Sexual selection and reproductive trade-offs in caring parents

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Abstract

Successful reproduction depends on individuals both obtaining mates and other reproductive resources, and producing offspring that are capable of surviving to maturity. However, for each of these investments they must draw on a finite pool of resources leading to trade-offs that can influence selection on both competitive and parental investment. As such, species are often characterised by one sex which focuses investment on reproduction and parental care and one which invests predominantly in intrasexual competition. However, there are important cases where males or females are selected to invest heavily in both reproduction and competition. In teleost fish, male-only parental care is the predominant parental strategy, but males typically also compete more intensely for mates than females. In polygynous species, females usually provide the majority of the parental care and invest in reproduction through eggs or foetal nourishment. However, female-female competition for resources that will improve reproduction can also be an important selective force in females and drive the evolution of competitive traits. How do the constraints imposed by a trade-off between reproduction and competition influence selection in these two cases? In this thesis I use a combination of experimental, observational and meta-analytical methods to investigate male-only parental care and female-female competition in fish. By analysing the costs and benefits of paternal care across species I show that care may be sexually selected in males, as females prefer males already guarding eggs, and that this preference in females may improve offspring survival. Using laboratory experiments and field observations on the harem-breeding cichlid, *Lamprologus ocellatus*, I find support for a trade-off between competition and reproduction in females and show that competition for male contributions to care may influence the outcomes of this trade-off. This work demonstrates that in some cases, relaxation of the trade-off between parental investment and intrasexual competition may select for parents that are both competitive and caring, but in other cases, this trade-off may still impose constraints on the evolution or expression of competitive or parental traits.

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Table of Contents

Chapter 1

General Introduction

For individuals to be successful at passing their genes directly to the next generation they must invest in traits that will assist them in producing viable offspring. Investing in competitive traits allows individuals to secure mates and other reproductive resources in the face of competition from same-sex rivals, while parental investment can improve the quality and survival of offspring (Andersson 1994; Smiseth et al. 2012). Since time and resources are limited, individuals are expected to face a trade-off between competitive and parental investment (Trivers 1972; Clutton-Brock 1991). In many species, this trade-off can explain divergent sex roles where one sex provides care and the other sex invests in intrasexual competition (Fromhage and Jennions 2016). However, these roles are not always clear and, in many species, individuals appear to allocate substantial time and resources to both competition and parental care (Ah-King and Ahnesjö 2013; Clutton-Brock and Huchard 2013). How does the trade-off between competition and reproduction influence selection when the principal care-giver, whether male or female, also benefits from investing in intrasexual competition? Investigating this question will help us to understand how reproductive trade-offs govern trait evolution in both sexes and the consequences for the evolution of mating systems and parental care. In this thesis I will look at this question from the perspective of both males and females.

1.1 Reproductive trade-offs

Organisms must invest in a multitude of phenotypic traits that will increase their ability to both survive and successfully reproduce. However, to express these traits, they must draw on a finite pool of resources and, therefore, investment in some traits will necessarily reduce the expression of other traits. This fundamental idea in life history theory demonstrates that evolution is often governed by trade-offs, as individuals attempt to maximise their fitness by expressing the most adaptive combination of traits (Williams 1966). When individuals attempt to breed more than once in their lifetime, investment decisions are often influenced by a trade-off between current and future reproduction (Stearns 1992). Individuals that invest in current reproduction by allocating resources to offspring production and parental care are predicted to face a cost to their future reproduction through increased mortality (Santos and Nakagawa 2012), restricted mating rates (Szekely and Cuthill 2000; Verboven and Tinbergen 2002), or a reduced ability to obtain resources needed for subsequent reproductive attempts (Fokkema et al. 2016; Richardson et al. 2020). This trade-off can help to explain differences in parental care strategies, both within and between species, with some individuals investing more in offspring care and others investing in reproductive competition (Clutton-Brock 1991).

Competition for limited reproductive resources is usually most intense between individuals of the same sex and can select for investment in traits that increase an individual's competitive ability (Clutton-Brock and Huchard 2013). These traits include morphological weapons that aid in direct conflict (Rico-Guevara and Hurme 2019), behaviours such as aggression or mate guarding (Jormalainen 1998), and signalling traits that communicate some aspect of an individual's quality to potential competitors or potential mates (Wiens and Tuschhoff 2020). In contrast, selection on offspring production results in traits that increase offspring number and viability including foetal nourishment, specialised care traits, or parental behaviours (Alonso-alvarez and Velando 2012; Smiseth et al. 2012). In addition to limits on the metabolic resources that can be allocated to competitive and reproductive traits, these investments may be further constrained by temporal limitations or conflicting mechanisms of trait expression, (Stiver and Alonzo 2009). For example, engaging in resource contests can detract from the time that individuals can spend in parental care, and the release of hormones that control territorial aggression, such as testosterone, can reduce the expression of care behaviours ("Challenge hypothesis" Wingfield et al. 1990; e.g. Saino and Møller 1995; McGlothlin et al. 2007).

1.1.1 Sexual selection theory

Maximisation of fitness under the constraints of a trade-off between intrasexual competition and parental investment is often related to the potential reproductive rate of each sex (Clutton-Brock and Vincent 1991). Sexual selection theory predicts that intrinsic differences between the sexes in their initial investment in reproduction, whereby males produce many small sperm and females produce few large eggs, results in males generally having a higher potential reproductive rate than females (Parker 1972; Schärer et al. 2012; Lehtonen et al. 2016). As a result males can maximise their reproductive success by investing in traits that confer competitive ability, and thereby increase their access to females (Bateman 1948). This investment in competition is thought to limit male investment in other components of reproduction, particularly parental care (Trivers 1972). In contrast, competition between females is not predicted to greatly increase their reproductive rate and, therefore, female strategies for maximising reproductive success are expected to focus on parental investment (Trivers 1972; Queller 1997).

Despite these general expectations, there are circumstances when the potential reproductive rates of females can be higher than males (Clutton-Brock 2017). For example, where males invest heavily in parental care, females may compete intensely for access to available males, as seen in some polyandrous birds (Goymann et al. 2004) and syngnathid fishes (Jones et al. 2001). Female reproductive rates may also be higher than males in cooperatively-breeding species, where reproduction can be monopolised by dominant females (Hager and Jones 2009). The best investment strategy for males and females is likely to depend on an interaction between several factors that affect the costs and benefits of caring versus competing, including the strength of sexual selection, the sex ratio and paternity assurance (Kokko and Jennions 2008; Liker et al. 2013; Fromhage and Jennions 2016; Janicke and Morrow 2018).

1.1.2 Competition vs care

The predicted trade-off between parental care and competition leads to the expectation that in each species one sex will evolve to be more competitive and the other will be selected to invest more in care (Fromhage and Jennions 2016). When one sex leaves the mating pool to provide care, their availability as sexual partners is reduced. As a result, competition for mates increases, which can generate stronger sexual selection in the more available sex (Clutton-Brock and Parker 1992; Amundsen 2018; Janicke and Morrow 2018). With greater investment in competition, the costs of caring will increase for the competitive sex resulting in increasing divergence between the sexes (Fromhage and Jennions 2016).

Although this process is exemplified by the many species exhibiting polygyny and classical polyandry (Andersson 1994; Janicke and Morrow 2018; Hare and Simmons 2019), sex roles are likely to be more dynamic than this logic suggests, and in many species those providing care may also benefit from investing in intrasexual competition (Ah-King and Ahnesjö 2013). For example, in many socially monogamous species there is selection for both sexes to be promiscuous (Griffith 2007; Slatyer et al. 2012; Parker and Birkhead 2013) and, even in polygynous species without male parental care, there is evidence for male mate choice (Edward and Chapman 2011). In this thesis I explore two additional cases where divergent sex roles are less clear and investigate how the trade-off between competition and reproduction influences selection when a) competitive males provide sole parental care and b) harem-breeding females invest in intrasexual competition.

First, despite the prediction that higher potential mating rates should select against parental care in males, males of many species contribute to parental care and sometimes provide more care than their female partner (Clutton-Brock and Vincent 1991). In some species, greater male investment in care results in greater reproductive rates in females and more female-female competition ("sex-role reversal"; Eens and Pinxten 2000; Owens 2002), but often males continue to be the more competitive sex, despite providing all of the parental care (Tallamy 2000; Ah-King et al. 2005; Rios-Cardenas 2005). How do these males evade the constraints that should limit their ability to invest more in both competition and care than females?

Second, although exaggerated sexual traits are less common in females, recent work has shown that females can increase their reproductive success through intrasexual competition for reproductive resources, which frequently results in selection for competitive traits (reviewed below). Sex differences in trait evolution can therefore not be solely explained by differences in mating competition as some theoretical frameworks suggest (Tobias et al. 2012; Clutton-Brock 2017). Although competitive traits are observed in many females that provide parental care, knowledge of how selection on competitive traits is shaped by reproductive trade-offs in females is still limited.

1.2 Male parental care

Instances of males contributing to care are found in all vertebrate groups (Reynolds et al. 2002) and some invertebrates (Zeh and Smith 1985; Tallamy 2001). How do males in these species escape the costs of missed mating opportunities when investing in parental care? Variation in male contributions to care might be explained by several factors. First, the benefits of care can outweigh the costs of missed mating opportunities. When the survival of offspring is dependent on care provided by both parents, male reproductive success will depend more strongly on care than mating investment. In many monogamous birds, altricial young can demand provisioning contributions from both parents and can be highly vulnerable to predation (Olson et al. 2008). In brown footed boobies for example, heavy predation of unattended nests results in obligate biparental care during the first five weeks (Tershy and Croll 2000). Second, the benefits of mating investment will depend on the opportunity for additional matings and the strength of competition for receptive females (Emlen and Oring 1977; Clutton-Brock and Parker 1992). Male investments in parental care have been shown to vary in response to the availability of females in some bird (Magrath and Elgar 1997) and fish species (Bjelvenmark and Forsgren 2003; Takeyama et al. 2013). In addition, male-biased adult sex ratios are associated with sex-role reversal across birds (Liker et al. 2013). Third, the strength of sexual selection acting on males will influence the costs of care. If there is limited variation in male mating success, the benefits of investing in competition will be reduced. Remeš et al (2015) show that in birds, the amount of male care relative to female care is influenced by the strength of sexual selection acting on males, and is reduced with male-biased sexual size dimorphism.

As well as variation in the relative benefits of investing in parental versus competitive strategies, investment in mating may not always be constrained by parental investment (Stiver and Alonzo 2009). Most theoretical models categorise individuals as either searching for mates ("time in") or providing parental care ("time out") (Jennions and Fromhage 2017), but if mating and parental behaviours are spatially associated, they can occur simultaneously at reduced costs. In species with external fertilisation, males can guard broods within their territories while continuing to engage in male competition and courtship, as observed in many frogs (Wells 2007) and fish (Ah-King et al. 2005), as well as some arthropods (Tallamy 2001). Furthermore, if some traits contribute to both parental and mating effort, there may be reduced mechanistic or energetic constraints. For example, contrary to the predictions of the challenge hypothesis, the same hormonal pathways can promote both competitive and parental behaviour, as seen in the California mouse (*Peromyscus californicus*; Trainor and Marler 2002). Finally, male traits that confer benefits to offspring may become objects of female choice. Female preference for males that provide care can relax the trade-off between mating and parental effort in males by increasing the mating success of caring males (Stiver and Alonzo 2009).

1.2.1 Exclusive paternal care in fish

In teleost fish, exclusive paternal care exists in more than half of families that display parental care and is more common than both female-only and biparental care. In the majority of these species sexual selection continues to act more strongly on males than females (Sargent 1997). Males compete with one another for territories on which to breed and, since body size correlates strongly with resource holding potential in fish, sexual size dimorphism is common (Balshine and Sloman 2011). Care behaviours in males are largely non-depreciable which means that males can care for the broods of multiple females simultaneously without incurring additional costs (Gross and Sargent 1985). Male-only care is closely associated with male territoriality in fish and as eggs are deposited into a male's territory, territory defence also functions as brood defence (Ah-King et al. 2005). Consequently, males do not need to abandon care in order to seek additional mates or limit future breeding until the current brood has been reared.

Female choice for male care may further promote the maintenance of paternal care in fish. There is evidence that female fish can respond to cues of male care, such as fanning behaviour (Östlund and Ahnesjö 1998; Lindström et al. 2006), courtship rate (Knapp and Kovach 1991) or nest quality (Östlund-Nilsson 2001). In particular, there are numerous examples of female preference for males already caring for the broods of other females or for larger broods (e.g. Unger and Sargent 1988; Kraak and Groothuis 1994; Goulet 1998; Fagundes et al. 2007). There are several hypotheses for why females might prefer to mate with these males. First, the presence of eggs may signal that a male is capable of providing sufficient care (Forsgren 1997). Although care is thought to have limited costs, the ability to fend off intruders (both brood predators and conspecific rivals) is likely to be crucial and to require males to be in good condition. The presence of eggs may indicate to females that a male is able to keep hold of a territory and provide sufficient defence. Second, the presence of eggs may indicate that a male is attractive to other females (Goulet and Goulet 2006). Mate choice copying can reduce the costs of mate sampling for females and is likely to be beneficial for less experienced females or when male quality is otherwise hard to assess (Pruett-Jones 1992). Third, by adding her eggs to an existing brood, females increase the size and, thereby, reproductive value of a male's brood (Sargent and Gross 1985). This may lead to greater investments of care by males (Clutton-Brock 1991). Males have been shown to increase care behaviour (Magnhagen and Vestergaard 1993; Karino and Arai 2006) and reduce total brood cannibalism (Lindström and Craig Sargent 1997; Manica 2002) when caring for large broods. Finally, the risk of predation or cannibalism of a female's offspring may be diluted in the presence of other young (Rohwer 1978). If male cannibalism doesn't increase with brood size then the likelihood of a female's eggs becoming victims of cannibalism will be reduced in larger broods (Kraak 1996). It is currently not clear which of these adaptive functions might drive selection for this preference in females.

In chapters 2 and 3, I use a series of comparative meta-analyses to investigate male-only care in fish. In Chapter 2, I analyse the costs and benefits of care in males to understand whether the prevalence of male care in fish is likely to be predominantly influenced by low costs associated with providing care, benefits in terms of offspring survival, or benefits in terms of mating success through female choice for care. I show that across species, both the condition costs to males and the benefits to offspring of male care are low, but that caring males have a mating advantage as females show a preference for males with eggs. In Chapter 3, I investigate whether partial filial cannibalism, a frequent behaviour of caring males, represents an investment by males in their own condition. I show that the average proportion that males cannibalise from their brood is positively correlated with the degree of sexual dimorphism in a species and therefore likely to be related to the energetic demands of intra-sexual competition. In both chapters I also test two of the above discussed hypotheses concerning female preference for males with eggs. In Chapter 2, I show that this preference is stronger in species where males increase their care effort when guarding larger broods, suggesting that by increasing the value of the brood females can promote better care for their offspring. In Chapter 3, I show that female preferences are also stronger in species where more males engage in partial brood cannibalism and suggest that this may be due to a dilution effect in large broods. To support this, I show that males cannibalise a lower proportion of their eggs when caring for large broods.

1.3 Female competition

Early observations that secondary sexual traits are rarer in females or often muted compared to those of their male counterparts, along with limited observations of overt aggression and contests among females, led to the initial hypothesis that the expression of competitive traits is likely to be non-adaptive in females and a result of genetic correlation with sexually selected male traits (Lande 1980; Rice 1984). The assumption that female-female competition is not an important process in female trait evolution has resulted in studies of intrasexual competition historically focusing disproportionately on males (Andersson 1994). Female competition is now accepted to be widespread and to have important benefits for female fitness, even in species that invest heavily in gamete production and parental care (Amundsen 2000; Tobias et al. 2012; Clutton-brock and Huchard 2013). However, since the mechanisms through which females maximise their reproductive success differ from those of males, the benefits of female competition are usually centred around different resources and this competition can select for traits that may or may not resemble those in males (Kraaijeveld et al. 2007).

1.3.1 Benefits of female-female competition

Although mates are not usually limiting for females, successful reproduction in females can be dependent on obtaining other ecological resources such as nest sites or food sources for offspring which may require females to compete (Woodroffe and MacDonald 1995; Robinson and Kruuk 2007; Dunn et al. 2015). For example, both the tree swallow (*Tachycineta bicolor*) and the parrot *Eclectus roratus* nest in pre-created cavities that are severely limited, leading to intense competition (Heinsohn et al. 2005; Rosvall 2008).

This competition has selected for aggressive behaviour in female tree swallows (Rosvall 2008), and colour signals in *E. roratus* that are distinct from their male counterparts (Heinsohn et al. 2005). Within social groups, resource limitation can drive conflict over which individuals can reproduce, resulting in competition for breeding dominance (Lazaro-Perea 2001; Kutsukake and Clutton-Brock 2006). Females may also compete for particular males. In species with female-only care, differences in the genetic quality of males can create mate limitation and drive competition for access to the best males (Sæther et al. 2001; Bro-Jørgensen 2002). For instance, at topi antelope (*Damaliscus lunatus*) leks, females aggressively compete for access to preferred males and will actively disrupt the copulations of rival females (Bro-Jørgensen 2002).

When males do contribute to care, or supply females with other resources, females might benefit from ensuring that they and their offspring are the sole recipients of this contribution (Slagsvold and Lifjeld 1994). In facultatively polygynous species, females have been shown to interfere with the settlement of additional females to maintain their monogamous position or reduce breeding synchrony (LaPrade and Graves 1982; Kempenaers 1995; Lutnesky and Kosaki 1995; Sandell 1998; Wong et al. 2008). For example, in the European starling (*Sturnus vulgaris*), the likelihood of a male attracting an additional female is related to female aggression towards intruding females rather than male attraction behaviour (Sandell 1998).

1.3.2 Reproductive costs of female-female competition

In females, the demands of offspring production and parental care are thought to constrain the evolution of competitive traits (Fitzpatrick et al. 1995). The expression and maintenance of these traits, which may include increased body size, weaponry and aggression, are energetically demanding, and may reduce the resources that can be turned into viable embryos (Emlen and Oring 1977). Time spent in contests with other females may detract from the time available for parental care of offspring, and physical injuries that can result from contests may reduce the ability to provide effective care (Stockley and Campbell 2013). Although female-female competition can play an important role in determining female reproductive success, this does not indicate whether the evolution of competitive traits is limited by costs to potential reproductive success through trade-offs with fecundity, parental care or offspring quality. In order to address this question, we need to examine the reproductive costs and benefits to females of competitive investment.

Recent observational studies have begun to examine the consequences of female competition for components of reproductive success, such as offspring number and quality. The costs of maintaining a dominant breeding position have been investigated in some communal and cooperative breeders. In pied babblers (*Turdoides bicolor*), reproductive skew is high, resulting in significant benefits for the dominant female, but competition with subordinates results in dominant females producing fewer successful broods (Nelson-Flower et al. 2013). Similarly, while high-ranking female baboons have higher infant survival and shorter intervals between breeding attempts, they also suffer more miscarriages and reduced fertility (Packer et al. 1995). These studies suggest that there may be some intrinsic costs to females of competitive investment. In banded mongooses, however, females invest more in their current offspring when competition is high with no consequences for maternal survival, suggesting that competition does not constrain investment in reproduction in this species (Inzani et al. 2016). In monogamous birds, investment in competition can be essential for securing nesting sites but may come at a cost of reduced female condition or maternal care. In a study of tree swallows (*Tachycineta bicolor*), Rosvall (2011) showed that aggressive females produce smaller offspring, with effects appearing upon hatching but also in response to reductions in provisioning behaviour. However, this pattern has not been found consistently. In both dark-eyed juncos (*Junco hyemalis*), and house wrens (*Troglodytes aedon*) aggressive females were not shown to put less effort into parental care or produce offspring of lower quality (Cain and Ketterson 2013; Krieg and Getty 2020). In fact, more aggressive female house wrens had higher fledgling success than their less aggressive competitors.

All of the studies discussed above rely on observations of wild populations and attempt to infer a causal link between competitive investment and reproductive success based on correlations between aggression and reproduction. However, since competitive traits are often condition dependent, the optimum investment strategy will likely differ between females of different quality which can conceal the presence of a trade-off (Hamel et al. 2009; Vedder and Bouwhuis 2018). For instance in tree swallows, larger females produce larger chicks but female aggression is positively correlated with female mass and therefore the costs of aggression will vary with female quality (Rosvall 2011). In addition, the costs and benefits of investment strategies are likely to change with varying ecological conditions. In superb fairywrens (*Malurus cyaneus*), more competitive females have been shown to have higher reproductive success in high quality habitat but lower reproductive success in low quality habitats (Cain et al. 2015; Cain and Langmore 2016).

Experimental manipulations of either the expression of competitive traits or reproductive investment can decouple phenotypic relationships and help to reveal the presence of a trade-off. Manipulations have been used successfully to investigate this trade-off in males. For example, in both collared flycatchers (*Ficedula albicollis*) and house sparrows (*Passer domesticus*) experimental increases of brood size, which force increased investment in parental care, reduce the subsequent expression of male colour signals (Gustafsson et al. 1995; Griffith 2000). On review of the literature I found that these methods have only twice been applied when looking at reproductive trade-offs in females. In their study on blue tits (*Cyanistes caeruleus*), Doutrelant *et al.* (2012) manipulated female investment in reproduction by removing nests and forcing pairs to lay a second clutch later into the season. They showed that manipulated females invested less in plumage colour signals than unmanipulated females, supporting a trade-off between reproduction and investment in competitive signals. Morales *et al.* (2009) took a different approach and identified dietary carotenoids as an essential resource for both egg production and colour signals in blue-footed boobies (*Sula nebouxii*). They show that supplementation with carotenoids increases both egg size and foot colour intensity, reversing the relationship observed under natural carotenoid levels.

Competition can lead to selection for prominent morphological traits in females that are similar to male traits, such as the horns used by female dung beetles (*Onthophagus sagittarius*) to acquire dung for brood balls (Watson and Simmons 2010), or the bright yellow patch on the throats of female rock sparrows (*Petronia petronia*), the size of which correlates with timing of access to food (Griggio et al. 2010). More commonly, female traits differ from male traits in ways that may make them less costly. In general, competitive traits in females exhibit plasticity beyond the condition-dependent expression of fixed morphological weapons and signals.

The expression of plastic traits can be limited to the times when competition is most intense and reduced when investment is needed elsewhere (Stockley and Campbell 2013). In both birds and fish, female aggression shows strong temporal variation, with high levels of aggression coinciding with periods of resource competition (Sandell and Smith 1997; Tubert et al. 2012; Bentz et al. 2019; Diniz et al. 2019). Similar adjustments are seen in other types of competitive traits. In female house mice (*Mus musculus*), the production of major urinary proteins used in competitive signalling are reduced when competition is relaxed (Stockley et al. 2013) and costly bill coloration in American goldfinches (*Spinus tristis*) is only expressed during the breeding season (Murphy et al. 2009). Although behavioural traits are often considered highly plastic, evidence for correlations both between behaviours, and within behaviours across contexts (behavioural syndromes) indicate that there can be limits to this plasticity (Sih et al. 2004). While this may lead to individuals behaving suboptimally in some contexts, resulting in constraints on selection, correlated behaviours may also reflect selection for traits that offer benefits across multiple contexts (Wolf et al. 2007). For example, aggressive defence against territory intruders may simultaneously enable individuals to maintain control of resources and protect offspring from predation (Cain et al. 2011). A trade-off between competition and reproduction may, therefore, select for competitive traits that have additional reproductive benefits. Determining when and how competitive traits are expressed and their fitness outcomes in different ecological contexts is therefore vital for understanding the costs and benefits of competition in females and the nature and extent of trade-offs between competitive trait expression and reproduction.

