* which followed their mated partner after the latter had been translocated to a neighbouring site ("Followed") or stayed at their home site ("Stayed"), for males (N=12) and females (N=12).

Discussion

This study shows that site attachment in *Sphaeramia nematoptera* is stronger than mate attachment, suggesting that site attachment is a primary driver of the social system in this group-living species.

While long lasting pair bonds and also strong, long lasting site attachment was found, mate removal and partner transplant experiments show that fish did not leave a site in search of a new partner, and do not follow transplanted partners to new sites, regardless of sex. I argue that it is strong site attachment and familiarity within social groups that facilitates the formation of long-lasting pair bonds, not vice versa.

The results agree with previous research that suggests mate attachment is mediated through strong site attachment or habitat selection. While several studies showed that in polygynous species females often choose males with the most desirable site (Jones 1981; Warner 1988), the role of site selection in socially monogamous and group living species is less clear. In cardinalfish it can be expected that mate attachment may outweigh site attachment for females, since males are the primary care giver and much of the reproductive output depends on the male providing quality care. However due to the high energetic investment and the high risk taken by males, it may be male choice, rather than female choice, operating in *S. nematoptera*. Neither males nor females followed their mate to a neighbouring site, demonstrating that they would not translocate, even though the move would result in mating with the same partner. This shows strong attachment to the site independent of any mate attachment. Additionally, both sexes returned to their familiar resting site, even after their mate was gone. Leaving the familiar site to look for the original partner and possibly having to find another partner altogether seems to bear too great a risk.

The Pajama cardinalfish lives in distinct aggregations, yet form pairs within these groups and is socially monogamous. The proportion of paired adults in *S. nematoptera* (76%) is similar to what has been found in monogamous reef fish species such as the goby *Valenciennea muralis* (72.7%) and the butterflyfish *Chaetodon lunulatus* (68.2%) (Pratchett *et al.* 2006). Pair bonds in *S. nematoptera* were stable over at least several breeding cycles and up to four months. Other cardinalfish species have been found to form bonds that last at least several weeks, such as *Apogon notatus* (Kuwamura 1985). Very few gregarious species have been found to form pairs and even fewer show potential for social monogamy (Whiteman & Côte 2004), though overall pair formation and duration is seldom studied in group-living reef fish. Unravelling this mating system of social monogamy with a gregarious species is indicative of the potential to find many more complex mating systems within apparent homogenous reef-fish groups.

The high site attachment for *S. nematoptera*, has also been found for other cardinalfish (Marnane 2000; Gardiner & Jones 2005; Rueger *et al.* 2014). However, in *S. nematoptera*, more than 80% of pairs remained together after 120 days (four months), higher than found in other cardinalfish, which range from 64.1% after 16 days (*Apogon notatus*, Kuwamura 1985) to 72% - 75% after 120 days (*Cheilodipterus artus* and *Cheilodipterus quinquelineatus* respectively, Marnane, 2000). This variation could be due to variance in mortality and locality, as longevity varies with latitude. In this study, fish that did not return to their home site and were not found in nearby groups of conspecifics were categorized as lost and presumed dead.

While sex and reproductive maturity had some effect on site attachment, individual heterogeneity in site attachment was best explained by whether a fish was paired or not. Solitary females showed the highest rate of movement, while subadults had the highest site attachment. Similarly, to *S. nematoptera*, in species of colonially nesting sea birds, solitary individuals tend to be less site attached than paired ones (Morse & Kress 1984; Cuthbert 1985; Cézilly & Johnson 1995). This has also been found for the goby *Gobiodon histrio* (Wall & Herler 2008). Solitary fish might be forced to move around more and take greater risks to find suitable mates, whereas paired individuals could utilize the advantages of staying in a familiar site, with a familiar mate. Higher mate and site attachment can lead to greater reproductive output (Vincent 1994) and less energy expenditure on courting. When mates of *S. nematoptera* were removed, partners left behind did not move more, but rather remated on the same site. High site attachment might facilitate mate attachment but at the same time it might facilitate familiarity with other close by adults which enables an individual to re-mate quickly if suitable partners are available.

It is not yet known whether any cardinalfish pairs are truly monogamous or not. *S. nematoptera* seems a likely candidate as it is socially monogamous, there is a high proportion of paired fish in any group, pair bonds are long- lasting and females stay with males during brooding. In contrast to other potentially monogamous species, the mate removal experiment herein showed that the pair bond in *S. nematoptera* can be easily broken and pairing seems opportunistic: most individuals remated shortly after their mate was removed and many were brooding again within a month. Furthermore, single females had the highest rate of movements indicating that extra-pair matings may occur. Determining the social nature of group living fish, like cardinalfish, requires a combined observational and genetic approach in which large parts of the breeding population are sampled. In the case of paternal mouthbrooders, questions of exclusive mating within and between breeding cycles can only be conclusively answered by testing broods for multiple maternity.

Conclusion

In the study of sexual selection, it is important to distinguish between mate choice and other factors that might influence an individual to reproduce in a particular time and place, such as selectivity for particular breeding sites. The results support the emerging view that the mating system and reproductive biology of animals can be closely linked to site fidelity. Here it is found that the Pajama cardinalfish form pairs within their social groups and stay with their mate as long as both partners are present on the same site, indicating social monogamy. Once individuals have formed pairs they are clearly less likely to move among groups. Pairing behaviour is mediated through strong site fidelity, which appears to facilitate both long-lasting pair bonds and the ability to re-mate when partners are lost. How they choose these partners needs to be addressed in future studies.

Chapter 3 : Size matters: male and female mate choice leads to size-assortative pairing in a coral reef cardinalfish

under review *Behavioural Ecology*

Abstract

Many animals exhibit size-assortative mating and matching theory predicts this occurs because both males and females prefer bigger mates. Monogamy and size-assortative pairing have been described for coral reef fishes, but the underlying behavioural mechanism has not been tested. Here I took a long-term observational and experimental study to resolve the causes of size-based pairing in the paternal mouthbrooding coral reef cardinalfish *Sphaeramia nematoptera*. For 65 pairs observed over a 23-month period, there was a strong size-correlation between paired males and females. This size-assortative mating was not a consequence of pairing at a young age as re-pairing was common, with only 7% juvenile pairs still found together after eight months. For adults that changed partners over this period, there was a strong correlation between the size of individuals and the size of their new partners. Following experimental removal of partners, both males and females quickly repaired with partners of similar or larger size. Together, these results suggest that size-assortative mating is explained by a mutual preference by both males and females for larger mates. I suggest that monogamous pairing occurs in cardinalfish because mouthbrooding restricts multiple mating by males. Size-assortative pairing follows as larger males likely prefer the more fecund larger females, and larger females prefer larger males because they can successfully brood all of their eggs. Mutual mate choice will likely explain size-assortative pairing in other fish species with paternal care.

Introduction

The characteristics of mate choice have been a topic of considerable interest and controversy in behavioural ecology and evolutionary biology (Darwin 1871; Trivers 1972; Parker 1983). Females are normally considered the choosy sex because of their greater energetic investment into individual gametes, and female choice can be a powerful selective force in the evolution of secondary sexual characteristics (Darwin 1871; Trivers 1972; Kraaijeveld *et al.* 2007). There is a voluminous literature on the characteristics of males and the resources they control that form the basis of female choice (Manning 1975; Downhower & Brown 1980; Berven 1981; Gwynne 1981; Loiselle 1982; Sargent *et al.* 1988; Alonzo & Warner 2000). One recurring factor of importance is male size, whether because females prefer larger males or larger males control critical resources sought after by females (Hixon 1980; Partridge *et al.* 1987; Hastings 1988; Wacker *et al.* 2012). In monogamous animals, where individuals often form reproductive pairs that can last many breeding seasons, it may be expected that choosing a partner carefully is especially important for both females and males since this single choice will have great influence on their reproductive success. A partner's body size may then be a factor that influences the fitness of both sexes (Warner & Harlan 1982; Kraaijeveld *et al.* 2007). The main characteristic and mechanism of mate choice is unknown for most animals.

Where females and males prefer similar characteristics such as larger body size, such preferences lead to *assortative mating*. This is a mating pattern in which individuals with similar genotypes and/or phenotypes mate with one another more frequently than would be expected under a random mating pattern (Jiang *et al.* 2013). It is important to understand the underlying cause of assortative mating as it has important implications both for population processes and the role of sexual selection in the evolution of pair-forming species. Assortative mating may, for example, increase fertility of both males and females (Boag & Grant 1978; Davies & Halliday 1978) and lead to higher variability in reproductive success within each sex (Wade & Arnold 1980). It may also lead to the preservation of phenotypic variation, by reducing the production of intermediate offspring (Wright 1921; Crow & Felsenstein 1968; Bulmer 1980; Lynch & Walsh 1998) and in extreme cases facilitate sympatric speciation by promoting the isolation of phenotypically distinct populations (Dickinson & Antonovics 1973; Udovic 1980; Bearhop *et al.* 2005; Jiang *et al.* 2013).

Size-assortative mating is one of the most common mating patterns across the animal kingdom (Arak 1983; Crespi 1989; Harari *et al.* 1999b). Mate choice might lead to size-assortative mating through direct selection, where fitness of both partners is higher when mated with an individual of near similar size (Crozier 1917, 1918). Size-assortative pairing may also evolve if pairing with a similar sized partner produced fitter offspring (Jiang *et al.* 2013). The most common hypothesis is that size-assortative pairing will arise because both sexes prefer to mate with the biggest partner possible to maximise reproductive output, and female choice, male choice, male-male competition or mate availability explain the mating pattern (Crespi 1989; Andersson 1994; Arnqvist *et al.* 1996). In this context *matching theory* has recently been successfully applied to mate choice and sexual reproduction, making predictions about the occurrence of mutual mate choice that have yet to be tested empirically and showing assortative mating is likely to evolve in a population with gene flow when mutual mate choice and the necessity for stable pairs exists (Puebla *et al.* 2011, 2012). An alternative explanation is that assortative mating is incidental, caused for example by temporal or spatial aggregation of similar-sized individuals or intra-sexual competition (Crespi 1989; Cézilly 2004; Jiang *et al.* 2013). It could be that, particularly in site attached species, individuals form size-assorted pairs because they pair with a close neighbour when they are very young and then stay with this partner into adulthood.

Size-assortative mating appears to be particularly common in teleost fishes. A recent review by Jiang et al. (2013) found that the strength of the correlation between male and female size among pairs tends to be particularly high. Many coral reef fishes form monogamous pair bonds. While female preference for larger males has been established for some polygynous species (e.g. Berghe & Warner 1989), little is known of size selection in monogamous species. Most studies that have examined the size of males and females in pairs have tended to show a positive relationship among pair sizes (Crespi 1989; Andersson *et al.* 1998; Harari *et al.* 1999a). Many appear to form pairs at a small size and it is possible that size-assortative pairing is a result of pairing at a young age and forming long-term relationships. It has also been shown in the coral dwelling goby, *Gobiodon histrio*, that adults may regulate their growth rate to achieve size assortment (Munda*y et al*. 2006). However, if individuals are frequently changing partners, then size-assortative mating is more likely to be explained by mutual choice for larger partners in both sexes.

Size-assortative mating is likely to be a feature of paternal mouthbrooding cardinalfishes (f. Apogonidae), where selecting a larger mate may be beneficial to both sexes. Males in this group have high parental investment because they cannot feed during brooding and their swimming abilities are compromised (Okuda & Yanagisawa 1996a). Males may therefore seek the biggest female possible because bigger females have higher fecundity and lay larger eggs, as demonstrated for the banggai cardinalfish, *Pterapogon kauderni* (Kolm 2002). For the females, a large male is desirable because reproductive output is largely restricted by the buccal cavity of the male (Kolm 2002), on the other hand the risk of filial cannibalism is higher when the male is larger (Okuda & Yanagisawa 1996b; Okuda 2000). Therefore, it can be predicted that size may play a great role for both males and female cardinalfish when choosing partners. This is an analogous situation to some pipefish and gobies, which also show paternal brood care and size-assortative pairing (Berglund *et al.* 1986; Kuwamura *et al.* 1993). Alternatively, if assortative mating exists in cardinalfish, it may simply be a result of juvenile pairing.

Here it is investigated, for the first time in a coral reef cardinalfish, size-assortative mating and the role of mate choice for similar or larger-sized partners. I focus on the pajama cardinalfish, *Sphaeramia nematoptera*, a group-living, socially monogamous fish with a large percentage of adult pairing and strong mate fidelity (Chapter 2). Both long-term observational and experimental removals were undertaken to establish size-assortative mating and distinguish two possible mechanisms leading to size-related pairing. Firstly, pairing may come about because they pair shortly after settlement and consequently grow up together. Secondly, pair formation may result from active choice for partners of near similar size or frequent shifts to partners of a larger size by both sexes. These two mechanisms are distinguished by long-term observations of natural partner switching and a mate removal experiment in which the formation of new pairs was observed. Specifically test the following hypotheses were tested: (1) If pairs are established through size assortative mating, there should be a strong correlation between the sizes of male and female partners. (2) If this correlation is explained by young juveniles pairing and staying together as they grow, re-mating should be uncommon. (3) If pairing is size-assortative, for both sexes, re-mating following natural partner losses should be with similar-sized or slightly larger fish. (4) If pairing is size-assortative, following experimental removal of partners, both sexes should re-mate with similar or slightly larger/smaller sized individuals.

Methods

Study location and species

The study was carried out between October 2012 and September 2014 on inshore reefs in Kimbe Bay, Papua New Guinea (5°30’S, 150°05’E) in the vicinity of Mahonia Na Dari Research & Conservation Centre. Data were collected from several survey trips (October-November 2012, February - March 2013 and July - August 2013) and an experiment conducted in April 2014. The study species, *Sphaeramia nematoptera* (Apogonidae) occurs in groups of 5-20 individuals on the sheltered inshore reefs of Kimbe Bay. It is a paternal mouthbrooder, which shows great site fidelity to its diurnal resting locations, usually associated with *Porites cylindrica* (pers. obs.). It is suspected to be socially monogamous due to its high percentage (75%) of paired adults and mate fidelity (Chapter 2). *Sphaeramia nematoptera* reach sexual maturity at a standard length (SL) of 38mm, and in Kimbe Bay a reproductive cycle lasts less than one month, with males brooding once or twice a month (pers. obs.).

Size-assortative mating

The potential for size assortative pairing was examined by a long-term tagging study over 23 months. In total, 253 *S. nematoptera* were tagged in 17 groups on five reefs in the study area. Fish were caught using diluted clove oil (Munday & Wilson 1997) and hand nets and their standard length was measured to the nearest millimetre. Visible Implant Elastomer (VIE) tags, six colours and five positions on the fish, were used to tag individual fish uniquely. VIE has been shown to last for at least several months and does not impact fish behaviour nor affect susceptibility to predation (Marnane 2000). Several studies have found little or no effect of VIE tags on behaviour of fishes (Whiteman & Côté 2002; Myhre *et al.* 2012).

During five observation periods (October-November 2012, February - March 2013 and July - August 2013, March- April 2014, September 2014) all individuals were located several times (every two to three days) over at least two breeding cycles and their partner noted. Each fish was included in the analysis once, with the first identified mate, in order to avoid temporal pseudoreplication. Partners were defined as individuals of opposite sex found in closer proximity than other individuals for the majority of observations in one survey period (Chapter 1). Actual courtship behaviour has only been observed for adult partners defined on this basis. Three pairs were observed with one individual smaller than the minimum size for adults, SL=38mm, and were excluded from the analysis.

On average 25% of adults in every social group are categorized as solitary, i.e. they are not observed to pair with a mated partner. These adults are not significantly different in size compared to the paired individuals (Appendix Table A-1).

Size-assortative pairing was established by correlating male and female standard length (SL) with Pearson's correlation coefficient (Wright 1921; Redden & Allison 2006), and tested using a linear model with male standard length (SL) as the response variable and female SL as the independent variable. I tested whether reef and site as random factors affected the pairing results by conducting a likelihood ratio test of the linear model without the random effect against the full mixed model. The coefficient of determination R^2 was calculated following Johnson (2014). I further analysed whether males or female were more likely to be the bigger partner within a pair and how big the size differences were.

Partner losses and remating

Individuals of all sizes commonly lost partners during the period of the study, either because individuals moved or died. Partners were categorized as lost when they did not return to their home coral colony for more than two observations. The frequency of remating after natural losses was recorded for all adults, subadults and juveniles. Thirty-six pairs of juveniles were followed for more than 8 months and their pairing status was confirmed in each observation period during that time.

Newly solitary individuals were closely observed over subsequent weeks and months, in order to observe which ones remated and the size of the new mate. The newly formed pairs were tested for size assortment by examining the correlation between the size of individuals and their new partners. Fish were recaptured and their standard length remeasured if several months had passed since the original size measurement. Size differences between the new and old partner were also calculated, to determine if new partners were consistently larger than the old ones. The new partners were categorized relative to the newly solitary fish with fish ±5% SL categorized as “similar”, fish >5% SL bigger categorized as “bigger” and >5% SL smaller categorized as “smaller”. 5% difference was chosen because this small variation leads to a perceptible size difference in the deep bodied *S. nematoptera*, and the variance in SL was small in the sample population with a range of SL from 38mm to 56mm for both males and females and a mean ± standard deviation of 45.7mm ± 4.5mm. I also categorized the new partners relative to other single or newly single adults of opposite sex available to the focal fish in the same group; the fish with largest SL was categorized as “biggest”, the smallest as “smallest” and fish in between as “medium”. To test whether one category was chosen significantly more often than expected by chance, exact binomial tests were performed. To analyse the difference between males and females Fisher’s exact test was performed.

Mate removal experiment

A mate removal experiment was conducted to test the hypothesis that both males and females exhibit size-assortative mating and compare the strength of the size-assortative mating in both sexes. Individuals were removed from 34 pairs (16 males and 18 females) from 8 different sites, with their partner remaining at the home site. The removed individuals were used in a different experiment and were returned to their original home site three to six weeks later. The partners and home sites were surveyed every day for 15 days before and 15 days after the removal and after that were part of the regular monitoring that continued through to September 2014. Size-assortative mating was established by examining the relationship between individuals and their new partners as well as whether there were significant differences in standard length between males and females. In addition, it was examined whether the new partner had left a previous partner or whether they had been solitary individuals. In case of partner switches the size of the previous and new partner was compared.

All statistical analysis was performed with R 3.2.3 (R Core Team 2015). Assumptions were tested and met for all tests. Visual inspection of residual plots did not reveal any obvious deviations from homoscedasticity or normality. Correlations were tested using the Hmisc package (Harrell 2015) and models were fitted and compared using the nlme package (Pinheiro *et al.* 2016).

Results

Size-assortative mating

Across all groups of fish, there was a significant positive relationship between the sizes of individuals in pairs (Pearson's r= 0.72, p<0.001; ANOVA $F_{1,60}$= 65.536, p<0.001; Figure 3-1). Overall, partners tended to be of near similar size, with female size explaining more than 52% of the variance in male size (R^2=0.522). However, for those with a size disparity, there was no consistent pattern in terms of which sex was the bigger partner. Males were the bigger partner in 23 pairs (37.1%) and females were bigger in 25 pairs (40.3%), with the remainder being of equal size in standard length (N=14 pairs). When females were bigger in a pair, they were an average (±SE) of 5.0% ±0.8% bigger, whereas the difference was slightly larger when males represented the bigger partner with an average (±SE) of 7.7% ±1.5%. The differences between reefs and sites did not play a major role in determining size-assortativeness between partners. Both were insignificant when added as random effects to the model (Likelihood ratio test; Reef: L-ratio=0.151, p= 0.697; Site: L-ratio= 2.360, p=0.124).

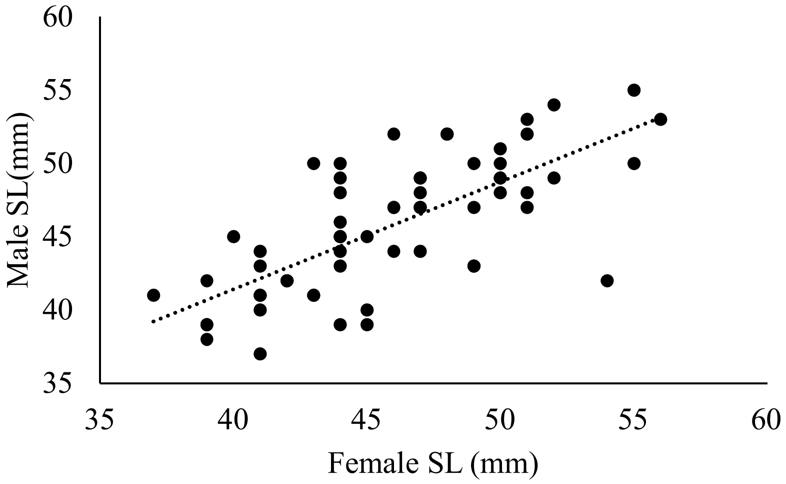

Figure 3-1 Sizes of males and females in pairs (standard length (SL) ± SE), in natural population of *S. nematoptera* (N=62).

Partner losses and remating

There was no evidence that size-assortative mating was explained by individuals forming long-term pair bonds as juveniles. Most juvenile pairs did not last into adulthood. Out of 36 observed pairs of juveniles, 10 were still together after four months (28%), which represents 50% of pairs of which both partners were still present on the site. After eight months only 2 pairs out of 28 (7%) were still found together, this corresponds to 13% of pairs for which it was possible to still be paired (N=16). Within the first four observation periods from October 2012 to April 2014, 21 instances of natural partner losses in adults were observed. Sixteen of the left behind partners remated, three were lost, one remained solitary on the same site and one changed site to a known location, where it remained solitary for the duration of the subsequent observations.

