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Extra-pair mating in a socially monogamous and paternal mouthbrooding cardinalfish

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Abstract

Many vertebrates form monogamous pairs to mate and care for their offspring. However, genetic tools have increasingly shown that many offspring arise from matings outside of the monogamous pair bond. Social monogamy is relatively common in coral reef fishes, but there have been relatively few studies that have confirmed monogamy or extra-pair reproduction, either for males or females. Here long-term observations and genetic tools were applied to examine the parentage of embryos in a paternally mouthbrooding cardinalfish, *Sphaeramia nematoptera*. Paternal care in fishes, such as

mouth brooding, is thought to be associated with a high degree of confidence in paternity. Two-years
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of observations confirmed that *S. nematoptera* form long-term pair bonds within larger groups.

However, genetic parentage revealed extra-pair mating by both sexes. Of 105 broods analysed from 64 males, 30.1% were mothered by a female that was not the partner and 11.5% of broods included eggs from two females. Despite the high paternal investment associated with mouthbrooding, 7.6% of broods were fertilised by two males. Extra-pair matings appeared to be opportunistic encounters with individuals from outside the immediate group. We argue that while pair formation contributes to group cohesion, both males and females can maximise lifetime reproductive success by taking advantage of extra-pair mating opportunities.

Keywords: Monogamy, extra-pair mating, parental care, Apogonidae

Introduction

Animals have adopted a great variety of mating systems, ranging from monogamy, in which males and females form pair bonds, to varying degrees of promiscuity, in which males and females engage with multiple partners (Breder & Rosen 1966; Emlen & Oring 1977; Smith 1984). It has been theorised that males have a greater propensity to mate with multiple partners due to the lower cost of gamete production and as a result, polygyny, in which only males have multiple partners, is more widespread than polyandry, where females mate with several males (Emlen & Oring 1977).

Monogamy is defined herein as heterosexual pairs exclusively mating with one another for at least one breeding cycle (Wilson 1975; Wittenberger & Tilson 1980). It is thought to arise when 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). However, in recent years the extent to which presumed monogamous species truly are monogamous has been questioned.

Most species of birds, mammals and fish considered to be monogamous were classified as such based on 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. Such species are best described as “socially monogamous” (*sensu* Whiteman & Côté 2003). In socially monogamous species, males and females associate with one another for prolonged periods of time, encompassing multiple mating seasons and non-reproductive periods (Wickler & Seibt 1983; Whiteman & Côté 2003). However, genetic parentage studies have now shown that the assumption of genetic monogamy based on prolonged association is often unfounded. Even in species with long-term pair bonds, extra-pair reproduction is more common than previously thought (Westneat 1987; Moller & Birkhead 1994; Sillero-Zubiri *et al.* 1996; Hughes 1998) and exclusively mating with one partner seems to be the exception rather than the rule. This raises the question as to how pair bonds are maintained in face of high levels of extra-pair mating. Despite the increasing evidence for extra-pair mating in socially monogamous species, 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 is relatively widespread in coral reef fishes compared to other marine fishes (Whiteman & Côté 2004), occurring in 14 of the most common coral reef fish families, most commonly small species closely associated with coral habitat (Barlow 1984, 1986; Thresher 1984). Common examples include anemonefishes *Amphiprion* spp. (Fricke & Fricke 1977; Buston 2003); and some species of coral crouchers *Caracanthus* spp. (Wong *et al.* 2005); hawkfishes *Cirrhites* spp. (Donaldson 1989); damselfishes *Dascyllus* spp. (Fricke 1980); coral gobies *Gobiodon* spp. (Cole & Hoese 2001; Thompson *et al.* 2007); *Paragobiodon* spp. (Lassig 1976; Kuwamura *et al.* 1993; Wong *et al.* 2008); butterflyfishes *Chaetodon* spp. (Fricke 1986; Hourigan 1989; Reavis & Copus 2011); and filefishes *Oxymonacanthus longirostris* (Barlow 1987). In many of these systems, mating appears to be monogamous and no extra-pair matings have been observed when males and females formed lasting pairs. However, molecular analysis of broods of socially monogamous coral reef fish species to confirm genetic monogamy or reveal extra-pair mating remain rare. Extra-pair mating describes a situation where a male or a female produces offspring outside the mated pair. In *Acanthochromis*

polyacanthus, a species without a larval phase and one of only a few reef fish species known to have biparental care of hatched young, one brood out of 30 was concluded to show evidence of extra-pair mating based on the number of alleles found (Miller-Sims *et al.* 2008). Such mixed broods may be the result of extra-pair mating activity by either sex. Distinguishing between social monogamy and genetic monogamy requires detailed genetic studies that measure the prevalence of pair and extra-pair matings.