1.3.3 The study system: Lamprologus ocellatus

The adaptive radiation of cichlid fishes in the African rift lakes (Lake Malawi, Tanganyika and Victoria) into over 1200 species has made them one of the largest and most phenotypically diverse animal families (Takahashi and Koblmüller 2011). Despite this diversity, parental care remains a defining characteristic of rift-lake cichlids (indeed, of all cichlids), with all species exhibiting some form of post-fertilisation brood care (Balshine and Abate 2021). Among the cichlid fauna of Lake Tanganyika, there has been significant focus on the Lamprologini tribe, which show great diversity in social behaviour and group structure, including monogamy, polygyny, coloniality and cooperative breeding (Kuwamura 1986; Sefc 2011) Unlike most other Tanganyikan tribes, which exhibit mouthbrooding, the lamprologines are substrate brooders (Sturmbauer et al. 1994). Within the tribe, a number of species has evolved to spawn in empty gastropod shells of the genus *Neothauma* that accumulate on the sand beds in varying densities (Bills 1997). Despite their dependency on a common resource, these species exhibit diversity in their mating and care systems including polygyny with predominantly female care (e.g. *Lamprologus ornatipinnus*), monogamy with biparental care (e.g. *Neolamprologus brevis*), and colonial breeding (e.g. *Neoloamprologus* *multifasciatus*), making them a valuable group for studies of social behaviour and parental care (Lein and Jordan 2021).

In chapters 4 and 5 of this thesis, I use the lamprologine cichlid *Lamprologus ocellatus* (Steindachner 1909) to investigate the costs and benefits of female competition. In this species, males defend territories containing multiple shells and form either pairs or harems with several females. Both females and males use empty shells as refuges and offspring are spawned and cared for inside the female's shell (Bills 1997; Figure 1.1). *L. ocellatus* are known to co-occur with 12 other lamprologine species and occupy habitats where shells are a limited resource (Lein and Jordan 2021). Both males and females bury shells and invest in territorial displays and aggression to defend their shells from both intraspecific and interspecific competitors (Bills 1997). As well as competing for shells, many heterospecific cichlids predate on fish larvae, and offspring defence against these predators is therefore an important aspect of parental care for female *L. ocellatus*.

Previous work on intrasexual competition in *L. ocellatus* has shown that females, as well as males, invest in territorial defence against same-sex rivals (Walter and Trillmich 1994). As well as preventing other females from accessing their own shell, shell-holding females attempt to prevent new females from settling in nearby shells and aggression between females continues even once both females are settled and breeding (Walter and Trillmich 1994). The benefits to females of intrasexual competition in this species are still unclear. Since breeding is dependent on shell access, more competitive females may have a breeding advantage. In the laboratory, larger females have been shown to settle and breed first (Walter and Trillmich 1994). In addition, rival females in a harem may pose a threat to juveniles, either through cannibalism (by females themselves or by their offspring) or by occupying shells that could be used by dispersing juveniles for shelter (Brandtmann et al. 1999). When given a choice of shells, females also try to settle closest to the male; the benefit of such behaviour is unknown, but may be related to contributions to territory defence provided by the male (Brandtmann et al. 1999).

Although we have some valuable information about the social structure and ecology of this species, we still have a limited knowledge of population and kin structure and important life history traits in the wild, including lifespan and breeding cycles. However, existing studies demonstrate that *L. ocellatus* is an excellent study system for investigating the trade-off between competition and reproduction in females. In particular, small territories that centre around a shell mean that natural harem sizes can be replicated in the laboratory allowing the study of social interactions at ecologically relevant scales, and that group composition and territory positions can be controlled to manipulate investment in competition. In addition, females experience threats from female competitors and brood predators during care, but male assistance in brood defence may be an important benefit of competition. By assessing the responses of both males and females to brood predators under different levels of competition we can investigate whether females experience a trade-off between competition and brood defence and how the effects of this trade-off may be reduced by male contributions to care.

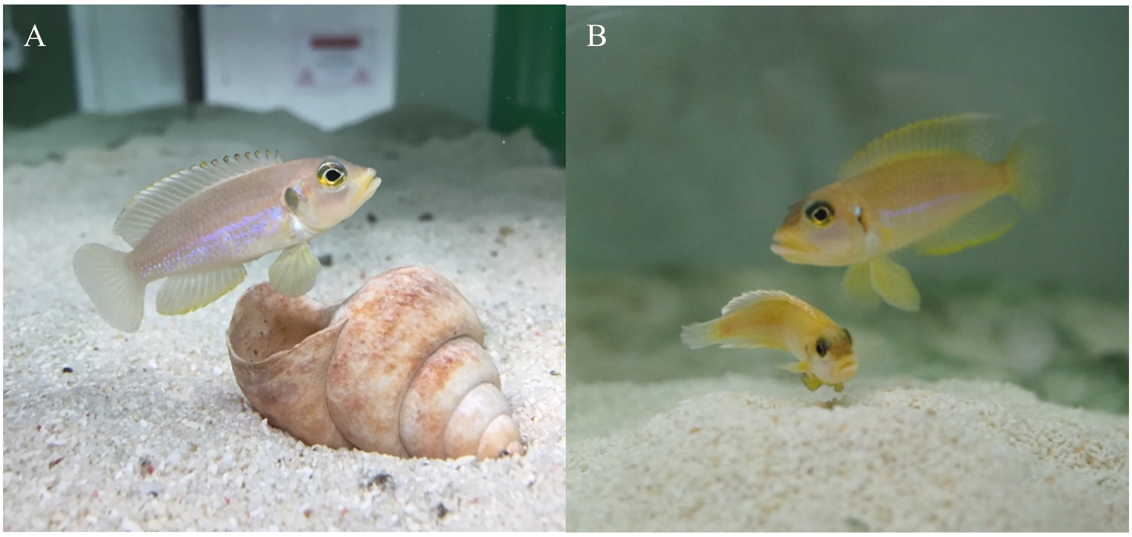

Figure 1.1. *Lamprologus ocellatus*. A) A male guards a *N. tanganycense* shell. B) A male and female court at the entrance to a partially buried shell. Images are my own.

In Chapter 4 I investigate the consequences of female competition for parental care and reproductive output by manipulating the intensity of competition experienced by females in controlled laboratory conditions. By observing maternal behaviour and reproductive investment through multiple brood cycles, I show that females suffer reduced hatching success when breeding under high competition, but that brood size and maternal behaviour are largely unaffected. In Chapter 5 I expose harem-breeding females to a model brood predator in the laboratory to investigate the effect of brood predation risk on female competitive behaviour. I find that females trade-off the time that they spend engaging with a predator and a competitor and become less responsive to competitor intrusions. I also show that, while aggression towards a competitor is correlated across contexts (with and without the threat of brood predation), aggression towards a predator is not correlated with aggression to conspecific competitors. I also investigate the role of males in brood defence and the potential benefits to females of settling near males. I expose males to the same model predator in the laboratory and show that males respond more aggressively to a predator in the presence of a female partner and her brood. Finally, I use preliminary field data to show that females receive more help from males against brood predator intruders when their territory is closer to the male's shell.

In the final chapter of this thesis I present a general discussion summarising the results of the four chapters described above. I also discuss potential directions for future research that could build on the work presented here to advance our understanding of sexual selection and reproductive trade-offs in caring parents.

Chapter 2

The costs and benefits of paternal care in fish: a meta-analysis

Rebecca Goldberg, Philip Downing, Ashleigh Griffin, Jonathan Green

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Author contributions: Jonathan Green and Ashleigh Griffin conceived of the study. Jonathan Green, Ashleigh Griffin and I designed the study. I ran the literature search and compiled the data. Philip Downing and I created the phylogeny and analysed the data. I wrote the manuscript and incorporated comments provided by all other authors.

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Abstract

Male-only parental care, while rare in most animals, is a widespread strategy within teleost fish. The costs and benefits to males of acting as sole carer are highly variable among fish species making it challenging to determine the selective pressures driving the evolution of male-only care to such a high prevalence. I conducted a phylogenetic meta-analysis to examine the costs and benefits of paternal care across fish species. I found no evidence that providing care negatively affects male condition. In contrast with other taxa, I also found limited evidence that male care has evolved as a strategy to improve offspring survival. Instead, I found that males already caring for a brood are preferred by females and that this preference is strongest in those species in which males work harder to care

for larger broods. Thus, in fish, investment in offspring care does not constrain a male's mating success but rather augments it, suggesting that the relatively high prevalence of male-only care in fish may be in part explained by sexual selection through female preference for caring males.

2.1 Introduction

Male-only parental care, where the male is the sole carer of his offspring, is a rare strategy in most animal groups. In birds, for example, the majority of species show biparental care with male-only care present in less than 1% of species (Cockburn 2006), while in mammals and reptiles male-only care is entirely absent (Reynolds et al. 2002). In these taxa, lower investment in parental care by males than females is thought to be a consequence primarily of sexual selection acting more strongly on males to increase their mating rate, although other factors such as paternity uncertainty and the influence of adult sex ratios on mate availability are also likely to play a role (reviewed in Kokko and Jennions 2008). In teleost fish, however, we see a contrasting pattern. In this group, male contributions to offspring care are unusually high, with the male acting as the sole carer in more than 50% of families with any parental care (Sargent 1997). The only other taxonomic group that show comparable levels are amphibians, where male-only care is equally as common as female-only care (Reynolds et al. 2002). Why male-only care should be so prevalent in teleost (and non-teleost) fish, including in species where sexual selection continues to act more strongly on males than females (Rios-Cardenas 2005), remains unclear.

Fish possess a number of traits less commonly found in other taxa that are likely to increase the benefits and decrease the costs of paternal care, potentially explaining its prevalence in this group. First, the care that male fish provide, which usually consists of guarding against predation, cleaning the substrate, and fanning the eggs to increase aeration, is shareable among offspring without depreciating and so the costs of care do not increase with increasing brood size. In contrast, depreciable care such as direct feeding of offspring is very rare - the only known examples are the nourishment that embryos receive within the brood pouches of male syngnathids (pipefish and sea horses) (Ripley and Foran 2009) and the production of epidermal mucus by both parents to feed offspring in some cichlid species (Noakes 1979). Second, while brood defence constitutes a critical aspect of parental care in fish, because of the substantial difference in size between adults and larvae, brood predators are rarely a threat to adults (Perrone and Zaret 1979). The non-depreciable nature of care and the low risks associated with brood defence are thus expected to reduce the energetic and survival costs of care in fish compared to other taxa (Balshine and Sloman 2011). Third, the trade-off between investment in care and investment in mating, which underpins sex differences in care strategies in birds and mammals, could be less important in fish (Magrath and Komdeur 2003). Male-only care is found exclusively in species with external fertilisation, and in many species eggs are deposited directly within the male's territory (Gross and Sargent 1985; Benun Sutton and Wilson 2019). Both territoriality and nest construction have been shown to be ancestral to male care, and brood defence may, therefore, be a by-product of territory defence (Ah-King et al. 2005), as such incurring no additional cost to the male (Baylis 1981; Smith and Wootton 1995). Furthermore, territorial males caring for broods may simultaneously be able to obtain further matings from additional females visiting the territory and so do not face a mating cost (Stiver and Alonzo 2009). Together, all of these factors can decrease the total costs associated with providing care for males (Blumer 1979). In addition, there is evidence that in some species, females prefer to mate with males already caring for eggs (Unger and Sargent 1988; Sikkel 1989), or with larger broods (Kraak and Videler 1991; Fagundes et al. 2007). Indeed, it has been suggested that a preference for males with broods may even drive males to raid one another's nests for eggs, which are then cared for in the kidnapper's nest (Rohwer 1978). Female preference for males with broods increases the benefits to males of providing care and indicates a possible role for sexual selection in maintaining male-only care in fish (Lindström and St. Mary 2008).

In an effort to determine the selection pressures favouring the evolution of male-only care in fish, numerous experimental studies have sought to quantify the relationship between paternal care and fitness parameters such as condition, offspring survival and mating success in a wide range of species. Collectively, the results of these studies paint a complex picture, with species showing diverse and often opposing effects of paternal care on components of reproductive success. For instance, increased male care leads to a loss of condition in both painted greenling (*Oxylebius pictus*) and pumpkinseed fish (*Lepomis gibbosus*), but in the former species male care increases offspring survival and the presence of broods is attractive to females, while in the latter, male care has relatively little effect on offspring survival and females find males caring for large broods unattractive (DeMartini 1987; Rios-Cardenas and Webster 2005). This variation, which is in part likely to reflect fundamental differences in reproductive biology and life-history among species, but also differences in methodology and sample size among studies, currently makes it challenging to determine what combination of costs and benefits have driven the evolution of male-only care to the relatively high levels that we observe in fish.

In this study, I quantify the costs and benefits of male-only care using phylogenetically-controlled meta-analyses. By synthesising the results of previous studies of paternal care from 48 teleost species, such an approach enables me to determine whether selection for male-only care in fish is shaped principally by a) low costs of offspring care, arising from the peculiarities of teleost reproductive biology; b) benefits to offspring in terms of enhanced survival, or, more intriguingly, c) benefits to the males themselves in terms of attracting additional mates. I quantified costs of care by determining the effect of paternal effort on male condition, while survival benefits to offspring were quantified by determining the effect of paternal effort on the survival of eggs to hatching. Finally, female preference for caring males was quantified by determining the preference shown by females either for males with versus without broods, or for males with large versus small broods. In addition, I determined the effect of brood size on paternal effort to test the hypothesis that variation in female preference for males with broods across species is driven by variation in the amount of care that males provide to larger broods. At the same time, my analyses controlled for other factors potentially shaping the payoffs to males of paternal care across species, including shared phylogeny, the brooding environment and differences in methodologies among studies.

2.2 Methods

2.2.1 Data collection and selection criteria

A literature search was conducted using Web of Science with the search terms ‘Fish AND (care* OR offspring OR egg* OR fry)’ to identify studies reporting the effect of paternal care on male condition and offspring survival, the effect of brood size on male care and the effect of brood size and brood presence on female choice. The search included all relevant papers up until June 2020. Studies were screened manually for four criteria: 1) The species studied exhibits predominantly male-only parental care; 2) a result was reported for one of the above five relationships; 3) enough statistical information was present to calculate an effect size; 4) the direction of the relationship reported was specified. The selection process is summarised in a PRISMA diagram (Figure 2.1). The search terms used were broad and so returned a high number of initial results. A large proportion of these studies were outside the field of behavioural ecology and could be easily excluded by title or abstract. For the remaining studies, abstracts were screened for evidence mainly of criterion 2 followed by full text screening to confirm all criteria and extract data. The full dataset included 253 effect sizes from 97 studies, across 48 species.

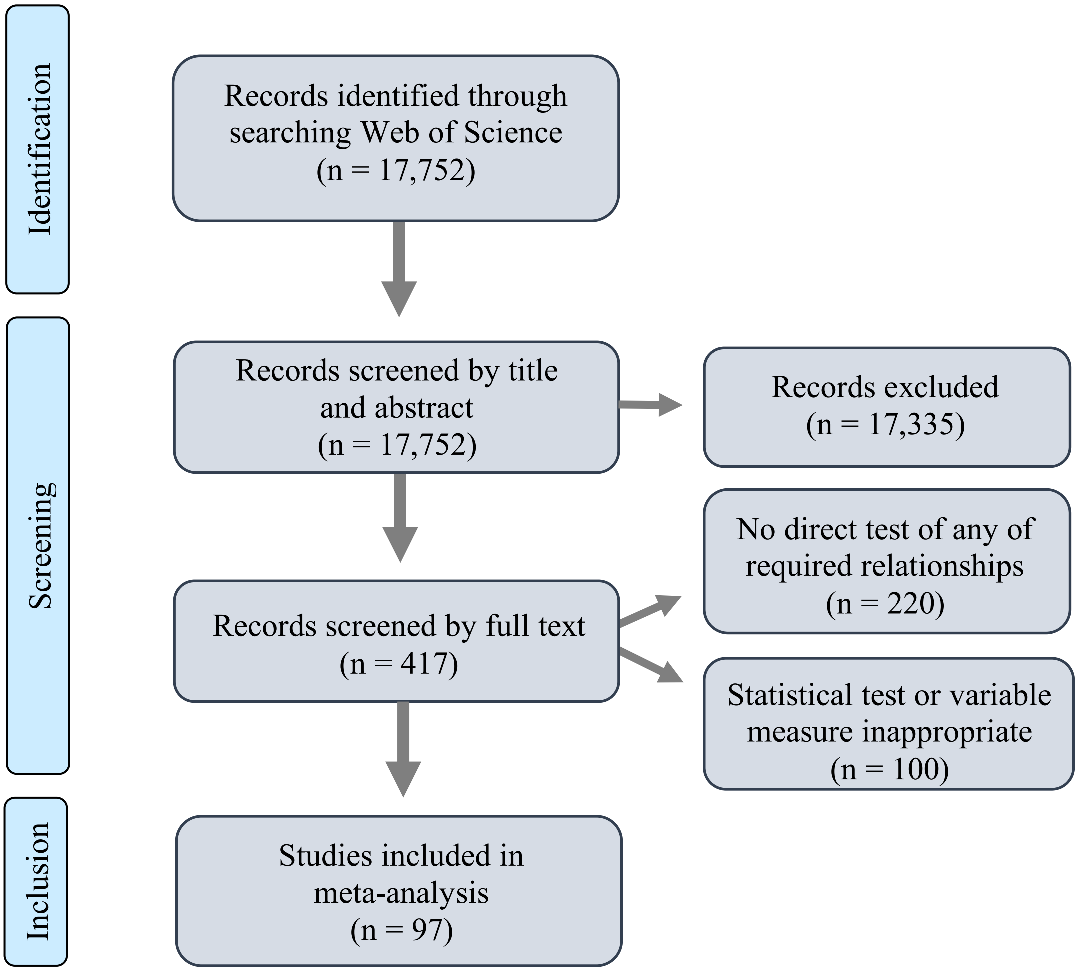

Figure 2.1. PRISMA flowchart showing the process of record identification and selection.

I used Fisher's *Z*-transformation of the correlation coefficient (*Zr*) as my effect size (Borenstein et al. 2007). Where possible, this was calculated from raw data presented in graphs and tables, otherwise test statistics (t, X^2, or F) or p-values were converted using the R package 'esc' (Lüdecke 2018) or published formulae (Lajeunesse 2013). The sampling variance of each effect size was calculated as $1/(n-3)$ where n is the number of individuals studied (estimated from the degrees of freedom of test statistics when not reported). Data from graphs were extracted using WebPlotDigitizer v4.1 (Rohatgi 2018). When studies reported multiple results for the same relationship, I extracted and used all results as separate data points. When results involved comparisons between more than two treatment groups I included all pairwise comparisons when these data could be obtained. I quantified the following five relationships:

1) Care and condition ($Zr_{condition}$)

To estimate the costs of care, 43 effect sizes were calculated from 25 studies on 19 species measuring the effect of parental care on male condition. In the absence of studies quantifying long-term costs of care, this analysis focuses on the short-term energetic costs of care. Positive values of $Zr_{condition}$ indicate that providing parental care increases a male's condition whereas negative values indicate that care decreases condition. My dataset includes studies that measured care effort in the following ways: the frequency, duration or rate of fanning behaviour (using fins to move water around the eggs to increase aeration); the duration of defensive behaviour; the number of broods in a season; the start compared to the end of the caring period; and whether a guarding male was with or without a brood. Measurements of male condition in these studies were: growth rate, weight change, hepatosomatic index, lipid mass, survival, and Fulton's condition factor or other condition factors based on a relationship between length and weight.

2) Care and offspring survival ($Zr_{offspring}$)

For my analysis of the effect of paternal care on offspring survival I calculated 36 effect sizes from 18 studies for 16 species. Positive values of $Zr_{offspring}$ indicate that greater care from males increases offspring survival. I included studies that estimated offspring survival as the number or proportion of eggs surviving to hatching. The care variables included were: the frequency, duration or rate of fanning behaviour; the duration or probability of defensive behaviour; the duration of care; nest attendance duration; and whether the male parent was present or absent. Partial filial cannibalism, whereby males consume a proportion of the brood, is a behaviour that has obvious negative consequences for the survival of some offspring but may simultaneously boost the survival of the remaining offspring, either through reinvestment of energy into parental care (Rohwer 1978) or by reducing disease (Bandoli 2016; Vallon et al. 2016) or competition among eggs for oxygen (Payne et al. 2002; Klug et al. 2006). The costs and benefits of partial filial cannibalism can therefore be difficult to determine and as a result I chose not to include it as one of the paternal behaviours that I analysed.

In species with obligate parental care, the effect of care on offspring survival is naturally expected to be large. Paternal care in the Syngnathidae (pipefish and seahorses) involves the male brooding offspring within a specialised pouch on his body, a mode of paternal care unique among teleosts (Stölting and Wilson 2007). Such brooding is commonly considered to be obligate, but to my knowledge this has not been demonstrated experimentally and there are no published data on the relationship between the presence or absence of care and offspring survival for this group. While my analysis of $Zr_{offspring}$ does include one pipefish species where the effect of duration of care on offspring survival has been reported (the broad-nosed pipefish, *Syngnathus typhle*), I therefore lack data from any Syngnathidae on the effect of the presence versus absence of care on offspring survival. The omission of this group from my analyses could lead me to underestimate the mean effect of paternal care on offspring survival across teleosts. To determine whether this was the case, I undertook analyses in which syngnathid species were included, specifying an almost perfect correlation between care and offspring survival as a means of capturing the likely obligate nature of care in this group (for details, see Appendix A).

3) Brood presence and female preference ($Zr_{preference1}$)

To estimate the effect of the presence of eggs in a male's nest on female mating preference I calculated 57 effect sizes from 34 studies for 23 species. Positive $Zr_{preference1}$ values indicate that females prefer to mate with males already caring for a brood over those without a brood. Female preference in these studies was measured as one of spawning choice, nest association preference, or the number of eggs received by the male.

4) Brood size and female preference ($Zr_{preference2}$)

In addition to brood presence, the size of a male's brood may provide information to females about the quality of the male or his nesting site. I therefore also investigated the effect of brood size on female mating preference. For this analysis I calculated 30 effect sizes from 15 studies for 11 species. Positive $Zr_{preference2}$ values indicate that females show a preference for males caring for larger numbers of offspring. Across studies, female preference was measured as spawning choice, nest association preference, or the number of eggs received by the male.

5) Brood size and care ($Zr_{brood\ size}$)

My analysis of the effect of brood size on male care effort included 87 effect sizes from 50 studies on 25 species. Measurements of care within these studies were: the frequency, duration or rate of fanning behaviour; the amount or probability of defensive behaviour; whether the brood was cared for or abandoned; and whether the brood was cared for or fully cannibalised. Measurements of brood size were the number of eggs or clutches.