Natural losses resulted in high rates of remating and there was a strong positive correlation between the size of individuals and their new partners (Pearson's r=0.67, p=0.0022, Figure 3-2a). Most individuals remated with a fish of similar size to themselves (56.3%, Figure 3-2b), but matings with both larger and small individuals were observed (Exact Binomial, p=0.0637). There was no significant preference for the biggest, smallest and medium sized fish available (N=6, N=7, N=3 respectively, Exact Binomial test, p= 0.292) (supplementary material Table A-1). On average (±SE) new partners were 8.2 % (±2.2 %) smaller than old partners. There was no significant difference between males and females in choice of partner size relative to their own size (Figure 3-2a, Fisher's exact, p=0.3171).

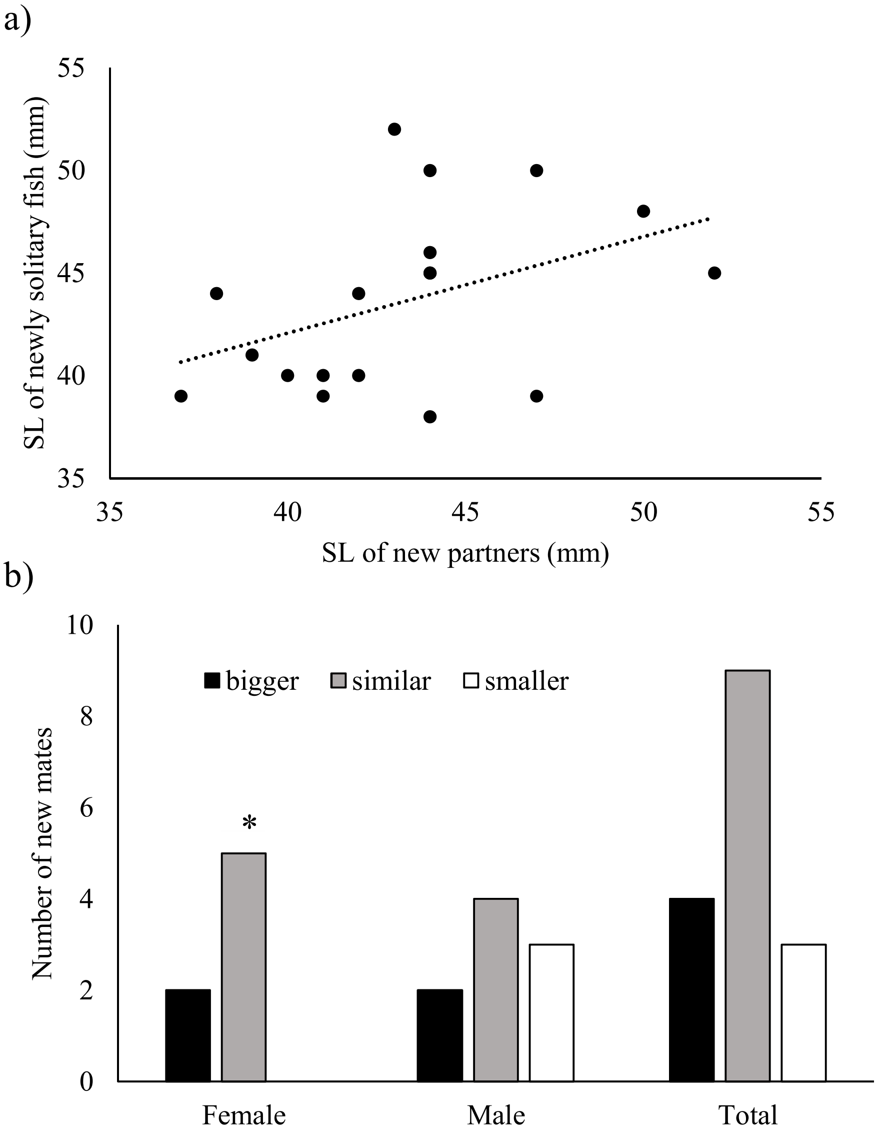

Figure 3-2 Remating patterns after natural losses in *S. nematoptera*. (a) Correlation between the standard length (SL) of the individual which experienced a natural partner loss ("newly solitary fish") and the new partner it remated with ("new partner") (N=16), (b) the number of new partners which are bigger (> 5% bigger in SL), smaller (> 5% smaller in SL) or of similar size (≤ 5% difference in SL) to females and males which experienced natural losses. Significant ($p<0.05$) differences according to Exact Binomial test are indicated with *.

Mate removal experiment

When their partner was removed, most fish (N=22, 64.7%) re-mated with a similar sized fish and there was a positive correlation between individuals and the size of their new partner (Pearson's r=0.48, p=0.0207). Similar sized fish (maximum of 5% difference in standard length) were chosen significantly more often than expected by chance (Exact Binomial, p=0.0003, Figure 3-3). New partners were either the largest (N=11) or smallest (N=7) single fish available, seldom of medium size (N=3) (Exact Binomial, p= 0.098). The new partners were very similar in size to the old partner, with an average (±SE) of 0.43% ±1.5% difference. Almost half of the new partners were of similar size as the old partner (N=10), and for the remainder, equal numbers were bigger and smaller (N=6). There was no significant difference between males and females in choice of partner size relative to their own size (Figure 3-3, Fisher's exact, p=0.327).

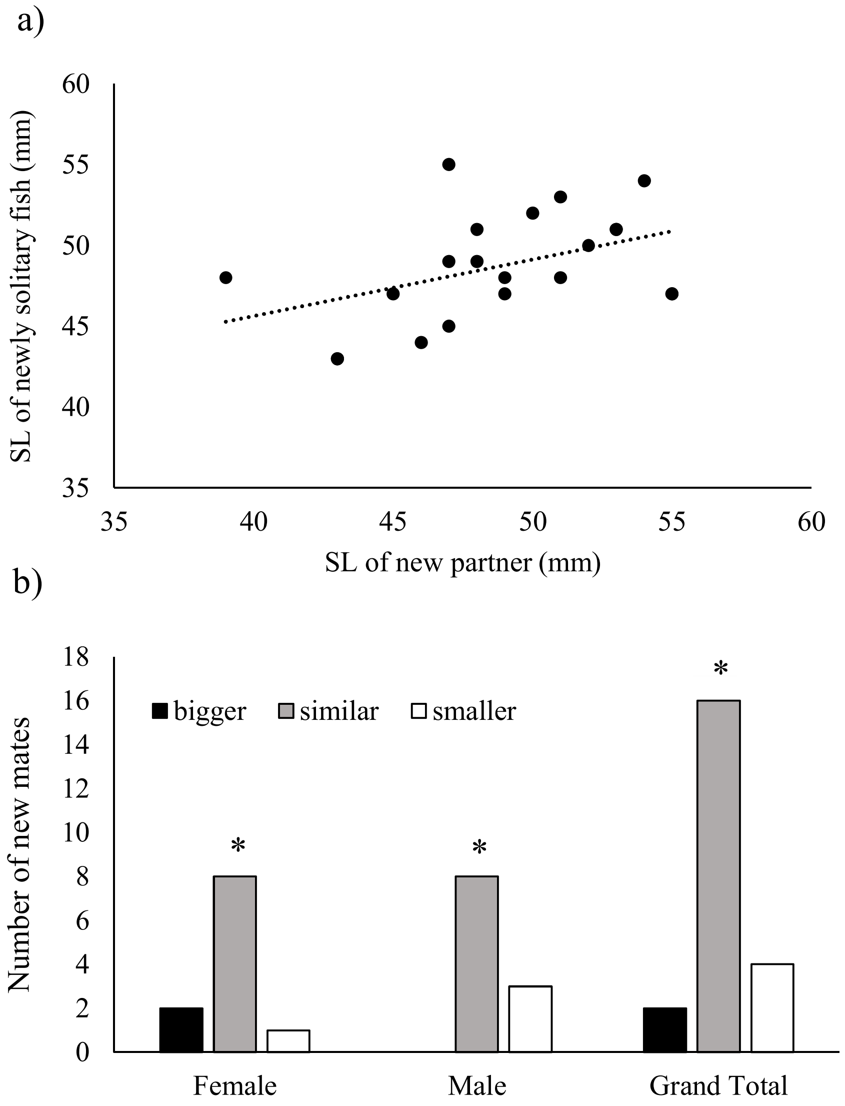

Figure 3-3 Remating after experimental mate removal in *S. nematoptera*. (a) Correlation between the standard length (SL) of the individual whose partner was removed ("newly solitary fish") and the new partner it remated with ("new partner") (N=22), (b) the number of new partners which are bigger (> 5% bigger in SL), smaller (> 5% smaller in SL) or of similar size (≤ 5% difference in SL) to females and males whose partner was removed. Significant ($p<0.05$) differences according to Exact Binomial test are indicated with *.

Out of the 22 new partners, 27.3% (N=6) had a partner prior to pairing up with the experimental fish. The rest were either single before (72.7%, N=14) or their partner was lost in the course of the experiment (9.1%, N=2). For five out of the six fish (83%) that switched partners, the new partner was larger. The one exception is a case of both partners being paired with a newly single fish, it might thus be that fish (a) left fish (b) for a new, bigger partner and fish (b) then had to pair with a slightly smaller fish.

Discussion

I found strong evidence of size-assortative mating in the coral reef cardinalfish, *Sphaeramia nematoptera*. Field observations showed a strong size-correlation between paired males and females, with no tendency for either sex to be consistently larger. Both males and females chose new partners of similar size when mates were lost, either through natural losses or following experimental removals. The preference for similar-sized mates appears to be mutual rather than due to the formation of long-term pair bonds by juveniles, since most juvenile pairs lasted only a few months. This is one of very few studies which investigate mutual mate choice and consequent size-assortative mating in coral reef fish.

Size-assortative mating: juvenile pairing versus mutual mate choice

This study clearly demonstrates positive size-assortative mating in *S. nematoptera*, implying that for both sexes, it is more desirable to mate with an individual of near similar size. Mate choice appears to be mutual, with both males and females showing a strong preference for partners of similar size. Both after natural losses and artificial mate removal, individuals remated with partners that were more similar in size both to themselves and their previous partner, than to other potential mates in the social group. Results support the general view that size-assortative mating is a common mating pattern in the animal kingdom and is particularly prevalent in fish. In the vast majority of cases, correlations are positive and among all the phyla, fish show the largest coefficient of size- correlation (mean of 0.55) (Jiang *et al*. 2013).

The mechanism causing the close size-relationship in *S. nematoptera* partners does not appear to be incidental through juvenile pairing, except for rare cases, but rather direct preferences for partners of similar size seems likely. Some reef fish have been shown to pair at a young age and mature together, such as *Gobiodon erythrospilus* (Hobbs *et al.* 2004). That this is not the case for most *S. nematoptera* is apparent since the size matching of partners remains consistent in spite of frequent repairing of both juveniles and adults. The strength of the correlation between the partner's size remains consistent both before and after natural losses as well as artificial mate removal and even in presence of potential partners of various sizes. A low number of juvenile pairs stay together through to adulthood, but most individuals are likely to re-form pairs numerous times as they grow, which does not reflect the high rate of adult pairing and strong mate fidelity demonstrated in previous studies (Chapter 2).

Why does size-assortative mating arise?

Size-assortative mating is likely to arise when potential reproductive success of both males and females increases with body size. In fish it is most often connected to particular reproductive strategies, particularly those with paternal care. I suggest that monogamous pairing occurs in cardinalfish because mouthbrooding restricts the ability of males to increase reproductive success by mating with multiple females. Size-assortative pairing follows as larger males prefer the more fecund larger females, and larger females prefer larger males because they can successfully brood an entire clutch. Mutual mate choice likely explains size-assortative pairing in other fish species with paternal care. For example, in the coral goby, *Paragobiodon echinocephalus* the male guards the eggs and both male and female fecundity are correlated with size, in which pairs are also size assorted (Kuwamura *et al.* 1993). Evidence for mutual mate choice has also been found in the pipefish *Syngnathus typhle* where, similar to *S. nematoptera*, males are responsible for brood care and fecundity increases with body size (Berglund *et al.* 1986). By contrast, a pipefish in which male fecundity does not change with size, *Nerophis ophidion*, shows one-sided, male mate choice (Berglund *et al.* 1986). In species where females brood, such as freshwater cichlids, female choice outweighs male choice (Keenleyside *et al.* 1985; Salzburger *et al.* 2006).

Support for matching theory

Matching theory predicts that a stable pairing system should arise if both sexes compete for the largest possible mate (Gale & Shapley 1962; Puebla *et al.* 2011). It follows that if every fish aims to find the biggest, most fecund partner possible, the population will be made up of stable, size-matched pairs. The only time a partner would leave a stable pair in such a system would be if a bigger, more desirable, partner became available. The current study supports this conclusion for *S. nematoptera*. One incident of a partner leaving a pair for a slightly smaller fish was observed, but in this case, the previous partner had left for a new, bigger, mate first, leaving its partner solitary and with no other choice than to go with the smaller available individual. The fact that newly solitary fish, either through natural losses or mate removal, re-mate with similar sized fish is explained when mutual mate choice is assumed as the mechanism for pairing. Even when presented with several options, *S. nematoptera* in this study tended to pair with a partner of similar size. This is in contrast to what has been found in the blister beetle *Lytta magister*, which mates assortatively but when males are presented with females of similar size to themselves or larger size, larger females are preferred (Brown 1990). Every individual in the group will try to mate with the biggest possible partner. However, since most adults are in pairs, it is not often possible to switch to a bigger partner and larger single fish might execute their choice by refusing to mate with a smaller partner. Mutual mate choice also explains the incidences where newly solitary individuals “choose” the smallest possible partner, since every one of the fish that are available tries to choose the most desirable mate, and already mated individuals only leave pairs for considerably bigger individuals, the newly solitary fish may have to pair with a less desirable small adult.

Other mechanisms of size-assortative mating

One alternative mechanism is indirect mate choice, where through intra or inter sexual competition size assortative pairs form. Indirect choice through intrasexual competition has been demonstrated in several arthropod and bird species (Crespi 1989; Cézilly 2004), and also in the size-assorted cichlid *Eretmodus cyanostictus*, where after mate removal, aggressive interactions decided on territory ownership in both sexes and in all cases winners of these interactions were accepted as partners (Taborsky *et al.* 2009). In cichlids, female choice is often associated with male-male competition (Keenleyside *et al.* 1985). This seems improbable in *S. nematoptera*, which shows no sexual dimorphism and low rates of aggressive behaviour (pers. obs) and can thus be hypothesized to have equal gender roles. Furthermore size assorted mating could be facilitated if fish school by size, thereby evoking a segregation by phenotype which may lead to assortative mating even under random mating, as has been shown for arthropods (Crespi 1989). In *S. nematoptera* no such segregation by size has been observed, with schools made up of individuals of various sizes (unpubl. data). Another mechanism leading to assortative mating commonly described is mate availability, where either only few females exist in the population or few partners are available due to temporal or spatial covariation (Crespi 1989; Arnqvist *et al.* 1996). Arrival time at mating sites might for example play a big role in mating patterns of migrating birds, as shown in the European blackcap *Sylvia atricapilla* (Bearhop *et al.* 2005). In cases where mate availability is low, animals may even regulate growth rates to achieve size assortative mating and maximise reproductive success, as has been shown in *G. historio* (Munday *et al*. 2006). No such temporal or spatial segregation was apparent in the sample population. Furthermore, in most cases newly single *S. nematoptera* in this study had more than one option for pairing with a new mate, thus the lack of available mates is an unlikely cause for size assorted pairing in *S. nematoptera*. More detailed observations of interactions between and among the sexes during mating and pair formation, as well as experimental studies should be conducted to confirm direct mate choice and to confirm whether cryptic processes correlated with size might play a role.

Further research is needed to discern whether direct or indirect selection acts on mate choice in *S. nematoptera*. Assortative mating could influence the fitness of a pairs' offspring, as demonstrated in *Heliconius* butterflies in which adults mate assortatively by colour morph to avoid maladapted colour patterns in offspring (Chamberlain *et al.* 2009), a form of indirect selection. On the other hand direct selection has been demonstrated for example in marine nudibranch where copulation is facilitated when partners are of similar size (Crozier 1918). Direct selection could be shown by demonstrating that fitness depends on the similarity of partners in *S. nematoptera*. This could be measured by comparing the number of offspring between assorted pairs, where partners are of similar size, and non-assorted pairs, with different sized partners. Further support of matching theory and mutual mate choice could be gathered by measuring aggression rates between and among the sexes, since mate defence might be expected (Wong *et al.* 2007).

Conclusions

The present results indicate size-assortative mating explained by direct preference by both male and females for similar sized partners in the pajama cardinalfish. It is also possible that in some cases, both sexes will compete for larger size partners when remating occurs, which will strengthen the size-assortative pattern. Size-assortative mating is most likely explained by the fact that reproductive success for both sexes increases with body size, with fecundity in females increasing with body size and larger males able to brood more eggs in their buccal cavities. The patterns found for *S. nematoptera* are likely to be applicable to many other species with paternal care.

Chapter 4 : A bit on the side: Extra-pair mating by males and females in a socially monogamous and paternal mouthbrooding cardinalfish

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Abstract

Many vertebrates form long-term monogamous pair bonds for the purposes of mating, caring for offspring together and they benefit from higher reproductive output and decreased courtship costs with familiar mates. However, increasingly, genetic molecular tools have shown that many offspring arise from extra-pair mating. The factors affecting the prevalence of extra pair mating and which sex is more likely to cheat are poorly understood. Social monogamy is also common in coral reef fishes, and while sneaker spawning has been observed, there has been no genetic confirmation of monogamy or extra-pair reproduction, either for males or females. Here long-term observations and genetic tools are applied to examine the paternity and maternity of embryos in the socially monogamous coral reef cardinalfish, *Sphaeramia nematoptera.* I hypothesized that because this species is a paternal mouthbrooder, females and males may have limited opportunities for cheating. Two-years of observations on tagged individuals suggests individuals form long-term pair bonds within larger social groups. However, genetic parentage tests revealed extra-pair mating and sneak mating by both sexes. Of 105 broods analysed from 64 males, 30.1% were mothered by a female outside the social pair and 11.5% of broods included eggs from two females. Despite the high paternal investment of mouthbrooding cardinalfishes, 7.5% of broods were fathered by two males, demonstrating male sneaking. Extra-pair matings were not related to body size or social status, but appeared to be opportunistic encounters with individuals from outside the immediate social group. I argue that pair formation and social group cohesion is maintained by social monogamy. However, both males and females, while having limited opportunity to cheat, will take advantage of extra-pair mating opportunities when individuals stray between social groups. These results provide evidence

for the existence of female sneaking and refute the theory that high-investment paternal care evolves in concurrance with guaranteed sole paternity.

Introduction

Animals have adopted a great variety of mating systems, from those in which individual males and females form long-term pair bonds (monogamy) to varying degrees of mating with multiple partners, by both males and females (promiscuity) (Breder & Rosen 1966; Emlen & Oring 1977; Smith 1984). According to theory, males have a greater propensity to mate with multiple partners, with polygyny (where only males mate with multiple partners) more common than polyandry (where females mate with several males) (Wade & Arnold 1980). However, monogamy is also expected to arise where there are life history constraints, such as the need for extensive biparental care, or ecological constraints that limit an individual's ability to acquire multiple partners (Wittenberger & Tilson 1980; Lukas & Clutton-Brock 2012). Most species have been described as monogamous on the basis of long-term observations on the association between a male and a female, rather than by direct observations of copulation or genetic evidence of maternity or paternity. However, new genetic tools have shown extra pair reproduction is more common than previously thought (Westneat 1987, Hughes 1998, Moller & Birkhead 1994, Sillero-Zubiri et al. 1996). This raises questions as to how social systems such as monogamy are maintained in face of high levels of extra-pair mating or "cheating". Despite the increasing evidence, the proportion of offspring arising from extra-pair mating and the propensity of either males or females to engage in this behaviour are poorly understood for most taxa.

Social monogamy appears to be unusually prevalent in coral reef fishes (Whiteman & Côte 2004), where it has been described in 14 families, such as gobies (f. Gobiidae), butterflyfish (f. Chaetodontidae) and damselfish (f. Pomacentridae) (see review Barlow 1984, 1986). Unlike birds, most fish have little or no parental care and it is unclear how social pairs form and are maintained.

While pairs most often consist of a male and a female, homosexual pairing has also been observed (Morris 1952; Brandl & Bellwood 2013). Similar to birds, social monogamy in coral reef fish has been largely defined on the basis of long-term observations, however mating is rarely observed because most fishes breed at dusk or during the night. The factors maintaining social monogamy in coral reef fishes remain poorly understood (but see chapter 2), and few genetic studies have been undertaken to examine the extent to which offspring arise from extra-pair mating by both males and females.

Sneak mating, where an individual gains reproductive success without the knowledge or consent of the mated pair, may be a random opportunistic behaviour or represent an alternate reproductive strategy to maximise reproductive success of the sneaker with low cost. The likelihood of sneak mating may also depend on an individuals' stage of development and place in social hierarchies (Gross 1996). Such differing strategies have been recorded in fish, and the evolution of such strategies is thought to be strongly influenced by interactions between the sexes (Taborsky 1994; Henson and & Warner 1997). Often it is body size that determines which alternative reproductive strategy within a species a male or female will take. For example, in wrasses, small males often spawn in large groups whereas larger males defend territories from other males (Warner 1987). The oscellated wrasse, *Syphodus oscellarus*, has four male alternative strategies that are staggered by size: small sneaker males; intermediate nest defenders; large nest builders ;and very large nest piraters (Warner & Lejeune 1985; Taborsky 1998). On coral reefs, sneak mating has been observed for damselfish (f. Pomacentridae) (De Boer 1981), butterflyfish (f. Chaetodontidae)(Lobel 1989) and wrasses (f. Labridae)(Berghe & Warner 1989). To date, extra-pair matings or ontogenetic changes in mating strategy have not yet been confirmed for monogamous coral reef fishes and female sneak mating has yet to be described. Defining mating strategies will require detailed genetic studies that look at the prevalence of extra pair matings and sneaking from adults at different life history stages.