The factors affecting opportunities for extra-pair mating in monogamous fishes are likely to depend on the mode of parental care. Monogamous fishes often exhibit paternal care, either by guarding developing embryos on the substrate, mouthbrooding, or by carrying the embryos on the body as in pipefishes and seahorses (Perrone & Zaret 1979). In species with paternal broodcare, the energetic expenditure may limit the males opportunity to sequester multiple mates, and increases the benefits of acquiring just one mate which is of high quality (Barlow 1984; Whiteman & Côte 2004).

Paternal care is thought to relate to confidence in paternity in externally-fertilising fishes, where there is a high probability that males are genetically related to the offspring they care for (Blumer 1979; Perrone & Zaret 1979; Gross & Sargent 1985). However, this would only apply if there were a low probability that other males had fertilised the eggs another male is caring for (Smith 1979; Jones & Avise 1997b). When males have all the parental care duties, opportunities for extra-pair fertilizations may be limited, with females having a greater propensity to seek extra-pair copulations, especially when they can produce more eggs than a single male can care for (Clutton-Brock 2009). Multiple paternity has been observed in fish with a range of mating systems including the cichlids *Pseudotropheus zebra* (Parker & Kornfield 1996), *Protomelas spilopterus* (Kellogg *et al.* 1998), and *Variabilichromis moorii* (Sefc *et al.* 2008), and the 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.

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Paternally mouth brooding cardinalfish exhibit a variety of mating systems, from social monogamy with lasting pairs, which stay together for entire breeding seasons or longer (*Apogon notatus* (Kuwamura 1983, 1985), *Pterapogon kauderni* (Vagelli & Volpedo 2004), *Ostorhinchus cyanosoma* (Rueger *et al.* 2014)); to promiscuity with transient pairs, which split up shortly after spawning (*Apogon niger* (Kuwamura 1985), *Ostorhinchus doederleini* (Kuwamura 1985; Okuda 2000), *Cheilodipterus quinquelineatus* (Kuwamura 1987), *Apogon imberbis* (Klein & Raventos 2007)). For the most part, these mating systems have been established by direct observations of reproductive behaviour. There have been no genetic studies on socially monogamous species and extra-pair mating has not been described in cardinalfish. Applying genetic studies to paternal mouthbrooders provides a useful test of whether a high level of paternal care is associated with the degree of confidence in paternity. There is the potential for extra-pair mating by females, since females can produce eggs faster than males can brood them (Okuda 1999; Okuda *et al.* 2003; Takeyama *et al.* 2007). On the other hand, extra-pair mating by males could be expected because of the possibility to cannibalise broods and increase their physical condition (Okuda 1999; Takeyama *et al.* 2007). Genetic studies can reveal the extent to which both sexes are involved in extra-pair reproduction.

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 cardinalfish, *Sphaeramia nematoptera*. Since male and female *S. nematoptera* have been found to form prolonged, size assortative pairs (Rueger *et al.* 2016b), the species would be assumed to be monogamous based on behavioural data alone. However, social monogamy does not ensure the absence of extra-pair matings. The small group sizes and paternal mouthbrooding in *S. nematoptera* provided the opportunity to sample clutches of developing embryos and determine the presence of either multiple maternity or paternity from genetic parentage analyses. We predicted that the majority of offspring would be derived from males and females in pairs, as this would be consistent with the occurrence of social monogamy in this species (Rueger *et al.* 2016b) and would support the hypothesis that mouthbrooding is associated with a high level of confidence in paternity.

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 1). Here, *Sphaeramia nematoptera* is common where it lives in small groups, within which most adults form male and female pairs that remain very close to each other, and have high site fidelity, during diurnal resting periods (Rueger *et al.* 2016a; b).