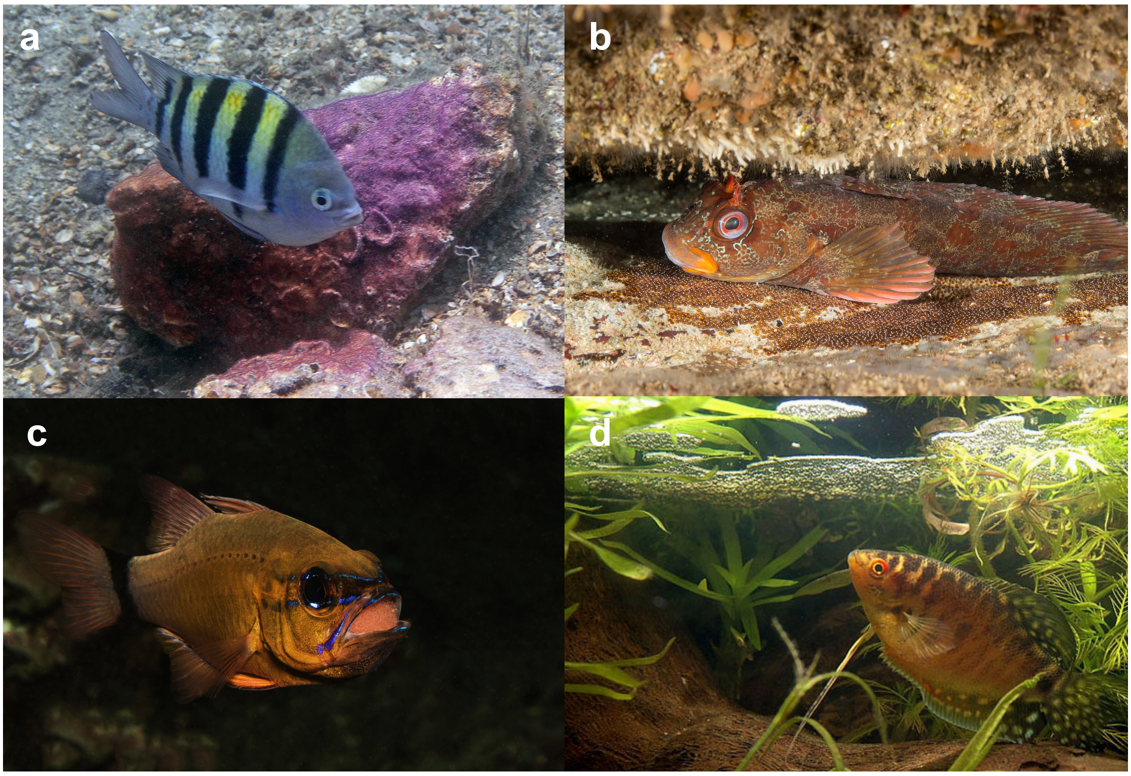

Figure 2.2. Diversity of paternal care in fish. Males care for their brood in different brooding environments. a) Substrate spawners, such as the sergeant major (*Abudefduf saxatilis*), guard their brood on an unaltered substrate in the open. b) Some males, such as the tompot blenny (*Parablennius gattorugine*) create simple nests by locating a rock crevice or bivalve shell and may alter it, for example by covering it in sand or algae. c) Mouth brooders, such as the ring-tailed cardinalfish (*Ostorhinchus aureus*), carry offspring (eggs and larvae) within their mouths. d) Gouramis from the family Ophronemidae build a more complex 'bubble nest' creating a bubble of air for each egg. Photo permissions and credits: a) Kevin Bryant b) Paul Naylor marinephoto.co.uk c) Antje Schultner d) Alberto Garcia. Images a, c and d are under licence CC BY-NC-SA 2.0 https://creativecommons.org/licenses/by-sa/2.0/legalcode, whilst b is under copyright with all rights reserved and was used with permission.

2.2.2 Moderator variables

1) Study design and measures

To explore the effect of study design on effect size estimates I recorded whether each study was conducted in the laboratory or the field and whether it was observational or experimental. For my analyses of $Zr_{condition}$ and $Zr_{offspring}$, I recorded whether studies measured differences in the amount of care between guarding males or differences between guarding and non-guarding males or guarded and unguarded broods respectively. Similarly, for $Zr_{brood\ size}$, I recorded if studies had looked at the effect of brood size on the amount of care males provided or whether they chose to care for or abandon or cannibalise their brood. For my analyses of $Zr_{preference1}$, $Zr_{preference2}$, and $Zr_{brood\ size}$, I recorded whether variation in brood size was natural or had been experimentally manipulated and for $Zr_{preference1}$ I recorded whether the "no-brood" treatment had been created by removing the brood from a male. In addition to exploring effects of study design, I also asked whether the size of the effects obtained depended on the way in which the variables (care effort, male condition, offspring survival, brood size and female preference) were measured.

2) Brooding environment

Although care behaviours are relatively consistent between species with male-only care (consisting primarily of defence and aeration of the brood), there is diversity in the brooding environment which may affect the outcomes of care (Figure 2.2). Differences in $Zr_{offspring}$ between exposed and covered brooding sites might signal a difference in the importance of male care behaviours for offspring survival; for instance, open nests are likely to require higher levels of defence while broods in closed nests are at greater risk of hypoxia and thus likely to require more fanning to maintain aeration. Similarly, if males must work harder to ensure the survival of broods in particular nest types (for example, open nests that may require greater defence against predators) then the effect of care on a male's condition ($Zr_{condition}$) may vary across brooding environments. Finally, differences between nest builders and substrate brooders might exist if environmental modifications by males influence the costs or benefits of subsequent male care. For each species I therefore classified their brooding environment as one of the following: covered substrate (e.g rock crevices), open substrate, covered nest, open nest, brood pouch or mouth brooder. This information was collected from the included studies and information on Fishbase (Froese and Pauly 2019).

2.2.3 Phylogenetic tree

I accounted for non-independence between species due to shared evolutionary history in my analyses by including a variance–covariance matrix that represents all pairwise distances between the 48 species in my data set in my statistical models. I calculated this matrix using the most comprehensive published molecular phylogeny of bony fishes (Betancur-R et al. 2017) as a backbone (17 of my species represented) to which I added 20 species from my sample which were represented at the genus level in this phylogeny and the remaining 11 species from my sample which were represented at the family level (Figure 2.3). I used the Gobiidae (Agorreta et al. 2013) and Blenniidae (Hundt et al. 2014) phylogenies to resolve species-level relationships among the added taxa.

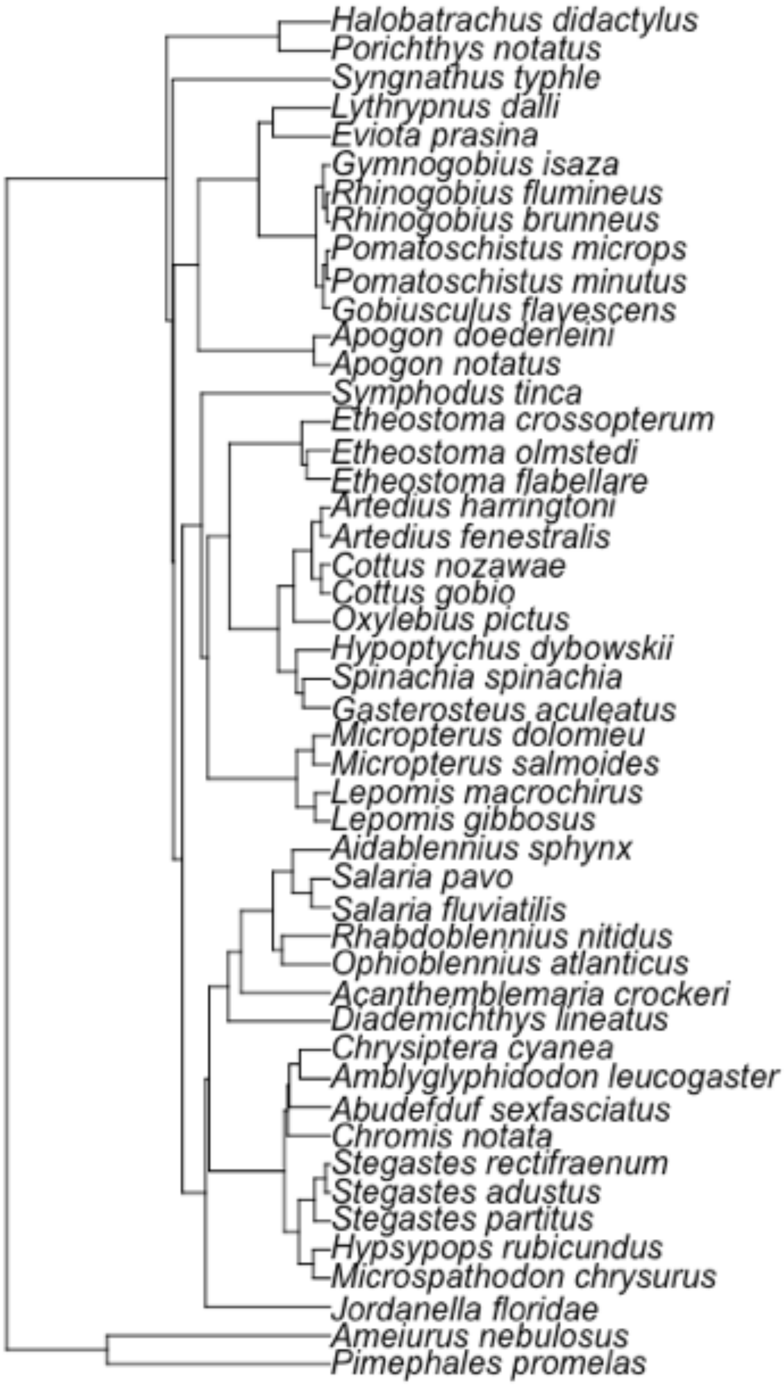

Figure 2.3. Phylogenetic tree used for phylogenetic analyses including all species from the five datasets. Adapted from Betancur-R et al. (2017).

2.2.4 Statistical analyses

I constructed five multi-level meta-analytic models to estimate the following mean effect sizes across species: 1) the effect of paternal care on male condition ($Zr_{condition}$); 2) the effect of paternal care on offspring survival ($Zr_{offspring}$); 3) the effect of brood presence on female preference ($Zr_{preference1}$) 4) the effect of brood size on female preference ($Zr_{preference2}$) and 5) the effect of brood size on paternal care ($Zr_{brood\ size}$). Each model contained random terms for phylogeny, study and species to account for statistical non-independence due to shared ancestry, repeated measures within studies and repeated measures within species respectively and accounted for sampling error variance:

$$Zr_i = \mu + a_{k[i]} + s_{k[i]} + u_{j[i]} + e_i + m_i \qquad (1)$$

Here, μ is the estimate of the mean effect size across species, $a_{k[i]}$ and $s_{k[i]}$ represent phylogenetic, and species-specific random effects for the kth species respectively, $u_{j[i]}$ represents the study-specific effect for the jth study, e_i is the within-study variance and m_i is the sampling error variance. Effect sizes were modelled using a Gaussian distribution and random effects were assumed to come from normal distributions. To explore how differences in study design, study measurements and brooding environment affected my estimates of each effect size, I added each moderator to the models described above as a fixed effect:

$$Zr_i = \beta X + a_{k[i]} + s_{k[i]} + u_{j[i]} + e_i + m_i \qquad (2)$$

where β is a vector of parameter estimates for each level of the fixed effect and X is the design matrix. I suppressed the global intercept in these models to estimate mean values of my effect size for each level of my fixed effect. The moderator variables included in each of these models for each effect size are shown in Table A.2.

I fitted these models in the MCMCglmm (Hadfield 2010) and metafor (Viechtbauer 2010; R Core Team 2021) packages. MCMCglmm uses Markov chain Monte Carlo methods for parameter estimation while Metafor uses Restricted Maximum Likelihood. I used inverse-Wishart priors (variance = 1 and belief parameter = 0.02) for all random effects in the MCMCglmm models and assessed model convergence by calculating the degree of auto-correlation between successive iterations in each chain, by inspecting traces of posterior distributions to evaluate chain mixing and by running each model three times and then comparing within- and between-chain variance (Gelman and Rubin 1992). Metafor does not estimate residual variance by default therefore I added an observation-level random effect to these models. The parameter estimates reported in the results are the mode and credible interval (CI) of posterior distribution of the mean effect size estimate from the MCMCglmm models and p values (pMCMC) are calculated from the Markov Chain and are either the proportion of posterior samples greater or less than 0 (for mean effect sizes) or the proportion of samples where one parameter is greater than the other (when comparing parameter estimates between two levels of a fixed effect).

I quantified heterogeneity using the I^2 statistic as described by Nakagawa and Santos (2012). I^2 quantifies the percentage of observed variation that is attributable to each of the random effects by dividing variance estimates for each effect by the total variance (combined variances of phylogeny, species, study, sampling error and residual error). These estimates are reported in Table 2.2.

Finally, to test whether the strength of female preference for brood presence is driven by the effort that males make when caring for larger broods, I calculated species-level effect sizes for the 13 species for which I had estimates of both $Zr_{brood\ size}$ and $Zr_{preference1}$. To estimate species-level effect sizes I first summarised effect sizes from the same study and then summarised across studies from the same species. The weighted mean effect size for each study was calculated as:

$$\mu_i = \frac{\Sigma(x * n)}{N} \tag{3}$$

Here, *x* is the effect size extracted from a given study, *n* is the sample size used to calculate that effect size and *N* is the total sample size used in that study. As the multiple effect sizes within a study could come from either the same or different samples of individuals, *N* was determined using information located within source papers and calculated separately for each study. Species averages were then calculated using formula (3) where *x* is the study effect size, *n* is the sample size for each study and $N = \Sigma n$. Using these data, I fitted a final $Zr_{preference1}$ model in MCMCglmm including $Zr_{brood\ size}$ as a fixed effect. I included sample size (log transformed) associated with each $Zr_{brood\ size}$ estimate as a covariate in this model to account for differences in sampling effort across studies. As the data used in this analysis were summarised to the species level, phylogeny was the only random term.

2.2.5 Publication bias

I tested for evidence of publication bias in each of the five effect sizes using trim and fill analysis and Egger's regression test using the 'metafor' R package. I found no evidence of publication bias in any of the effect sizes using Egger's regression (Table 2.1). For

Zr$_{condition}$, *Zr*$_{offspring}$ *and Zr*$_{preference1}$, there was some evidence of funnel plot asymmetry, but not for the other two analyses (Figure 2.4).

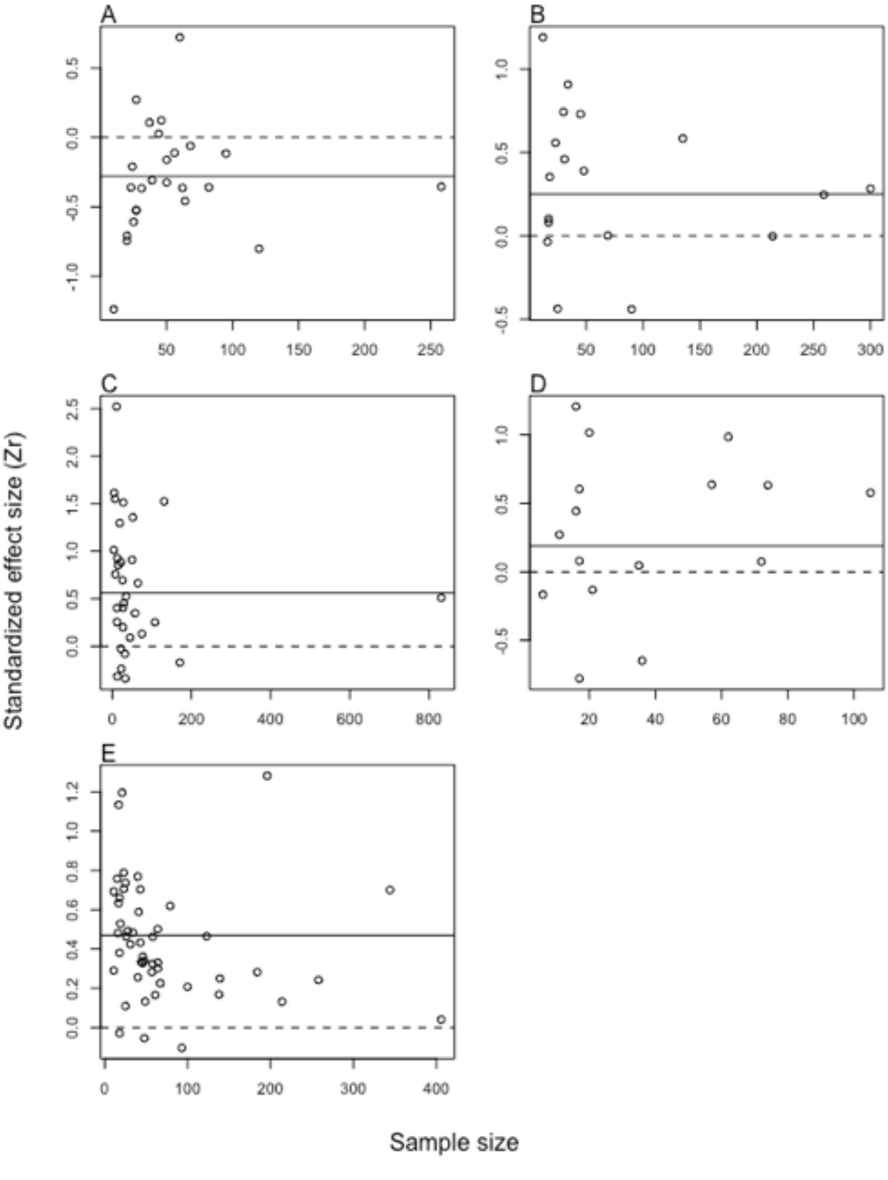

Figure 2.4. Funnel plots of publication bias using study-level data for the five relationships tested by meta-analysis. A) Zr$_{condition}$ B) Zr$_{offspring}$ C) Zr$_{preference1}$ D) Zr$_{preference2}$ E) Zr$_{brood\ size}$. Solid lines represent overall effect size estimates from phylogenetic MCMCglmm models and dashed lines indicate 0.

Table 2.1. Results of trim and fill analysis and egger’s regression tests for publication bias for each of the five effect sizes

	Trim and Fill	Egger's Regression				
Effect Size	*N* missing studies	parameter	estimate	se	*t*	*P* value
Zr condition	9	intercept	-0.31	0.42	-0.93	0.36
		slope	0.07	0.07	0.99	0.32
Zr offspring	10	intercept	0.15	0.35	0.43	0.67
		slope	-0.03	0.05	-0.49	0.63
Zr preference	12	intercept	0.16	0.19	0.84	0.40
		slope	-0.03	0.03	-1.03	0.31
Zr brood size	0	intercept	0.09	0.22	0.43	0.67
		slope	-0.01	0.03	-0.50	0.62

2.3 Results

2.3.1 The effect of care on male condition

Providing care had a negative effect on male condition in 9/19 of the species in my dataset while in one species condition increased while caring (Figure 2.5a). When averaged across species, caring does not have an effect on male condition ($Zr_{condition}$ mean effect = -0.28, 95% credible interval (CI) = -0.62 to 0.08, pMCMC=0.12; $N_{species}$ = 19, $N_{studies}$ = 25, $N_{effect\ sizes}$ = 43). Differences in brooding environment failed to explain variation between species in the effect of care on condition (Figure A.1a; Table A.3).

2.3.2 The effect of male care on offspring survival

Care had a positive effect on offspring survival in 8/16 species (Figure 2.5b). Across species I found no significant effect of male care on offspring survival across species ($Zr_{offspring}$ mean effect = 0.23, CI = -0.27 to 0.67, pMCMC = 0.23; $N_{species}$ = 16, $N_{studies}$ = 18, $N_{effect\ sizes}$ =36). When including syngnathids where paternal care is considered obligate, the mean effect size increased but remained nonsignificant (see Appendix A). I found no difference in the effect of male care on offspring survival between different brooding environments (Figure A.1b; Table A.4).

Table 2.2. Results of intercept only models fitted in the MCMCglmm and metafor packages for each of the five effect sizes.

Effect size*	*N* species	*N* studies	*N* effect sizes	R package	lwr CI	Posterior mode	upr CI	I^2 study	I^2 repeated	I^2 phylogeny
Zr condition	19	25	44	MCMCglmm	-0.62	-0.28	0.08	11%	8%	10%
				Metafor	-0.39	-0.26	-0.13	37%	0%	0%
Zr offspring	16	18	37	MCMCglmm	-0.18	0.25	0.76	4%	4%	5%
				Metafor	0.03	0.25	0.46	12%	7%	0%
Zr preference1	22	32	54	MCMCglmm	0.04	0.51	1.38	17%	23%	11%
				Metafor	0.26	0.5	0.74	23%	51%	0%
Zr preference2	10	15	29	MCMCglmm	-0.32	0.19	0.7	7%	7%	10%
				Metafor	-0.35	0.14	0.63	3%	11%	56%
Zr brood size	25	48	92	MCMCglmm	0.19	0.47	0.86	20%	15%	18%
				Metafor	0.23	0.48	0.72	23%	20%	25%

* intercept only models i.e. Zr ~ 1

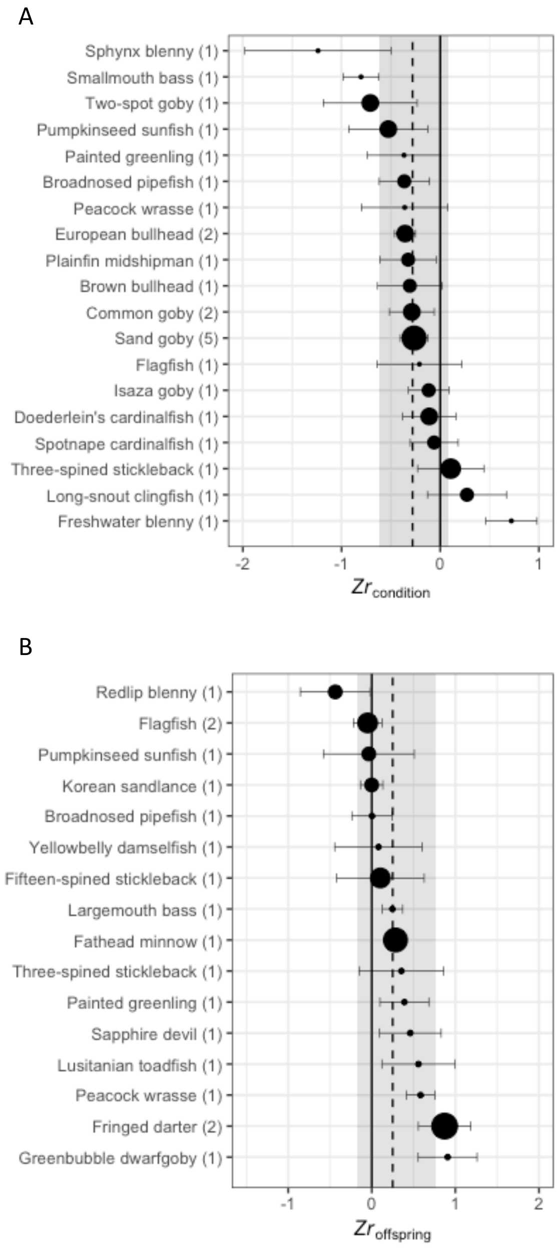

Figure 2.5. The effect of paternal care on A) male condition and B) offspring survival. Points are species-level, weighted mean effect sizes with 95% confidence intervals. The dashed line indicates the mean effect size across species with credible intervals donated by the shaded bar. Point sizes are scaled to the total number of effect sizes while the number of studies is given in brackets beside the species names.

2.3.4 Female preference for caring males

Females showed a preference for males with broods in 14/23 species, while in two species females avoided these males (Figure 2.6a). Across species, females show a significant preference for males already caring for broods ($Zr_{preference1}$ mean effect = 0.51, CI = 0.04 to 1.38, pMCMC = 0.04; $N_{species}$ = 23, $N_{studies}$ = 34, $N_{effect\ sizes}$ =57). In contrast I found no effect of brood size on female mating preference ($Zr_{preference2}$ mean effect = 0.19, CI = -0.32 to 0.7, pMCMC = 0.42; $N_{species}$ = 10, $N_{studies}$ = 15, $N_{effect\ sizes}$ = 29; Figure 2.6b).