The factors affecting opportunities for extra-pair mating in monogamous fishes are likely to depend on the mode of parental care. Fishes in general often exhibit paternal care, either by guarding developing embryos on the substrate, mouth brooding or by carrying the embryos on the body as do pipefishes and seahorses (Perrone & Zaret 1979). Paternal care is thought to relate to confidence in paternity in externally fertilizing fishes, but this would only apply if there is a low probability that other males have fertilized the eggs a male is caring for (Smith 1979, Jones and Avise 1997). Where males have all the parental care duties, opportunities to cheat may be limited, with females having a greater propensity to seek extra pair copulations, especially when they can produce more eggs than one male can care for (Clutton-Brock 2009a). Multiple paternity has been observed within broods of mouthbrooding females of *Pseudotropheus zebra* (Parker & Kornfield 1996) and other species who show biparental care (Kellogg *et al.* 1995; Sefc *et al.* 2008). Broodmixing is extremely common in mouthbrooding cichlids from lake Malawi (Keenleyside 1991), and in *Protomelas spilopterus* the proportion of offspring sired by the brooding female ranged from 5 to 65% in four out of six broods examined (Kellogg *et al.* 1998). Evidence for multiple paternity has also been found in 90% of broods in live-bearing mosquito fish, *Gambusia holbrooki* (Zane *et al.* 1999). However, the relative frequency of multiple mating by males and females in socially monogamous, paternal caring coral reef fishes has not been examined.

Some coral reef cardinalfish are socially monogamous, but the relative frequency of pair and extra-pair mating is unknown (Kuwamura 1985; Kume *et al.* 2000b). Paternal mouthbrooding clearly creates a disparity between males and females in the opportunity for sneak and extra-pair matings. Since the brooding takes place orally, the male cannot feed during the breeding cycle and must recover before he can brood again (Okuda 2001). Females can potentially produce eggs faster than partnered males can brood them and so may look for other mating opportunities. On the other hand, the reproductive success of males is dependent on the number of eggs they can hold, and how quickly they can recover after each brood. Males may potentially benefit from eating part of each brood to recover faster, and take on a second brood (Takeyama *et al.* 2007). However, whether this complex mating system leads to extra-pair mating or sneaking in either sex has not yet been investigated.

The aim of this study was to combine a long-term behavioural study with genetic tools to examine the extent of pair and extra-pair mating in the socially monogamous, paternal mouthbrooding pajama cardinal fish, *Sphaeramia nematoptera*. This species lives in small groups of 2 to 40 individuals, within which most adults form male and female pairs, and females stay with the brooding males at their daily resting sites (Chapter 2). The small group sizes and unique mating system provides the opportunity to sample clutches of developing embryos and determine the presence of either multiple maternity or paternity from genetic parentage analysis. It was predicted that the majority of offspring would be derived from males and females in pairs, as this would explain the occurrence of social monogamy in this species. However, it is also possible for both males and females to be involved in extra-pair mating, since mating takes place during dusk or after nightfall (Kuwamura 1985) and outside their daily resting sites. Sneak mating might be expected from males because this provides opportunities for acquiring fertilizations without the investment into brood-care. Female sneaking might be a possibility because female cardinalfish can increase their reproductive output by giving clutches to males as soon as they are developed, instead of waiting for their male partner to finish the lag phase between brooding.

The specific objectives of this study were to: (1) Use long-term observational data of tagged adults to identify pair bonding in multiple social groups. (2) Use DNA parentage analyses to identify the true mother and father from embryos carried by brooding males, and measure the frequency of extra-pair mating by males and females. (3) Examine the size of parents exhibiting sneak mating to assess whether the propensity to sneak changes with ontogeny. (4) Examine the frequency of extra-pair spawning with additional partners from within or among different social groups.

Methods

Study site and species

The study was conducted on inshore reefs near Mahonia Na Dari Research and Conservation Centre, Kimbe Bay, Papua New Guinea (5°30’S, 150°05’E), from October 2012 to September 2014 (Figure 4-1).

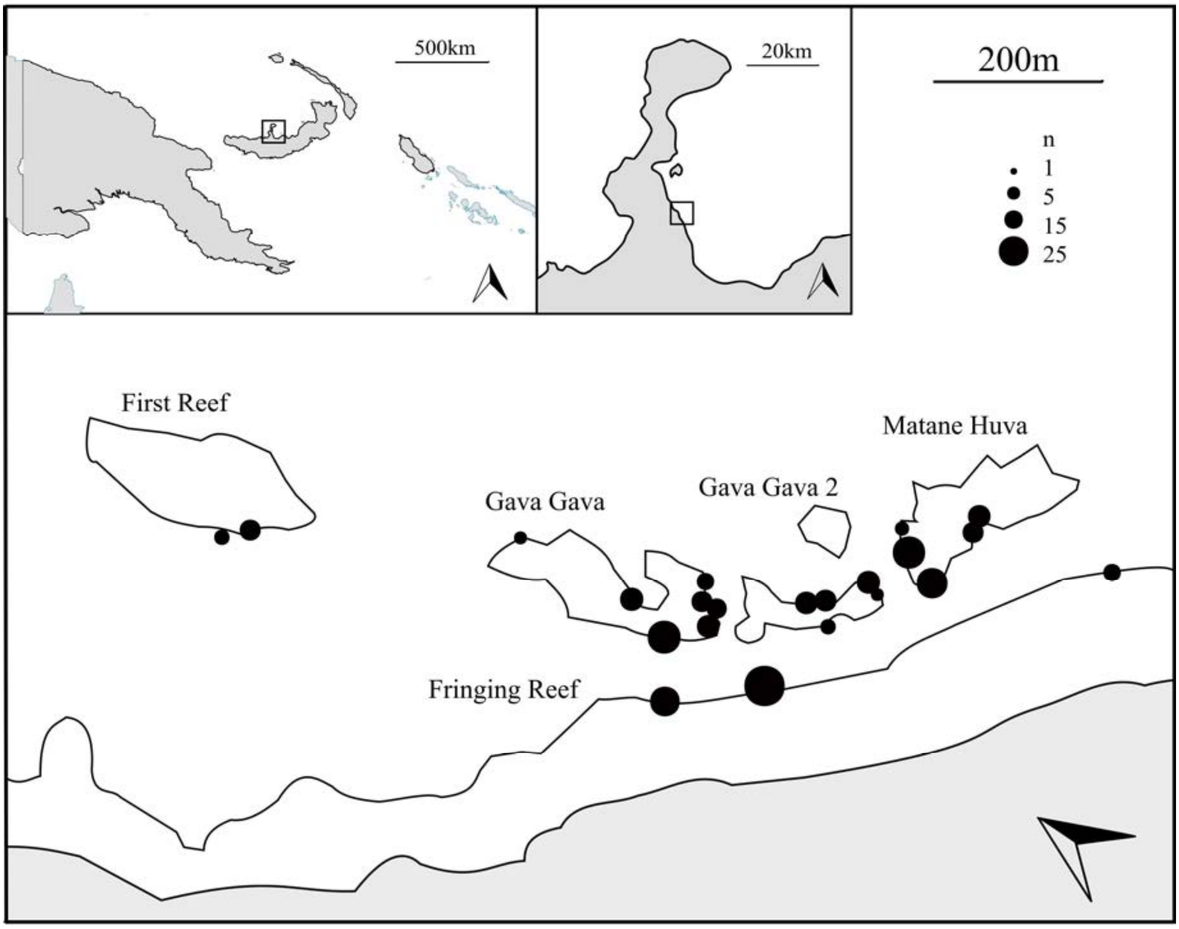


Figure 4-1 Map of study area near Mahonia Na Dari, West New Britain, Papua New Guinea. Location of social groups of *Sphaeramia nematoptera* are indicated as black dots (N=22), whose size indicates the number of individuals in each group.

Observational studies were conducted October-November 2012, February - March 2013, July - August 2013, March-April 2014 and September 2014. Fish were caught using diluted clove oil (Munday & Wilson 1997) and hand nets from five reefs (N=544). All individuals from 22 groups were sampled. Social groups varied in size from 4 to 32 individuals (Figure 4-1).

Tagging and long-term behavioural observations

All fish were physically marked using Visible Implant Elastomer (VIE) tags with unique combinations from six colours and five positions on the fish. VIE has been shown to last for several months and does not impact fish behaviour nor affect susceptibility to predation (Marnane 2000). The standard length of all sampled fish was measured to the nearest millimetre. During each of the five observational periods all individuals were located every two to three days via visual census, and information recorded on the most likely mate. Pairs were determined as described in Chapter 2. Courtship behaviour was only observed between partners thus defined and all pairs were found to be heterosexual. Sex was determined by distended buccal cavity during brooding (male) and bulging abdomen shortly before brooding (female). Overall, 500 hours of observational data and social pair formation was collected over 28 months.

DNA sampling and genetic analyses

Within the study periods, all broods found in observed males and broods from nearby groups were collected. 1056 embryos of 105 broods from 64 males were assayed (10-20 embryos per clutch). To take into account the possibility of multiple mothers in each clutch, eggs were sampled from different parts of the egg mass, including several points on the surface and the centre of the congealed egg mass. Binomial probability theory predicts that a random sample of size n will include eggs from a female which laid some proportion p of eggs in the nest, with 95% certainty if $n \geq \ln(0.05/\ln(1-p)$ (Ross 1997). If two mothers have an equal number of embryos in a clutch (p=0.5), a sample of 5 eggs would be sufficient to include at least one egg from each mother. And 10 eggs were considered sufficient to detect a proportion of 0.25 (DeWoody & Avise 2000).

DNA extractions were performed from fin tissue samples preserved in 95% ethanol using the Nucleospin-96 Tissue kit (Macherey-Nagel). All individuals were genotyped at 23 microsatellite markers in four multiplex PCRs following the procedures described in Appendix D. Genotyping error was assessed using repeat samples from 43 individuals and calculated as the ratio between mismatches in alleles and the number of replicated alleles (Pompanon *et al.* 2005).

Loci were amplified using the Type-it Microsatellite PCR kit (Qiagen), PCR products were screened on an ABI 3370xl DNA analyser (Applied Biosystems), and individual genotypes were scored in Genemapper v4.0 (Applied Biosystems). Unique alleles were distinguished using marker-specific binsets in the R package *MsatAllele* (Alberto 2009). 3- 34 alleles per locus were observed, with a mean (±SE) of 15.1 (±1.8), in this population of *S. nematoptera* (Supplemental Material Table 1). The mean (±SE) observed heterozygosity was 0.666 (±0.060), the mean (±SE) expected heterozygosity was 0.733 (±0.058). Significant deviation from Hardy-Weinberg equilibrium was found in 4 loci. Four markers which showed high genotyping error (≥6%) were dropped from the analyses. The remaining 19 loci had an average genotyping error (±SE) of 2.2 (±0.4) %.

Parentage assignments were conducted with the software COLONY v2.0 (Jones & Wang 2010) to identify the most likely mother or mothers of a selection of eggs carried by male cardinalfish. Analyses were performed using the full-likelihood method with a medium precision, long run and low probability threshold. All putative males and females were included as candidate parents and both sexes were considered polygamous in the analysis. A simulation test was conducted to assess the accuracy of COLONY settings. A population of 1000 offspring and 250 mothers and fathers was simulated in Mykiss (Kalinowski 2009) and measured the frequency of type I and type II errors following methods described in Harrison et al. (2013). The parameter set yielded the highest overall accuracy, with most parents (93.5%; type-I error 2%, type-II error 4.5%) of simulated offspring being assigned to the correct parent pair (Appendix B & D). Multiple matings were defined as clutches of embryos which include more than one genetic father or mother (termed partial broods).

Male sneakers were defined as all males which, with molecular methods, were identified as having sired offspring which was sampled from another males' buccal cavity. Female sneakers were defined as females which are identified as the genetic mother of offspring from a male which during the same time was found to be behaviourally paired with another female.

Ontogenetic patterns in pair and extra-pair matings

As size is likely an influential factor directing the evolutionary benefits of cuckoldry and extra pair matings, I compared the body size of sneakers, brooders and other paired fish. Using paired t-tests, the sizes of sneaker males and brooders were compared, as well as female sneakers and paired females. Among male-female pairs we compare sizes of brooders and both their behavioural females and any corresponding sneakers. I also compare the overall size range of males to the average sizes of brooders and male sneakers and the overall size range of females to the average size of paired females and female sneakers.

Pair and extra-pair matings within and among social groups

I determined whether male and female sneakers are part of the same social group as the breeding pair or whether they came from a different site. If sneakers were not sampled in the course of this study, I deemed them to be from a different social group, since sampling of individuals of known groups was exhaustive. To check for any locational biases in mating behaviour, the instances of sneaking were compared between sites and reefs using Fisher's exact test. Similarly, sneaking frequencies were compared by density of social groups. This was to check whether larger social groups had differential mating frequencies or not. Density of social groups was assessed by counting the number of fish present and dividing it by the size of the coral colony. Coral colony size was taken by measuring width and length of the coral colony to the nearest 10cm.

Results

Social monogamy

I followed the social pairing of 64 brooding male cardinalfishes throughout a 28-month period and collected 1056 embryos from 105 clutches with an average (±SE) of 10.5 (±0.28) (5-20) embryos per clutch. Each clutch represents one successful mating event, with each male mating one to three times over the study period.

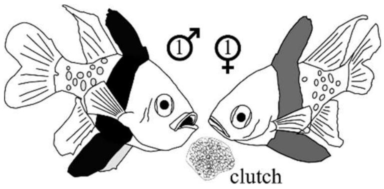
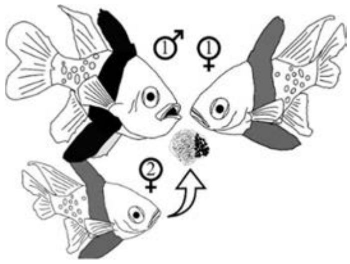
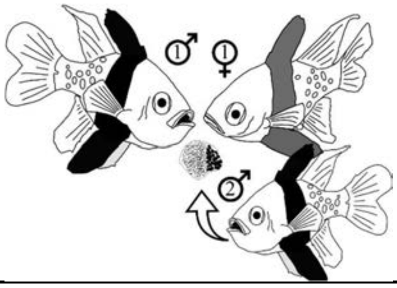
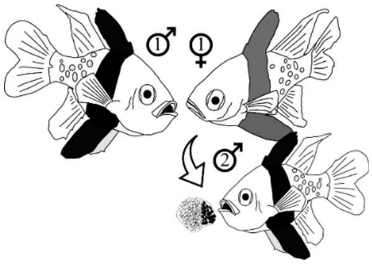
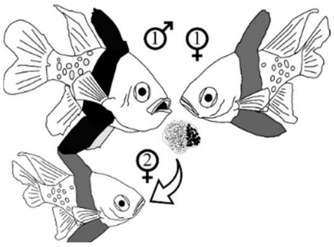
Behavioural data on social pairs was available for four weeks prior to brooding in 70 of 105 mating events, and in 94.3% (66 of 70) of cases, the brooding males could be identified as paired with its social partner. In the other four cases males were observed but no partner could be identified 20 pairs could be observed to produce multiple clutches during this period.

Extra-pair & sneak-mating

Using genetic parentage analysis on brooding males, embryos and putative mothers, I was able to identify the true parents of 105 clutches of eggs. It was found that the brooding male was the sole father in 97 clutches (92.4%), but 8 clutches (7.6%) contained eggs that had been fathered by a male other than the brooding male (Table 4-1), evidence of sneak mating by male cardinalfishes. Each of these 8 clutches was sampled from a different male and all of the embryos had the same yolk colourations, indicating that all embryos were of similar age. On average (±SE) male sneakers sired 25.9% (±6.9%) of the analysed embryos in a clutch. Four of the eight sneaking males could be identified as part of the sampled population. Half of identified sneaker males (2 of 4) were found to be brooding with another female in a different breeding cycle.

Of the 66 clutches from known social pairs, 46 clutches (69.7%) were the progeny of an observed social pair (Table 4-1). Another seven clutches (10.6%) were mothered by two different females, demonstrating evidence of extra-pair mating by male cardinal fishes. In each case the number of embryos by the extra-pair female ranged from 1-8 embryos with a mean of 32.1% (±7.3%) of the clutch. One of these clutches had clear morphological differences between embryos, where yolks were of different colour and which corresponded to the different mothers: this clutch was the result of separate matings at different stages of the breeding cycle. In an additional 13 clutches (19.7%) the brooding male was carrying an entire clutch from a female other than its social pair. Clearly, males in social pairs take part in partial or complete extra-pair mating. Extrapair mating by females was also found. Seven of the sneaking females could be identified to be part of the sampled population. Out of the sneaking females which could be identified, a third (2 of 7) were observed to be paired within the sampling period (Table 4-1).

Table 4-1 Frequency and total number of clutches that monogamy/ sneaking or extra-pair mating by either males or females was inferred from 105 clutches, sampled from 64 males. Behavioural data was available for 66 clutches; these were used in assessing monogamy. Monogamy is defined as complete match between social pair and genetic parentage. Sneaking is defined as an individual being assigned maternity or paternity of embryos in a clutch which was sampled from a social pair that the sneaker is not part of, thereby gaining offspring by copulating with a member of a pair but not being part of that pair and not caring for the offspring. Female extra-pair mating was defined as mother assigned to female not in the social pair. Male extra-pair mating occurred when males carried embryos whose maternity does not match their social partner. Males are depicted with a black vertical stripe, brooding males with an extended buccal cavity and open mouth. Females are depicted with a grey vertical stripe.

Mating pattern		% of clutches	# clutches
Monogamy		69.7	46/66
Sneaking		31.4	33/105
Female sneaking		23.8	25/105
Male sneaking		7.6	8/105
Extrapair mating		33.6	46/66
Female		3.3	2/66
Male		30.3	20/66

Ontogenetic shift

Male sneakers tended to be smaller than the corresponding brooder male, while female sneakers tended to be bigger than- or of the same size- as the paired females however, there was no significant difference in size between behavioural parents and sneakers in either gender. Of the four tagged sneaker males, three were smaller than the brooder they were sneaking on, and one was same size (paired t-test, t = -2.038, df = 3, p-value = 0.134) (Figure 4-2). Male sneakers were an average (±SE) of 3 (±1.5) mm smaller than the brooders they were sneaking on. For females, in two thirds of cases the cuckolder was bigger than the paired female (paired t-test, t = -1.626, df = 6, p-value = 0.155) (Figure 4-2). Female sneakers were an average (±SE) of 2.4 (±1.5) mm bigger than the paired female they were cuckolding.

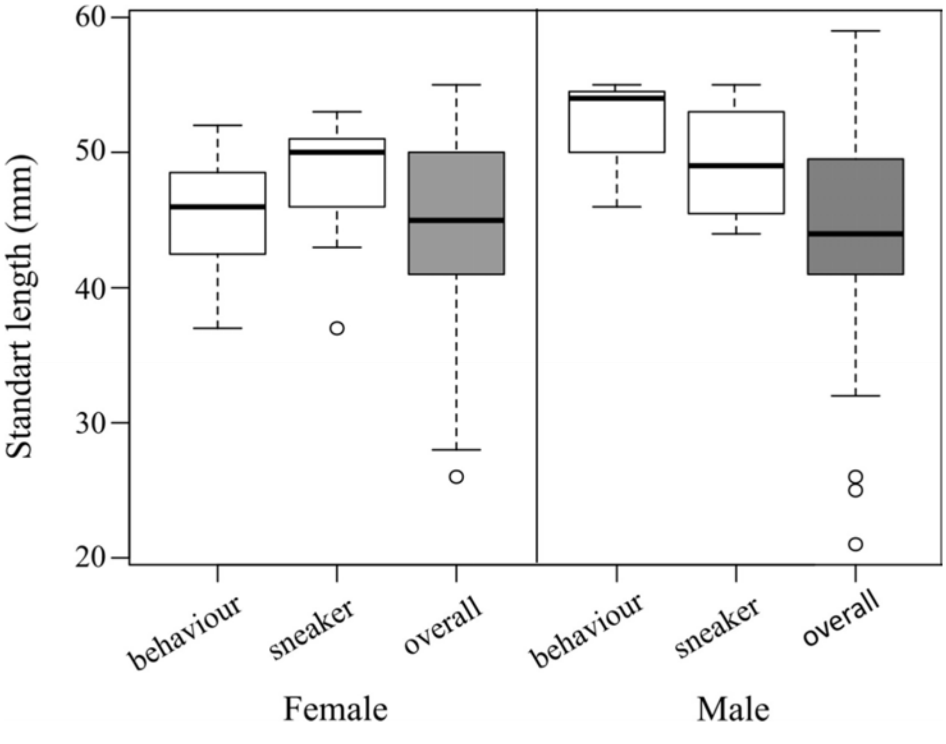


Figure 4-2 Box and whiskers plot of standard length (mm) of *Sphaeramia nematoptera*; behavioural parents which where cuckolded (behaviour), sneakers, and overall sizes of reproductive individuals sampled, by sex.

Pair and extra-pair matings within and among social groups

In most cases sneakers were found to be from a different social group than the breeding pair. In total, 17 of 28 sneakers did not belong to any sampled social group (13 females, four males). It is unlikely that fish escaped observation as sampling of groups was exhaustive. Nine of 28 sneakers were identified from within the social group, and the remaining two (one male, one female) were identified from social groups 80m and 330m distant respectively. There was no difference in number of sneak matings between the five reefs or the 13 social groups. The social groups sampled on different coral colonies ranged from 5 to 32 fish with differences in density of 0.7 to 12.4 fish/m^2 (mean ±SE 3.9 ± 1.7 fish/ m^2). The number of broods with multiple parents did not differ by reef (Fisher's exact, p=0.547) or site (Fisher's exact, p=0.579).