Pair formation and mating behaviour

We observed groups of tagged cardinalfish for two years to identify potential pair bonds within the groups and contrasted our observations with genetic parentage analyses to identify the true mother and father from embryos carried by brooding males. All fish were physically marked using Visible Implant Elastomer (VIE) tags (Northwest Marine Technology) with unique combinations from six colours and five positions on the fish. VIE has been shown to last for at least several months, often years, and does not impact cardinalfish behaviour nor affect susceptibility to predation (Marnane 2000). All fish tags in this study remained clearly visible over the two- year period. The standard length of all fish was measured to the nearest millimetre and caudal fin clips taken for genetic analyses. During each of the five observational periods all individuals were located every two to three days via visual census, and information recorded on their most likely mate. Overall, 500 hours of observational data and social pair formation was collected over 24 months.

Observational studies were conducted October - November 2012, February - March 2013, July - August 2013, March-April 2014 and in September 2014. Fish were caught, using diluted clove oil (Munday & Wilson 1997) and hand nets, from 22 groups of fish located across five different reefs (N = 544 individuals). A group is defined as an aggregation of fish on a distinct patch of habitat, in this case all colonies of *Porites cylindrica*. Groups varied in size from 4 to 32 individuals (Figure 1). All individuals within each of the 22 groups were tagged and DNA tissue samples taken.

Pairs were determined when two fish were consistently observed in close proximity ($\leq 20\text{cm}$) to one another over one or more breeding cycles. Pairs typically rest within the same coral branches and have high site fidelity. A breeding cycle last for approximately 14 days, during which pairs would be observed on 5-7 separate occasions. Courtship behaviour was only observed between partners thus defined and all pairs were found to be heterosexual. Sex was determined by observing the distended buccal cavity during brooding (male) and bulging abdomen shortly before brooding (female). Sex change is not known to occur in cardinalfish (Kume *et al.* 2000a; Pisingan & Takemura 2007; Choi *et al.* 2012). Our previous work has established that the species appears to be socially monogamous as pairs are typically together for at least one month, 50% of pairs were found together after 4 months, which represents multiple brooding cycles, and the females remain with the males during brooding (Rueger *et al.* 2018).

Pairs were monitored regularly to observe courtship, mating and males taking up broods. The location and any signs of breeding behaviour from un-paired tagged individuals were also monitored. Tagged juveniles were observed throughout the observational periods. If juveniles matured and formed breeding pairs, they were included in the breeding observation and sampling. All observed groups on the five reefs were sampled thoroughly, although the rare appearance of new, untagged, adult *S. nematoptera* during the 24- month study period might suggest there was movement into the area from unsampled aggregations elsewhere. When new fish appeared in this manner, they were caught and tagged and their location and incidences of pairing were henceforth observed across time. Membership of groups was relatively stable. There were only a few recorded movements of fish between groups. Out of over 544 fish, 25 were found to switch groups permanently and a total of 91 movements between groups were recorded within the 24- month period.

DNA sampling and genetic analyses

We undertook genetic parentage analyses to identify the true mother and father from embryos carried by brooding males. From these analyses, we measure the frequency of extra-pair mating by males and females within or among different groups. Within the study periods, all broods found in observed males were collected ($n = 64$ brooding males). To do this, brooding males were caught with hand nets and diluted clove oil. Males would usually expel the brood, either immediately or within a few minutes. 105 clutches with approximately 6000-10000 embryos in total were collected from the 64 males. From each of these clutches, 5-20 embryos were subsampled. In total 1056 embryos were assayed. To consider the possibility of multiple mothers in each clutch, embryos were sampled by extracting the eggs 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 five eggs would be sufficient to include at least one egg from each mother. A sample of 10 eggs would be 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 Rueger *et al.* (2015).

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). A range of 3 to 34 alleles was observed per locus, with a mean of 15.1 ± 1.8 SE, in this population of *S. nematoptera* (Supplemental Material Table 1).

The mean observed heterozygosity was 0.666 ± 0.060 SE and mean expected heterozygosity was 0.733 ± 0.058 SE. Significant deviation from Hardy-Weinberg equilibrium was found in four loci.

Four markers that showed high genotyping error ($\geq 6\%$) were excluded from the analyses. The remaining 19 loci had an average genotyping error of $2.2\% \pm 0.4$ SE.

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) to measure 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 of simulated offspring being assigned to the correct parent pair (99.9%; type-I error 0.1%, type-II error 0%). Multiple matings were defined as clutches of embryos that included more than one genetic father or mother. Extra-pair males were defined as any male that sired offspring held in another males' buccal cavity.