To investigate whether the strength of female preference for males caring for broods is driven by the effort that males make when caring for larger broods, I first examined how males adjust care in relation to brood size. I found that males significantly increased their care effort when guarding larger broods ($Zr_{brood\ size}$ mean effect = 0.47, CI = 0.19 to 0.86, pMCMC = 0.006; $N_{species}$ = 25, $N_{studies}$ = 50, $N_{effect\ sizes}$ =87; Figure 2.7). Across 13 species for which I had effect sizes for both $Zr_{brood\ size}$ and $Zr_{preference1}$, I found that female preferences for males with broods were stronger in those species where males increase care with larger broods (estimate = 1.24, CI = 0.24 to 2.35, pMCMC = 0.028; Figure 2.8).

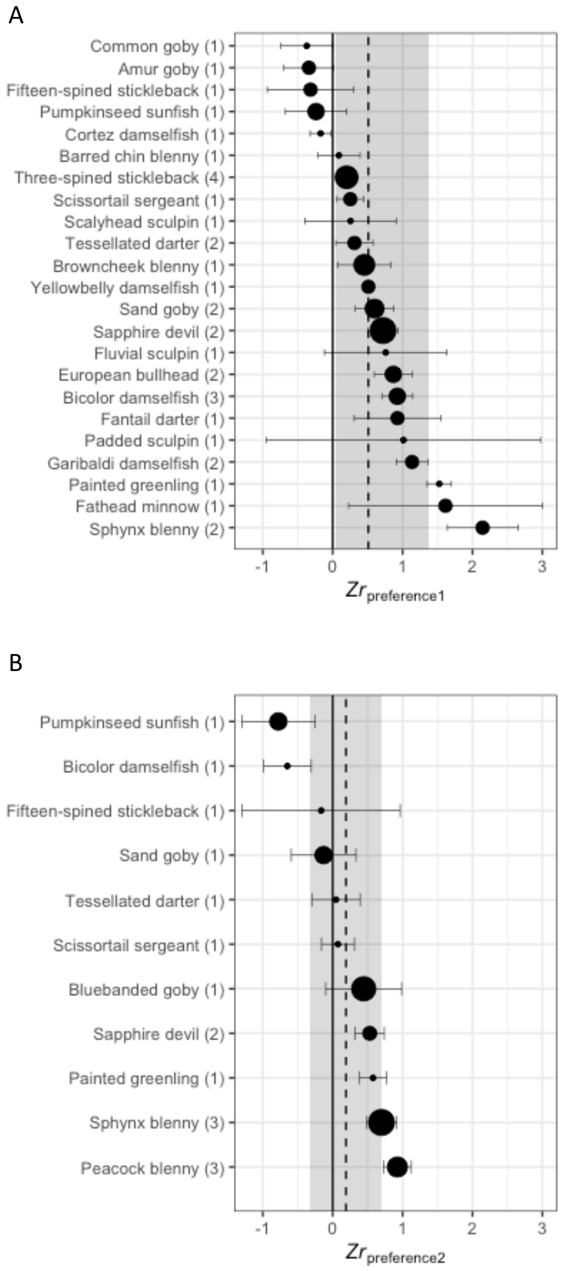

Figure 2.6. Female preference in relation to A) brood presence and B) brood size. Points are species-level, weighted mean effect sizes with 95% confidence intervals. The dashed line indicates the mean effect size across species with credible intervals donated by the shaded bar. Point sizes are scaled to the total number of effect sizes while the number of studies is given in brackets beside the species names.

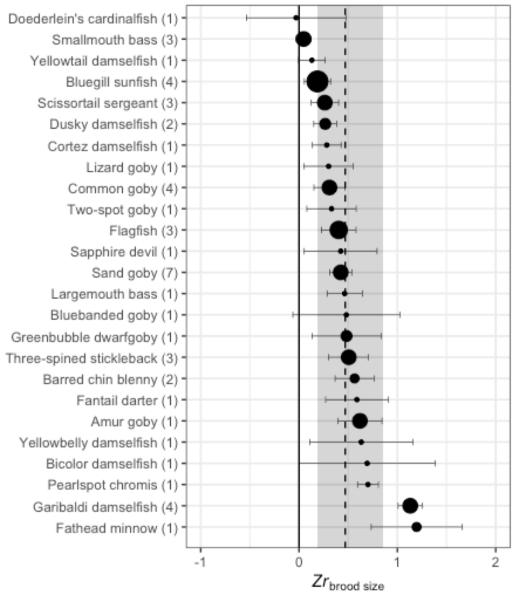

Figure 2.7. The effect of brood size on male care effort. Points are species-level, weighted mean effect sizes with 95% confidence intervals. The dashed line indicates the mean effect size across species with credible intervals donated by the shaded bar. Point sizes are scaled to the total number of effect sizes while the number of studies is given in brackets beside the species names.

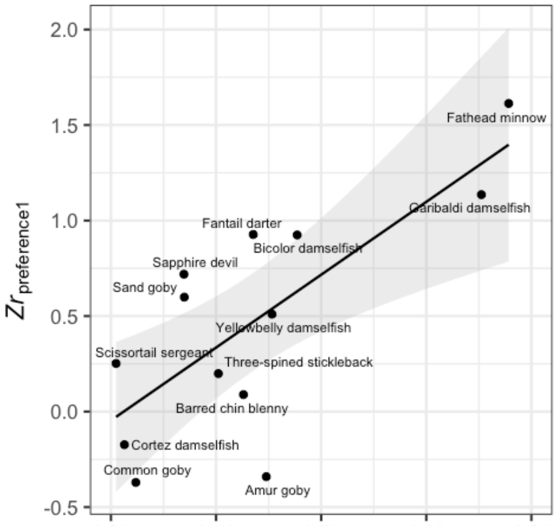

Figure 2.8. Correlation between the effect of brood presence on female preference and the effect of brood size on care. Points are species-level, weighted mean effect sizes. The line and confidence intervals (shaded bar) are predicted from the phylogenetic model.

2.3.5 Study methodology

Variation in the five effect sizes I examined ($Zr_{brood\ size}$, $Zr_{condition}$, $Zr_{offspring}$, $Zr_{preference1}$, $Zr_{preference2}$) was not explained by differences in study design or the way in which the variables were measured (for a detailed breakdown of these results, see Appendix A)

2.4 Discussion

Compared to other taxa, paternal care in fish appears to have relatively little impact on hatching success. Instead, I find that the benefit from providing care comes from increased mating success. Females prefer caring males, particularly when males increase care with more offspring. This supports the hypothesis that caring males do not suffer a mating opportunity cost and indicates that sexual selection through female choice is likely to be an important mechanism maintaining the prevalence of male-only care in fish (Alonzo 2012). In addition to making males attractive to females, care has relatively little impact on condition, either because the care that males provide is not energetically demanding, or they are able to access sufficient resources to sustain the investment required. In summary, male fish, unlike male birds and mammals, have little to lose by investing in the care of their offspring, and much to gain.

Despite the results of my analyses revealing on average little cost to care, and little benefit in terms of survival of offspring, there is evident variation in the effects of care on both male condition and offspring survival across species. However, this variation was not explained by methodological differences among studies, including differences in the way in which care and its costs and benefits were quantified. Thus, my results show that the estimation of the costs and benefits of parental care are robust to the specific methodology that a study employs. In addition to differences in study design, I considered whether this variation is influenced by the environment in which offspring develop. I predicted that the brooding environment would influence the effects of care on both offspring survival and male condition where different nest types reduce or increase the value of care or the care effort required by males. Contrary to this prediction, I found no differences in the effects of care on male condition or offspring success between any of the environments. This may indicate that, across species, selection and modification of brooding environments represent important adaptations that successfully mitigate risks to offspring and thus reduce the costs of active male care. Moreover, this may also help to explain why I failed to find any effect of care on offspring survival or male condition across species.

The relationship between care and condition, and care and offspring survival, may be influenced by additional factors which I was unable to account for. First, partial brood cannibalism, whereby parents consume some of their offspring, is common among the species in my analysis and a number of studies have investigated how this can affect both offspring survival and male condition, producing mixed results (Hoelzer 1992; Marconato et al. 1993; Payne et al. 2002; Manica 2004; Okuda et al. 2004; Klug and St Mary 2005; Klug et al. 2006; Gomagano and Kohda 2008). If males regularly compensate for energy lost during care through filial cannibalism, the observable effect of care on male condition would be reduced. Alternatively, if males cannibalise some eggs to improve the survival of their remaining brood, this positive effect of care will not be reflected in measures of hatching success. Second, the lack of studies measuring the effects of care beyond hatching means I was unable to assess the longer-term consequences of care for offspring survival and care may therefore have a greater positive effect on offspring than is evident from my study. Further work that focuses on assessing the longer-term effects of care as well as the adaptive function of filial cannibalism across species would help us to understand more fully the costs and benefits to males of offspring care, as well as the benefits to females of mating with caring males. In Chapter 3 of this thesis, I investigate whether partial brood cannibalism is likely to be driven by opportunities for future reproduction in males.

I found that, across species females prefer to mate with males already caring for the clutches of other females. Why should this be? My results showed that female preferences for males with broods were particularly strong in species where males substantially increased their care for large broods. This could indicate that females are selected to choose males already caring for broods when this will result in greater care for their offspring or reduce the likelihood of abandonment or cannibalism. However, if offspring survival is not significantly increased by male care, as my results indicate, females would not benefit from increased male care effort. An alternative explanation for this correlation is that the presence of a brood acts as an honest signal of male quality. If providing care is costly, only high-quality males will be able to raise a brood. Although I find that care does not reduce condition, this does not necessarily contradict the idea that care is a condition-dependent signal of quality, since observed levels of care may reflect what males can afford to invest without losing condition. Experiments that manipulate the costs of care, for example by increasing the perceived risk of brood predation, for males of differing quality will be necessary to determine the extent to which paternal care reliably reflects male quality in fish. In addition to using the presence of broods as a cue to male quality, females may also benefit from choosing males with broods if this dilutes the risk to their offspring of predation or filial cannibalism by the male (Rohwer 1978; Kraak and Weissing 1996; Lindström 2000). In Chapter 3 I investigate whether female choice for males with eggs may reflect a counter-strategy against paternal egg cannibalism.

A positive effect of brood size on male care indicates that males increase investment in care when mated to more females. As each female’s clutch is cared for as part of one brood, all females will receive an increase in care for their eggs and female interests are not in conflict. This contrasts with other polygynous mating systems where male care is often diluted by additional females in the group, such as in facultatively polygynous harems where females provide most of the parental care (Kempenaers 1995; Desjardins, Fitzpatrick, et al. 2008). In these species females often compete for male care creating sexual conflict over harem size which may reduce male mating success (Slagsvold and Lifjeld 1994). In Chapter 5 I investigate if mating competition between females is driven by male contributions to brood defence in the harem-breeding cichlid *Lamprologus ocellatus* and how this may influence harem size. In this species contributions to brood defence by males may alleviate some of the costs of a trade-off between competition and care in females.

Although my results suggest that, on average, females prefer males already providing care, this preference was not universal. In addition, across species there was no evidence that females preferred males with large broods to those with small broods. Large broods may be associated with increased egg mortality due to reduced oxygen availability (Jones and Reynolds 1999), increased risk of disease transmission (Lehtonen and Kvarnemo 2015), or filial cannibalism by males if the demands of care are increased by larger broods (Kvarnemo et al. 1998; Klug et al. 2006). Larger broods also often contain older clutches which are more valuable to the male leaving younger clutches vulnerable to cannibalism or abandonment (Petersen and Marchetti 1989; Sikkel 1994). Such effects may reduce the benefits to females of choosing males with large brood sizes, thus helping to explain why we see a general preference for males with broods but not for males with larger versus smaller broods. A clear example of this is seen in the painted greenling, where male care significantly improves offspring survival and where hatching success shows an initial increase with brood size but then declines sharply beyond eight clutches (DeMartini 1987). In this species, females prefer males with a brood to those without but do not discriminate between brood sizes.

In general, we expect selection to favour use of the most cost-effective cues that permit accurate assessment of males (Candolin 2003). In this respect, the presence or absence of a brood is simpler information to process than continuous brood number, since the latter would require a mechanism for quantity discrimination (Agrillo et al. 2008). By choosing males with any number of eggs, females can quickly identify caring males without incurring high processing costs. It has also been hypothesised that female choice on the basis of brood presence/absence reduces sampling costs by copying the choices of other females (Pruett-Jones 1992). Other traits used by female fish to assess males include body-size (Hanson and Cooke 2009; Stiver and Alonzo 2010), colour (Haley et al. 2004), nest quality (Hoelzer 1990) and courtship displays (Knapp and Kovach 1991; Haley et al. 2004). In some species, these traits may provide more accurate information about a male's quality; in the fifteen-spined stickleback (*Spinachia spinachia*), for example, females show no preference based on brood size but instead prefer males that demonstrate better care (fanning of eggs) during courtship (Östlund and Ahnesjö 1998; Ostlund-Nilsson 2002).

Male-only care is more common in fish than in any other taxonomic group, but examples of this strategy are nonetheless widespread. In arthropods, species with male-only care share similar traits to those observed in fish including association between the male and the eggs and simultaneous paternal care and mating (Tallamy 2000). Female choice for caring males has also been found in some species, such as in the harvestman *Iporangaia pustulosa* (Requena and Machado 2015) and the assassin bug *Rhinocoris tristis* (Gilbert et al. 2010). Similarly, in amphibians, external fertilisation combined with male defence of nest sites within territories has been linked to the evolution of male care (Wells 2007). Simultaneous care and mating has also been shown in a number of species, although it is generally limited to species that attend to but do not transport eggs or larvae (Hoskin 2004; Summers and Tumulty 2014). It is possible that similar selective processes are responsible for male-only care in these groups but further studies are needed for both arthropods and amphibians before solid comparisons can be drawn.

In contrast, male-only care in birds appears to result through different processes. Bird species with male-only care often exhibit classical polyandry, where males do not benefit from additional matings when providing care (Andersen 2005). A study by Owens (2002) showed that bird families with male-only care were characterised by low remating opportunities for both sexes compared to families with female-only care. This suggests that the trade-off between care and mating opportunity is important in determining the costs of parental care for male birds. Similarly, sex-role reversal is common within polyandrous birds, whereby females compete for access to males, in contrast to many fish where males remain the more competitive sex and compete for access to territories that will attract females (Rios-Cardenas 2005). Parental care in birds is thought to be highly demanding and the risk of adult predation increased in contrast to the low condition costs observed in fish (Montgomerie and Weatherhead 1988; Peterson et al. 1990). However, male-only care is predominantly found in bird species with precocial young where the demands of care are reduced. Additionally, in some bird groups, such as the ratites and megapodes, there are species where males care for the eggs of multiple females which resembles more closely the situation observed in fish (Cockburn 2006).

In summary, this study provides support for the hypothesis that male care in fish has evolved by sexual selection, and not as a strategy to simply increase offspring survival as in other taxa. The simple nature of parental care in fish and a territorial system, whereby males guard territories that attract females, creates an environment in which caring for offspring and continuing to mate can be achieved simultaneously. Although a similar situation is found in some arthropods and amphibians, this contrasts to the majority of taxa where males lose mating opportunities by providing care. In addition to this, males are shown to increase the amount of effort they put into caring for larger broods which may encourage females to choose males that are already guarding a brood. These results suggest that sexual selection does not always act predominantly on the less caring sex, but can select for increased care investment in males and lead to largely different parental care patterns in different taxonomic groups.

Appendix A

Supplementary material for Chapter 2

A.1 Inclusion of the Syngnathidae in the calculation of Zr$_{offspring}$

Among teleosts, pipefish and seahorses (family Syngnathidae) exhibit a unique form of paternal care, in which offspring are cared for within a specialised internal pouch (Stölting and Wilson 2007). This form of care is likely to be vital for offspring development and survival; however, there are no published estimates of the effect of brooding by males on offspring survival. To explore the influence of obligate paternal care in the Syngnathidae on my estimate of the mean effect of paternal care on offspring survival (Zr$_{offspring}$), I performed two additional analyses, in which I added either 1 or 99 species of syngnathid to my existing dataset (this approach was taken to investigate the effect of varying the number of representatives on the estimate of the mean effect size). Since offspring are assumed to be unable to survive outside of the father's brood pouch, I assigned a coefficient of 0.999 for the correlation between care and offspring survival, giving an effect size (Zr) of 6.1 (It is not possible to assign a correlation coefficient of 1, since this would result in a Zr value of infinity). As there was no sample size associated with this effect size from which to calculate the sampling variance, I used the mean sampling variance from the main dataset. Species were added to the phylogeny at the position of *Syngnathus typhle*.

The addition of effect sizes for syngnathid species increased the mean effect size for $Zr_{offspring}$ and this increase was greater when 99 species were added compared to 1 species. However, in both MCMCglmm analyses there was no significant effect of care on offspring survival across species (Table A.1).

Table A.1. $Zr_{offspring}$ model results with the addition of 1 and 99 species representatives of the Syngnathidae family.

No. added syngnathids	R package	Mean $Zr_{offspring}$	Lwr CI	Upr CI	I^2 phylo	N (species / effect sizes)
1	metafor	0.44	0.07	0.82	0%	17/37
	MCMCglmm	0.43	-0.52	1.33	8%	
99	metafor	0.83	-0.97	2.62	91%	115/135
	MCMCglmm	0.61	-0.84	2.92	92%	

A.2 Moderator variables

Table A.2. Moderator variables analysed for each of the five effect sizes

	Effect Size				
	$Zr_{condition}$	$Zr_{offspring}$	$Zr_{preference1}$	$Zr_{preference2}$	$Zr_{brood\ size}$
Study design					
Laboratory vs. field	Yes	Yes	Yes	Yes	Yes
Observational vs. experimental	Yes	Yes	Yes	Yes	Yes
Brood size: Natural vs. manipulated	-	-	Yes	Yes	Yes
Brood Size: Natural vs. removed	-	-	Yes	-	-
Care: Continuous vs. binary	Yes	Yes	-	-	Yes
Study measure					
Care measure	Yes	Yes	-	-	Yes
Condition measure	Yes	-	-	-	-
Offspring measure	-	Yes	-	-	-
Brood size measure	-	-	-	-	Yes
Preference measure	-	-	Yes	Yes	-
Brooding environment	Yes	Yes	-	-	-

A.2.1 Differences in $Zr_{condition}$ and $Zr_{offspring}$ between brooding environments

I found no difference in $Zr_{condition}$ between species brooding in brood pouches (1 species; mean = -0.27, CI = -1.2 to 0.62), covered nests (4 species; mean = -0.08, CI = -0.62 to 0.41), covered substrates (7 species; mean = -0.30, CI = -0.8 to 0.11), open nests (3 species; mean = -0.42, CI = -1.16 to 0.06), or open substrates (2 species; mean = -0.46, CI = -1.1 to 0.46), or mouth-brooding (2 species; mean = 0.03, CI = -0.76 to 0.69; Figure A.1a; Table A.3). I also found no difference in $Zr_{offspring}$ between species brooding in brood pouches (1 species; mean = -0.1, CI = -1.33 to 1.51), covered nests (1 species; mean = 0.63, CI = -0.92 to 2.04), covered substrates (6 species; mean = 0.5, CI = -0.33 to 1.12), open nests (5 species; mean = 0.26, CI = -0.63 to 0.86), or open substrates (3 species; mean = -0.02, CI = -0.74 to 0.91; Figure A.1b; Table A.4).

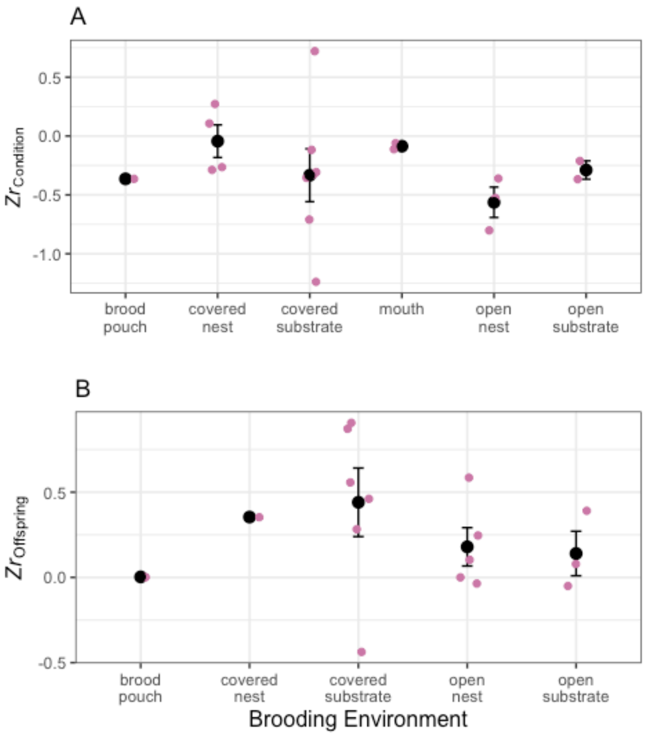

Figure A.1. Estimates of A) $Zr_{condition}$ and B) $Zr_{offspring}$ from species with different brooding environments. Means ± SE across species are in black and coloured points indicate species means.

Table A.3. P values comparing the results for $Zr_{condition}$ between different brooding environments.

Brooding Environment	Brood pouch	Covered nest	Covered substrate	Mouth	Open nest	Open substrate
Brood pouch	-	0.30	0.44	0.29	0.65	0.47
Covered nest	-	-	0.74	0.45	0.90	0.70
Covered substrate	-	-	-	0.29	0.81	0.56
Mouth	-	-	-	-	0.89	0.70
Open nest	-	-	-	-	-	0.30
Open substrate	-	-	-	-	-	-

Table A.4. P values comparing the results for $Zr_{offspring}$ between different brooding environments.

Brooding Environment	Brood pouch	Covered nest	Covered substrate	Open nest	Open substrate
Brood pouch	-	0.59	0.77	0.61	0.53
Covered nest	-	-	0.57	0.52	0.55
Covered substrate	-	-	-	0.44	0.41
Open nest	-	-	-	-	0.44
Open substrate	-	-	-	-	-

A.2.2 Study design

1. Field vs. lab studies

$Zr_{condition}$ was calculated from 24 effect sizes from field studies and 19 from laboratory studies and there was no difference between these in mean effect (field = -0.16, CI = -0.67 to 0.18; lab = -0.27, CI = -0.84 to 0.19; pMCMC=0.74; Figure A.2a). $Zr_{offspring}$ was calculated from 13 effect sizes from field studies and 23 from laboratory studies and there was no difference between these in mean effect (field = 0.17, CI = -0.31 to 0.80; lab = 0.30, CI = -0.25 to 0.92; pMCMC=0.86; Figure A.2b). $Zr_{preference1}$ was calculated from 30 effect sizes from field studies and 27 from laboratory studies and there was no difference between these in mean effect (field = 0.43, CI = -0.08 to 1.47; lab = 0.58, CI = -0.1 to 1.43; pMCMC=0.87; Figure A.2c). $Zr_{preference2}$ was calculated from 19 effect sizes from field studies and 11 from laboratory studies and there was no difference between these in mean effect (field = 0.42, CI = -0.92 to 1.38; laboratory = 0.09, CI = -1.31 to 1.16; pMCMC=0.38; Figure A.2d). $Zr_{brood\ size}$ was calculated from 51 effect sizes from field studies and 36 from laboratory studies and there was no difference between these in mean effect (field = 0.36, CI = 0.13 to 0.89; lab = 0.54, CI = 0.15 to 0.90; pMCMC=0.66; Figure A.2e).