Discussion

The observations of social pair formations of cardinal fishes and the genetic parentage analysis of embryos provides a unique insight into the reproductive strategies in a mouth-brooding coral reef fish. My findings demonstrate that neither social monogamy nor high-investment mouth-brooding indicates exclusive mating since both males and females took part in extra pair matings. As was predicted, the majority of offspring was from mated pairs. However, some brooding males were shown to take multiple clutches from separate females within their immediate social group and also mated with females from other social groups. I found that males sometimes carry eggs fertilised by other males, which contradicts the expectation of assured paternity being a pre-requisite for the evolution of such high cost parental care systems (McCoy *et al.* 2001). I also conclude female sneaking behaviour, which is exceptionally rare in the animal kingdom (but see Eadie & Fryxell 1992). These findings provide the first evidence of extra pair mating in a coral reef fish and imply that mating systems may be much more complex than behavioural observations imply and need to be viewed from the male as well as the female perspective.

Clearly, social monogamy does not exclude the existence of extra-pair mating in coral reef fish. Many coral reef fish have been found to form long lasting pair bonds (Thresher 1984) and for many species, monogamy is assumed to last for at least one breeding cycle (Barlow 1984; Whiteman & Côte 2004). However, detailed studies of other animal groups such as birds have shown that such pairing behaviour can be deceiving and complex mating strategies, including extra-pair copulations, often underlie cases of social monogamy. In this study 30% of broods were found to include either multiple females or females other than the social partner, in a paternal mouthbrooding fish. The average proportion of extra-pair paternity in birds is 15%, but rates of 30% are not uncommon (Griffith *et al.* 2002; Sardell *et al.* 2010; Gerlach *et al.* 2012). In mammals the equivalent extra-group paternity exceeds 20% in almost half of the species studied, and is lower in the other half (Isvaran & Clutton-Brock 2007). In another teleost system, the socially monogamous cichlid *Variabilichromis moorii*, both parents defend the nest and while all offspring in a nest share the same mother, 2-10 fathers have been found in every brood (Sefc *et al.* 2008).

My study provides a unique case of female sneak mating, where females deposited eggs to males that were already carrying eggs from another female. Since the buccal cavity limits the number of embryos being carried (Okuda 2001), the male has to cannibalise the socially paired females' eggs, at least partially, to take up the new brood. Female cardinalfish can potentially produce eggs quicker than the males can brood them (Okuda 1999b). This gives females the opportunity to be in a pair but also maximise their reproductive output by performing extrapair matings. Multiple maternity has been described for pipefish, for example *S. floridae* (Jones & Avise 1997a), but in pipefish the females do not remain with the brooding male (Vincent *et al* 2011). In goldeneye ducks, *Bucephala islandica*, older females lay eggs in younger females' nests without the latter being aware of the process (Eadie & Fryxell 1992). Cases of female fish giving eggs to other pairs exist, but only for maternal care systems and not the paternal system seen in cardinalfish. Possible female sneaking has been observed in the paternally caring damselfish *Chromis multilineata,* with females seen depositing eggs in the nest of a male that had previously denied them (Johnston 1996), however this behaviour had not yet been satisfactorily verified.

For the first time in science, I found embryos fertilized by another male in the clutch of a brooding father. These results are in contrast to findings in classic teleost models for the evolution of reproductive systems with high parental costs. In pipefish and seahorses males brood in external or internal brood pouches, which is a costly investment. The assurance of sole paternity is thought to have led to the evolution of these high investment strategies (Clutton-Brock 1991). In all five syngnathid species surveyed with genetic methods, the brooder has proven to be the sole sire of its clutch, even in the pipefish *Nerophis ophidion*, in which clutches are brooded externally (Jones & Avise 1997a,b; Jones et al. 1998a, 1999a, 2000; review in Jones & Avise 2001, McCoy et al. 2001). By contrast, cuckoldry by other males does occur in nest-tending species such as sunfish (Gross 1991; DeWoody et al. 1998, 2000b; Neff et al. 2000), darters (DeWoody et al. 2000a), sand gobies (Jones et al. 2001) and sticklebacks (Jones et al. 1998b). In these systems paternal investment is lower and thus the cost of tending a few extra eggs is negligible. In *S. nematoptera* the low rate of male sneaking explains why the paternal care system is still stable. In only 7.6% of broods male sneaking was found, and even then only 10-20% of embryos were fathered by the sneaker male.

While size data were not available for all the sneaking males, the results indicate that there is no conditional strategy, where males perform sneak mating to a certain threshold size before brooding themselves, but rather that sneak matings performed by males are context dependent. This is in contrast to other studies. For example in wrasses such as *S. oscellarus*, smaller males exclusively mate by sneaking and larger males defend territories and tend to nests (Warner & Lejeune 1985). In the case of *S. nematoptera*, because pairs have been found to be size assorted (Chapter 3), smaller males might sneak to get an opportunity to fertilize high quality offspring of big females that they otherwise have no access to. However, whether sneaking is performed might depend more on the relative size of sneaker and mated pairs than on an ontogenetic switch-point. Similarly, in

S. nematoptera it seems to be that large females successfully cuckold females of equal or smaller size, but because many sneakers could not be identified, the differences were not significant. For the male, accepting eggs from the larger females is probably advantageous because larger females usually produce larger or better provisioned eggs (Perrone 1978; Berglund *et al.* 1986). It may also benefit males to accept such eggs because it provides them an additional food source and ensures their offspring to have high genetic diversity, as has been suggested for *Syngnathus typhle* (Jone*s et al* 1999). Male cardinalfish do not forage during their brooding phase and often cannibalise a portion of their broods (Okuda & Yanagisawa 1996a). By accepting a sneaker's eggs, they would need to partially cannibalise either their current brood, or the new packet or part of both. In this study, only one of the 12 clutches with multiple females showed different aged embryos. This indicates that the sneak-mating by the female, which also constitues an extra-pair mating by the male, is most likely to occur on the same night that the social partners spawn. Since time and energy is invested in embryos he has already been brooding for a few days, this might be expected. In the one case of different sized embryos the sneaking female was a lot bigger than the partner female, so the partners' quality might have convinced the male to make an exception.

Sneaking and cheating with individuals from a different social group may help maintain social stability and high heterozygosity in *S. nematoptera*. Relatively few instances of extrapair mating and sneaking were being performed within the same social group that the mated pair lives in. In most instances, I could not identify sneaker males and females and due to the extensive sampling effort, this lead to the conclusion that sneakers were from social groups not sampled. In birds, most extra-pair mating is done with direct territorial neighbours, which is thought to promote cooperation (Suter *et al.* 2007; Sardell *et al.* 2010; Taff *et al.* 2013; Eliassen & Jorgensen 2014). However, exceptions exist, such as a study on the blue tit, *Cyanistes caeruleus*, which found 22% of young produced as a result of extra-pair mating were fathered by an unknown, or non-resident, male (Schlicht & Kempenaers 2013). Mating with non-residents may be beneficial because it reduces the risk of detection and increases heterozygosity within the brood compared to extra-pair offspring which is produced with a neighbour (Foerster *et al.* 2003; Stapleton *et al.* 2007). In fish the genetic benefits of mating with non-resident individuals and the avoidance of aggression within the social group may outweigh the possibility of cooperation and thus lead to higher rates of extrapair-mating and sneaking behaviour among non-neighbours.

Parental investment seems to play a big role in shaping the reproductive system of *S. nematoptera*. The high investment of the male and the potential equal or higher reproductive output of the female are driven by male mouthbrooding and lead to a complex mating system. Mutual parental care has been found to be one of the main drivers of monogamy in fishes, and paternal care, which is common in teleosts, may lead to polyandry (Whiteman & Côte 2004). Cardinalfish are paternal mouthbrooders, and since the males cannot feed during brooding and have to have a lag phase between broods, females in this group may have a higher potential reproductive output (Okuda 2001). However the male can offset the females advantage by cannibalising partial or whole broods (Kume *et al.* 2000c; Takeyama *et al.* 2007). Here paternal care is such a high investment it seems to lead to a seemingly monogamous system with female as well as male sneaking. Polygynandry has been found in aquarium populations of the pipefishes *S. typhle* and *S. floridae* (Jones & Avise 1997a), but has not yet been observed in wild populations.

The many advantages gained by extrapair mating and the occurrence of both male and female sneaking shown in this study, begs the question of why individuals pair at all. Most adults of *S. nematoptera* have been found in pairs, most of which last for more than one breeding cycle (Chapter 2). The females stay with the males during the brooding (Chapter 2), which may indicate a role of the females in protecting the male from potential egg thieves, be it other cardinalfish or predators. Another reason to stay in a pair is to ensure a reproductive opportunity every breeding cycle and to prevent a high quality partner from taking advantage of extrapair mating opportunities. In the monogamous goby, *Valenciennea strigata*, abundant resources and mating opportunities for males were found to create an advantage to mate guarding for females (Reavis & Barlow 1998). From the females' perspective in *S. nematoptera*, it is likely beneficial to stay with the brooding male to ensure that other females do not offer their broods, which would then be an incentive for the male to cannibalise partial or whole broods. Furthermore, long lasting pairs have been shown to be beneficial for the reproductive output of individuals in several species of birds (Ismar *et al.* 2010).

To conclude, this study confirms that for this coral reef fish, social monogamy is largely congruent with genetic monogamy, with the majority of offspring attributed to social partners. However, I show that social monogamy in a brood-caring reef fish does not guarantee exclusive mating, as has been observed in birds and other animals. Females have the potential as well as the drive to perform extrapair matings if they have the opportunity to increase their reproductive output. To the best of my knowledge, I describe the first cases of female sneak-mating in a coral reef fish. Males which invest highly into offspring care seem to accept low levels of male sneaking and offset the females advantage by cannibalising broods and perform extrapair matings in their own right. Molecular as well as long term behavioural studies are clearly necessary to understand the often complex pairing dynamics in coral reef fish.

Chapter 5 : Self-recruitment and kin association in social groups of a coral reef cardinalfish

Abstract

Social groups of coral reef fishes are often assumed to form from unrelated individuals that settle into the same coral habitats. However, local-scale self-recruitment and attraction between sibling larvae create the potential for inbreeding and kin associations within social groups. While there is increasing evidence that larval reef fishes may return home and kin remain associated during the larval phase, few cases of true kin group structures in marine fish have been described. Here I examined the relatedness of individuals within and among reefs, and within and among social groups of the pajama cardinalfish, *Sphaeramia nematoptera*, in Kimbe Bay, Papua New Guinea. In addition, I used parent and sib-ship analyses to examine local-scale self-recruitment and association among siblings. Pairwise relatedness and genetic distance was quantified among 527 individuals from 41 social groups on 7 reefs within an area of just 3.9km^2, using 19 microsatellite markers. I found that individuals on the same reefs were 1.25x more closely related than would be expected by chance and 1.3x more closely related when compared to individuals on other reefs. On the same reef, individuals within social groups were 1.3x more closely related than to individuals in other groups. Overall there was a strong negative relationship between pairwise relatedness and distance over a scale of less than 3km. Parent-offspring and sib analyses showed high levels of both fine-scale self-recruitment and siblings settling together, suggesting these processes account for high levels of kin association within groups. However, there is some evidence of ontogenetic shifts where related juveniles move to other groups which would then avoid inbreeding. Together, the findings suggest a mechanism by which coral reef fish larvae can disperse and recruit in close kin-groups. Assumptions that social groups in coral reef fishes are made up of unrelated individuals may need to be re-evaluated.

Introduction

The evolution of social organization in terrestrial animals is closely linked to the association between kin in social groups, including parent-offspring and sibling-sibling relationships. According to kin selection theory, the evolution of many social behaviours can be explained by the degree of relatedness of individuals within groups of animals (Hamilton 1963, 1964; West-Eberhard 1975). Indeed, many social systems such as eusociality are thought to have evolved through related individuals recognizing each other and helping their kin raise offspring (Hamilton 1964; Queller 1994; Hughes *et al.* 2008). Kin association can lead to increases in reproductive output, social group stability, reduced competition and more effective cooperation (Hamilton 1964; Milinski 1987; Ward & Hart 2003; Frommen & Bakker 2004). However, the close association of related individuals comes with a risk of inbreeding, and in most social systems, mechanisms have evolved to avoid mating with relatives (Gerlach & Lysiak 2006; Clutton-Brock 2009b; Leclaire *et al.* 2013). In terrestrial animals whether kin groups form, and the pattern of kin selection, depends on the extent to which offspring are dispersed (Hamilton 1964; Taylor 1992a; b; Queller 1994; West *et al.* 2002; Johnstone & Cant 2008). Most marine fish have a pelagic larval phase that lasts several weeks at which time juveniles are assumed to be transported away from natal populations (Leis 1991; Gaines & Kinlan 2003; Fuiman & Werner 2009; Leis *et al.* 2011). Because of this dispersive pelagic larval phase it has been assumed that social groups consist of unrelated individuals and kin selection is unimportant as an evolutionary mechanism (Kolm *et al.* 2005). Clearly, our understanding of the basis of animal societies will benefit greatly from having detailed knowledge about the genetic composition of groups of animals. However, for many taxa including most marine fishes, this information is scarce.

Coral reef fishes commonly form discrete social groups that are thought to arise because they are highly site attached, and also because unrelated individuals can benefit from living in groups (Fricke 1977; Sale 1977). Early genetic studies of coral reef fish emphasized genetic continuity over large geographic scales, propagating the idea that long-distance larval dispersal is the norm (e.g. Doherty et al. 1995, Shulman & Bermingham 1995, Dudgeon et al. 2000, Bernardi et al. 2001, Ovenden et al. 2002, Planes & Fauvelot 2002, Hickford & Schiel 2003, Van Herwerden et al. 2003). However, over the last decade, a number of different approaches have indicated that a significant proportion of juveniles settling on individual reefs are returning to natal populations (self-recruitment). This information includes genetic differentiation among populations on adjacent reefs (Gerlach *et al.* 2001; Planes *et al.* 2001), natal chemical signatures on otoliths (Swearer et al 199), larval tagging studies (Jones *et al.* 1999a, 2005; Almany *et al.* 2007), and most recently, genetic parentage analyses (Jones *et al.* 2005; Planes *et al.* 2009). The percentage of self-recruitment can be extremely high (up to 60%, Almany *et al.* 2007) and in some cases, juveniles have been recorded settling only meters from their parents (Jones *et al.* 2005; Buston *et al.* 2009). This raises the possibility that related individuals may choose to join or avoid social groups containing related individuals.

There have been few studies that have addressed the fine-scale genetic structure or degree of relatedness of individuals in social groups of coral reef fishes. Buston *et al.* (2009) found no evidence of cohabitation of parents and offspring in orange clownfish (*Amphiprion percula*). However, higher than average relatedness has been observed within groups of the humbug damselfish *Dascyllus aruanus*, which may be a consequence of closely related juveniles dispersing and settling together (Buston *et al*. 2009). If fish actively choose to settle with kin, they need to be able to recognise them. There is no published evidence for this for coral reefs, although this is well established for freshwater fishes (Arnold 2000; Frommen & Bakker 2004; Frommen *et al.* 2007; Hain & Neff 2007; Gerlach *et al.* 2008; Le Vin *et al.* 2010). In freshwater fishes, which generally do not have a dispersive larval stage, high levels of relatedness within social groups has been documented (Piyapong *et al.* 2011). Subordinate Atlantic salmon have been shown to increase their territory sizes and foraging by associating with dominant relatives (Griffiths & Armstrong 2002), and juvenile zebrafish *Danio rerio* were found to grow 33% more in kin groups than groups of unrelated individuals (Gerlach *et al.* 2007b). On the other hand, offspring from closely related parents has been shown to have reduced hatching success and survival in fish, for example in salmonids (Waldman & McKinnon 1993). In three-spine sticklebacks, *Gasterosteus aculeatus*, females have been shown to prefer to mate with unrelated males rather than brothers, demonstrating non-random mate choice as a possible mechanism to avoid inbreeding (Frommen & Bakker 2006; Mehlis *et al.* 2008). Kin competition similarly needs to be avoided by reproductively active adults living with close relatives (Johnstone 2008). To date, none of these issues have been addressed for social groups of coral reef fishes.

The paternal mouthbrooding coral reef cardinalfishes (f. Apogonidae) form social groups with potentially high levels of relatedness. Some cardinalfishes have direct development, while others have a pelagic larval phase lasting 20-24 days (Fisher & Bellwood 2003). The banggai cardinalfish, *Pterapogon kaudernii*, which has no pelagic larval phase, exhibits population differentiation between sites less than 5km apart (Hoffman *et al.* 2005), although no kin groups have been documented (Kolm *et al.* 2005). For cardinalfish species with a larval phase, larvae are able to distinguish olfactory cues between reefs (Atema *et al.* 2002; Gerlach *et al.* 2007a) and show a preference to orient towards familiar lagoon water at settlement stage (Atema *et al.* 2002), and prefer the water from "home" reefs once settled (Gerlach *et al.* 2007a). The cardinalfish *Ostorhinchus doederleini* exhibits genetic substructure among adjacent reefs, and self-recruitment can be high, with 36% of juveniles assigned with 95% confidence to parents in the same lagoon population (Gerlach *et al.* 2007a). Additionally, other mouthbrooding fish, freshwater cichlids, aggregate with kin and show signs of inbreeding (Pouyaud *et al*. 1999; van Dongen *et al.* 2014). All of these examples indicate a measure of choice is employed by larval stage cardinalfish and that relatedness within social groups may occur. To date however, there have been no studies linking relatedness in social groups, and evidence for self-recruitment and cohabitation in coral reef fishes.

One of the limitations of examining genetic relatedness in social groups of fishes has been the lack of appropriate molecular tools to resolve relationships among individuals, since this takes both many allelic markers as well as high polymorphism (Harrison *et al.* 2013). To date, most studies have employed 3-7 microsatellite markers (Herbinger *et al.* 1997; Pouyaud *et al.* 1999; Gerlach *et al.* 2001), which may not be enough to reliably identify parent-offspring or sibling relationships (Fraser *et al.* 2005). In addition, to fully describe social group structure, it is necessary to get genetic profiles of whole social groups. In order test the reliability of estimates of parent offspring, sib-sib, or sib-half-sib relationships, simulations must be undertaken to calculate error rates (e.g. Fraser *et al.* 2005), although this is not always done.

The aim of this study was to test whether social groups of the pajama cardinalfish *Sphaeramia nematoptera* exhibit high genetic relatedness, and determine whether this is can be explained by either high levels of self-recruitment in local populations or sib-sib relationships that persist after settlement. The likely reproductive consequences of high relatedness within social groups are also addressed by measuring relatedness among reproductive partners to evaluate the potential for inbreeding or a mechanism for inbreeding avoidance. I used 19 highly variable microsatellite markers to test the following hypotheses: (i) individuals in the sampled population are more related than would be expected by chance; (ii) individuals in social groups are more related to each other than to individuals in other social groups; (iii) relatedness is higher within populations on the same coral reef, compared to populations on different reefs; and (iv) genetic relatedness declines in relation to distance between social groups. In order to explain mechanisms of high relatedness within social groups, I also tested: (v) whether the presence of full sib or half-sib relationships is greater within reefs than among reefs, and (vi) whether levels of self-recruitment is high enough to explain levels of relatedness within social groups. In order to test for potential inbreeding avoidance, it was examined (vii) whether there was an ontogenetic decline in the association between related individuals, and (viii) whether breeding pairs were less related to each other than others in the population.

Methods

Study location and sample collection

The study was conducted on inshore reefs near Mahonia Na Dari Research and Conservation Centre, Kimbe Bay, Papua New Guinea (5°30’S, 150°05’E), from October 2012 to September 2014 (Figure 5-1).

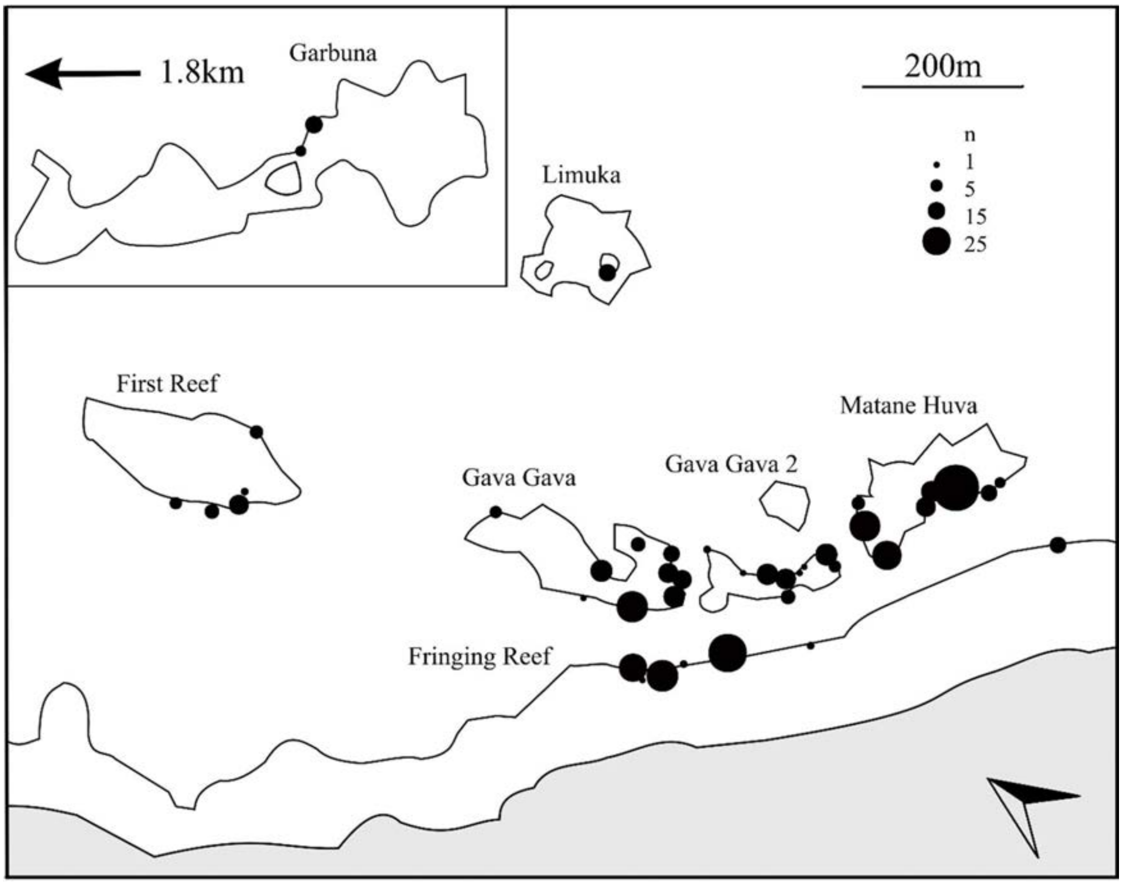


Figure 5-1 Map of sampling area in Kimbe Bay, Papua New Guinea. Size of black circles indicates the relative number of fish sampled from each of the 41 social groups in the sampled area. Light grey indicates land.