Pair and extra-pair matings within and among groups

We determined whether extra-pair male parents were part of the same group as the breeding pair or whether they came from a different group. In cases where the extra-pair individual's identity was not known, because they were not one of the fish sampled in the study, we deemed them to be migrants from outside of the study area. Sampling of resident individuals within the study area was complete, as far as we could tell, during the two- year study period. To check for any locational biases in mating behaviour, the instances of extra-pair mating were compared between groups on the same or different reefs using Fisher's exact test.

Results

Social monogamy

Each of the 105 clutches represents one successful mating event, with each male observed brooding one to three times over the two- year study period, although other mating events are likely to have taken place between observational periods.

Behavioural data on social pairs was available for four weeks prior to brooding in 70 of the 105 mating events. In 94.3% (66 of 70) of cases where behavioural data was available, the brooding males could be identified as paired with a social partner. In the other four cases males were observed, but no social partner could be identified. In these cases, partners could not be identified due to frequent changes in position of the male in the group or due to lack of consistent proximity to any females.

Overall, 20 pairs were observed to produce multiple clutches during the study.

Mating observations

Mating displays were only observed between pairs and occurred throughout the day, although most active courting happened starting at dusk and throughout nightfall. Mating takes place within coral branches. Both males and females perform s- bends and males/females display by presenting their side and twitching pectoral fins. Males and females line up side by side and the male flicks his caudal fin. The female drops a cohesive, neutrally buoyant clutch, during which the male quivers. The male then moves back and scoops the clutch up in his buccal cavity within seconds. During nightfall, *S. nematoptera* typically begin to move out of the coral colony to migrate to their nightly feeding sites, similar to what has been observed in other species of coral reef dwelling cardinalfish (Marnane 2000).

When a mating event is occurring however, conspecifics as well as other cardinalfish species were observed to stay close to the mating pair, with both mating partners spending considerable time displaying aggressive behaviours towards them. During brooding, a single clutch fills the male's buccal cavity completely (Kume *et al.* 2000). Brooding in *S. nematoptera* lasts for 8 days and happens

year-round. Males have been observed to brood two clutches in one month, however, brooding a maximum of one clutch per month was more common in the study population.

Pair and extra-pair mating

Using genetic parentage analysis on brooding males, embryos and putative mothers, we identified the parents of 105 clutches of eggs. We found that the brooding male was the sole father in 97 clutches (92.4%), but 8 clutches (7.6%) contained some eggs that had been fathered by a male other than the brooding male (Table 1). In all cases, sires were excluded on the basis of mismatches at multiple loci. On average, non-brooding males sired 18.4% ($\pm 2.1\%$ SE) of the analysed embryos in these clutches (Supplemental Material Table 2). Four of the eight non-partner males could be identified as part of the sampled population, of which two 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 the paired male and female (Table 1). In the other 20 clutches, males were carrying eggs mothered by different females, demonstrating evidence of extra-pair mating by male cardinalfishes. Seven of these (10.6%) were mothered by two different females. In each of these seven cases, the proportion of embryos belonging to the extra-pair female ranged from 10-80% of sampled embryos with a mean of 32.1% ($\pm 7.3\%$) of the clutch. One of these clutches had clear morphological differences between embryos, in which yolks were of different colour corresponding to the different mothers, indicating that this clutch was in fact merged from two clutches and the result of separate matings on different days. In the remaining 13 clutches (19.7%) with extra-pair matings by the male, 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.

Extra-pair mating by females was also found. Seven of the extra-pair females could be identified to be part of the sampled population. Of these, 2 females, which produced 2 clutches, were in a social pair during the sampling period (Table 1).

Pair and extra-pair matings within and among groups

In most cases extra-pair partners were from a different group than the breeding pair. Of the 8 extra-pair males and 20 extra-pair females, only nine (32%) were from the same group. Two were from distant groups with one male from a group 80m away and a female from a group 330m away. The remaining 17 extra-pair partners did not belong to any sampled group (13 females, 4 males). It is possible, however unlikely, that individual parentage could not be matched to known groups members due to genotyping error. It is unlikely that fish escaped from observation as sampling of known groups inhabiting the small area was thorough. There were no locational differences in the number of extra-pair matings between the five sampled reefs (Fisher's exact, $p=0.547$) nor between groups (Fisher's exact, $p=0.579$).