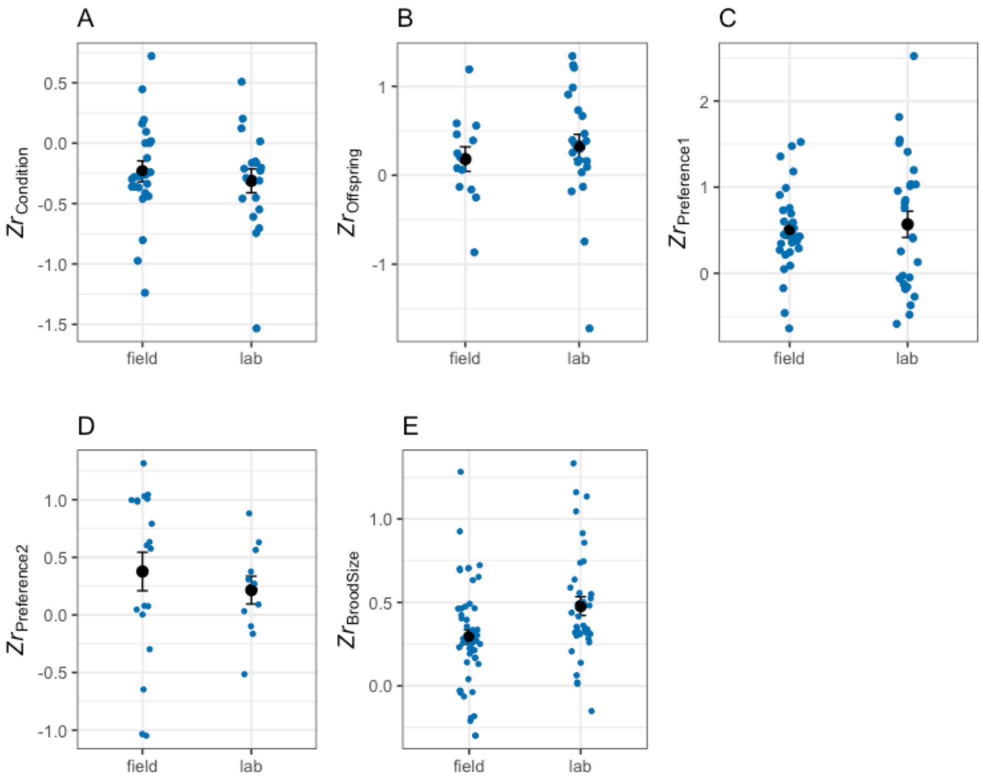

Figure A.2. Estimates of $Zr_{condition}$ (A), $Zr_{offspring}$ (B), $Zr_{preference1}$ (C), $Zr_{preference2}$ (D) and $Zr_{brood\ size}$ (E), from field and laboratory studies. Means ± SE are shown in black.

2. Observational vs. experimental studies

$Zr_{condition}$ was calculated from 24 effect sizes from observational studies and 19 from experimental studies and there was no difference between these in mean effect size (observational = -0.25, CI = -0.58 to 0.14; experimental = -0.30, CI = -0.74 to 0.10; pMCMC=0.62; Figure A.3a). $Zr_{offspring}$ was calculated from 11 effect sizes from observational studies and 25 from experimental studies and there was no difference between these in mean effect size (observational = 0.17, CI = -0.31 to 0.80; experimental = 0.30, CI = -0.25 to 0.92; pMCMC = 0.51; Figure A.3b). $Zr_{preference1}$ was calculated from

11 effect sizes from observational studies and 46 from experimental studies and there was no difference between these in mean effect size (observational = 0.63, CI = -0.1 to 1.47; experimental = 0.48, CI = -0.08 to 1.32; pMCMC=0.49; Figure A.3c). $Zr_{preference2}$ was calculated from 5 effect sizes from observational studies and 25 from experimental studies and there was no difference between these in mean effect size (observational = 0.42, CI = -0.92 to 1.38; experimental = 0.09, CI = -1.31 to 1.16; pMCMC=0.66; Figure A.3d). $Zr_{brood\ size}$ was calculated from 24 effect sizes from observational studies and 63 from experimental studies and there was no difference between these in mean effect size (observational = 0.36, CI = 0.13 to 0.89; experimental = 0.54, CI = 0.15 to 0.90; pMCMC=0.76; Figure A.3e)

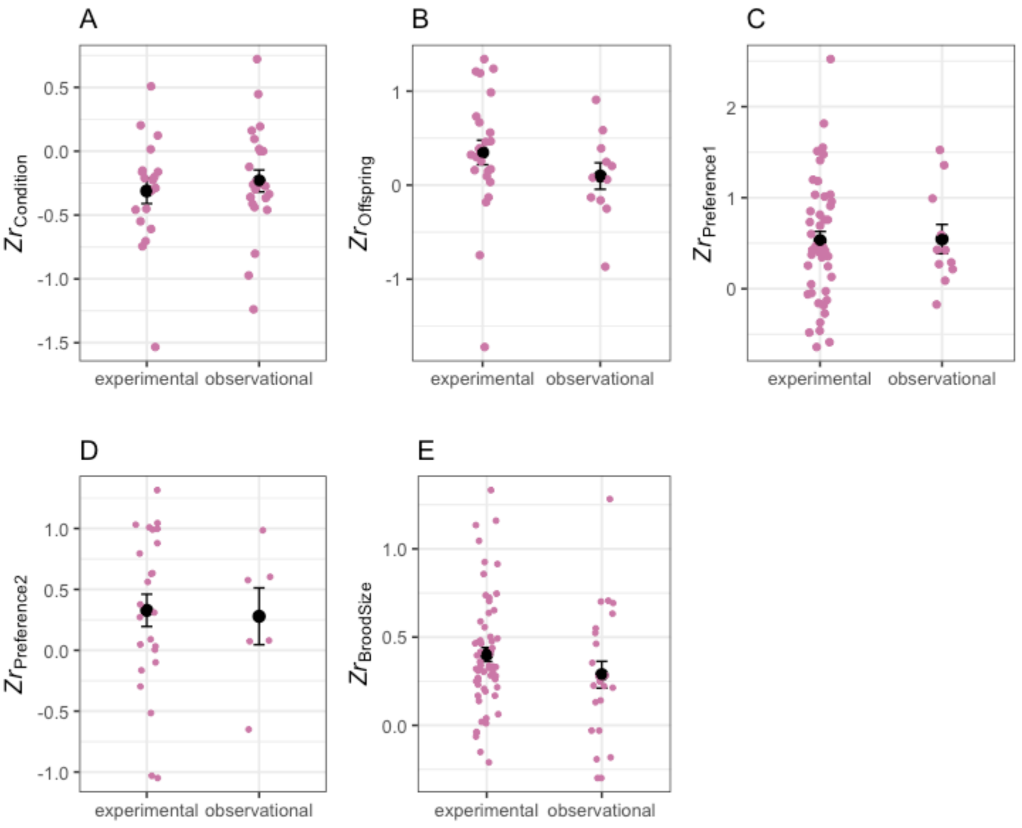

Figure A.3. Estimates of $Zr_{condition}$ (A), $Zr_{offspring}$ (B), $Zr_{preference1}$ (C), $Zr_{preference2}$ (D) and $Zr_{brood\ size}$ (E), from experimental and observational studies. Means ± SE are shown in black.

3. Continuous vs. binary care measure

$Zr_{condition}$ was calculated from 32 effect sizes measuring the amount of care and 11 effect sizes comparing carers and non-carers and there was no difference between these in mean effect (continuous = -0.29, CI = -0.63 to 0.08; binary = -0.22, CI = -0.63 to 0.22; pMCMC = 0.76; Figure A.4a). $Zr_{offspring}$ was calculated from 16 effect sizes measuring the amount of care and 20 effect sizes comparing broods with and without a carer and there was no difference between these in mean effect (continuous = 0.30, CI = -0.32 to 0.77; binary = 0.30, CI = -0.24 to 0.87; pMCMC = 0.75; Figure A.4b). $\mathrm{Zr}_{\mathrm{brood\ size}}$ was calculated from 56 effect sizes measuring the amount of care and 31 effect sizes comparing care vs. abandonment or full cannibalism and there was no difference between these in mean effect (continuous = 0.42, CI = 0.12 to 0.85; binary = 0.48, CI = 0.2 to 0.95; pMCMC = 0.49; Figure A.4c).

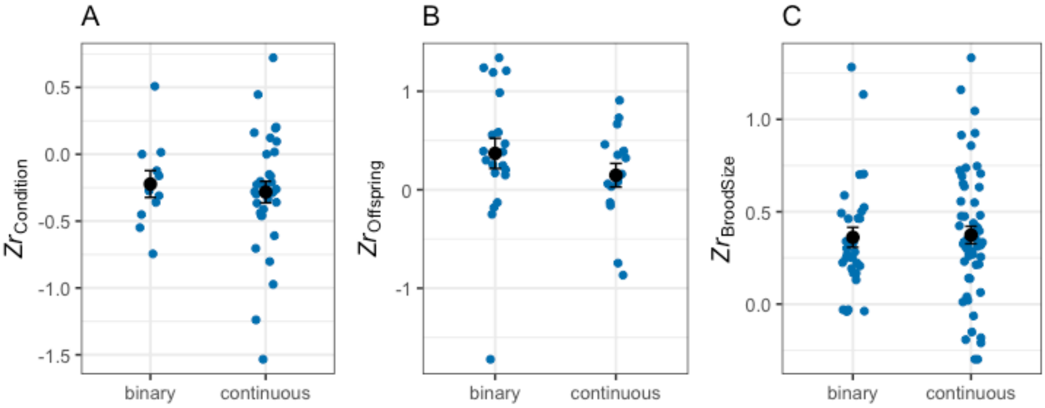

Figure A.4. Estimates of $Zr_{condition}$ (A), $Zr_{offspring}$ (B) and $Zr_{brood\ size}$ (C), from studies using a continuous or a binary measure of care. Means ± SE are shown in black.

4. Natural vs. experimental brood size manipulations

$Zr_{preference1}$ was calculated from 18 studies with natural variation in brood size and 39 studies where brood size was experimentally manipulated and there was no difference between these in mean effect (manipulated = 0.70, CI = -0.07 to 1.33; natural = 0.48, CI = 0.01 to 1.52; pMCMC = 0.97; Figure A.5a). $Zr_{preference1}$ was also calculated from 27 effect sizes where the no brood treatment had been created by removing a male's brood and 30 effect sizes where these males had never had a brood and there was no difference between these in mean effect (brood removal = 0.55, CI = -0.10 to 1.42; no brood removal = 0.51, CI = -0.06 to 1.41; pMCMC = 0.80; Figure A.6).

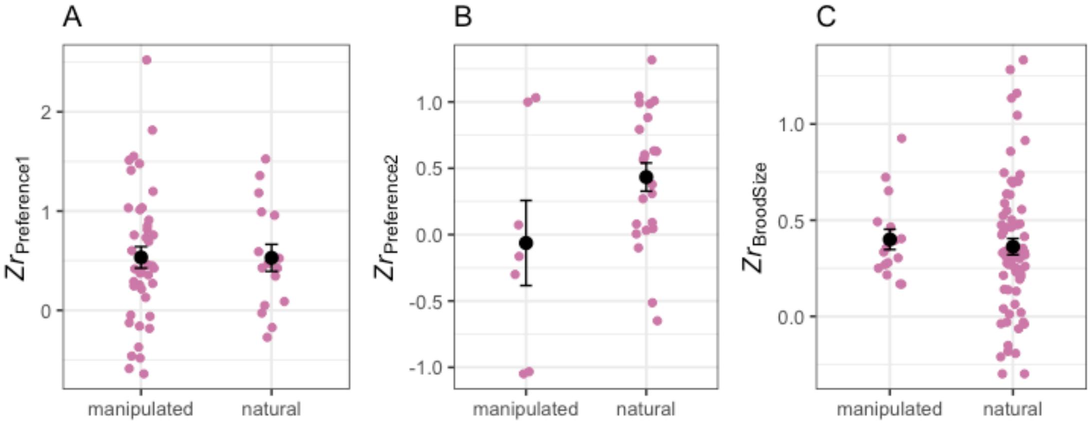

Figure A.5. Estimates of $Zr_{preference1}$ (A), $Zr_{preference2}$ (B) and $Zr_{brood\ size}$ (C), from studies where differences in brood size between males were natural or manipulated. Means ± SE are shown in black.

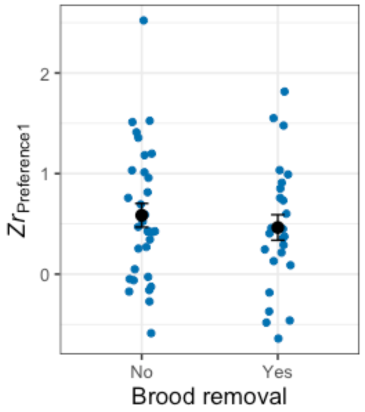

Figure A.6. Estimates of $Zr_{preference1}$ from studies where a male's brood was removed to create a no-brood treatment and those where it was naturally absent. Means ± SE are shown in black.

$Zr_{\text{preference2}}$ was calculated from 23 studies with natural variation in brood size and 7 studies where brood size was experimentally manipulated and there was no difference between these in mean effect (manipulated = 0.12, CI = -0.82 to 1.26; natural = 0.11, CI = -1.22 to 1.17; pMCMC = 0.56; Figure A.5b). $Zr_{\text{brood size}}$ was calculated from 71 studies with natural variation in brood size and 16 studies where brood size was experimentally manipulated and there was no difference between these in mean effect (manipulated = 0.45, CI = 0.18 to 1.00; natural = 0.48, CI = 0.14 to 0.87; Figure A.5c).

A.2.3 Study measures

1. $Zr_{\text{condition}}$

I found no differences in $Zr_{condition}$ between studies measuring male condition as condition factor (15 effect sizes; mean =-0.10, CI = -0.55 to 0.20), growth rate (4 effect sizes; mean =-0.15, CI = -0.93 to 0.41), HSI (6 effect sizes; mean =-0.25, CI = -0.73 to 0.16), weight change (14 effect sizes: mean = -0.34, CI = -0.79 to 0.01), lipid mass (3 effect sizes; mean

= -0.24, CI = -0.90 to 0.39), or survival (1 effect size: mean = -0.30, CI = -1.00 to 0.63) (Figure A.7; Table A.5).

Table A.5. P values comparing the results for $Zr_{condition}$ between different levels of condition measure.

Condition measure	Condition factor	Growth rate	HSI	Lipid	Survival	Weight change
Condition factor	-	0.59	0.77	0.61	0.53	0.88
Growth rate	-	-	0.57	0.52	0.55	0.66
HSI	-	-	-	0.44	0.41	0.64
Lipid	-	-	-	-	0.44	0.68
Survival	-	-	-	-	-	0.66
Weight change	-	-	-	-	-	-

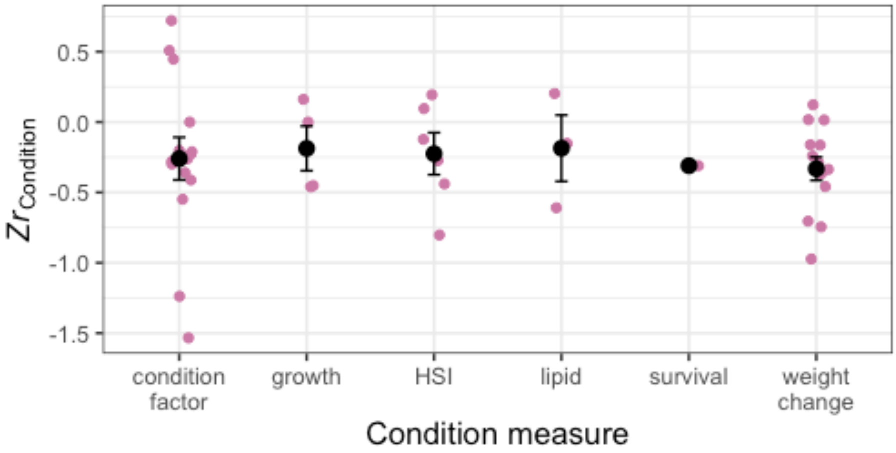

Figure A.7. Estimates of $Zr_{condition}$ from studies using different measurements of male condition. Means ± SE are shown in black.

Similarly, there was no differences in $Zr_{condition}$ between studies measuring male care as presence/absence of a brood (11 effect sizes; mean =-0.12, CI = -0.75 to 0.23), care period (17 effect sizes; mean = -0.37, CI = -0.76 to 0.14), defence (5 effect sizes; mean = -0.01, CI = -0.72 to 0.60), fanning (7 effect sizes; mean = -0.32, CI = -0.77 to 0.42), or number of broods in a season (3 effect sizes; mean = -0.07, CI = -1.12 to 0.90) (Figure A.8; Table A.6).

Table A.6. P values comparing the results for $Zr_{condition}$ between different levels of care measure.

Care measure	Brood P/A	Care period	Defence	Fanning	No. of broods
Brood present/absent	-	0.75	0.26	0.54	0.43
Care period	-	-	0.14	0.33	0.34
Defence	-	-	-	0.73	0.59
Fanning	-	-	-	-	0.43
Number of broods	-	-	-	-	-

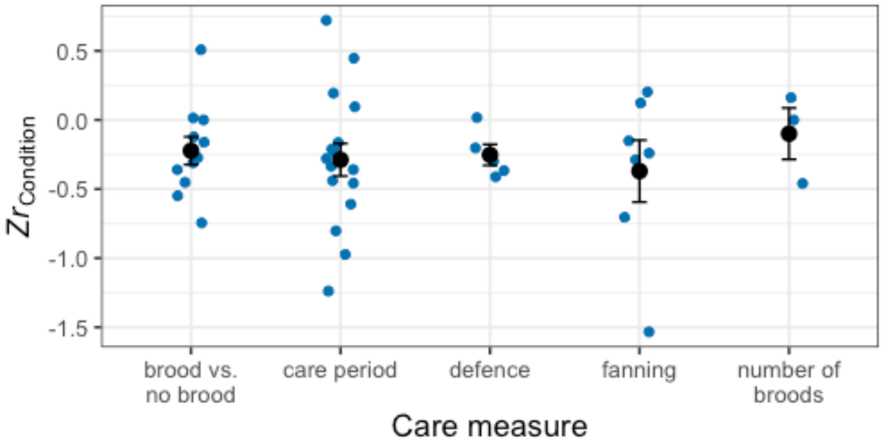

Figure A.8. Estimates of $Zr_{condition}$ from studies using different measurements of male care. Means ± SE are shown in black.

2. $Zr_{offspring}$

I found no differences in $Zr_{offspring}$ between studies measuring offspring survival as the number of eggs hatched (7 effect sizes; mean =0.32, CI = -0.32 to 0.98), the proportion of eggs hatched (28 effect sizes; mean =0.32, CI = -0.33 to 0.72), or the probability of a clutch surviving (1 effect size: mean = 0.36, CI = -0.15 to 1.58) (Figure A.9; Table A.7).

Table A.7. P values comparing the results for $Zr_{offspring}$ between different levels of offspring survival measure.

Offspring survival measure	Number of eggs hatched	Proportion of eggs hatched	Probability of clutch hatching
Number eggs hatched	-	0.57	0.73
Proportion eggs hatched	-	-	0.53
Probability of clutch hatching	-	-	-

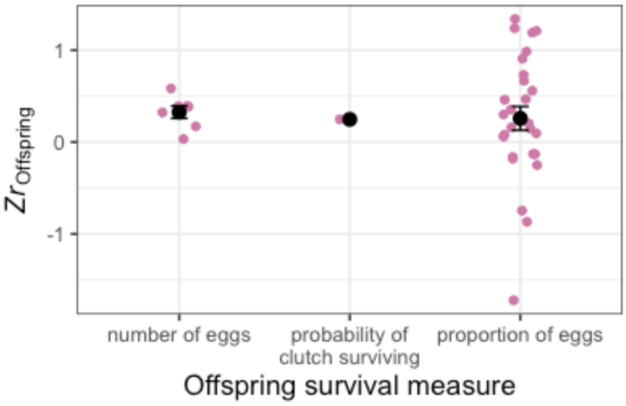

Figure A.9. Estimates of $Zr_{offspring}$ from studies using different measurements of offspring survival. Means ± SE are shown in black.

I also found no differences in $Zr_{offspring}$ between studies measuring male care as presence/absence of a male (21 effect sizes; mean =0.33, CI = -0.26 to 0.83), care duration (1 effect sizes; mean =0.05, CI = -1.29 to 1.42), defence (5 effect sizes; mean = 0.00, CI = -0.64 to 0.81), fanning (8 effect sizes; mean = 0.31, CI = -0.32 to 1.17), or care index (1 effect sizes; mean = -0.11, CI = -1.55 to 1.33) (Figure A.10; Table A.8).

Table A.8. P values comparing the results for $Zr_{offspring}$ between different levels of care measure.

Care measure	Nest attendance	Defence	Care duration	Fanning	Male p/a
Nest attendance	-	0.45	0.50	0.34	0.34
Defence	-	-	0.54	0.26	0.23
Care duration	-	-	-	0.34	0.34
Fanning	-	-	-	-	0.48
Male present/absent	-	-	-	-	-

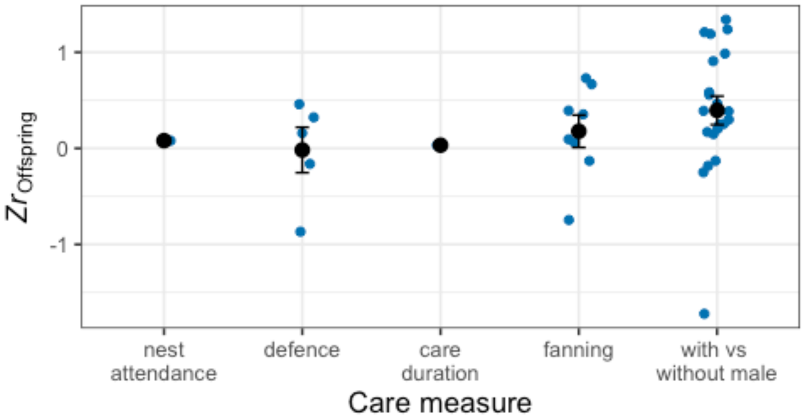

Figure A.10. Estimates of $Zr_{offspring}$ from studies using different measurements of male care. Means ± SE are shown in black.

3. $Zr_{preference1}$

I found no differences in $Zr_{preference1}$ between studies measuring female preference as spawning choice (27 effect sizes; mean =0.68, CI = -0.02 to 1.50), association preference (9 effect sizes; mean =0.56, CI = -0.10 to 1.47), or brood size (21 effect sizes; mean =0.58, CI = -0.07 to 1.42) (Figure A.11; Table A.9).

Table A.9. P values comparing the results for $Zr_{preference1}$ between different levels of preference measure.

Preference measure	Association preference	Brood size	Spawning choice
Association preference	-	0.55	0.54
Brood size	-	-	0.48
Spawning choice	-	-	-

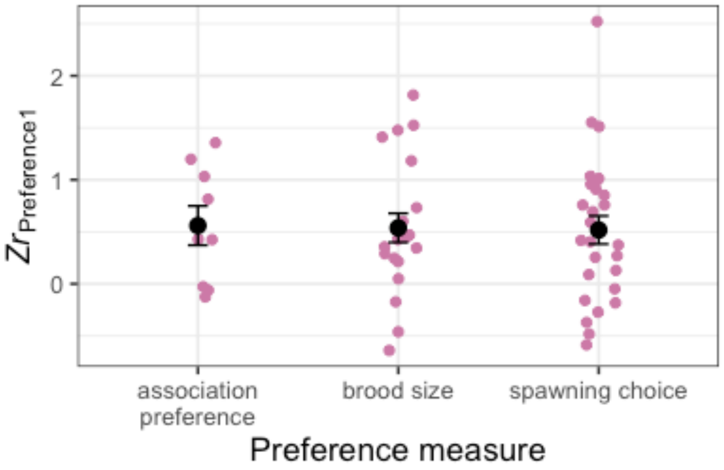

Figure A.11. Estimates of $Zr_{preference1}$ from studies using different measurements of female preference. Means ± SE are shown in black.