I focused on the pajama cardinalfish, *Sphaeramia nematoptera*, comprehensively sampled from 5 different reefs and a location on the fringing reef (Fig. 1). *Sphaeramia nematoptera* forms pairs that last for at least one breeding cycle within small social groups of 5-35 individuals (Chapter 2). A paternal mouthbrooder, *S. nematoptera* incubates eggs for about 8 days (pers. obs). The larval phase was measured to be 24-25 days in a laboratory study (Fisher & Bellwood 2003). At hatching larvae measure ~3mm with a critical swimming speed of ~2cm s-1 and they grow to ~12mm with swimming speeds increasing to ~15cm s-1 (Fisher et al 2000).

A total of 527 *Sphaeramia nematoptera* were caught using hand nets and diluted clove oil as a mild anaesthetic (Munday & Wilson 1997). Each fish was measured underwater (Standard Length SL) and a fin clip was taken from the caudal fin. Tissue samples were preserved in 99% ethanol for genetic analysis. All fish were categorized as either adult (≥38mm SL), subadult (33-37mm SL) or juvenile (<33mm SL), as assessed by gonad histology (Chapter 2). Fish were caught from 41 different social groups on 7 inshore reefs (Figure 1). Each social group was characterised as fish on the same distinct coral colony.

Genetic analyses and locus characteristics

Genomic DNA was extracted from ~2 mm^2 of fin tissue collected from each individual and screened at 23 microsatellite markers (Appendix D). DNA extractions were performed following procedures described in the Nucleospin-96 Tissue kit (Macherey-Nagel, Germany) and microsatellites were amplified in four multiplexes of 3 loci. Selected primer pairs were combined in a primer premix for in-reaction concentrations ranging from 0.02 to 0.06 µM, adjusted for even amplification. All four multiplex reactions were performed using the QIAGEN Microsatellite Type-it kit (QIAGEN, Germany) in a total volume of 10 µl containing 5 µl of QIAGEN Multiplex Master Mix (2x), 1 µl QIAGEN Q-solution, 1 µl of distilled water, 2 µl of primer premix, and 1 µl template DNA. PCR products were screened on an ABI 3370xl DNA Analyzer (Applied Biosystems) with the GeneScan 500 LIZ (Applied Biosystems) internal size standard following a 1:15 dilution. Individual genotypes were scored in GENEMAPPER v4.0 and unique alleles were distinguished using marker specific binsets in MSATALLELE (Alberto 2009).

Allele frequencies, linkage disequilibrium and deviation from Hardy-Weinberg equilibrium were estimated with Genepop (Raymond and Rousset, 1995) and the data was checked for the presence of null alleles with Microchecker (van Oosterhout et al 2004). Genotyping error was assessed using repeat samples from 43 individuals and calculated as the ratio between mismatches in alleles and the number of replicated alleles (Pompanon et al. 2005). For further analysis, we used the 19 markers with the lowest genotyping error, <6%.

Estimating pairwise relatedness within and amongst groups

The relatedness of any two of sampled individuals were assessed using COANCESTRY (Wang 2007). To identify the most accurate estimate of pairwise relatedness, I first simulated 1000 individuals from the estimated allele frequencies at each locus. The rate of missing allele was set to 0.01 for all loci and locus-specific genotyping error rates were used. The parameters tested included estimators described by Wang (2002, 2007), Lynch (1988), Lynch & Ritland (1999), Ritland (1996), Milligan (2003) and Queller & Goodnight (1989). The triadic likelihood estimator (Wang 2007) showed the highest correlation with the true values (Pearson's $r = 0.994$, $p < 0.001$) and was consequently used in all subsequent analyses.

To assess whether individuals in the sampled population were more related than expected by chance, I simulated 526 unrelated individuals based on allelic frequency and genotyping errors found in the sampled population. This allowed us to compare overall relatedness in the sampled population with the relatedness that would be expected by chance, if individuals were not related. For the comparison, Wilcoxon rank sum tests with continuity correction were used, which have been shown to perform best if the sample is non-normally distributed, as is the case here (Higgins 2003).

With the aim of studying whether individuals in a social group were more related to each other than to others in the population, pairwise relatedness was compared within groups to the average relatedness of any two fish not within the same social group. I compared the two relatedness values using Wilcoxon rank sum tests, to account for non-normal distribution of samples. The same procedure was followed to compare relatedness within and between distinct reefs, comparing the average relatedness of dyads on the same reef with the average relatedness of dyads with individuals on separate reefs. In order to assess a reliable measure of relatedness 5000 each of first degree relative dyads, second degree relative dyads and non-related dyads were simulated in COANCESTRY and calculated the relatedness threshold as the midpoint of the means of first or second degree and non-related dyads (following Fraser et al 2005) (Appendix C). The error rates were then calculated according to this threshold, Type I as related dyads being identified as unrelated and Type II error as non-related dyads identified as related. Mid-points and corresponding errors were calculated between first degree relatives and unrelated dyads (r_{xy}=0.236, Type I=3.92%, Type II= 0.34%) and between second degree relatives and unrelated dyads (r_{xy}=0.119, Type I=21.5%, Type II=4.1%). Accordingly, r_{xy}=0.236 was chosen as the most accurate threshold to assess relatedness in dyads in the population.

With the aim of determining whether genetic relatedness declined in relation to distance in the study area, the distance between each possible dyad was calculated by comparing Cartesian coordinates of all social groups. I plotted the mean distance for all dyads above a given relatedness threshold (100 steps between r_{xy} =0 and r_{xy} =0.99) to account for the skew in the data, i.e. the number of dyads with r_{xy}<0.1 was 125773, whereas the number of dyads with r_{xy}>0.2 was 1937.

Self-recruitment & sib-ship

To determine whether the presence of full sib or half-sib relationships is greater within reefs than among reefs, and whether levels of self-recruitment are high enough to explain levels of relatedness within social groups, I used the same 19 microsatellites (Rueger *et al.* 2015, Appendix D) to match juveniles and subadults to potential parents. Parentage was assessed with COLONY, with the following parameters; Full likelihood, medium likelihood precision, long run. These parameters were shown in (Chapter 4) to yield a total accuracy of 93.7% for this marker set identifying true parent-offspring pairs (as per Harrison *et al*. 2014).

In order to compare the presence of full-sib and half-sib relationships among reefs, full sibs were assessed by finding juveniles with the same assigned father and same assigned mother and half siblings were juveniles that had the same assigned father or mother. The number of half siblings that were sampled from a reef with at least one other half sibling was tested against an expected distribution of 0.2 for each of the five reefs sampled with Pearson's Chi-squared test. Two reefs were excluded from these analyses (Limuka and Garbuna) due to low sample sizes ($N_{Limuka}= 6$, $N_{Garbuna}= 4$).

To assess whether levels of self-recruitment could explain the high levels of relatedness within groups, the juveniles that returned to their natal reef and natal social group were identified. I tested whether the number of juveniles that were found on the same reef as their assigned parents was different than expected by chance with a Fisher's exact test.

Inbreeding avoidance

I hypothesised that related individuals in a group avoid inbreeding via an ontogenetic switch in the association of related individuals. To test this, an ANCOVA was used so as to analyse the relationship between pairwise relatedness (dependent), body size ratio (continuous) and social group (factorial: same social group or different social group). Specifically, I was interested in the interaction between size ratio and social group in order to understand whether a relationship, such as parent-offspring relationship, is more likely to occur within the same social group. Following Buston *et al.* (2009) I predicted that if siblings tend to recruit together, high relatedness within social groups would be associated with similar sized dyads, with a standard length (SL) ratio of > 75, and if dissimilar dyads which are closely related are more common in the same social group (SL ratio < 75), this would indicate parent-offspring relationships to be contributing to the relatedness structure. Those relationships are not mutually exclusive, since if offspring are indeed attracted to their parent's reef or social group, it might also be expected some siblings to be present close to one another. I further looked at juveniles and subadults (SL ≤ 39) and adults (SL > 39) separately and conducted a full factorial ANOVA on pairwise relatedness (dependent), size (large or small) and social group (same or different). This was done to understand whether siblings settle together (expected; high relatedness among juveniles in the same group), and whether they also tend to stay in the same social group to adulthood (expected; high relatedness among adults in the same group). Again I was specifically interested in the interaction between size and group.

To test the hypothesis that individuals in a breeding pair were less related to each other than to others in the population, the average genetic relatedness and variance of relatedness of breeding pairs was compared to the average for the population. First, the pairwise relatedness of 67 known reproductive pairs of *S. nematoptera* was calculated. These pairs had been identified by sampling broods from the males' mouth and conducting parentage analysis on a subsample of an average of 10.2 embryos per brood. The variance of relatedness among breeding partners was compared to the variance of relatedness in the general population using Bartlett's test (Snedecor & Cochran 1967).

Results

Pairwise relatedness of individuals

Relatedness amongst sampled individuals was 1.25x higher than would be expected by chance, indicating the presence of highly related individuals in the study population (Wilcoxon Rank test: W = 9956100000, $p<0.001$). Fish were more likely to be related to individuals on the same reef than fish on different reefs. Among related individuals ($r_{xy}>0.236$), the relatedness-coefficient within reefs was 1.3x higher than the coefficient between reefs (within reefs $r_{xy}= 0.387 \pm 0.015$ versus between reefs $r_{xy}= 0.299 \pm 0.003$, Wilcoxon Rank test: W = 101280, $p<0.001$). This is reflected in the relatedness scores of all dyads (overall mean $r_{xy}= 0.032 \pm 0.0001$, within reefs $r_{xy}= 0.033 \pm 0.0003$ versus between reefs $r_{xy}= 0.031 \pm 0.0002$, Wilcoxon rank test: W = 112101.5, $p<0.001$). Comparisons of individuals within social groups and between social groups showed that social groups within reefs represented the most highly related individuals. Among related individuals ($r_{xy}>0.236$), relatedness-coefficient within social groups was 1.3 times higher than between social groups (within groups $r_{xy}= 0.468 \pm 0.035$ versus between groups $r_{xy}= 0.361 \pm 0.015$, Wilcoxon Rank test: W = 38471, $p<0.001$). This is reflected in the relatedness scores of all dyads (within groups $r_{xy}= 0.035 \pm 0.0008$ versus between groups $r_{xy}= 0.032 \pm 0.0004$, Wilcoxon Rank test: W = 39480, $p<0.001$).

The majority of individual fish had relatives in the sampled population, many of them on the same reef or even in the same social group. 980 dyads (0.7%) were above the relatedness threshold, including 347 individuals (65.8%) from all seven reefs and almost all social groups (N=40, 97.6%). A third of sampled fish had a relative on the same reef (N=168, 31.8%) and more than a tenth had a relative in the same social group (N=66, 12.5%).

Relatedness vs distance

Relatedness among dyads showed a strong, negative, non-linear relationship with distance on a small scale (Figure 2). Average relatedness (± SD) among all 138 576 dyads was 0.032 ± 0.05. Most dyads (90%, N = 125795) showed low relatedness $r_{xy} \leq 0.1$. However, relatedness ranged from 0 to 0.97. While the average (±SE) distance between fish was 557.77±1.96m, those dyads closely related ($r_{xy} > 0.35$) were almost twice as close to one another (310.93 ± 35.51m). Mean distance (±SE) among related ($r_{xy} \geq 0.236$) dyads was 5% smaller than that of unrelated dyads (529.2 ± 23.3m cf 557.96 ± 1.96m).

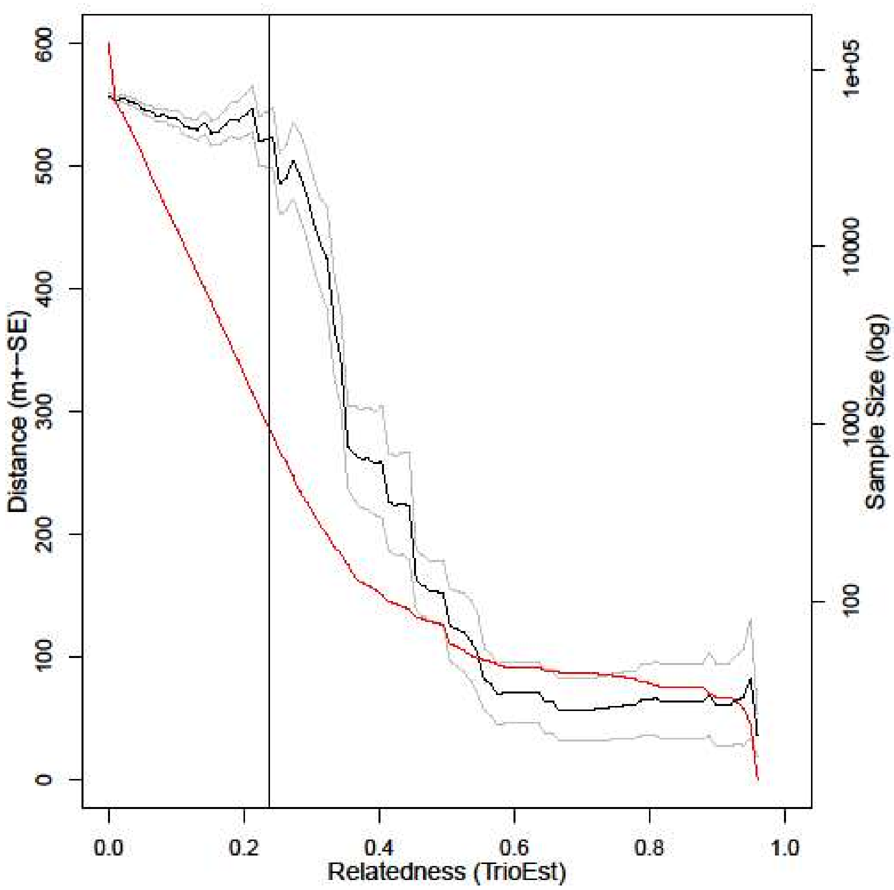


Figure 5-2 Mean distance (m ± SE) between dyads for all dyads above a relatedness threshold, 100 steps iterated between r_{xy} 0 to 1, in *Sphaeramia nematoptera*. Red line indicates sample size for each relatedness value (log scale). Vertical black line indicates relatedness threshold $r_{xy}=0.236$.

Sibling relationships

No full siblings, but significantly more half siblings than expected by chance, were found in the same social group or on the same reef (Figure 3: χ^2-test, $\chi^2=14.002$, df=1, $p<0.001$). 8 juveniles (3.5%) had full siblings with the same assigned father and mother, in the sample, 168 juveniles (75.3%) had half siblings, with either the same assigned father or the same assigned mother in the sample. In total a third (N=53) of half sibling dyads were found within the same reef; one sixth were found on the same reef but in different social groups, one seventh were found in the same social group (Figure 3).

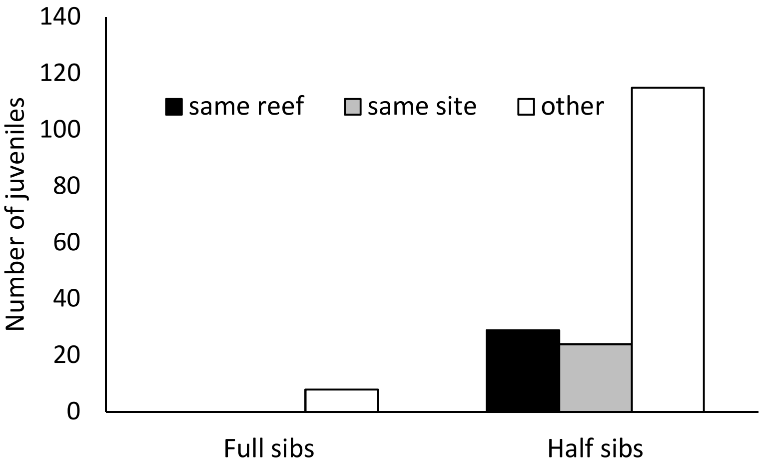


Figure 5-3 Sites at which full sibs and half sibs were sampled; same social group (black), different social group but same reef (grey), different reef (white).

Self-recruitment

Out of 233 juveniles and subadults collected, 34 (15%) could be assigned either to a single parent (N=27, 12 %), or to a parent pair (N=7, 3%) within the sampling area (Figure 4). Out of the assigned individuals, a third (N=11, 32%) were sampled on the same reef as their parents, which is significantly more than expected by chance (Fisher's exact test, p=0.017).

A twentieth (N=11, 4.7%) of sampled juveniles self-recruited back to their reef of origin, five of them were either found in the same social group as their parents (N=3) or in a neighbouring group, 5- 20m away (N=2). The average (±SE) distance between offspring and their assigned parents was 378 ± 90m, which is closer by a third compared to the average distance between all fish sampled (558 ± 2m).

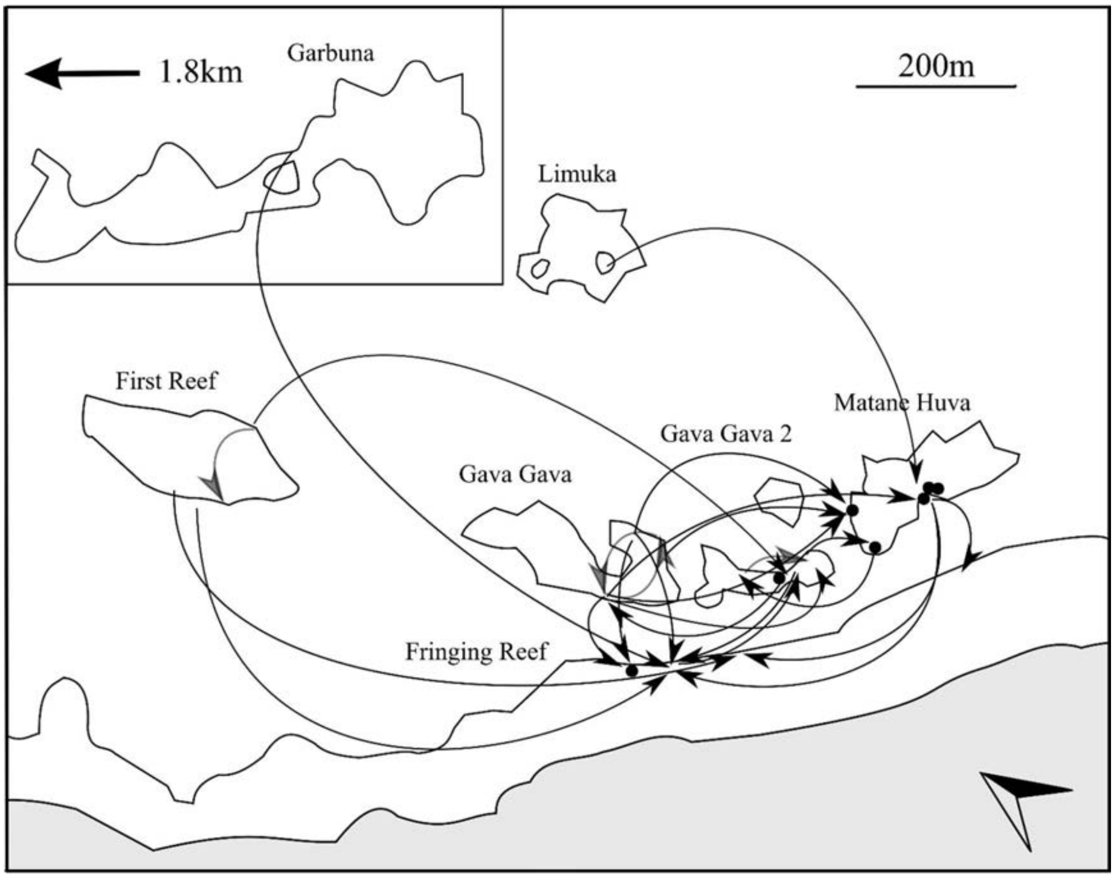


Figure 5-4 Recruitment of *Sphaeramia nematoptera* on inshore reefs in Kimbe Bay, PNG. Each assigned recruit is represented by an arrow from its' origin (location of parents) to its' current position (location of juvenile when sampled). Black points indicate recruitment into the same or a neighbouring social group, grey arrow indicates recruitment into a social group on the parental reef, black arrows indicate recruitment to a neighbouring reef.

The number of juveniles that could be assigned to a parent in the sampled population differed among reefs (Figure 5, Fisher's exact test, p=0.274). Similarly, the amount of self-recruitment differed among reefs (Figure 5, Fisher's exact test, p= 0.373). However, none of these differences were statistically significant.