Discussion

The combination of observations of social pair formation and the genetic parentage analysis of embryos provided unique insight into the reproductive strategies in a mouth-brooding cardinalfish. These 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 (70%) were from mated pairs. However, 9% of brooding males were shown to take multiple clutches from separate females within their immediate group and 21% also mated with females from other groups. We also found cases where males carried eggs that were fertilised by other males. These observations contradict the long held assumptions that monogamy is a pre-requisite in the evolution of parental care systems that have a high cost to either parent (Clutton-Brock 1991; McCoy *et al.* 2001, Sheldon 2002, Kokko and Jennions 2008). These findings provide one of the first cases of concrete evidence of extra-pair mating in a monogamous coral reef fish and confirm that mating systems may be much more complex than expected based solely on behavioural observations.

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 pairing behaviour can be deceiving and complex mating strategies, including extra-pair copulations, are often associated with social monogamy. In this study, 30% of broods, cared for by the paternal mouthbrooding fish, included either multiple females or a female other than the seemingly established partner. In comparison, the average proportion of extra-pair paternity in birds is 15%, though rates of up to 30% also occur (Griffith *et al.* 2002). In mammals, the equivalent extra-group paternity exceeds 20% in almost half of the species studied (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 100% of broods (Sefc *et al.* 2008).

Our study provides evidence of an unusual female mating strategy, during which females give eggs to males that are already carrying eggs from another female. Female cardinalfish can potentially produce eggs quicker than the males can brood them (Okuda 1999). This gives females the opportunity to be in a pair, but also maximise their reproductive output by performing extra-pair matings. It is possible that some small females lay small clutches (Kolm 2001, 2002; Kume *et al.* 2002), so males might have the potential to take more eggs from other females. However, since the buccal cavity limits the number of embryos being carried (Okuda 2001), it is possible that the male cannibalises the socially paired female's eggs, at least partially, to take up additional broods. Filial cannibalism has been described in several species of cardinalfish (Okuda 1999, 2001; Kume *et al.* 2000b), and may allow males to maintain condition during the mouthbrooding period when feeding is normally restricted (Takeyama *et al.* 2002, 2007). Multiple maternity has rarely been described, but is known for the pipefish *Syngnathus floridae* (Jones & Avise 1997a) where females do not remain associated with the brooding male (Vincent *et al.* 2011).

Here we provide one of the first example of embryos fertilised by another male in the clutch of a brooding father. Clearly, oral brooding by males does not equate with complete confidence in paternity. The idea that high investment, male paternal care is associated with the assurance of sole paternity does seem to apply to pipefish and seahorses (Syngnathidae), in which males brood embryos in external or internal brood pouches. In all 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.* 1999, 2000; 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; DeWoody *et al.* 2000a; Neff 2004), darters (DeWoody *et al.* 2000b), sand gobies (Jones *et al.* 2001) and sticklebacks (Jones *et al.* 1998). 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 multiple paternity may explain why the paternal care system persists. In only 7.6% of broods two sires were found, and even then, only 10-30% of embryos were fathered by the extra-pair male.

Extra-pair mating with individuals from a different group may help maintain social stability and heterozygosity in *S. nematoptera*. Relatively few instances of extra-pair mating occurred within the same group that the mated pair occupied (10%). In most instances, we could not identify the extra-pair males and females despite the extensive sampling effort, which suggests they belonged to unsampled groups. 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: a study on the blue tit, *Cyanistes caeruleus*, found that 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 group may outweigh the possibility of cooperation and thus lead to higher rates of extra-pair mating behaviour among non-neighbours. An important gap in our knowledge that remains, is how mating

pairs behave at night and during the migration to the feeding sites, which might represent an opportunity for extra-pair mating outside the group to occur.

Parental investment seems to play a major role in shaping the reproductive system of *S. nematoptera*.

The high investment of the male and the potential equal or higher reproductive output of females are driven by male mouthbrooding and lead to a complex mating system. Mutual parental care has been proposed as 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 than males (Okuda 2001).

However, the male can offset the females' advantage by cannibalising partial or whole broods, if mature females are available (Kume *et al.* 2000; Takeyama *et al.* 2007). Here, paternal care is such a high investment it seems to lead to a seemingly monogamous system with male and female extra-pair-mating. Polygynandry, where both males and females acquire multiple mating partners, has been found in populations of the pipefishes *S. typhle* and *S. floridae* (Jones & Avise 1997a; Jones *et al.* 1999). In these systems however, multiple paternity has not been described.