4. $Zr_{preference2}$

I found no differences in $Zr_{preference2}$ between studies measuring female preference as spawning choice (5 effect sizes; mean =0.28, CI = -1.08 to 1.39), association preference (7 effect sizes; mean =0.35, CI = -1.05 to 1.65), or brood size (18 effect sizes; mean =0.25, CI = -1.12 to 1.11) (Figure A.12; Table A.10).

Table A.10. P values comparing the results for $Zr_{preference2}$ between different levels of preference measure.

Preference measure	Association preference	Brood size	Spawning choice
Association preference	-	0.76	0.74
Brood size	-	-	0.42
Spawning choice	-	-	-

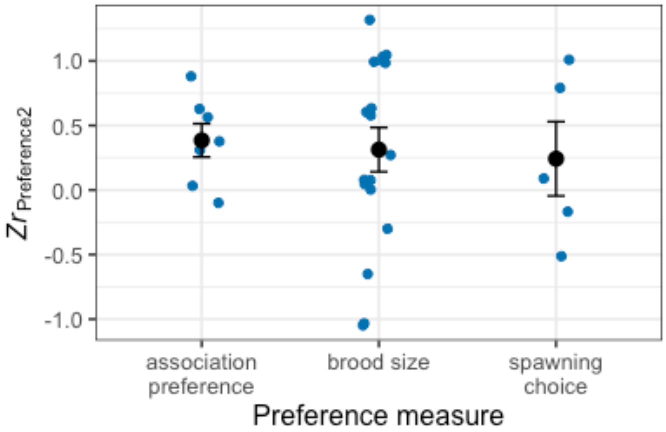

Figure A.12. Estimates of $Zr_{preference2}$ from studies using different measurements of female preference. Means ± SE are shown in black.

5. $Zr_{\text{brood size}}$

I found no differences in $Zr_{brood\ size}$ between studies measuring brood size as brood area (1 effect size; mean =0.74, CI = -0.26 to 1.58), absolute number of eggs (54 effect sizes; mean =0.45, CI = 0.18 to 0.88), or size category (32 effect sizes; mean =0.45, CI = 0.16 to 0.91) (Figure A.13; Table A.11).

Table A.11. P values comparing the results for $Zr_{brood\ size}$ between different levels of brood size measure.

Brood size measure	Brood area	Absolute egg number	Brood size category
Brood area	-	0.69	0.73
Absolute egg number	-	-	0.62
Brood size category	-	-	-

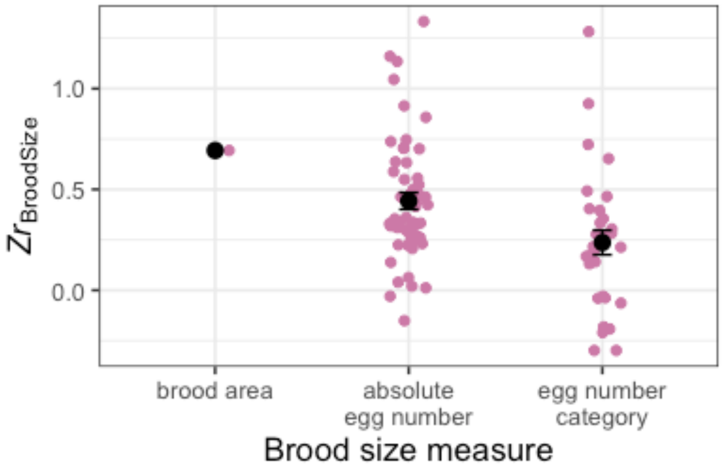

Figure A.13. Estimates of $Zr_{brood\ size}$ from studies using different measurements of brood size. Means ± SE are shown in black.

Similarly I found no differences in $Zr_{brood\ size}$ between studies measuring male care as care provided or brood abandoned (11 effect sizes; mean = 0.52, CI = 0.17 to 1.04), care provided or brood cannibalised (20 effect sizes; mean = 0.49, CI = 0.15 to 0.94), defence (24 effect sizes; mean =0.37, CI = 0.04 to 0.83), fanning (12 effect sizes; mean = 0.49, CI = 0.06 to 0.95), or care index (20 effect sizes; mean = 0.39, CI = 0.09 to 0.91) (Figure A.14; Table A.12).

Table A.12. P values comparing the results for $Zr_{brood\ size}$ between different levels of care measure.

Brood size measure	Care or abandon	Care or cannibalise	Defence	Fanning	Care index
Care or abandon	-	0.62	0.86	0.64	0.74
Care of cannibalise	-	-	0.79	0.56	0.64
Defence	-	-	-	0.31	0.30
Fanning	-	-	-	-	0.57
Care index	-	-	-	-	-

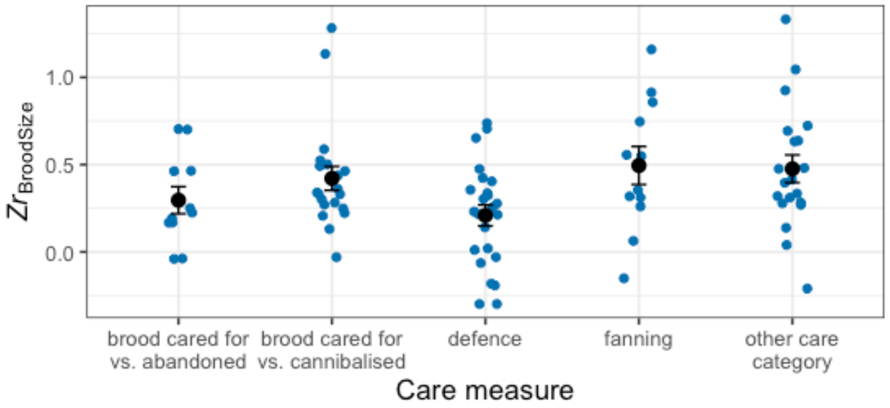

Figure A.14. Estimates of $Zr_{brood\ size}$ from studies using different measurements of male care. Means ± SE are shown in black.

Chapter 3

Sexual conflict over offspring care in fish: filial cannibalism by males and female counter-strategies

Rebecca Goldberg, Ashleigh Griffin, Jonathan Green

Author contributions: Jonathan Green, Ashleigh Griffin and I conceived of and designed the study. I ran the literature search and compiled and analysed the data. I wrote the chapter and incorporated comments provided by Jonathan Green and Ashleigh Griffin.

Abstract

Through abandonment, infanticide and cannibalism, parents often play an active role in determining which of their offspring do and do not survive. In teleost fish, parental care is most often provided by the male alone and filial cannibalism by the father is common. If partial brood cannibalism improves survival of the remaining offspring, mothers should favour egg-eating by males. If, however, males cannibalise eggs to obtain energy for future reproductive attempts, this will result in sexual conflict between parents. Using a comparative analysis of twenty-eight fish species, I show that males under stronger sexual selection cannibalise more offspring, suggesting that the opportunity for future reproduction is an important driver of this behaviour. Furthermore, I find that females prefer to spawn with males that are already caring for broods in species where the risk of

cannibalism by fathers is relatively high. Finally, I show that by adding eggs to existing broods, mothers can dilute the risk of their eggs being cannibalised. Female preference for caring males, therefore, represents a strategy by which females can minimise reproductive costs arising from selfish investment by males in future mating success.

3.1 Introduction

In many species, parental care is essential for offspring survival, yet offspring are not always safe in their parents' care. Abandonment, infanticide and cannibalism by parents is widespread across animals (Hausfater and Blaffer Hrdy 1984) but the adaptive value of these behaviours is often unclear. If the costs of continuing care outweigh potential fitness benefits, abandoning the brood, or recuperating energy invested in reproduction through cannibalism, may be beneficial. In birds, intense predation or food shortages can trigger the desertion of eggs or nestlings (Magrath 1990). Additionally, broods of low reproductive value are often abandoned, particularly when opportunities for remating are high (FitzGerald 1992). Alternatively, by killing or consuming part of a brood, parents may improve conditions for offspring that remain, in which case offspring destruction can be considered a form of parental care (Mock and Parker 1986; Mock et al. 2009; Davenport et al. 2019). For instance, when care is depreciable across offspring, infanticide can be a means of brood reduction when sufficient resources to rear all offspring are not available (O'Connor 1978; Trumbo 1990; Elwood 1992).

Although observed in numerous taxa, infanticide through filial cannibalism is particularly widespread in teleost fish species exhibiting male-only care (Manica 2002). In these species, males often care for clutches from multiple females simultaneously, over several brood cycles, and fathers have been observed to consume a proportion of the clutch while continuing to care for the remaining eggs (Gross and Sargent 1985; Ah-King et al. 2005). Where paternal care imposes energetic costs, egg consumption may allow males to maintain their condition for future breeding attempts or to continue caring for the current brood until independence (Rohwer 1978; Manica 2002). In several species, cannibalism has been shown to improve male condition when food availability is low (Lindström and Craig Sargent 1997; Manica 2004; Takeyama et al. 2013). Alternatively, cannibalism may represent a care behaviour that improves the survival of remaining offspring e.g. through the targeted removal of diseased eggs (Kraak 1996; Bandoli 2016; Vallon et al. 2016).

From the mother's perspective, filial cannibalism by the father is only beneficial if it increases the success of her offspring; she is not likely to have an interest in a male's prospects of future matings with other females (Lindström 2000). In addition, although her offspring may benefit from the care of a father sustained through egg cannibalism, since broods often consist of clutches from multiple females, there is likely to be conflict over whose eggs are sacrificed. As a result, if energetic benefits are driving filial cannibalism, selection is expected to favour female counter-strategies that minimise the loss of offspring to cannibalistic males. However, it currently remains unclear whether partial brood cannibalism in fish is primarily in the shared interests of all parents or likely to be a source of conflict.

Here I use a comparative analysis across fish species with male-only care to investigate whether partial brood cannibalism may be driven by the energetic demands of territoriality in males and whether female mate choice for males with eggs represents a strategy for diluting the risk of egg cannibalism. In fish, male-only care is closely associated with male territoriality, with mating and offspring care occurring within the male's territory (Gross and Sargent 1985; Ah-King et al. 2005). Males that can acquire and defend territories, therefore, receive a greater number of matings, resulting in strong male-male competition for territories. This can generate, *inter alia*, selection for large body size (an important determinant of fighting ability in fish) and male-biased sexual size dimorphism (Pyron et al. 2013; Horne et al. 2020). If cannibalism is driven by energetic demands, males would be expected to cannibalise more when they are under stronger intrasexual selection, since these males would require more resources to invest in competition.

In this analysis I use sexual size dimorphism as an easily measured proxy for the strength of sexual selection acting on males. In their comparative analysis of over 600 fish species, Horne et al. (2020) show that sexual size dimorphism is significantly predicted by the intensity of sexual selection for increased male size. In fish, as in other taxa, sexual size dimorphism therefore provides an indirect measure of the strength of sexual selection acting on males through competition for mates (Soulsbury et al. 2014; Janicke and Fromonteil 2021). In the first part of my analysis I tested whether cannibalism by fathers is associated with stronger sexual size dimorphism, which is expected if cannibalism is an energetic investment in territory defence, but not if it does not have energetic benefits for males.

In Chapter 2 I showed that females preferentially spawn with males that are already caring for the offspring of other females, which may represent a strategy to reduce the costs of selfish filial cannibalism by males. If male egg cannibalism primarily serves to improve the condition of males, by adding her clutch to an existing brood a female may dilute the risk to her offspring of being cannibalised (Rohwer 1978; FitzGerald 1992; Kraak and Weissing 1996). Additionally, by augmenting a male's clutch, females may also increase the value of the brood to the male, reducing the benefit of cannibalism and the redirection of resources to future reproduction. If females seek to reduce the risk of cannibalism by mating with males that already have broods, we would expect a stronger preference for mating with such males in species where cannibalism is more frequent (Lindström 2000). In contrast, if cannibalism is a targeted care behaviour, preferences for males with eggs are not expected to correlate with cannibalism risk. To distinguish between these scenarios, in the second part of my analysis, I test whether a) the strength of female preference for males with eggs is predicted by the intensity of filial cannibalism, and b) the risk of cannibalism to a female's eggs is reduced when they develop within larger broods.

3.2 Methods

3.2.1 Data Collection

I conducted a literature search using Web of Science with the topic search terms "partial cannibal* OR clutch cannibal* OR filial cannibal* OR brood cannibal*", including all results up to February 2022. This search produced 1080 results, 123 of which were found to be relevant and data from 56 of these papers contained cannibalism rates and were used in my analyses (Figure 3.1). I extracted data for two metrics of partial brood cannibalism: a) the percentage of males engaging in cannibalism (N = 25 species), determined through observations of egg losses or identification of eggs in the father's stomach, and b) the mean proportion of eggs cannibalised (N = 14 species). All available cannibalism measures for a species were extracted from the text or tables or calculated from plots using WebPlotDigitizer V. 4.1 (Rohatgi 2018) and were used to generate a species average weighted by sample size (Table 3.1). I used the species mean effect sizes for the strength of female preferences for males with eggs that were calculated in Chapter 2. Sexual size dimorphism was calculated as mean male size/mean female size and, therefore, values greater than one indicate that males are larger than females while values below one indicate larger females; in all but two species (*Stegastes leucostictus* and *Porichthys notatus*), size data for males and females came from the same population. Results for the effect of brood size on the number and proportion of eggs cannibalised were converted to z-transformed correlation coefficients with sampling variance calculated as 1/(N-3).

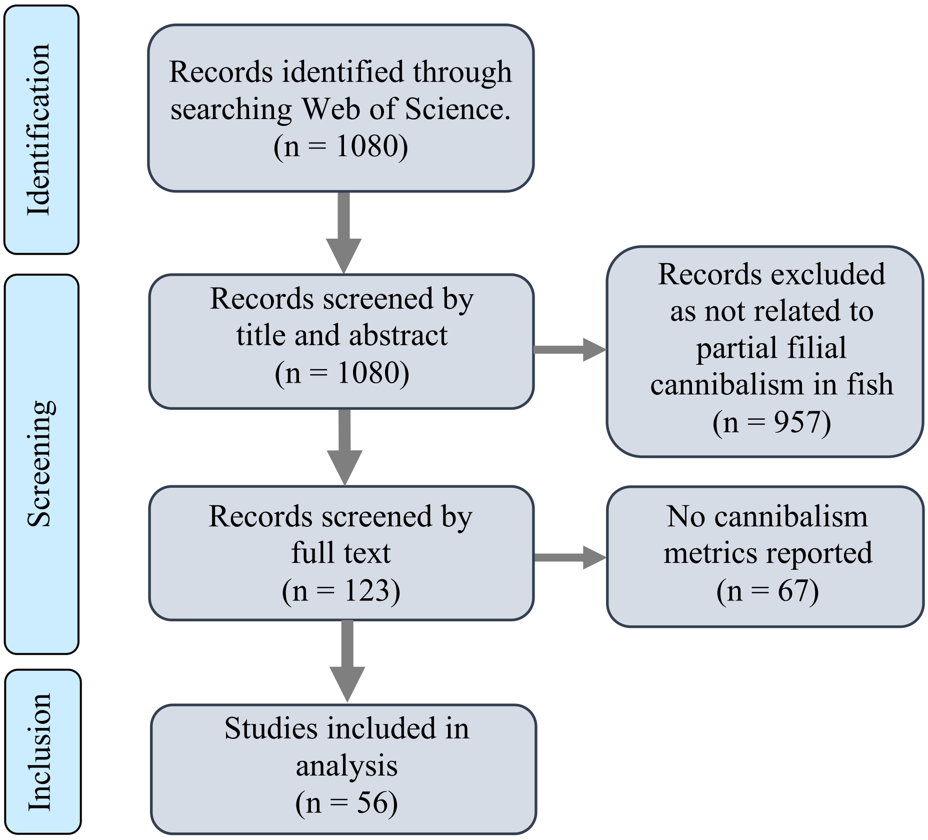

Figure 3.1. PRISMA flowchart showing the process of record identification and selection.

3.2.2 Statistical analysis

I calculated species means for the two measures of cannibalism weighted by sample size using the following formula:

$$\mu_i = \frac{\Sigma(x * n)}{N} \qquad [1]$$

Here, x is the cannibalism rate extracted for a given species from a given study, n is the sample size used to calculate that rate and N is the total sample size for that species. As only 5/14 and 6/25 species had repeated measures for the proportion of the brood cannibalism and for the propensity to cannibalise respectively, I passed these means to the model rather than using a species-level random effect.

I ran phylogenetic mixed-effects models in R version 4.0.4. using the MCMCglmm package (Hadfield 2010; R Core Team 2021). All models included a phylogenetic (co)variance matrix as a random effect to account for non-independence among species due to shared ancestry. The phylogeny used to calculate the (co)variance matrix was adapted from the teleost phylogeny in Betancur-R et al. (2017), to which I added 23 additional species (Figure 3.2). For this random term and the residual variance term, I specified inverse-Wishart priors (variance = 1 and belief parameter = 0.002). Each model was run for 1200000 iterations with a burn-in of 100000 and a 1000 thinning interval. Model convergence was assessed by calculating auto-correlation between successive iterations in each chain, inspecting traces of posterior distributions mixing and running each model three times and comparing within- and between-chain variance. I also ran each model using the metafor package (Viechtbauer 2010), which uses Restricted Maximum Likelihood for parameter estimation, to compare estimates. Reported parameters are the mode and credible intervals of posterior distributions from MCMCglmm models. The results of both model types are presented in Table 3.2.

To explore the relationship between cannibalism and the intensity of sexual selection on males, I fitted models with either propensity to cannibalise or the mean proportion of the brood cannibalised as the response variable and sexual size dimorphism as the predictor. I repeated these analyses with the absolute size of males as the predictor to determine whether any effect of sexual size dimorphism simply reflected an effect of male size. To test whether female preference for males with eggs is related to the risk of cannibalism, I fitted models with the strength of female preference for males with eggs ($Zr_{\mathrm{Preference}}$) as the response and either propensity to cannibalise or the mean proportion of the brood cannibalised as the predictor. To determine how cannibalism varies with brood size, I calculated a species mean effect of brood size on the number ($Zr_{\mathrm{BroodNumber}}$) or proportion ($Zr_{\mathrm{BroodProportion}}$) of eggs cannibalised using formula [1] and fitted intercept-only models with either $Zr_{\mathrm{BroodNumber}}$ or $Zr_{\mathrm{BroodProportion}}$ as the response to estimate the mean effect across species.

Methodological differences between the studies used in these analyses could influence cannibalism rates. In particular, some studies have recorded the cannibalism rates of captive animals while others have assessed cannibalism in wild populations. Since food availability and energetic demands are likely to differ between wild and captive populations, cannibalism rates may vary between laboratory and field-based studies, particularly if cannibalism is driven by energetic benefits. I investigated whether the relationship between cannibalism and sexual size dimorphism was affected by study methodology by rerunning the Bayesian models for each cannibalism metric using a subset of the data that included studies from one study type (laboratory or field). Species means were re-calculated from studies of the most represented study type for each metric: for the proportion of the brood cannibalised I used only laboratory studies (10 species) and for the propensity of males to cannibalise I used only field studies (13 species).

To investigate whether the relationship between male propensity to cannibalise and $Zr_{\text{Preference}}$ was influenced by study type I refitted the Bayesian model using species means calculated only from field studies (8 species). Sample sizes were too small to analyse the relationship between the percentage of the brood cannibalised and $Zr_{\text{Preference}}$ separately for field (2 species) or laboratory (6 species) studies.

Finally, to test whether the effect of brood size on cannibalism differed between laboratory and field studies I fitted two models with $Zr_{\text{BroodNumber}}$ or $Zr_{\text{BroodProportion}}$ as the response and included study type (laboratory or field) as a fixed effect. Although these two effect sizes are species means, there was only one effect size that combined results from studies with different methodology ($Zr_{\text{BroodProportion}}$ for the common goby), this species was therefore excluded from this analysis.

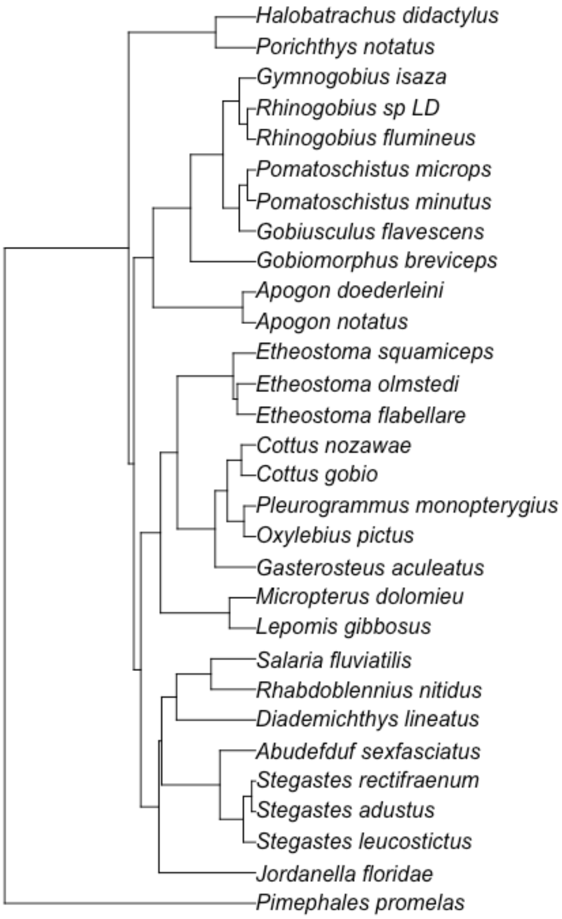

Figure 3.2. Phylogenetic tree including species with either of the two cannibalism rates reported used for all phylogenetic models. Adapted from the phylogeny presented in Betancur-R et al. (2017).

3.3 Results

In species exhibiting stronger sexual size dimorphism, males consumed a higher proportion of the brood (estimate = 57.23, CI = 14.90 to 95.76, pMCMC = 0.006, species N = 11; Figure 3.3) but they were not more likely to be cannibals (estimate = -5.80, CI = -60.1 to 58.9, pMCMC = 0.977, N=20). These results remained consistent when the models were run on data from only laboratory or field studies (proportion of the brood cannialised: estimate = 55.74, CI = 16.42 to 88.99, pMCMC = 0.006, N=9; propensity of males to cannibalise: estimate = -17.8, CI = -58.67 to 48.5, pMCMC = 0.849, N=13). This relationship was not explained by larger males eating more eggs, since the proportion of the brood cannibalised was not correlated with absolute male size in these species (estimate = -1.45, CI = -5.44 to 5.56, pMCMC = 0.96). There was also no relationship between absolute male size and the probability of cannibalism (estimate = -0.13, CI = -0.50 to 0.23, pMCMC = 0.505).

My analyses also revealed an association between cannibalism and mate choice by females: in species where males were more likely to engage in cannibalism, females showed stronger preferences for males caring for broods (estimate = 0.015, CI = 0.002 to 0.030, pMCMC = 0.020; N = 11; Figure 3.4a). This relationship was not significant when only cannibalism rates from field studies were used (estimate = 0.001, CI = -0.009 to 0.031, pMCMC = 0.176; N = 8). In contrast, the strength of preference for males with broods was not related to the proportion of the brood cannibalised (estimate = 0.021, CI = -0.035 to 0.086, pMCMC = 0.320; N = 9).