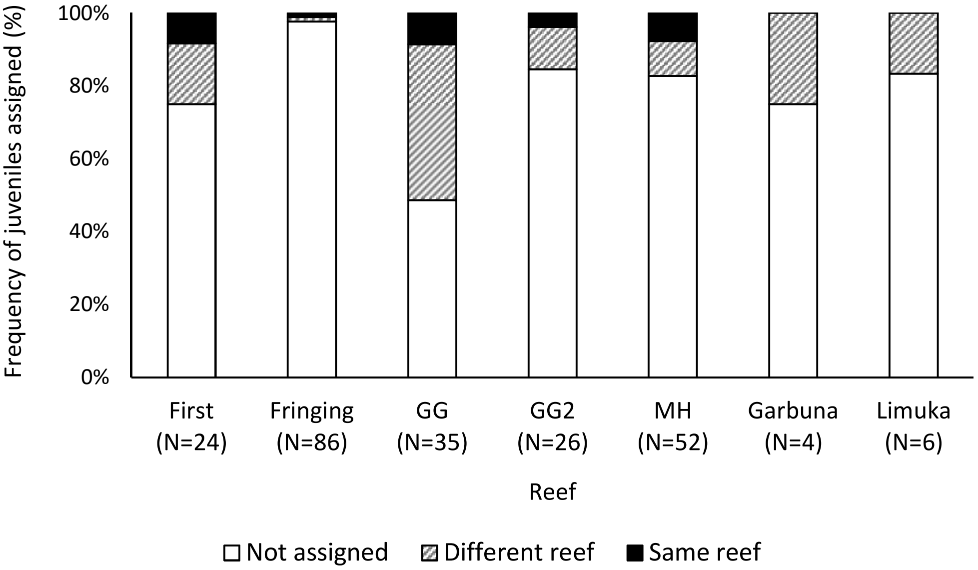


Figure 5-5 Frequency of offspring assigned to a sampled parent by reef of origin, divided by offspring not assigned (white), offspring assigned to a parent on a different reef than its' origin (pattern) and offspring assigned to a parent on the reef it was collected from (black).

Inbreeding avoidance

I found evidence of an apparent ontogenetic shift in social groups and the relatedness of individuals within these. Within a social group, parent-offspring relationships were more common than sibling relationships (Table 5-1a, Figure 5-6a). Within groups, relatedness was lower among similarly sized fish and higher among fish of different sizes. While similar sized fish (SL ratio ≥ 0.75) tended to be closely related if they were on the same social group, rather than in different groups, even greater relatedness within groups could be found among dissimilar sized dyads, with those SL ratios smaller than 0.75. There was also an indication for an ontogenetic shift in relatedness within groups. When separating juveniles and subadults (SL ≤ 39mm) from adults (SL > 39mm), small individuals in the same social group tended to be more closely related amongst themselves, compared to relatedness among adults, which was 1.2 times less and not different whether individuals were in the same site or in different social groups (Table 5-1b, Figure 5-6b).

Table 5-1 a) ANCOVA. Pairwise relatedness with size ratio (continuous, SL Individual 1 / SL Individual 2) and social group (factorial, different or same social group) of *Sphaeramia nematoptera* in Kimbe Bay, Papua New Guinea. **b)** Two-way ANOVA. Pairwise relatedness with body size (small (SL ≤ 39mm) or large (SL > 39mm)) and social group (different or same social group) of *Sphaeramia nematoptera* in Kimbe Bay, Papua New Guinea.

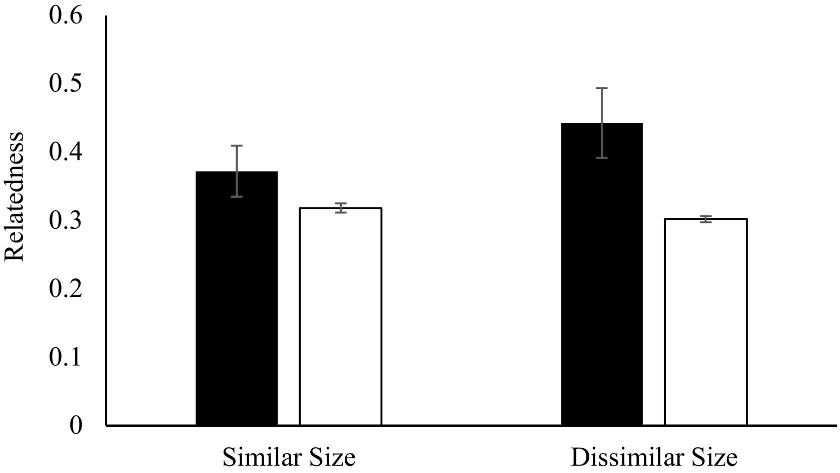
a)

Relatedness~	DF	SS	MS	F	p
Size ratio	1	0.049	0.049	3.100	0.078
Social group	1	0.451	0.451	28.008	<0.001
Size ratio X Social group	1	0.223	0.223	13.831	<0.001
Residuals	896	14.417	0.016		

b)

Relatedness~	DF	SS	MS	F	p
Size (small/large)	1	0.010	0.010	0.632	0.426
Social group	1	0.459	0.459	28.556	<0.001
Size X Social group	1	0.283	0.283	17.623	<0.001
Residuals	896	14.388	0.016		

a)



b)

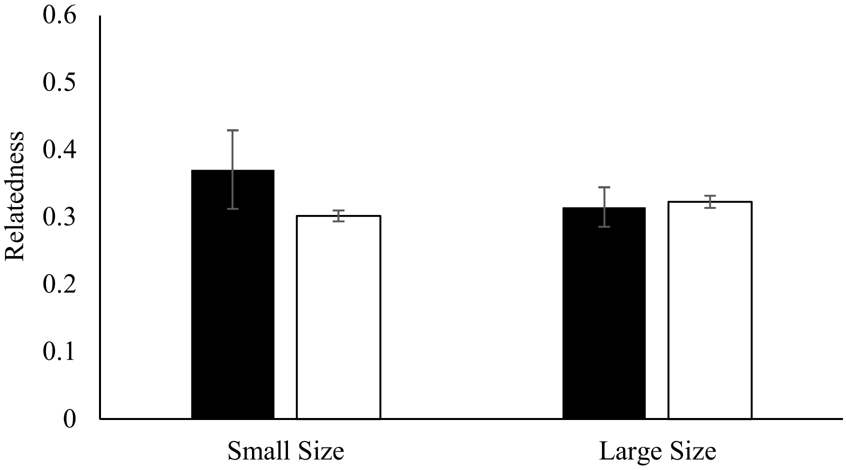


Figure 5-6 a) Relationship between pairwise relatedness and size similarity (similar size, standard length (SL) ratios ≥ 0.75; dissimilar size, SL ratios < 0.75); and **b)** Pairwise relatedness in relation to total size for juveniles and subadults (small size SL ≤ 39) and adults (large size SL > 39mm); of the cardinalfish *Sphaeramia nematoptera* from the same social group (black bars) and different social groups (white bars).

None of the reproductive partners sampled were above the relatedness threshold. Relatedness could be calculated for 67 mated pairs of *S. nematoptera* that produced offspring together. Mean (± SE) pairwise relatedness among reproductive partners was lower than the average of other dyads within social groups, although this difference was not significant (r_{xy} among partners = 0.033 ± 0.006 versus r_{xy} non partners= 0.035 ± 0.001, Wilcoxon rank test; W= 4663700, p= 0.945). However, the variance of relatedness was significantly lower among mated partners, than in the general population (Bartlett's test, p = 0.003), with r_{xy} only ranging from 0.000 to 0.215.

Discussion

This study refutes the long-held assumption that social groups of coral reef fishes are made up of unrelated individuals. I demonstrated that social groups of *Sphaeramia nematoptera* exhibit greater than expected genetic relatedness, reflecting fine-scale genetic structure within and among reefs on a scale of less than 1km. I not only found high relatedness within reefs, but also within social groups. Pairwise genetic relatedness declined with distance over scales of 100s of meters. High relatedness appears to be explained by both high levels of self-recruitment to natal populations and recruitment back into the same social group as the parents. In addition, evidence of siblings settling together was found, which may lead to kin-group structure within the population. Within social groups, genetic relatedness was higher among individuals of different body sizes and breeding adults were not more closely related to each other than to others in the population. These two results indicate that related individuals may be re-assorting among social groups after settlement and before maturity to avoid inbreeding and kin competition. Understanding the genetic relatedness of individuals provides insight into how social groups are formed and maintained in this species.

Are juveniles attracted to social groups with kin?

I found substantial evidence that *S. nematoptera* preferentially live with kin. Relatedness was higher within reefs than between reefs and even higher within social groups on the same reef. Furthermore, a clear relationship is described between pairwise relatedness and distance among dyads on an extremely small scale. All of which is indicative of the pajama cardinalfish being attracted to and seeking the proximity of kin, or being attracted to its' site of origin. There were indications of juveniles being attracted to parents; high levels of self-recruitment and high relatedness among dissimilar sized individuals in social groups, as well as evidence for juveniles being attracted to siblings, namely many half-sibs being found in close proximity and especially high relatedness between similarly sized individuals within social groups. On the other hand, no full sibs were found within the same social groups and few full-sibs within the sampled population over all. In mammals, kin groups are mostly the consequence of offspring staying in close proximity to their parents after birth, cooperative breeding and differential sexual dispersal where only males leave the group when they become sexually mature (Clutton-Brock 2009b). It is now known that juveniles settling close to their parents is not as unlikely in marine organisms as the pelagic larval phase might make us predict (Jones *et al.* 1999a; Swearer *et al.* 2002). There is also evidence for attraction to kin among teleost fish from experimental studies (Mehlis *et al.* 2008). Whether the proximity to relatives found here is due to a direct attraction to kin or alternatively due to a strong attraction to the home sight, the smell of which might have been imprinted in the egg and early larval stage, as shown for other teleost species (Gerlac*h et al*. 2008), is unknown and needs to be subject to further experimentation. As the present results suggest, living in close proximity to close relatives might be much more common for marine fish than currently believed.

Is the level of relatedness a consequence of fine-scale self-recruitment?

Both parent-offspring and sibling relationships contributed to the high relatedness within social groups in *S. nematoptera*. Dyads were found to be more closely related within the same social group than were individuals on different social groups. The contribution of parent - offspring relationships to fine scale genetic structure has also been shown for the cichlid *Neolamprologus pulcher* (Dierkes *et al.* 2005). Evidence of siblings recruiting together has been found in the kelp bass *Paralabrax clathratus* (Selkoe *et al*. 2006) and the coral reef damselfish *Dascyllus aruanus* (Buston *et al.* 2009). The close kin associations found here could be a consequence of *S. nematoptera* returning close to their birth social group after the pelagic larval stage, or, alternatively, of the larvae staying in close proximity to their site of origin throughout the pelagic larval stage.

S. nematoptera showed high self-recruitment, which may be a contributing factor to the high relatedness within social groups. As suggested by the size-dissimilarity data, within a very small area (max distance ~3.1km, average distance ~0.5km) many (15%) of the juveniles were assigned to sampled adults. A relatively large proportion of them were found on the same reef as their parents and some were even found in the same social group. 58% of juveniles were assigned to parents in a small study area (~12km) in another cardinalfish, *Ostorhincus doederleini* (Gerlach *et al.* 2007). *O. doederleini* has a longer larval phase and is a stronger swimmer than *S. nematoptera* (Fisher & Bellwood 2003), which contradicts the theory that these characteristics are directly indicative of levels of dispersal (Fisher 2005). However, the rate of self-recruitment found for *O. doederleini* might have been overestimated because of the low number of markers used in that study (Fraser *et al.* 2005), as well as the unique, lagoonal environment the study was conducted in.

Do siblings settle together?

Many half-siblings were found in close proximity to one another, contributing to the kin group structure found in *S. nematoptera*. Supporting the evidence of size-similarity being connected to high relatedness within social groups, many more half-siblings were found than expected by chance on the same reef and in the same social group. None of the full-sibling dyads identified were found in close proximity. Since only eight full-siblings were found, this might indicate an issue of sample size rather than contradicting the hypothesis. If there is a strong attraction of juveniles to parental reefs and even social groups, as shown above, siblings settling together might be a consequence of self-recruitment. Siblings staying in close proximity to one another seems to be quite common in teleost fish, e.g. in the guppy *Poecilia reticulata* (Piyapong *et al.* 2011) and the three spined stickleback *Gasterosteus aculeatus* (Frommen *et al.* 2007; Mehlis *et al.* 2008). Evidence of siblings or closely related individuals of similar size settling together after a pelagic larval stage is less common, but has been found for the damselfish *D. aruanus* (Buston *et al.* 2009). The fact that many more half- than full siblings were found in the sample might indicate that larvae do not stay together through the entire larval stage but rather that related juveniles find one another during settlement stage. Furthermore, settling with half- rather than full siblings might reduce the potential for kin competition and inbreeding among adults, whilst still promoting cooperative behaviour among relatives. This should be considered and tested in future research, since it might give insight into the evolution of cooperative group living in animals through kin selection.

Is there evidence of inbreeding avoidance as individuals reach reproductive size?

While siblings tended to settle together in *S. nematoptera*, it seems that there was an ontogenetic shift and they were not necessarily in the same social group anymore as adults. In this study small individuals within the same social group were on average closer related than large individuals. This would narrow down the reasons for kin group structures and may be indicative of an evolutionary mechanism to gain advantages of living with kin but avoiding inbreeding. Juvenile *S. nematoptera* settled together and seek close proximity to their parents, possibly in order to grow in a peaceful environment. Juvenile teleosts have been shown to grow quicker when surrounded by siblings, and groups of kin show lowered levels of aggression (Gerlach *et al.* 2007b). Thus small pajama cardinalfish may choose relatives in early stages of their lives in order to gain a developmental advantage. However, when growing up, they might change social group in order to find a reproductive partner, as is suggested in a study showing juvenile pairs of *S. nematoptera* break up before they reach maturity (Chapter 3) and by the current results finding no relatives among reproductive partners. This would be an effective mechanism to avoid inbreeding as well as kin competition among adults, but is subject to further investigations.

What are the ecological and evolutionary consequences?

One of the main ecological and evolutionary consequences of kin associations is fine scale population genetic structure. Population genetic structure on a small scale, albeit very rarely as small as in the case of *S. nematoptera*, has been found for other teleost fishes. One of the only examples for fish schooling with relatives comes from the migratory char, *Salvelinus fontinalis,* in which fish were found in the same school as close relatives more often than expected by chance (Fraser *et al*. 2005). When it comes to reef fish, there are some examples of fine scale genetic structure. The damselfish *Acanthochromis polyacanthus*, which does not have a larval phase, shows differences in relatedness between reefs as close as 3km (Gerlach *et al*. 2007). Similarly, the Banggai cardinalfish, *Pterapogon kauderni*, also with no larval phase, shows meaningful differences of population structure on a small scale (2-5km) (Hoffman *et al*. 2005), when tested for within group relatedness however, no kin- group structure was found (Kolm *et al.* 2005). The differentiation of populations within a small area might be contributing to the high diversity we see among some taxa in ecosystems such as coral reefs. Also local adaptation might favour self-recruitment and consequent high relatedness within social groups and reef populations. While *S.*

nematoptera has a relatively unusual parental care strategy, it being a small bodied, group living, habitat dependent, paternal brood-carer does make it representative of a large number of reef fish species in this context.

Conclusion

I found kin-group structures in S. *nematoptera*; individuals are more likely closely related within social groups than between groups and relatedness was higher within than between reefs. Generally, pairwise relatedness showed a strong, negative relationship with distance, even though the study area was very small compared to other studies concerning population genetics. Causes for this fine scale genetic structure are high ratios of self-recruitment as well as siblings settling together. The fact that relatedness among adults does not follow the same trend, and adults were no more related within a group than between groups, might indicate an ontogenetic shift of trying to be close to relatives when young in order to benefit from low aggressiveness and more stable social orders, but avoiding inbreeding when it comes to searching for reproductive partners. The current study suggests we might be vastly underestimating the degree of relatedness among groups of coral reef fishes and other marine taxa and thus missing important trends in population genetic structure and the dynamics of social group formation and maintenance.

Chapter 6 : Discussion

Although social relationships influence all aspects of ecology and evolution of sexually reproductive animals, many taxa are still excluded from the detailed study of social interactions. Questions concerning reproduction, parent-offspring relationships and group structures remain controversial. On coral reefs, social relationships play a great role in shaping the ecology and evolution of many habitat dependent and group- or pair-living fish species, but issues concerning the relationship between pair bonds and site attachment, in particular which direction this relationship takes; the nature of mate choice, for example which sex chooses and which characteristics are important in mate choice; the difference between social and genetic mating systems; and the relatedness among groups of individuals, which can reveal much about population dynamics, are all still understudied. I investigated a habitat dependent, group living and pair forming coral reef fish, the cardinalfish *Sphaeramia nematoptera*, and showed that for this species 1) site and mate attachment are tightly linked and it is site fidelity that facilitates pair formation, 2) pairs are size-assorted through mutual mate choice, 3) while many pairs are observed to be monogamous, molecular tools reveal extrapair mating and sneaking by both sexes occurs, and 4) groups are made up of closely related individuals, through high levels of self-recruitment and associations among siblings at settlement.

One major question concerning social relationships, which has eluded ecologists thus far, is which direction the apparent relationship between site and mate fidelity might take and whether one facilitates the other. I show that, at least for *S. nematoptera*, site attachment is a major driver of the reproductive system. Firstly, I established that pairs are socially monogamous for the breeding cycle, and often over multiple cycles. A fifth of the breeding pairs remained together for eight months, such that I conclude long-term pairing is relatively common for this species. I then go on to show that pairing is facilitated by site attachment. It was known from other reef fish studies that reproductive characteristics such as sexual maturity and paired/solitary status can influence movement between coral colonies. For example in the goby *Gobiodon histrio*, single adults showed the highest rate of movement (Wall & Herler 2008). In my thesis I show for the first time that not only do sex and pairing status influence site attachment but it is site attachment which facilitates pairing in aggregations of these reef fish. Almost all fish stayed in place after their partner was removed, even though the fishes' natural movement would enable them to search neighboring social groups. What is more, most fish re-mated within only a few weeks following mate removal.

The site attachment I found in this species is unusually high. Other cardinalfish also have high site attachment (Marnane 2000) as do reef fish of other families such as the goatfish, *Parupeneus porphyreus* (Meyer & Holland 2000), the goby *Valenciennea strigata* (Reavis & Barlow 1998), and the damselfish *Dascyllus aruanus* (Sale 1971). Loyalty to a particular site or habitat is common in the animal kingdom overall, and is often associated with reproductive activities. Some taxa show a trend of paired individuals having high site attachment, such as colonially breeding sea birds (Cézilly *et al.* 2000). The relationship between site and mate attachment I found is known for other taxa, but the direction of the interaction was studied here for the first time. Since living at a familiar site has advantages for all animals, for example predator avoidance and more efficient foraging, it is likely that site attachment is a driving factor in shaping the reproductive system of many habitat dependent taxa.

Site attachment might also be a major driver for group and population structures in *S. nematoptera*. While self-recruitment and settler sibship, according to existing literature, seem to be the most probable explanation for the relatedness patterns found here, there is also a less explored possibility that larvae do not venture far from their social group of origin after hatching. Site attachment very early in their development might lead to high relatedness within social groups even in the absence of kin attraction.

I show that assortative mating in the pajama cardinalfish is a product of mutual mate choice. This might be explained by the apogonids' unusual parental care. While the males brood and cannot feed during this process, the females invest in eggs and stay with the male during brooding, which likely acts to protect the male from egg thieves and rival females. Thus the sex's investment is equal and both of them would be expected to aim for the biggest partner possible. Reproductive output for both sexes in cardinalfish is linked to size: the males' buccal cavity limits reproductive potential for both sexes, since it can hold less eggs than are typically in a clutch (Kume *et al.* 2000c); and bigger females produce bigger eggs which are likely to have a fitness advantage (Perrone 1978; Berglund *et al.* 1986). While size being tightly linked to reproductive quality is a usual phenomenon, this type of equal mate choice is rarely observed in animals. Other examples include monogamous seahorses and pipefish, where males brood in internal pouches, and some species of birds, in which mutual parental care is common (Wittenberger & Tilson 1980; Sogabe & Yanagisawa 2007).

I demonstrate size-assortative mating occurs in *Sphaeramia nematoptera*. Size-assortative mating is particularly common in teleost fish and while the reason for this is unknown, it seems to be connected to the many cases of sympatric speciation found in this group of animals (Jiang *et al.* 2013). In many taxa, females are the choosy sex, since eggs are costlier to produce than sperm and it is typically the female which provides care (Emlen & Oring 1977; Kokko & Jennions 2008). In teleost fish however, paternal care is common and the differing grades of investment between males and females are reflected in myriad reproductive systems (Breder & Rosen 1966; Thresher 1984). Many reef fish live or at least reproduce in pairs and in most species reproductive output is linked with the size of both males and females. In these pairs males often provide care, which leads to similar investment between the sexes (Whiteman & Côte 2004). All of these characteristics enable assortative mating and since assortative mating is thought to lead to the preservation of phenotypic variation and even sympatric speciation, it may explain some of the huge diversity of species in coral reef ecosystems.

The single individuals found in almost all social groups could be explained by the high propensity to look for a similarly sized partner and an unwillingness to settle for a partner too small, as explored in Chapter 3. If we take the possibility of sneaking for both males and females into account, as demonstrated in Chapter 4, then it seems plausible that individuals might try to sneak on an existing couple, rather than invest energy into a too-small mate. Yet another explanation, which could be considered in combination with the size argument, is that high relatedness among group members might deter some individuals from forming a pair within their social group.

While *S. nematoptera* is socially monogamous, microsatellite markers revealed extrapair and sneak mating. In very few cases is molecular data available to study reproductive systems of coral reef fish. For many species only short term behavioural data is available and based on this many of the pair forming species, e.g. butterflyfish, damselfish, and gobies, are assumed to exclusively mate with one another (Fricke 1986; Kuwamura *et al.* 1993). My data shows however, that reproductive systems can be much more complex than can be observed. By employing genetic tools as well as long term behavioural studies we can gain insight into sexual selection and the evolution of parental care.