The many advantages gained by extra-pair mating and the occurrence of both male and female extra-pair mating shown in this study raises 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 (Rueger *et al.* 2016b). The females stay with the males during the day while brooding lasts (pers. obs.), which may indicate a role of the females in protecting the male from potential egg thieves, such as other cardinalfish or other predators. Another reason to stay in a pair is to ensure a reproductive opportunity at every breeding cycle and to prevent a high-quality partner from taking advantage of extra-pair mating opportunities. In the monogamous goby, *Valenciennea strigata*, abundant resources and plentiful mating opportunities for males create an advantage for mate guarding by 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 (Manica 2002; Takeyama *et al.* 2007).

Furthermore, long lasting pair bonds may benefit reproductive output, as known 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, we show that social monogamy in a brood-caring reef fish does not guarantee exclusive mating, similar to what has been observed in birds and other animals. We found evidence that both males and females participate in extra-pair matings. Females clearly have the potential to increase their reproductive output with extra-pair matings. Males which invest highly into offspring care seem to bear low levels of other males fertilizing their clutches and offset the females' advantage performing extra-pair matings of their own. Molecular as well as long term behavioural studies are necessary to understand the often- complex mating systems coral reef fish.

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Data accessibility

The data associated with this study is available at Data Dryad, doi:10.5061/dryad.557br15.

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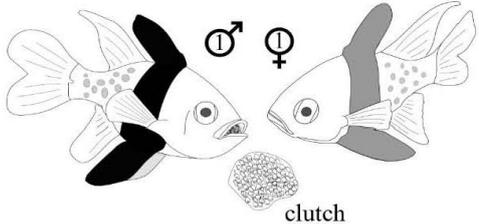
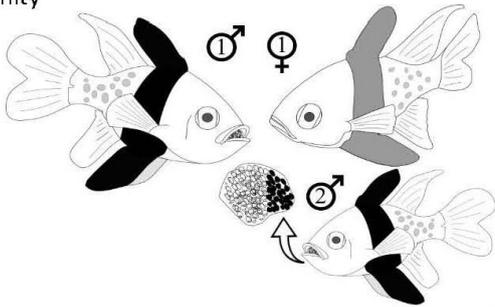
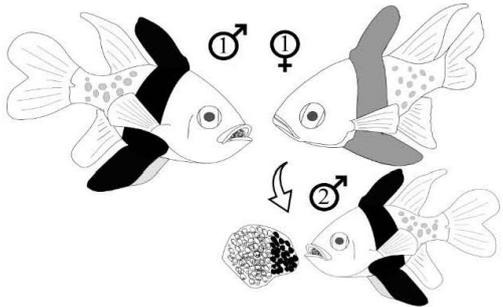
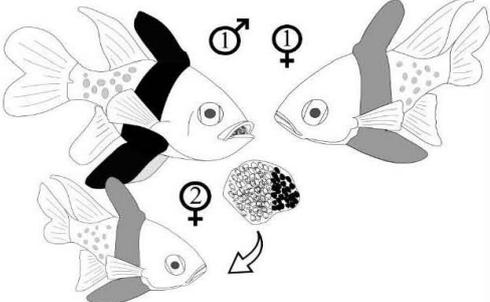
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Table 1. Summary of mating patterns found in *Sphaeramia nematoptera*. 105 clutches were collected and a mean (\pm SE) of 10.5 (\pm 0.28) embryos were assayed per clutch. For 66 clutches sufficient behavioural data was available in the given breeding cycle and pairs of one male and one female were identified. Monogamy: the same two individuals which were found to be a pair by behavioural observations at the time of clutch collection, are the sole two genetic parents of the clutch. Multiple paternity: Two males are found to be genetic sires of one clutch. Extrapair mating: The two individuals which were found to be a pair by behavioural observations at the time of clutch collection are not the sole two genetic parents. Female extrapair mating: A female is the genetic mother of a clutch collected from a male, while at the same time she is identified to be behaviourally paired with a different male. Male extrapair mating: A female other than the behavioural partner is found to be the genetic mother of the whole clutch or part of the clutch.

Mating pattern	% of clutches	# clutches
Monogamy 	69.7	46/66
Multiple Paternity 	7.6	8/105
Extrapair mating	33.3	22/66
Female 	3.03	2/66
Male 	30.3	20/66