The decision by females to spawn with males already caring for eggs will result in an increase in the size of the brood cared for by the male. With increasing brood size, I found no change in the number of eggs males consumed (estimate = -0.06, CI = -0.19 to 0.18, pMCMC = 0.902, N = 8), leading to a smaller proportion of eggs consumed when broods were large (estimate = -0.32, CI = -0.55 to -0.14, pMCMC = 0.006; N = 10, Figure 3.4b). There was no difference in mean effect size between field and laboratory studies for $Zr_{\mathrm{BroodNumber}}$ (estimate = -0.06, CI = -0.53 to 0.27, pMCMC = 0.475; N = 8) or $Zr_{\mathrm{BroodProportion}}$ (estimate = -0.08, CI = -0.46 to 0.52, pMCMC = 0.869; N = 9).

Table 3.1. Fish species with cannibalism rates measured as a) the percentage of males engaging in cannibalism (weighted average), and/or b) the mean percentage of eggs cannibalized (weighted average).

Species name	**Common name**	**a) Ave. % cannibal males**	**b) Ave. % Brood cannibalised**	$Zr_{Preference}$ [1]	**Male size (cm)**	**Female size (cm)**	**Dimorphism Female/male size**
Abudefduf sexfasciatus	Scissortail sergeant	100 [2]	12 [2,3]	0.25	10 [4]	9.6 [4]	1.042
Apogon doederleini	Doederlein's cardinalfish	48 [5]	NA	NA	NA	NA	NA
Apogon notatus	Spotnape cardinalfish	48 [6,7]	NA	NA	NA	NA	NA
Cottus gobio	River bullhead	32.8 [8,9]	35 [9]	NA	8.1 [8]	7.1 [8]	1.141
Cottus nozawae	Fluvial sculpin	86 [10]	NA	NA	9.1 [10]	8 [10]	1.1375
Diademichthys lineatus	Long-snout clingfish	100 [11]	NA	NA	3.7 [12]	3.3 [12]	1.121
Etheostoma flabellare	Fantail darter	100 [13]	45 [13]	0.93	5.6 [14]	3.9 [14]	1.436
Etheostoma olmstedi	Tessellated darter	73 [15]	NA	0.31	NA	NA	NA
Etheostoma squamiceps	Spottail darter	NA	32 [16,17]	NA	5.1 [18]	4.8 [18]	1.063
Gasterosteus aculeatus	Three-spined stickleback	68.9 [19-22]	19 [22]	0.2	5.9 [23]	6.4 [23]	0.922
Gobiomorphus breviceps	Upland bully	100 [24]	38 [24]	NA	5.5 [25]	5 [25]	1.1
Gobiusculus flavescens	Two-spot goby	93 [26]	NA	NA	4.3 [27]	4.2 [27]	1.024
Gymnogobius isaza	Isaza goby	6 [28]	NA	NA	6.3[29]	6.2 [29]	1.016
Halobatrachus didactylus	Lusitanian toadfish	44 [30]	NA	NA	26.5 [31]	23.8 [31]	1.114
Jordanella floridae	Flagfish	86 [32,33]	38 [32]	NA	5 [34]	4.5 [34]	1.111
Lepomis gibbosus	Pumpkinseed sunfish	18 [35]	NA	-0.24	16.3 [36]	8.2 [36]	1.988
Oxylebius pictus	Painted greenling	98 [37]	NA	1.53	17.9 [38]	19.2 [38]	0.932
Pimephales promelas	Fathead minnow	NA	45 [39]	1.61	5.1 [40]	NA	NA

Pomatoschistus microps	Common goby	32.6 [41,42]	19 [41-44]	-0.37	4.7 [45]	5 [45]	0.94
Pomatoschistus minutus	Sand goby	63.8 [46-55]	24 [46-50, 56-58]	0.6	3.8 [59]	3.7 [59]	1.027
Porichthys notatus	Plainfin midshipman	64.5 [60,61]	NA	NA	16 [62]	12.2 [62]	1.312
Rhabdoblennius nitidus	Barred chin blenny	100 [63]	52 [63]	0.09	8.3 [64]	5.6 [64]	1.482
Rhinogobius flumineus	Lizard goby	100 [65]	28 [65]	NA	6.1 [66]	5.5 [66]	1.109
Rhinogobius sp LD	Rhinogobius sp LD	32 [67]	NA	NA	NA	NA	NA
Salaria fluviatilis	Freshwater blenny	24 [68]	NA	NA	4.7 [69]	4.3 [69]	1.093
Stegastes adustus	Dusky damselfish	NA	9 [70]	NA	15 [64]	NA	NA
Stegastes leucostictus	Beaugregory damselfish	43 [71]	NA	NA	8.5 [72]	6.7 [73]	1.269
Stegastes rectifraenum	Cortez damselfish	72 [74]	13 [75]	-0.17	13.5 [64]	NA	NA

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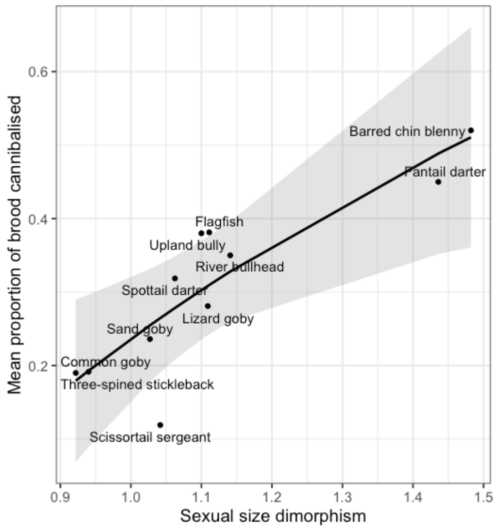

Figure 3.3. Correlation between sexual size dimorphism and the average proportion of the brood cannibalised. Species means plotted with regression line and 95% CIs (shaded bar) predicted from the phylogenetic model.

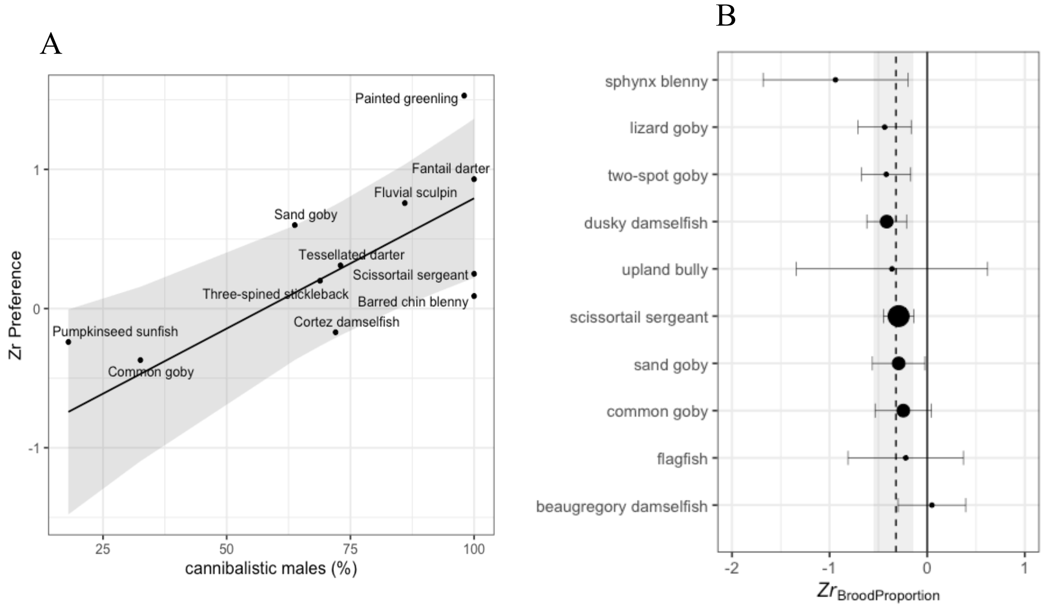

Figure 3.4. a) The correlation between the percentage of males that engage in partial brood cannibalism and the strength of female preference for males with eggs ($Zr_{Preference}$). Species means plotted with regression line and 95% CIs (shaded bar) predicted from the phylogenetic model b) Effect of brood size on the proportion of eggs cannibalised by males ($Zr_{BroodProportion}$). Points are species-level, weighted mean effect sizes with 95% confidence intervals. Point size represents the number of effect sizes used to calculate the species mean. The dashed line and shaded bar indicate the mean effect size across species with 95% credible intervals.

Table 3.2. Estimates and credible/confidence intervals from all MCMCglmm and metafor models.

Response	Predictor	N species	R package	lwr CI	Estimate	upr CI
Proportion of brood cannibalised	Sexual size dimorphism	11	MCMCglmm	14.9	57.2	95.8
			metafor	18.7	57.8	96.9
Proportion of brood cannibalised	Male size	11	MCMCglmm	-5.44	-1.45	5.56
			metafor	-4.98	-0.36	4.26
Propensity to cannibalise	Sexual size dimorphism	20	MCMCglmm	-60.1	-5.80	58.9
			metafor	-81.6	-20.8	40.0
Propensity to cannibalise	Male size	20	MCMCglmm	-2.97	-0.82	1.58
			metafor	-3.12	-0.94	1.24
Preference for males with eggs (*Zr Preference*)	Proportion cannibalised	11	MCMCglmm	-0.04	0.02	0.09
			metafor	-0.02	0.02	0.05
Preference for males with eggs (*Zr Preference*)	Propensity to cannibalise	9	MCMCglmm	0.002	0.02	0.03
			metafor	0.01	0.02	0.03
Effect of brood size on number of eggs cannibalised (*Zr BroodNumber*)	Intercept only	8	MCMCglmm	-0.19	-0.06	0.18
			metafor	-0.13	-0.01	0.12
Effect of brood size on proportion of eggs cannibalised (*Zr BroodProportion*)	Intercept only	10	MCMCglmm	-0.55	-0.32	-0.14
			metafor	-0.41	-0.33	-0.24

3.4 Discussion

When mating success depends strongly on possession of breeding territories, as is the case for many fishes with male-only care, energetic gains from filial cannibalism may support continued territory defence and hence increase future mating opportunities, as well as enabling continued care of the current brood. Across fish species, I found a significant correlation between the proportion of the brood cannibalised and the degree of sexual size dimorphism, suggesting that filial cannibalism is related to the intensity of sexual selection acting on males. This can be illustrated by a species in my dataset, the long-snout clingfish (*Diademichthys lineatus*), which is characterised by strong male-male competition for territories and large sexual size dimorphism. In this species, cannibalism of eggs increases male condition, potentially enabling males to retain territories to continue care or enjoy further matings over the four-month breeding season (Gomagano and Kohda 2008).

In addition to the energetic benefits of cannibalism for competition, there may be other explanations for a positive relationship between size dimorphism and the amount of cannibalism by males. In species with females that are a similar size or larger than males, females may be better able to police male cannibalism. In the paternal mouthbrooding cardinalfish *Apogon doederleini*, females that had lost a brood to cannibalism were observed attacking their male partner and males were more likely to cannibalise broods from non-resident females (Okuda and Yanagisawa 1996). Females in biparental species have also been observed to exclude males from directly tending the brood which may represent an attempt to reduce cannibalism (Lavery and Keenleyside 1990; Cunha-

Saraiva et al. 2018). However, in most paternal species, females do not remain in the male's territory after spawning and interference with cannibalism has not been reported.

I found no relationship between male size and the proportion of the brood cannibalised which supports the conclusion that the correlation with sexual size dimorphism is not simply a result of greater cannibalism in species where males are larger. However, variation in egg size and number between species may influence the energetic contributions of a given brood proportion. In particular, egg size is predicted to increase with female size (Hendry et al. 2001), particularly in species with demersal eggs (Einum and Fleming 2002). However, evidence for this relationship across fish species is mixed (Elgar 1990; Kamler 2005; Braga Goncalves et al. 2011; Kasimatis and Riginos 2016). If larger species do produce larger eggs, large males could gain similar energetic benefits to small males by consuming a smaller proportion of their brood. Egg size has also been shown to correlate positively with the quality and duration of parental care provided, across some fish taxa, which may influence the relationship between partial brood cannibalism and the energetic demands of care (Gross and Sargent 1985; Kolm and Ahnesjö 2005). More information about egg sizes and the factors influencing variation in egg size across species with male-only care would inform our understanding of the energetic benefits of brood cannibalism.

In addition to competition for territories, mate availability would also be expected to influence filial cannibalism by affecting the potential for future reproduction (Deal and Wong 2016). So far, mate availability has been shown to vary with cannibalism in some fishes but not in others (Bjelvenmark and Forsgren 2003; Pampoulie et al. 2004; Myint et al. 2011; Takeyama et al. 2013). Outside of fish, the potential for future reproduction has been linked to decisions to abandon whole broods in birds (Pilastro et al. 2001; Szentirmai et al. 2007) and insects (Ward et al. 2009). Partial brood reduction through abandonment or infanticide is also commonly observed in non-piscine taxa (Hausfater and Blaffer Hrdy 1984; Magrath 1990), but the extent to which this represents an investment in the current reproductive attempt or future attempts remains to be determined.

Even in species where sexual size dimorphism is absent or reversed, partial brood cannibalism persists, suggesting that factors other than sexual selection also influence cannibalism decisions. For instance, cannibalism has been observed in response to paternity uncertainty in some species (Neff 2003; Mehlis et al. 2010; Bose et al. 2019). Brood reduction has also been shown to improve the survival of remaining offspring in a number of species, with likely mechanisms including the removal of diseased eggs and a reduction in brood density leading to improved oxygen availability (Payne et al. 2002; Klug et al. 2006; Bandoli 2016). Cannibalism may, therefore, also function as paternal care (Davenport et al. 2019). However, if brood protection was the primary driver of cannibalism in males, we would expect more eggs to be cannibalised in larger broods, yet I find no relationship between cannibalism and brood size. My results suggest that the decision to cannibalise is influenced by the energetic demands that males face as they seek to maximise reproductive success across the breeding season. Indeed, theory predicts that filial cannibalism is most likely to evolve when parents derive energetic benefits through consumption of offspring (Klug and Bonsall 2007).

I found that the average number of offspring cannibalised did not vary with brood size, leading to a smaller proportion cannibalised when males care for large broods. By contributing eggs to existing broods, thereby increasing total brood size, females are thus able to dilute the risk to their offspring of being cannibalised. In addition, by augmenting broods, females may increase their value to males. In other taxa, brood size is known to influence parental investment decisions, with small broods more likely to be cannibalised or abandoned if the parent can expect to achieve a greater brood size in the future (Verboven and Tinbergen 2002; Ward et al. 2009). Similarly, the youngest, weakest offspring are usually preferentially sacrificed to increase the survival of their siblings (Mock 1984; Mock and Parker 1986; Afonso and Santos 2005). In the case of fish, if caring males did not value large broods more highly, we would expect the number of offspring cannibalised to increase with brood size, as the cost of cannibalising each offspring decreases (Manica 2002). Instead, my finding that it does not change suggests that males place a higher value on large broods, consuming proportionally fewer offspring. This supports the results of Chapter 2 showing that males invest more in care when broods are large, which in turn increases their attractiveness to females.

Differences in conditions between laboratory and field studies may influence parental behaviour, including partial brood cannibalism (Campbell et al. 2009). For instance, if cannibalism contributes to condition, differences in food availability may influence resource budgets and affect the benefits of eating eggs. Cannibalism rates have also been shown to respond to changes in environmental conditions, such as oxygen availability (Olsson et al. 2016) and the presence of pathogens (Lehtonen and Kvarnemo 2015). In addition, in wild populations the proportion of cannibalistic males was usually calculated from the number of guarding males found with eggs in their stomachs, whereas, in most captive populations brood sizes were monitored to determine whether eggs were lost.

When only field studies were included in the analysis of female preference for males with eggs there was no significant positive correlation with the proportion of cannibalistic males. In the original analysis, two of the species means combined cannibalism rates from laboratory and field studies (sand goby and three-spined stickleback), when these were recalculated from only field studies, rates were lower for both species. This result may indicate that this relationship is influenced by the methodology used to determine cannibalism rates. However, running this analysis also required reducing the sample size from eleven to eight species which is likely to have influenced the power of this analysis to detect a significant relationship. Further studies on both captive and wild populations, particularly within species, would help us to understand whether cannibalism rates differ consistently between wild and captive populations.

Overall, my results provide support for the energy-reserves hypothesis that partial brood cannibalism by male fish has evolved primarily as a result of energetic benefits that help males to maintain their condition. This will particularly benefit males that experience strong male-male competition and are selected to invest in competitive traits such as increased size. Whether cannibalism benefits males through increasing survival of the current brood or improving opportunities for future reproduction is not clear. However, sexual conflict resulting from male egg cannibalism may play a role in shaping female mate preferences, with female choice for caring males representing a counter-strategy by which females can protect their offspring from cannibalistic fathers.

Chapter 4

The reproductive consequences of female competition in a harem-breeding cichlid

Rebecca Goldberg, Jonathan Green

Author contributions: Jonathan Green conceived of the study. Jonathan Green and I designed the experiment. I collected the data. I analysed the data with guidance from Jonathan Green. I wrote the chapter and incorporated comments from Jonathan Green.

Abstract

While sexual selection in males frequently results in the evolution of elaborate competitive traits, the evolution of competitive traits in females is predicted to be limited by their greater investment in reproduction and parental care. Despite this prediction, few studies have manipulated investment in females to understand whether females experience a trade-off between intrasexual competition and reproduction. In this chapter I manipulate female investment in intrasexual aggression in the harem-breeding cichlid *Lamprologus ocellatus* to investigate the consequences of competitive investment for reproduction (egg number and hatching success) and parental care. I find that females invest more in aggressive displays when breeding in close proximity to another female compared to when their territories are dispersed. In these close harems, females did not produce smaller broods but they had significantly lower hatching success than in dispersed harems or in the absence of a rival female. This demonstrates that a trade-off

between reproduction and competition may impose constraints on the evolution of competitive investment in females.

4.1 Introduction

When the resources required for reproduction are limited, the reproductive success of individuals often depends on outcompeting other individuals. As the resources that limit reproduction generally differ between the sexes, intrasexual competition is common and can result in sexual selection for competitive traits (Clutton-Brock and Huchard 2013). For males, reproductive success is usually most limited by their mating success, and therefore males compete with one another for access to females (pre-copulatory competition) and for paternity through sperm competition (post-copulatory competition) (Andersson 1994; Birkhead and Pizzari 2002). Heavy investment in this competition results in the expression of exaggerated secondary sexual traits in the males of many species that increase their competitive ability. In contrast, female reproductive success is less likely to be constrained by their access to males, and mating competition between females appears to be less common.

However, reproductive success in females may be limited by their ability to acquire other resources (Clutton-Brock 2009; Tobias et al. 2012), such as nest sites (Heinsohn et al. 2005; Rosvall 2008), high quality males (Sæther et al. 2001; Bebié and McElligott 2006) or males that provide parental care (Summers 1989; Smith et al. 1994). Despite the potential for competitive investment to have reproductive benefits for both sexes, competition often appears to be less intense in females and to involve less exaggerated traits compared to males. This observation has led authors to hypothesise that the expression of competitive traits in females is limited by their greater investment in gamete production and parental care than males, reducing the resources available for competition (Fitzpatrick et al. 1995). However, it remains unclear whether females do actually experience such a trade-off, that is, whether greater investment in competition imposes a cost on reproduction through physiological constraints or interference with parental care.

In females, resources gained during competition can enhance reproduction, but energetic losses and physiological changes resulting from competition may limit reproductive output or parental care. Competitive investment can therefore both positively and negatively influence the same reproductive attempt. Observational studies investigating the influence of competitive phenotypes on care and reproduction have demonstrated differing effects. For example, less aggressive females are shown to have higher reproductive output in some birds (e.g. tree swallows; Rosvall 2011) but not in others (e.g. dark-eyed juncos and house wrens; Cain and Ketterson 2012; Cain and Ketterson 2013; Krieg and Getty 2020). Although these studies demonstrate an effect of aggressive phenotypes on reproductive success, environmental variation and differences in resource acquisition between females can alter the observed relationship between these investments (van Noordwijk and de Jong 1986; Descamps et al. 2016). In addition, observations of breeding females may form a biased subset of the population that differs in age, quality or phenotype from the population mean (Bolund 2020). For instance, if strong competition for nest sites excludes some females from breeding, only the outcomes of large investments in competition can be assessed. Experimental manipulations of competition are needed that control for environmental covariates and decouple any relationship between aggression and resource acquisition to identify the effects of competitive investment on reproduction that might be masked when looking at phenotypic correlations. To date we lack experimental evidence based on manipulations of levels of competition. How changes to the competitive environment affect investment decisions and reproductive success in females is therefore unknown.

As well as individual differences in trait expression among females, variation in the competitive environment can lead to the facultative adjustment of investment in competitive traits by individual females (Stockley et al. 2013). Behavioural traits such as aggression and competitive displays are often plastic traits that can be modulated in response to social cues (Clutton-brock and Huchard 2013). For example, in both males and females testosterone-mediated aggression has been shown to peak during mating behaviour and territory establishment and to decline during parental care (Oliveira et al. 2002; Rosvall et al. 2020). In addition to changes between breeding stages, the composition of the social environment may drive adjustments in competitive investment that have reproductive consequences. For instance, changes in female aggression have been observed in response to changes in the operational sex ratio in Japanese medaka (*Oryzias latipes*; Clark and Grant 2010) and the density of females in Soay sheep (*Ovis aries*; Robinson and Kruuk 2007) and chimpanzees (*Pan troglodytes*; Pusey and Schroepfer-Walker 2013). By manipulating the intensity of competition experienced by females during breeding and offspring care we can, therefore, demonstrate how changes in competitive investment influence reproductive output and isolate the intrinsic costs of investment in competitive traits within females.

The facultatively polygynous cichlid, *Lamprologus ocellatus*, is an excellent system for investigating the costs of female competition. Native to Lake Tanganyika, populations of *L. ocellatus* are found on shallow sand beds where empty snail shells from the Neothauma family have accumulated as a result of the alkaline conditions. *L. ocellatus* is one of several species that has evolved as an obligate shell-dweller with eggs spawned inside shells and both sexes using them for shelter (Bills 1997). Females compete aggressively for empty shells within a male's territory in which they breed and care for their offspring. Males typically defend shells for up to four females and control when they are allowed to settle, but they are unable to move shells and the territories of females are partly dependent on the natural distribution of shells in the male's territory. Settled females aggressively defend the territory against new females and attempt to evict other settled females from their shells, particularly those in close proximity (Walter and Trillmich 1994). In the laboratory, larger and more aggressive females are more likely to settle first and to settle closer to the male (Brandtmann et al. 1999). These females may benefit from being able to begin breeding sooner, or from territorial or parental behaviour provided by the male; however, the relationship between aggression and reproductive success in females has not been quantified in laboratory or natural populations.

Although shells are an essential resource for reproduction, females must also invest in egg production and parental care, including fanning and cleaning eggs in the shell and defending the brood against predation. Since females can breed in either monogamous pairs or in harems with multiple females, and the distances between female territories can vary, there is natural variation in the intensity of competition experienced by females (Bills 1997). As a result, females are expected to adjust their investment in competitive displays and aggression in response to their social environment. This variation can be replicated in the laboratory by altering the number of breeding females in a harem and the distances between female shells, enabling the manipulation of investment in competition within females. In this study, I measured the behaviour and reproductive output of females under three different levels of competition to quantify the fitness consequences to both females and males of female investment in competition. If females face a trade-off, we would expect to see a reduction in reproductive success under higher levels of competition. I predicted that with higher levels of competition, females would a) produce smaller brood sizes, b) spend less time in parental behaviours and c) have lower hatching success.