Even though male cardinalfish invest a lot into offspring care, as they brood clutches in their buccal cavity, and current evolutionary theory predicts sole paternity for such a case, I show that there is multiple paternity within one clutch, demonstrating the occurence of male sneaking. Thus far paternal care strategies have been thought to evolve in a context of guaranteed sole paternity (Jones & Avise 1997b). Detailed studies on seahorses and pipefish, even those species in which brooding is done in external pouches, have not found any fertilizations by sneaker males. Here I show that sneaking is possible, probably as a status-dependent conditional strategy with which smaller males gain offspring with little investment until they are large enough to court females and brood embryoes themselves (Gross 1996).

The female sneaking I found is one of the first cases such a strategy has ever been described. I found 30% of clutches with a sneaking female present, half of which were partial clutches, meaning there were multiple mothers involved. Throughout the animal kingdom can be found examples of females competing with one another for resources and also mates. This leads to female ornamentation and in some cases even reversed gender roles (Clutton-Brock 2009a). However, the description of alternative strategies within the same species is very rare. The most common form is found in cooperative breeders, where only dominant females have the oportunity to reproduce. The only other case in which female sneaking is described, even though the authors do not describe it in these terms, is in goldeneye ducks, where older females lay eggs in younger females' nests in addition to their own (Eadie & Fryxell 1992). This represents a conditional strategy, with ducks of a certain age using sneaking to maximise their reproductive output. I found no analogous connection of sneaking with size of the females in *S. nematoptera*. The female sneaking in *S. nematoptera* might represent a facultative strategy which is used when the female can either not find a mate, or her mate is not ready to brood when she produced the next clutch of eggs. Facultative alternate reproductive tactics are very rarely described in the animal kingdom at all, let alone for females (Gross 1996).

I found that *S. nematoptera* often live in groups of closely related individuals. This is the first case of kin group structure described for coral reef fish in the wild. Closely related to the unusual reproductive system of *S. nematoptera* is their group structure, since fish are strongly site attached and pair within groups. The close, negative relationship between distance and pairwise-relatedness I found is not unusual and in many populations of teleosts and other taxa the same relationship can be found (Ovenden *et al.* 2002; Coltman *et al.* 2003; Schunter *et al.* 2011). However, the small scale at which this relationship is found is unique. In the damselfish *Acanthochromis polyacanthus*, which develops directly with no larval phase, population sub-structures have been found between reefs in an area of approximately 15km (Gerlach *et al.* 2007a). In the banggai cardinalfish

(*Pterapogon kauderni*) small scale genetic structure was evident at distances as short as 3-5km (Hoffman *et al.* 2005), but no kin group structure despite the lack of larval phase in this mouthbrooder (Kolm *et al.* 2005). *Sphaeramia nematoptera* on the other hand had significant kin relationships among groups and at a scale < 2.1km. There was higher relatedness within than between reefs and also higher relatedness within groups compared to between groups

The fine scale genetic structure I found can be explained by high rates of self-recruitment and siblings settling in close proximity to each other. The rate of self-recruitment in *S. nematoptera* is high when taking into account the small scale of the study area. In lakes and estuaries it is not uncommon for juvenile fish to stay close to one another and to their parent‘s group. For instance, siblings of the migratory brook charr *Salvelinus fontinalis* stay in close proximity, leading to kin group structure (Fraser 2005). Similarly, siblings of the reef fish, *Dascyllus aruanus*, are also thought to settle together even though no kin group structure has yet been found (Buston *et al.* 2009). This is similar to what was found for *S. nematoptera*, where siblings and half-siblings settle in close proximity and alongside high self-recruitment leads to fine scale genetic structure in this species.

While small individuals of *S. nematoptera* tend to be more closely related to other small individuals in the same social group and on the same reef, this trend does not continue into adulthood, where similarly sized fish are no more closely related within than between groups. This is evidence for an ontogenetic shift, which might be a mechanism to avoid inbreeding. Indeed I found adults, especially single females, ocassionally moving between social groups. It is possible that *S. nematoptera* enjoy the benefits of living with close kin, such as lower aggression and more stable heirarchies, when they are young but once they reach sexual maturity they tend to look for a partner outside that social group in order to avoid breeding with siblings or other close relatives.

Future research

In this thesis I answer many questions and I discover many new ones. I found that site fidelity is a major driver of the reproductive system, but is this true for all habitat dependent, pair-living animals, or even for all reef fish with such characteristics? Understanding the connection between reproductive system and habitat dependence will be a major topic as habitat degradation through anthropomorphic influences, be it rising sea levels or temperature, pollution or deforestation, affects more and more species of all taxa. It will be impossible to predict how populations will demise or thrieve in a changing environment without understanding how their reproduction may be influenced by these changes. Similarly, the pairing and mate choice patterns I found for *S. nematoptera* may be able to explain some of the huge diversity that is found in habitats such as coral reefs by providing a mechanism how phenotypic heterogeneity might be stable and speciation might be promoted, but future research is needed to confirm this hypothesis. Knowing population genetic structure and dispersal allows us to understand many aspects of an animal‘s ecology, such as group living, as well as make predictions about future population trends. My study is one of the first showing kin group structures in wild fish, but as methods become more reliable, with the development of genetic markers becoming cheaper and more analysis tools being broadly available, it is very well possible that it will soon be found that this type of fine scale genetic structure is not an exception. The topics covered in the studies presented here provide examples of topics which can only be understood by studying animals in detail and looking at aspects of ecology from several different perspectives.

Conclusion

The relationship between site fidelity and mating was found to operate in the direction of site attachment facilitating pair formation in *Sphaeramia nematoptera*. Knowing this pattern allows us to better understand which consequences habitat degradation might have on the species. The pairs that form are size-assorted and form by mutual mate choice, an unusual case in the animal kingdom, which is explainable by the unusual mode of brood care employed by this species. Patterns of reproduction are often understudied in coral reef fish, which stands in sharp contrast with the multitude of reproductive systems found in such habitats. Similarly, molecular tools, while now widely available, are still not used to an extent where knowledge of reef fish mating patterns can be updated. In the case of *S. nematoptera* alone, long-standing theories such as the absence of female sneak-mating and sole paternity in paternal brooders, are now being challenged. Molecular methods allowed the discovery that the species is attracted to and lives with closely related individuals and this, applied to more reef species, has consequences for our understanding of how populations might differ from one another. Social relationships remain a central topic of ecology, indispensable in understanding behaviour, population dynamics and habitat dependency. In this thesis I used an unusual model organism and combined several methodological approaches, which allowed me to shed some light on classic theories as well as ask new questions.

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Appendix A: Supplementary material for Chapter 3

Table A-1. Standard lengths (mm) of *Sphaeramia nematoptera*, involved in remating after natural losses and after a mate removal experiment, in Kimbe Bay, Papua New Guinea.

Natural losses

FF SL (mm)	Sex	OP SL (mm)	NP SL (mm)	Other potential mates SL (mm)			
42	F	42	40	41	43	39	
44	F	45	45	39	40		
44	F	50	38	42	47	48	
44	F	49	50	42	47	48	
43	F	50	52	46			
44	F	43	46	39	44		
41	F	44	39	44	39		
40	M	41	40	44	39		
42	M	42	44	44	44		
38	M	38	39	40	41	43	
44	M	47	45	41			
39	M	45	41	45			
38	M	39	44	44	44		
50	M	50	48	47	50		
41	M	41	40	39	50	42	
47	M	49	50	50	49		
Mate removal							
49	F	47	48	49	48	48	50
48	F	51	51	45	49	47	
43	F	49	43	39			

46	F	44	44	42	47		
49	F	52	47	42	44		
52	F	46	50	43			
53	F	51	51				
47	F	46	55	51			
53	F	56	51	55			
54	F	52	54	55			
47	M	47	45	51	49	47	
51	M	47	48	49	48	48	50
48	M	44	49	48	48	48	50
39	M	39	48	43			
43	M	41	43	40			
55	M	50	47	54			
45	M	45	47	46	49		
47	M	48	49	46	47		
47	M	48	49	42	44		
50	M	51	52	50			
51	M	52	53	49			
55	M	55	47	55			

FF= focal individual, OP= original partner, NP= new partner.

Appendix B: Supplementary material for Chapter 4

Table B-1 Characteristics of 23 microsatellite markers of *Sphaeramia nematoptera*. Number of alleles (N_a), observed (H_o) and expected (H_E) heterozygosities, deviation from the exact test of Hardy-Weinberg-Equilibrium with significant departures depicted in bold, fixation index (F_{is}) and genotyping error are presented for each locus.

Primer name	N_a	H_o	H_E	HWE	F_{is}	Genotyping error	GenBank accession no.
Sphae02	34	0.915	0.995	0.184	0.021	0.058	KP790099
Sphae07	16	0.920	0.905	0.591	-0.017	0.012	KP790101
Sphae13	18	0.913	0.915	0.224	0.002	0.005	KP790103
Snema04	7	0.147	0.151	0.999	0.029	0.035	KP790108
Snema05	7	0.385	0.664	0.001	0.420	0.005	KP790112
Sphae17	8	0.758	0.806	0.674	0.059	0.012	KP790104
Sphae18	27	0.892	0.936	0.283	0.047	0.023	KP790105
Sphae28	17	0.659	0.820	0.013	0.196	0.047	KP790107
Snema03	3	0.294	0.299	0.708	0.017	0.005	KP790111
Snema08	10	0.750	0.715	0.155	-0.049	0.005	KP790113
Sphae08	14	0.868	0.872	0.177	0.005	0.023	KP790102
Snema11	11	0.580	0.721	0.013	0.196	0.023	KP790115
Snema15	11	0.589	0.730	0.331	0.193	0.035	KP790117

Snema16	12	0.113	0.168	0.453	0.330	0.023	KP790118
Sphae06	23	0.641	0.915	0.029	0.300	0.023	KP790100
Sphae20	18	0.886	0.888	0.424	0.002	0.012	KP790106
Sphae31	28	0.922	0.943	0.391	0.023	0.012	KP790109
Snema19	15	0.760	0.787	0.144	0.034	0.047	KP790120
Snema20	7	0.670	0.764	0.107	0.123	0.047	KP790121

Appendix C: Supplementary material for Chapter 5

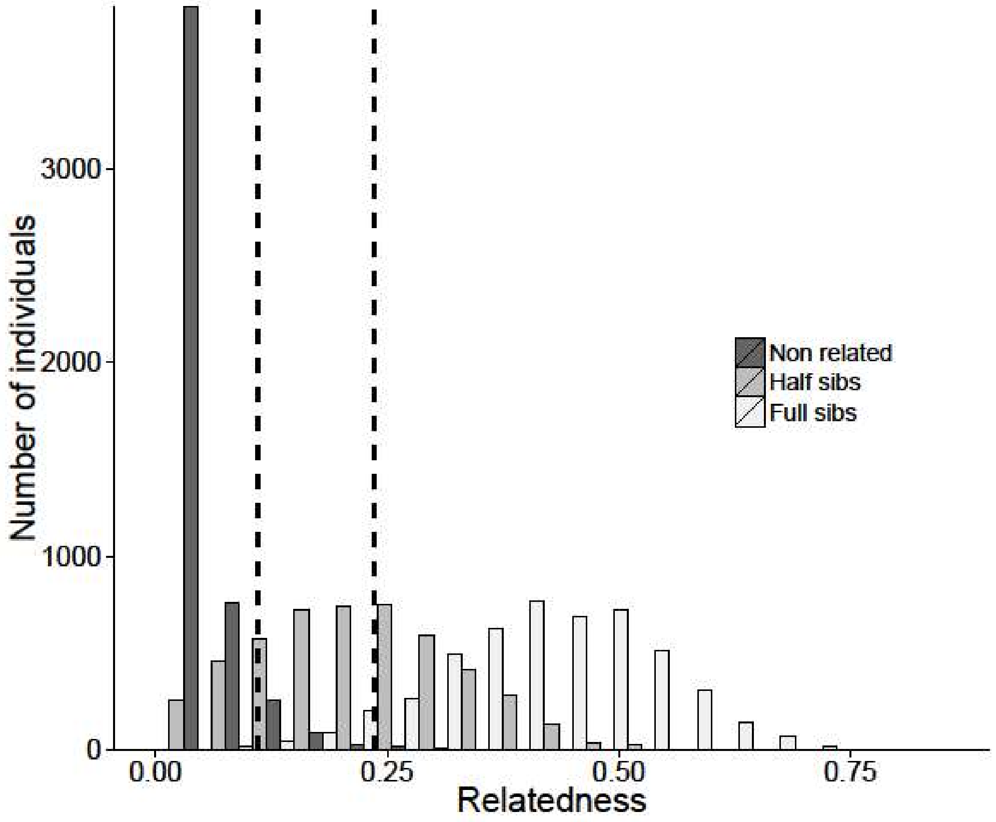

Figure C-1 Relatedness in simulated groups of first degree relatives (full sibs), second degree relatives (half-sibs) and unrelated individuals of *Sphaeramia nematoptera*. Dashed lines indicate thresholds between unrelated individuals and second degree relatives (r_{xy}=0.119) and unrelated individuals and first degree relatives (r_{xy}=0.236).

Appendix D: Resolving genealogical relationships in the in the Pajama cardinalfish, *Sphaeramia nematoptera* (Apogonidae) with 23 novel microsatellite markers

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Abstract

Many coral reef fishes exhibit unique reproductive strategies that can play a central role in conservation programs. Cardinalfishes (f. Apogonidae) are all paternal mouthbrooders, where the male holds the fertilised eggs in his mouth until they hatch. Males may fertilise the eggs of multiple females resulting in polygyny and skewed reproductive success. Here we present 23 tetranucleotide microsatellite loci in four multiplexes to identify breeding strategies in the Pajama cardinalfish, *Sphaeramia nematoptera* (Bleeker, 1856). All markers were polymorphic with a mean of 14.39 ± 1.61 SE alleles per locus and an average observed heterozygosity of 0.624±0.054 SE across 384 genotyped individuals. This marker set provides a rare opportunity to investigate mating behaviour, reproductive success, kin group structure and larval dispersal in natural populations of a coral reef fish targeted by the aquarium trade.

Note

Not much is known about the reproductive biology of the group-living Pajama cardinalfish, *Sphaeramia nematoptera*. Ongoing observational studies in Papua New Guinea suggest it has a monogamous mating system, where breeding pairs stay together over multiple brooding cycles. However, only molecular studies can confirm true monogamy. Since paternal mouthbrooding also leads to short pelagic larval durations, the limited dispersal potential and connectivity between isolated coral reefs is likely to result a high degree of inbreeding. Here we describe the isolation and characterisation of 23 microsatellite markers in four multiplex PCRs suitable to identify extra-pair mating and genealogical relationships in populations of the Pajama cardinalfish. Understanding the unusual mating system of cardinalfish and connectivity between isolated reefs will help the conservation of this species, which is regularly targeted by the aquarium trade.

Tissue samples for 384 adult *S. nematoptera* were collected from inshore reefs in Kimbe Bay, Papua New Guinea (5°30'S, 150°05'E). All tissue samples were collected between October 2012 and July 2013. Fish were captured using diluted clove oil as an anaesthetic and hand nets. Small fin clips were removed and preserved in 95-100% ethanol. Genomic DNA was extracted from fin tissue following procedures described in the NucleoSpin 96 Tissue kits (Macherey-Nagel, Germany).

Microsatellite markers were identified from a microsatellite-enriched library and a 454 pyrosequencing method performed on genomic DNA using the Genome Sequencer FLX following the manufacturer's instructions (Roche 454 Life Sciences, Basel, Switzerland). Primers were designed using MsatCommander (Faircloth 2008) with melting temperatures of 60˚C to minimising heteroduplexing in multiplex PCRs and tertiary structure formations.

Selected marker were labelled with one of the fluorescent dies 6-FAM, NED, PET or VIC and integrated to four multiplex PCRs (Table D-1). The primer pairs were combined in premixes with in reaction concentrations adjusted for even amplification ranging from 0.03 to 0.1 µM. QIAGEN Microsatellite Type-it kit (QIAGEN, Germany) was used to perform the multiplex reactions in a total reaction volume of 10 µl containing 5 µl of QIAGEN Multiplex Master Mix (2x), 3 µl of distilled water, 1 µl of primer premix, and 1 µl template DNA (34-97ng). Multiplex PCRs were performed on Veriti thermal cyclers with the following sequence: 15min initial denaturation at 95˚C, 5 cycles of 30 seconds at 95˚C, 90 seconds at 62˚C, and 60 seconds at 72˚C, then 5 cycles of 30seconds at 95˚C, 90 seconds at 60˚C, and 60 seconds at 72˚C, then 20 cycles of 30 seconds at 95˚C, 90 seconds at 58˚C, and 60 seconds at 72˚C, followed by 30 minutes at 60˚C. After a 1:15

dilution PCR products were screened with an ABI 3370xl DNA Analyzer (Applied Biosystems) with the GeneScan 500 LIZ (Applied Biosystems) internal size standard. Genotypes were scored in GENEMAPPER v4.0 (Applied Biosystems) and unique alleles were distinguished using marker specific binsets in MSATALLELE (Alberto 2009).

The number of alleles per loci for 384 *S. nematoptera* ranged from three to 31 with an average observed heterozygosity (±SE) of 0.624±0.054. Out of 23 markers, nine did not conform to Hardy-Weinberg expectations and no loci showed significant pairwise linkage disequilibrium, as determined in GenePop on the web (Rousset & Raymond 1997; Rousset 2008). Significance levels of 0.05 were adjusted for a given false discovery rate of 10% to account for multiple testing. Thus the three multiplexes are suitable to study a range of ecological and evolutionary questions and identify breeding strategies and reproductive success. Simulations confirmed this suite of markers will resolve parent offspring relationships with an accuracy of correct assignments and correct exclusions of 99.03% ± 0.24% SE (Harrison *et al.* 2014). *In situ* population studies will thus provide accurate information on breeding strategies and dispersal patterns that will serve to improve our understanding of these important ecological processes.

Table D-1 Description of 23 microsatellite markers isolated from a novel genomic library of *Sphaeramia nematoptera*. Number of alleles (N_a), observed (H_o) and expected (H_E) heterozygosities, deviation from the exact test of Hardy-Weinberg-Equilibrium with significant departures depicted in bold and fixation index (F_{is}) are presented for each locus.

Primer name	Sequence 5’-3’	Repeat Type	Size range (bp)	N_a	H_o	H_E	HWE	F_{is}	Reaction concentration (µM)	GenBank accession no.
	Multiplex 1									
Sphae02	F: GGTATGACTGCCTCTTGAAAGTATAG R: AACGACTACAAACTCATACCAGATTC	$(ATCT)_{14}$	200-310	25	0.808	0.916	0.184	0.118	0.05	KP790099
Sphae07	F: AAGGCTACTGAATACTTGAATTTCCC R: AGCTCAATAAATAGTTGAAGGGTCTG	$(ATCT)_{16}$	140-200	14	0.912	0.906	0.591	-0.006	0.05	KP790101
Sphae13	F: TCTTCTTGTACTGCTACTGTTGTAAC R: TTAACAAGTTGTAACCCTGACATACC	$(ATCT)_{19}$	140-230	11	0.848	0.917	0.224	0.075	0.05	KP790103
Snema04	F: TCCTCAGGTGCAAGGATGAC R: CGGTCTTCTGCATCACACTC	$(AATG)_6$	400-440	6	0.138	0.185	0.999	0.253	0.03	KP790108
Snema05	F: CAGGCTGAATGTGGAACCTG R: GAGGACTGATGGTGGAGGTC	$(CATT)_7$	400-440	11	0.388	0.678	0.001	0.428	0.05	KP790112
	Multiplex 2									

Sphae17	F: TTTAACTTTACACAGTCCCACAATCC R: GTTAAATGTGTGCACTCCGTATAAAC	(AATG)$_{12}$	100-140	10	0.715	0.799	0.674	0.105	0.03	KP790104
Sphae18	F: GAGTATTTCAGACACACAGGTTCTAC R: TCAAAGAACGACTCACTGATCAATAC	(AGAT)$_{13}$	130-240	24	0.876	0.925	0.283	0.055	0.05	KP790105
Sphae28	F: CCTAGTACTCATTTCTTCCTTGTGTC R: GTAACACTAGAGTAACACTGGACAAC	(AAAC)$_{13}$	240-320	16	0.734	0.839	0.013	0.125	0.03	KP790107
Snema03	F: GCTGCTCTGTCCCATTCAAC R: ATGATGTGTCCTGGCTTTGG	(ATTT)$_{6}$	280-320	6	0.259	0.298	0.708	0.131	0.05	KP790111
Snema08	F: GTCTGTTTGGAATGGGAGGAC R: AGATGACAAGGCGCATTTGG	(AAAT)$_{10}$	320-370	8	0.724	0.709	0.155	-0.022	0.05	KP790113
Snema09	F: CCCATTTCCCACAAACCCTG R: GACAGACGTTTCCTGTTGCC	(AAAC)$_{6}$	390-450	7	0.284	0.378	0.024	0.249	0.1	KP790114
	Multiplex 3									
Sphae08	F: TTGACATTAAAGACATGAGAGGACAC R: AATGTTTACTGATGAAATGCACAACC	(CTTT) $_{17}$	140-230	14	0.878	0.869	0.177	-0.010	0.05	KP790102
Sphae29	F: TGGGTAATCACTTCACATGTCAAATC R: TTGACTCTGTTGACTATCGATTTCTG	(AGAT)$_{12}$	120-240	26	0.525	0.899	0.001	0.416	0.05	KP790108
Snema11	F: AAAGACAAGTGGCGTGCG	(GCGT)$_{6}$	200-250	9	0.576	0.724	0.013	0.204	0.05	KP790115

	R: CCAAAGCGTTCTGGCCATAG									
Snema14	F: TCTGTGTGAAACTTGCGTGG R: TGCTCCAAACGCACTGATAC	$(AGAT)_9$	280-370	31	0.863	0.934	0.001	0.076	0.05	KP790116
Snema15	F: GAACTGCATGACACGTGAAAC R: ACTGCCGTCTGTATCCACTG	$(ATTT)_7$	330-370	11	0.591	0.748	0.331	0.061	0.05	KP790117
Snema16	F: ATTCTGGGTCACTGGCAATC R: AGTGGTGAGTGCATCTGAGG	$(AAAC)_6$	330-360	9	0.169	0.543	0.001	0.689	0.05	KP790118
	Multiplex 4									
Sphae06	F: CTCCAATTACTTCACACAAACTGATG R: AATTTATACAGTTTCCCAGTTTCGGG	$(AGAT)_{16}$	120-210	17	0.524	0.900	0.029	0.418	0.05	KP790100
Sphae20	F: GGTCCATTTCCTTTAGTTCTACTGTC R: GAAGGAAAGCATCAAATGACTTACAC	$(ATCC)_{14}$	190-280	18	0.895	0.876	0.424	-0.022	0.05	KP790106
Sphae31	F: AATGTTAAAGCAGCAGGATGTTAAAG R: ATAGGATATGGGAAAGGCGTAAATTC	$(AGAT)_{17}$	120-250	27	0.873	0.944	0.391	0.075	0.05	KP790109
Snema17	F: CCCTGGGAGAAATTTGGCTTAG R: AGTTAGGTGCAGCTACCAAATG	$(ACAT)_6$	230-260	3	0.268	0.253	0.999	-0.063	0.05	KP790104
Snema19	F: TCAACAGCAAATGGGAACGG R: GAGTGTGGACCAGAGTGAAC	$(ATGT)_9$	320-410	16	0.803	0.852	0.044	0.058	0.03	KP790120
Snema20	F: CAATAGCTGAAGGGTTGGCG R: AAGAGGGAAGTCTGGGCATC	$(ATCC)_8$	380-440	8	0.895	0.876	0.107	0.083	0.05	KP790121

Appendix E: Homing is not for everyone: displaced cardinalfish find a new place to live

in print *Journal of Fish Biology*

Abstract

It was tested whether the pajama cardinalfish, *Sphaeramia nematoptera* (f. Apogonidae) could home by displacing individuals up to 250m within and among isolated reefs. Contrary to expectations only 2 of 37 (5.4%) displaced *S. nematoptera* returned home and another 16 (43.2%) were found to have joined other social groups and did not home after 26 months of observations, while over the same period, 94% of control *S. nematoptera* remained associated with home corals, demonstrating strong site attachment. Hence, while this species has the potential to return home, being able to do so may not be as critical as previously assumed.

Introduction

Homing behaviour has been observed in a wide range of animal groups, including insects, fishes, reptiles, birds and mammals (Papi 1992). Individuals may return home from daily feeding migrations, seasonal migrations between feeding and breeding areas or return to natal sites once in a life-time (Williamson 1988; Papi 1992; Nørgaard *et al.* 2012). Much of the literature has focussed on the sensory mechanisms used to navigate back to home sites (Qiu 2004; Leis *et al.* 2011; Zeil 2012). The ability to home has several potential benefits associated with familiarity with the local environment, including increased foraging success and predator avoidance (Noda *et al.* 1994; Brown & Dreier 2002). Homing is often associated with strong mate fidelity, with mate familiarity an important factor in determining reproductive success (Bried *et al.* 2003; Naves *et al.* 2006). The potential to return home from great distances is often experimentally tested using displacement experiments (Luschi *et al.* 1996; Marnane 2000; Devine *et al.*

2012; Thyssen *et al.* 2014). However, not all individuals home and the fate of individuals that move elsewhere is not always known.

Many marine animals are well-known for their ability to home, whether this is associated with trans-oceanic migrations (Thorrold *et al.* 2001) or localized homing following diel migrations (Monteiro *et al.* 2005; Thyssen *et al.* 2014). Fishes find their way back to home sites using a variety of sensory mechanisms including olfactory cues (Cooper & Hasler 1974), visual cues and landmarks (Warburton 1990). Many coral reef fishes display strong site fidelity (Marnane 2000; Meyer & Holland 2000) and can home to particular shelter sites over a range of distances. For example, the small (max. 3.5cm total length (TL)) goby *Gobiodon histrio* (f. Gobiidae) (Valenciennes, 1837) exhibited a high homing success after being displaced distances of 2-4 meters (Wall & Herler 2008), while the grouper *Epinephelus tauvina* (f. Serranidae) (Forsskål, 1775) showed a mean homing rate of 67% when displaced 0.5km to 2.6km (Kaunda-Arara & Rose 2004). Homing in coral reef fishes is thought to relate to a strong association with particular habitats and shelter sites, and sometimes familiar mates, which may be in short supply (Wall & Herler 2008).

Coral reef cardinalfish (f. Apogonidae) are extremely site attached and to date have been universally shown to home. They migrate between their daily resting sites and nocturnal feeding sites, and are known to stay loyal to their familiar territory over long periods of time (Greenfield & Johnson 1990; Marnane & Bellwood 2002). Homing has been demonstrated using displacement experiments, where tagged individuals were moved fixed distances and observed returning to home sites. For example, 33% of the banggai cardinalfish, *Pterapogon kauderni* (Koumans, 1933), returned home within 24h after 50m translocation (Kolm *et al.* 2005). In another study *Cheilodipterus quinquelineatus* (Cuvier,1828) returned from 1km (56-81%) and many even from 2km distance (33-63%) (Marnane 2000). While some cardinalfish are clearly very good at homing, what happens to those individuals that do not return home is often unknown.

The aim of this study was to test the homing ability of pajama cardinalfish, *Sphaeramia nematoptera* (Bleeker, 1825), and test whether homing ability was affected by displacement distance or whether they were displaced on the same reef or to a different reef. It was further investigated whether individuals take a specific direction after displacement, whether they, for example, move homewards even if they do not return to their home site. As individuals of this species form small social groups on discrete coral heads, it was hypothesized that displaced *S. nematoptera* would return to their home site within a few days of displacement regardless of displacement distance and reef, and that control *S. nematoptera* (which were not displaced) would continue to return each night to the same home site.

Methods

S. nematoptera lives in small groups of 10-30 individuals in branching coral colonies, it shows great site fidelity and most adults (76%, N_{total}=103) form long lasting pairs (Chapter 2). In order to test its' homing ability, a displacement experiment was conducted in March 2013 - May 2015 on the inshore reefs near Mahonia Na Dari Conservation and Research Centre in Kimbe Bay, Papua New Guinea (5°30'S, 150°05'E). A total of 37 adult *S. nematoptera* from six groups in branching *Porites cylindrica* were caught using diluted clove oil (Munday & Wilson 1997), uniquely tagged with VIE tags (Okuda 1999a), and displaced into a similar sized *P. cylindrica*, void of conspecifics, either on their home reef ("within reef") or a neighbouring reef ("between reefs") (Figure E-1). As a control, a total of 33 *S. nematoptera* were caught, their VIE tags were identified, and they were released back onto their respective home site. Two groups were displaced 80-90m (within reef, $N_{treatment}$=4, $N_{control}$=2; between reefs, $N_{treatment}$=14, $N_{control}$=13), two groups 120-150m (within reef, $N_{treatment}$=3, $N_{control}$=2; between reefs, $N_{treatment}$=7, $N_{control}$=6), and two groups 220-250m (within reef, $N_{treatment}$=5, $N_{control}$=7; between reefs, $N_{treatment}$=4, $N_{control}$=3). The transport to the displacement site was done via boat and *S. nematoptera* were left in mesh holding cages for acclimation for 20 minutes before release. Release and home sites were visually surveyed every day for 10 days for approximately 20 minutes, recording all tagged individuals. Surveys of the 6 home sites as well as other groups of *S. nematoptera* on the same inshore reefs continued every six months for up to 26 months. The number of *S. nematoptera* relocated was compared between each of the 6 home sites using Fisher's exact test. Distances were calculated using GPS points and compared using Student's t-test (Winter 2013). Uniformity of directions taken after release, based on the position *S. nematoptera* were found in relative to their home site and displacement site, and dispersion between vectors were compared between groups using Rao's Spacing Test (Rao 1972), implemented in the *circular* -package (Agostinelly & Lund 2013). All analyses were done using R statistical program version 3.0.0. (R Core Team 2015).

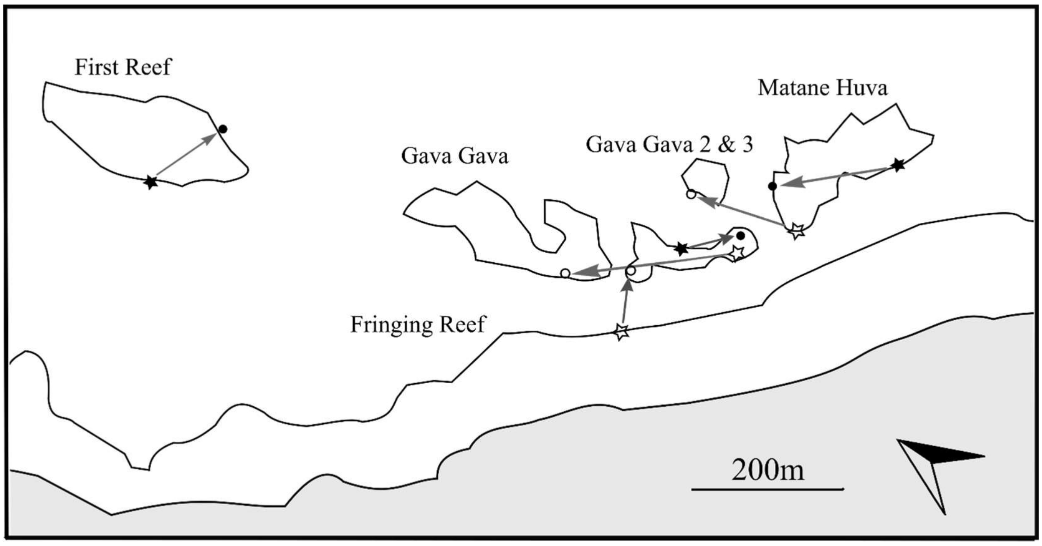

Figure E-1 Overview of inshore reefs near Mahonia Na Dari, Kimbe Bay. Indicated are home sites (stars) of 37 displaced *S. nematoptera* and the corresponding sites they were released in (circles) after displacement. Empty symbols indicate displacement between two different reefs, full symbols indicate displacement within the same reef.

Results & Discussion

Contrary to expectations, most *S. nematoptera* did not return to their home site, either in the short –term or after 26 months of observation, regardless of displacement reef and distance. Thus this experiment shows a much lower propensity to home in *S. nematoptera* than seen for similar displacement experiments for other cardinalfish (Marnane 2000; Munday & Dixson 2009; Devine *et al.* 2012). Only two individuals returned to home sites, and did so within two days after release. These two *S.*

nematoptera were part of the shortest within reef displacement. Most control *S. nematoptera* (N=31, 93.9%) were still found on their home site 10 days after the experiment, demonstrating limited impact of experimental procedures. Many control *S. nematoptera* continued to stay at their home site for 6 to 12 months and thus showed great site fidelity (supporting information E-3 a).

Over the course of the 26 months after the experiment, 16 of the displaced *S. nematoptera* were found living in different social groups other than their home site (supporting information E-3 b). All 16 *S. nematoptera* were found in groups of varying sizes of conspecifics in branching *P. cylindrica*. 10 *S. nematoptera* were found on a different reef than the one they were released on. No difference was found in number of *S. nematoptera* rediscovered or homed, whether they were released on the same or a different reef (Fisher's exact test, p>0.05) and no significant difference according to displacement distance (Fisher's exact test, p>0.05). Although *S. nematoptera* have the ability to home during their night time foraging excursions, and control *S. nematoptera* continued to be found at home sites, homing may not be critical as they clearly have the ability to join other groups.

The direction the 18 displaced *S. nematoptera* took in this experiment varied and, in all but five *S. nematoptera*, showed no indication of moving towards their home site or a specific direction relative to North (Figure E-2 a, b). Directions taken relative to their home site did not differ significantly between treatment groups (Rho's test statistic=0.199, d.f.=3, p>0.05). Similarly, there was no difference in direction (Rho's test statistic=1.854, d.f.=3, p>0.05) relative to North, between individuals from different treatment groups (Figure E-2 b). It has been suggested, e.g. for parrotfish, that reef fish use a sun compass to home (Winn *et al.* 1964), but for the pajama cardinalfish this does not seem to be the case, since the results showed no indication of them swimming homewards.

The displaced *S. nematoptera* moved a considerable distance from the release site before joining a social group for longer periods of time, moving an average (±SE) of 240.88m (±61.81) from their displacement site. This distance was different between the three distance based experimental groups, and found to be significantly different between the 80m and 250m group (Paired t-test, t = -2.43, df = 6.96, $p<0.05$). Individuals displaced 80m between reefs moved more than twice as far (265.00 ±49.72m) as individuals from the group displaced 250m between reefs (118.33±34.19m) (Figure E-2 b). The two individuals found from the group displaced 120m within the same reef had travelled the longest distance of any *S. nematoptera* (890m and 800m), whereas the four *S. nematoptera* displaced 90m within the same reef travelled the shortest distance (65±31.75m), with two of them reaching their home site within two days. This difference is despite the fact that all *S. nematoptera* had to pass closely to a number of groups of conspecifics to reach their final settling site in the context of observations and more than half of *S. nematoptera* crossed to a different reef.

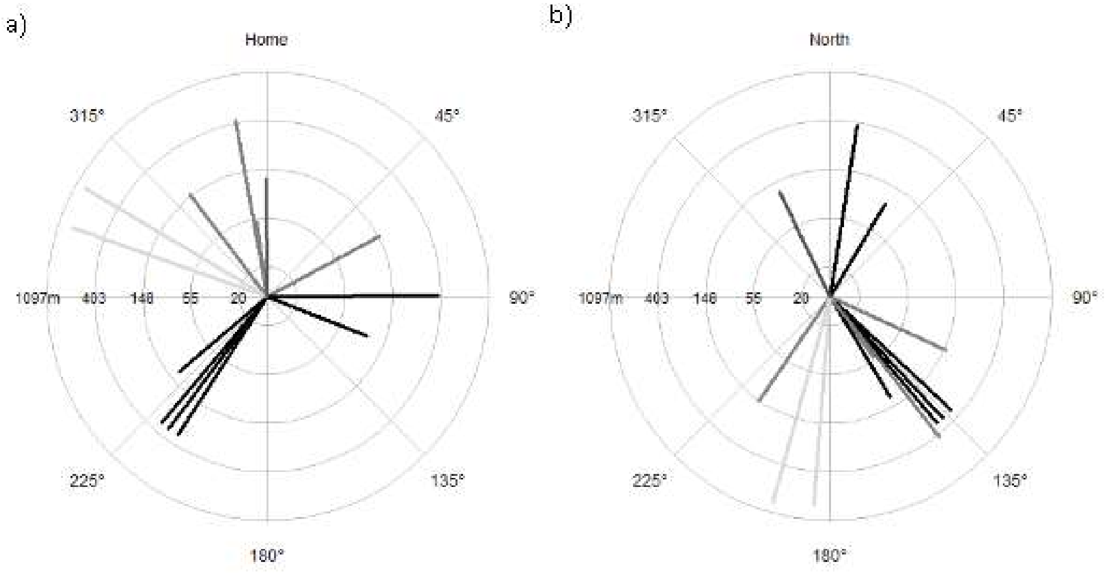

Figure E-2 Direction of movement and distance travelled by displaced fish after release. **a)** "Home" is set as North and the direction between release site and site individuals were rediscovered at is plotted as degrees from "Home". Vector length indicates distance travelled (in meter, log scale). **b)** Direction is indicated as degrees relative to true North. Length of vector represents distance moved from the release site (in meter, log scale). Treatment groups are represented as black (displaced 80m, between reefs), dark grey (250m, between reefs), medium grey (90m, within reef) and light grey (120m, within reef).

There may be a lower tendency to home in *S. nematoptera* if habitat and mates are not in short supply. While most *S. nematoptera* adults are found in pairs (75%, N_{total}=102), there are solitary individuals in most groups (Chapter 2). These "singles" may present a valuable choice as new partners, allowing displaced individuals to join a new social group without consequences to their reproductive output which otherwise would drive stronger homing behaviour. Also, the preferred coral species of many cardinalfish species in Kimbe bay, *Porites cylindrica*, is relatively common (Gardiner & Jones 2005). In contrast, for other habitat dependent reef fishes which home, e.g. *Gobiodon histrio* (Wall & Herler 2008), preferred coral hosts are often in short supply. Hence good habitat and mate availability might limit motivation to home for this species, although further experiments are necessary to investigate these underlying mechanism.

While *S. nematoptera* in the experiment showed the capacity to swim long distances, locate groups of conspecifics and stay alive in presumably unfamiliar surroundings, most of them did not find their way back to their home sites. In most other displacement experiments animals that do not return home are not found again and assumed lost or deceased. An alternative hypothesis, based on the results, might be that such individuals do not have the ability or motivation to home after displacement and simply choose a random direction, or possibly a specific direction connected to their daily habits, find a suitable social group or site and go on to lead a typical adult life.

In conclusion, the results indicate that not all cardinalfish home when displaced and that failure to home does not necessarily lead to any negative consequences. In *S. nematoptera*, individuals appeared to move in random directions, across variable distances, and were able to join other social groups. It can be hypothesized that if they cannot immediately locate their home site after translocation, they look for other groups of conspecifics, for as long as it takes, to find a suitable single individual with whom they can mate. For these individuals, there does not appear to be any long-term cost of being displaced from their former home.

Supporting Information

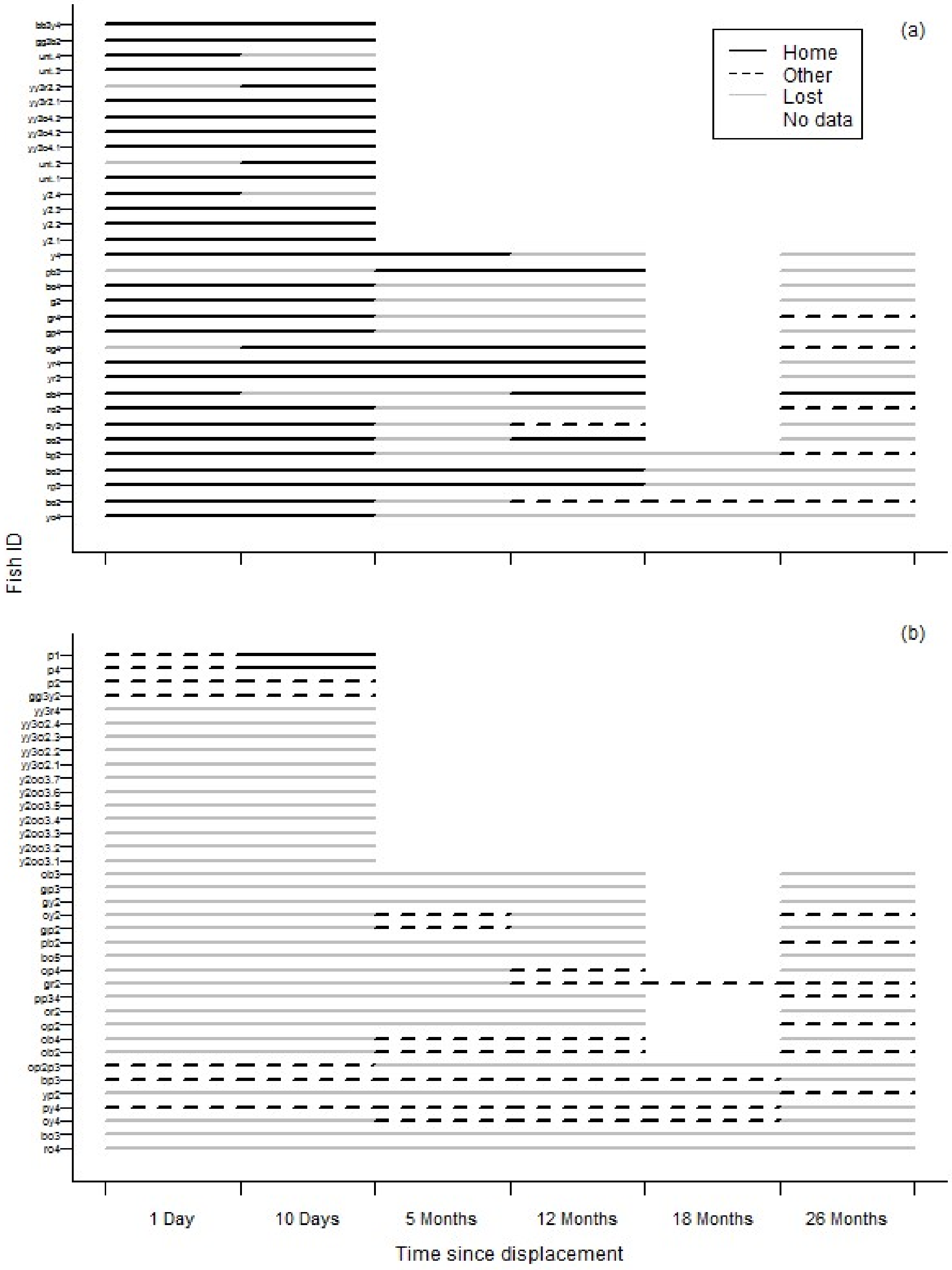

Figure E-3 Position of *Sphaeramia nematoptera* after displacement experiment in Kimbe Bay, Papua New Guinea. Solid black line indicates individuals at home sites, dashed line indicates individuals identified at another site, grey line indicates individuals lost, i.e. could not be found, no line indicates no data is available for the individual. a) Control group (N=33). b) Displaced S. nematoptera (N=37).

THE END