4.2 Methods

4.2.1 Experimental animals and housing

Fish used in experiments were from six tank-bred lines (A-F; generations in captivity not known) obtained from independent breeders. When not in experiments, fish were housed in groups of 1-12 in tanks measuring 60 x 30 x 30cm (40L). Tanks contained 1cm of sand but no shells or other features to avoid territorial aggression and breeding. Water was maintained at a temperature of 26 ± 1°C, pH at 8.4, gH at 22-26 and kH at 8-11. The first round (hereafter round 1) of experiments took place between April 2019 and March 2020 followed by a second round (hereafter round 2) from June 2020 to August 2021 (experiments were paused for three months from March 2020-June 2020 due to national coronavirus lockdown restrictions). For round 1 fish were fed twice daily with flake in the morning and frozen mysis or brine shrimp in the afternoon. As part of husbandry refinements for the entire population, this diet had been changed for round 2 to flake for both feeds on six days and one day with shrimp in the afternoon.

4.2.2 Experimental set up

The experimental set-up is shown in Figure 4.1. Experimental tanks measuring 40 x 90 x 30cm (90 litres) were partitioned into three compartments: a long compartment housing the male, and two adjacent compartments each housing one female. Each of the three fish came from different stock populations to ensure that all individuals within a trio were unrelated and unfamiliar. This was done to control for any reduction in aggression in response to perceived kinship, which is expected when aggression towards kin results in inclusive fitness costs. Although we presently have no knowledge of either the kin structure of wild *L. ocellatus* populations or the extent of kin discrimination in this species, changes in behaviour in response to kinship have been recorded in other fish species, including a reduction in aggression when neighbouring territory holders are closely related (Brown and Brown 1993; Hesse and Thünken 2014; Makowicz et al. 2016).

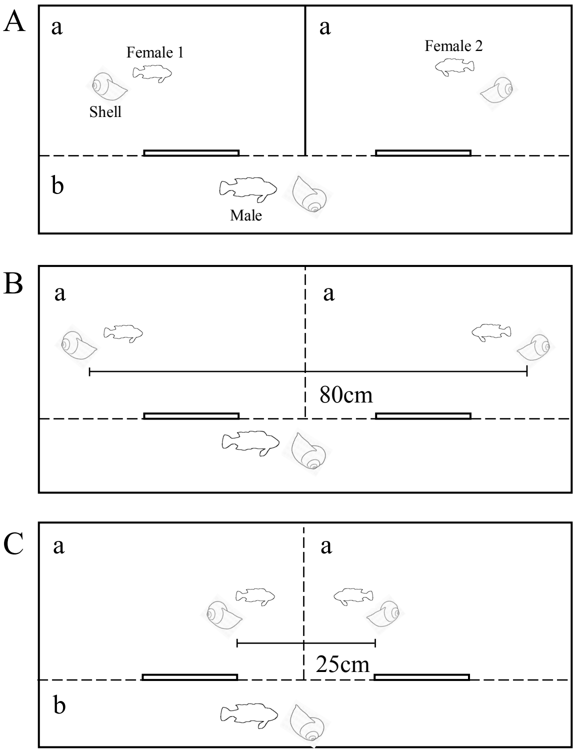

Figure 4.1. The three competition treatments. Solid lines indicate opaque barriers whilt dashed lines are transparent barriers. a - female compartment containing one female fish and her shell, b - male compartment containing one male fish and his shell. A) Pair: females breed with the same male but with an opaque barrier between them so they have no visual cues to the presence of another female. B) Dispersed Harem: females breed in a harem 80cm apart with a transparent barrier between them. C) Close Harem: females breed in a harem 25cm apart with a transparent barrier between them.

Each fish was given a glass shell (Robert DuGrenier Glass Art, www.dugrenier.com), modelled on the *Neothauma tanganyicense* shells that are used by this species in the wild. This enabled me to accurately determine when a female had spawned and to count eggs and hatched larvae without removing them from the shell or causing excessive disturbance to females. The bottom of the tank was covered in 4cm of sand, enabling males to fully bury shells (part of the natural repertoire of behaviours shown by territorial males in the wild).

I controlled male access to females via removable barriers between their compartments. Barriers between males and females prevented shell evictions and protected fry from cannibalism while still allowing females to be influenced by competitive cues and engage in aggressive displays. This enabled me to create stable harems that could persist over multiple brood cycles and to isolate the reproductive consequences of investment in aggression from the impacts of female territory loss and brood predation.

I arranged shells and barriers to create three treatments in which the intensity of competition between females differed (Figure 4.1):

1) Pair - both females breed with the same male but were restricted from viewing the other female due to an opaque barrier between their territories. Although I am unable to rule out the possibility that females perceived the presence of another female in the tank during this treatment e.g. through olfactory cues, I did not observe any behaviour that would indicate this.

2) Dispersed Harem- females breed with the same male in shells 80cm apart with a transparent barrier between territories. Distances of 80cm between female territories

correspond with those frequently observed in field populations (Bills 1997; Brandtmann et al. 1999; personal obs.)

3) Close Harem – females breed with the same male in shells 25cm apart with a transparent barrier between territories. Distances of 25cm have not been reported in the wild (minimum reported distances: 50cm, Brandtmann et al. 1999; 65cm, pers. obs.) and were designed to inflate competition levels with the aim of revealing trade-offs between investment in competition and reproduction.

I aimed for each female to produce two successful broods in each of the three competitive treatments, run in series, and treatment order was randomised across groups. Females were replaced if they failed to breed within one month of entering the experiment, and if neither female had bred within one month the male was replaced. I created eleven harems of two females and one male. In six harems, one of the two females either died (3 harems) or stopped breeding partway through the experiment (3 harems) and was replaced with a new female. This occurred following the Pair treatment in two cases, the Close Harem treatment in three cases and the Dispersed Harem treatment in one case. Females spent on average 86 ± 28 days (mean ± SD) in a treatment.

If a female was not immediately ready to spawn she was usually evicted from her shell by the male, which would completely bury the shell. Spawning events could be identified when a female was newly guarding a shell that had previously been buried. Following spawning, shells were checked for eggs, which were clearly visible through the glass. Eggs were counted and the door between the male and female compartment closed. Shells were checked again on day four to see if the brood had been successful (i.e. one or more eggs had hatched) and count the number of larvae. When fry reached 20 days post-spawning they were removed from the tank and females were given access to the male to breed again. The treatment was changed once each female in the harem had completed two successful brood cycles with at least 15 days per brood where the other female was settled in her shell. I measured the wet weight of each female to the nearest 0.001g at the end of each brood cycle in a 50ml centrifuge tube filled with tank water. I measured standard length to the nearest 0.1mm using a calliper while females were in a net held up against the glass of the tank. In round 2, I increased the number of times that female length was measured from once at the start of the experiment to once during the first brood cycle of each treatment to account for growth during the experiment. As repeated measures of length were only taken in round 2, analyses involving length or condition as a variable only use data from round 2.

4.2.3 Video recording and analysis

Females were filmed for 30 minutes on five days during the brood cycle: once during the egg stage (day 0-3), twice during the larval stage (day 4-11), and twice during the free-swimming stage (day 12-20). Videos were analysed using the Behavioural Observation Research Interactive Software (BORIS; Friard and Gamba 2016). For each female I recorded the duration and number of aggressive behaviours. These included competitive displays (all fins erect and body tilted upwards or downwards facing perpendicular to the rival female; Figure 4.2a) and barrier contact (pushing her mouth against the barrier that divides the female compartments). I also recorded the time that females spent in their shells, guarding their shell (within two body lengths of the shell; Figure 4.2b) or away from their shell. Finally, I also recorded the brood stage and brood size at the time of filming for each female.

Table 4.1 shows the number of females used in each harem and summarises the reproductive and behavioural data available for each female. In two cases (1B_F1_12 and 2A_F1_13), reproductive data were available while whole cycle behavioural data were missing due to missed recordings during at least one stage of the cycle. In one case (2A_F1_13), behavioural data were recorded but reproductive data were missing due to an error in the initial calculation of brood size.

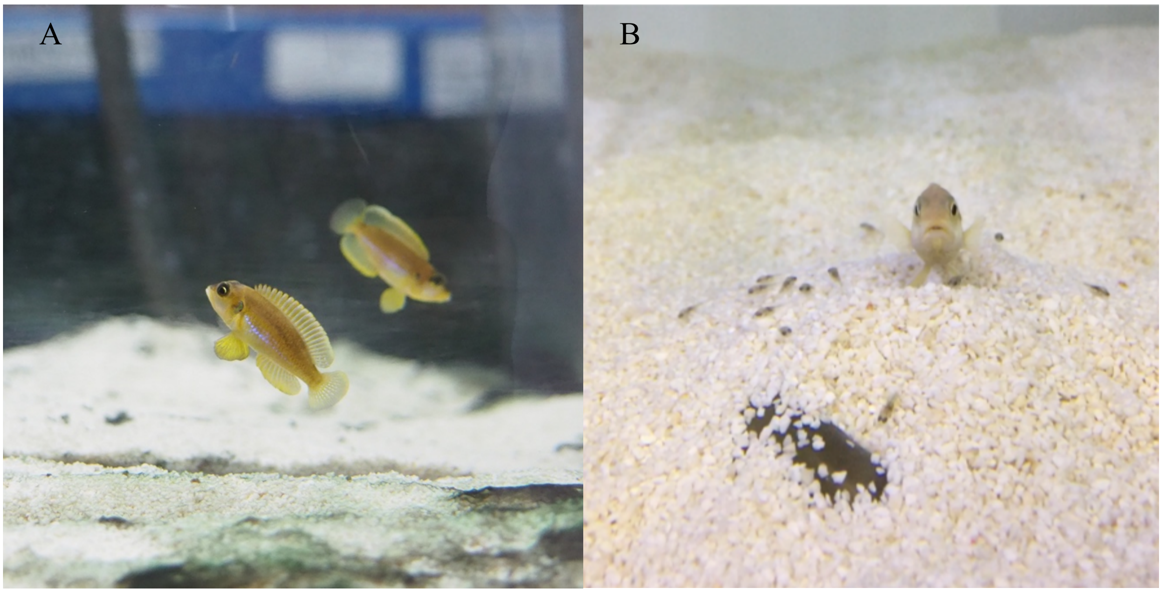

Figure 4.2. Female behaviour. A) Two females engage in lateral displays across the transparent barrier between their two territories. B) A female *L. ocellatus* guards the entrance of her shell as her offspring begin to explore the territory. Images are my own.

Table 4.1. Data available from the 11 harems and 27 females in this experiment. R = reproductive data and behaviours from the egg stage. B = behavioural data from the whole brood cycle. Green boxes indicate data is available while shaded grey boxes indicate data is missing. Female ID includes information about tank number (1A-4B), number within pair (F1 or F2) and a unique individual number (2-35).

Harem	Female ID	Pair		Dispersed Harem		Close Harem	
		R	B	R	B	R	B
1	2B_F1_6	🟩	🟩	🟩	🟩	🟩	🟩
1	2B_F2_7	🟩	🟩	🟩	🟩	🟩	🟩
3	3A_F1_5	🟩	🟩	🟩	🟩	🟩	🟩
3	3A_F2_10	▨	▨	🟩	🟩	🟩	🟩
4	1B_F1_12	🟩	🟩	🟩	🟩	🟩	▨
4	1B_F2_11	🟩	🟩	🟩	🟩	🟩	🟩
5	4B_F1_2	🟩	🟩	▨	▨	🟩	🟩
5	4B_F2_19	🟩	🟩	▨	▨	▨	🟩
6	2A_F1_13	▨	🟩	🟩	🟩	🟩	▨
6	2A_F2_14	🟩	🟩	▨	▨	▨	▨
6	2A_F2_4	▨	▨	🟩	🟩	▨	▨
8	3B_F1_15	🟩	🟩	🟩	🟩	🟩	🟩
8	3B_F2_20	🟩	🟩	▨	▨	▨	▨
8	3B_F2_9	▨	▨	🟩	🟩	▨	▨
9	1B_F1_22	🟩	🟩	▨	▨	▨	▨
9	1B_F2_24	🟩	🟩	🟩	🟩	▨	▨
10	4A_F1_23	🟩	🟩	🟩	🟩	🟩	🟩
10	4A_F2_26	🟩	🟩	▨	▨	▨	▨
10	4A_F2_35	🟩	🟩	🟩	🟩	🟩	🟩
11	4B_F1_25	🟩	🟩	🟩	🟩	🟩	🟩
11	4B_F2_27	🟩	🟩	🟩	🟩	🟩	🟩
12	3A_F1_28	▨	▨	▨	▨	🟩	🟩
12	3A_F2_29	🟩	🟩	🟩	🟩	🟩	🟩
12	3A_F1_32	🟩	🟩	🟩	🟩	▨	▨
13	3B_F1_30	▨	▨	🟩	🟩	🟩	🟩
13	3B_F2_31	▨	▨	▨	▨	🟩	🟩
13	3B_F2_33	🟩	🟩	🟩	🟩	🟩	🟩

4.2.4 Statistical analysis

Unless specified, all analyses were run on data from the second successful brood produced by each female in each treatment. Changing females between treatments caused disruption to groups through moving shells and territory boundaries which may have impacted female investment and behaviour in the first brood cycle following a change. In addition, prior experiences can influence subsequent reproduction through a variety of mechanisms, including delayed effects of resource trade-offs ('carryover effects'; Harrison et al. 2011). By sampling from the second brood in each treatment and distancing females from their experience of other treatments, I aimed to reduce the contribution of these effects. All females, therefore, had previously experienced one full brood cycle in the treatment and may have produced some unsuccessful broods before producing their second successful brood.

I ran linear mixed-effect models (LMMs) in R version 4.0.4. (R Core Team 2021) using the packages lme4 (Bates et al. 2015), lmerTest (Kuznetsova et al. 2017) and glmmTMB (Brooks et al. 2017). Bonferroni post hoc pair-wise comparisons were run using emmeans (Lenth 2021). Unless specified, all analyses were two-tailed statistical tests in which $\alpha = 0.05$. The inclusion of additional fixed or random effects was tested by comparing model fit with and without these variables using a likelihood ratio test. Residual analysis was performed using diagnostic plots for LMMs and non-parametric overdispersion tests for generalised LMMs (GLMMs) in the DHARMa package (Hartig 2021). Transformations were used when variables did not conform to the assumptions of linear regression (see below). Outliers were identified using Rosner's test in the EnvStats package (Millard

2013). Analyses were run with and without outliers present: if the significance of the results differed between these models, both results are reported, otherwise results are reported from models that include all data. The distributions of several behaviours included a high proportion of zeros which resulted in overdispersion, therefore these were modelled using zero-inflated distributions. Unless specified, all models included the unique identifier for each female (female ID) as a random effect due to the repeated sampling design where the same females experienced multiple treatments.

To confirm whether female competitive behaviour differed between the two harem treatments I ran a series of GLMMs. Two models with a zero-inflated negative binomial error distribution were fitted to test the effect of treatment on the duration of displays and the duration of barrier contact, and two GLMMs with Poisson error distribution were fitted to test the effect of treatment on the number of displays and barrier contacts. In all models, brood stage (Eggs, Larvae or Free-swimming fry) and brood number were included as fixed effects. Since females experienced both harem treatments with the same rival I investigated whether females displayed evidence of habituation to the rival, through reduced aggression, which may influence the effect of treatment on aggression. I compared the average time that females spent displaying to the rival and the average time spent in contact with the barrier in the first brood cycle to times from the second brood cycle in each harem treatment. No variables other than brood cycle differed between these data, I therefore used one-tailed Wilcoxon signed-rank tests, analysing data from Close Harems and Far Harems separately. This analysis only included data from harem treatments where females had experienced two successful brood cycles (16 Far Harem treatments and 15 Close Harem treatments).

As well as increases in competitive aggression, I predicted that females would reduce the time spent caring for offspring under higher competition. I therefore modelled the effect of treatment on the time females spent in their shells during the egg stage to understand whether changes to care behaviours might directly influence hatching success. I fitted an LMM with time spent in shell (log-transformed) as the response variable and treatment and brood number as the predictors. I also modelled the effect of treatment on the time females spend guarding their shells across the whole brood cycle to understand how competitive threats influence female nest defence. To do this, I fitted an LMM with time guarding shell as the response variable and treatment, brood stage and brood number as the predictors.

To investigate the effect of treatment on hatching success I modelled hatching success as a binary variable using a GLMM with binomial error distribution, with treatment, brood number and round as predictors. I also included time spent in shell as an additional predictor to test for an effect of maternal behaviour on hatching success. Upon finding a significant difference in hatching success between rounds, I identified that one of the key differences between rounds was that the majority of females belonged to different populations. I therefore tested for an effect of population by fitting a further hatching success model which included population as an additional predictor. Female weight was identified as another potentially important predictor of hatching success, since larger females may have more resources to allocate to offspring. However, weight measurements were only available for 38/57 broods. I therefore fitted a separate hatching success model on this reduced dataset that included the same predictors as the main model with the addition of female weight.

I found that competitive treatment had an effect on both aggressive behaviour and hatching success. I therefore investigated whether there was a correlation between hatching success and previous or current investment in aggressive behaviour. I fitted a GLMM on a reduced dataset that included data from broods in harem treatments that had been preceded by a successful brood (rather than a failed brood). This approach then allowed me to test for a relationship between the time that females spent in aggression during the prior brood cycle and hatching success in the current brood cycle. This model included the significant predictors identified in the main hatching success model (treatment, round and brood number), plus three additional predictors: previous display time (the mean time that females spent in display during the prior brood cycle, controlling for brood stage); current display time (display time during the egg stage of the current brood); and inter-brood interval (the interval between removal of the first and spawning of the second brood). Inter-brood intervals may influence hatching success if longer intervals allow females to gather more resources to allocate to eggs. This variable was not included in the main model since the majority of second broods were preceded by a failed brood. Since the data used for this model included only broods preceded by a successful brood I could test for an effect of brood interval. Including brood interval in this model also enabled me to account for variation in the time between measurements of display time and subsequent hatching success.

To investigate the effect of treatment on brood size I used an LMM with brood size as the response and treatment, round and brood number as predictors. Again, female weight was expected to be an important predictor of brood size but was only available for 38/57 broods. I therefore fitted a second version of this analysis on this reduced data set, including female weight as an additional fixed effect.

To investigate the effect of treatment on the probability of a brood failing, I modelled the fate of the first brood spawned following the first successful brood cycle in a treatment. I used a GLMM with binomial error distribution and set the fate of the brood (failed or successful) as a binary response variable and treatment as the only predictor. Although female weight is also predicted to influence brood failure, in the reduced data set there were only 3 instances of brood failure and, therefore, I could not model the effect of female weight for this variable.

To investigate how competitive investment affects female condition, I calculated Fulton's condition factor (condition (K) = weight (W) / length (L)^3) for females at the end of each brood from round 2, for which I had measures of both length and weight for each female during each treatment, and modelled this against treatment using a linear model. For this model I removed the random effect for female ID as 6/15 females had only experienced one treatment, which prevented the model from accurately estimating variance within females. Finally, I modelled the effect of treatment on inter-brood interval using a GLMM with a Poisson error distribution, with treatment, brood number and female weight as fixed effects. Here, I calculated the inter-brood interval as the number of days between when the first successful brood in a treatment was removed (day 20) and the following brood was spawned, regardless of the fate of this brood.

4.2.5 Ethics statement

This experiment was approved by the Animal Welfare & Ethics Review Board in the University of Oxford's Department of Zoology.

4.3 Results

4.3.1 Breeding behaviour

A total of 206 broods were spawned during the course of the experiment. Of these, 70 failed completely (34%), with most eggs disappearing or detaching from the shell surface prior to the day of hatching. Of the failed broods, 32 were spawned by females when access to the male was being withheld, indicating lack of fertilisation as one cause of brood failure. The remaining 38 broods failed following courting between males and females and the causes of these failures are presently unknown. Females spawned again on average 13 ± 2.53 (Mean ± SD) days following brood failure. Mean ± SD hatching success was 39 ± 32% across all broods where fertilisation was assumed to have occurred and 50 ± 28% across successful broods. Brood size across all broods was 29 ± 12 eggs.

4.3.2 The effect of competition on female behaviour

Females spent more time displaying, and displayed more frequently, in Close Harems than in Dispersed Harems (display time: GLMM, estimate ± SE = 1.057 ± 0.18, Z = 5.90, p <0.0001, N =23; display number: estimate ± SE = -0.66 ± 0.15, Z = -4.299, p = <0.0001, N =23; Figures 4.3a and 4.3b; Table 4.2). There were two significant outliers for display time (1800s and 1750s) but the results did not change with these outliers removed. Females spent more time displaying when they had free-swimming fry than when they had eggs (GLMM, estimate ± SE = 1.051 ± 0.23, Z = 4.666, $p < 0.0001$) or larvae in the shell (estimate ± SE = 0.767 ± 0.17, Z = 4.500, $p < 0.0001$). There was no difference in display times between the egg and larval stages (estimate ± SE = 0.284 ± 0.244, Z = 1.163, p = 0.2449). Escalated aggression, in the form of barrier contacts, was also more frequent in Close than Dispersed Harems (barrier contact time: GLMM, estimate ± SE = -0.54 ± 0.21, Z =-2.560, p = 0.0105, N = 23; barrier contact number: estimate ± SE = -0.428 ± 0.19, Z = -2.206, p = 0.0274, N = 23; Figures 4.3c and 4.3d; Table 4.2).

Females showed some evidence of habituation to the rival between broods: There was no difference in the amount of time females spent displaying to the rival between their first and second brood in Close Harems (Wilcoxon signed-rank test: W = 123 p = 0.3413, N = 15) or Far Harems (W = 118 p = 0.6519, N = 16). There was also no difference in the amount of time females spent at the barrier between brood cycles in Far Harems (W = 178 p = 0.0029, N = 15). However, females spent less time at the barrier during their second brood cycle in Close Harems (W = 137 p = 0.3743, N = 16).

There was a significant effect of treatment on the amount of time that females spent in their shells during egg stage (LMM: $\chi^2 = 8.36$, df = 2, p = 0.0285; Figure 4.4; Table 4.2). Females spent significantly more time in their shells in Close Harems than when in Pairs (estimate ± SE = 0.399 ± 0.19, T = 26.88, p = 0.0128, N =27), but there was no difference between Close Harems and Dispersed Harems (estimate ± SE = -0.241 ± 0.17, T = -1.40, p = 0.1742, N =27) or between Dispersed Harems and Pairs (estimate ± SE = 0.257 ± 0.18, T = 2.668, p = 0.1564, N =27). There was one significant outlier for shell time (1514s) but the results did not change with this outlier removed.

There was a significant effect of treatment on the time that females spent guarding their shells during the whole brood cycle (LMM, $\chi^2 = 13.75$, df = 2, p = 0.0010; Figure 4.5). Females spent significantly more time guarding in Close Harems than in both Pairs (estimate ± SE = 206.73 ± 72.3 , t = 2.86, p = 0.0046) and Dispersed Harems (estimate ± SE = 256.17 ± 71.3, t = 3.59, p = 0.0004). There was no difference in time guarding between Pairs and Dispersed Harems (estimate ± SE = 49.44 ± 65.1, t = 0.76, p = 0.4483).

Table 4.2. Mean ± SD for the number of incidences or the time spent performing different behaviours by females in each of the three treatments. For behaviours across the whole brood cycle (all except time in shell), values were averaged within brood stage within individual female and then across all females. Behaviour times are given in seconds.

	Treatment		
Behaviour	Pair	Dispersed Harem	Close Harem
Time spent displaying	NA	140 ± 197	454 ± 366
Number of display bouts	NA	5 ± 5	10 ± 8
Time spent at barrier	NA	18 ± 28	28 ± 30
Number of barrier bouts	NA	3 ± 4	5 ± 5
Time spent in shell (Egg stage)	167 ± 131	111 ± 42	108 ± 64
Time spent guarding shell	779 ± 373	698 ± 402	887 ± 477

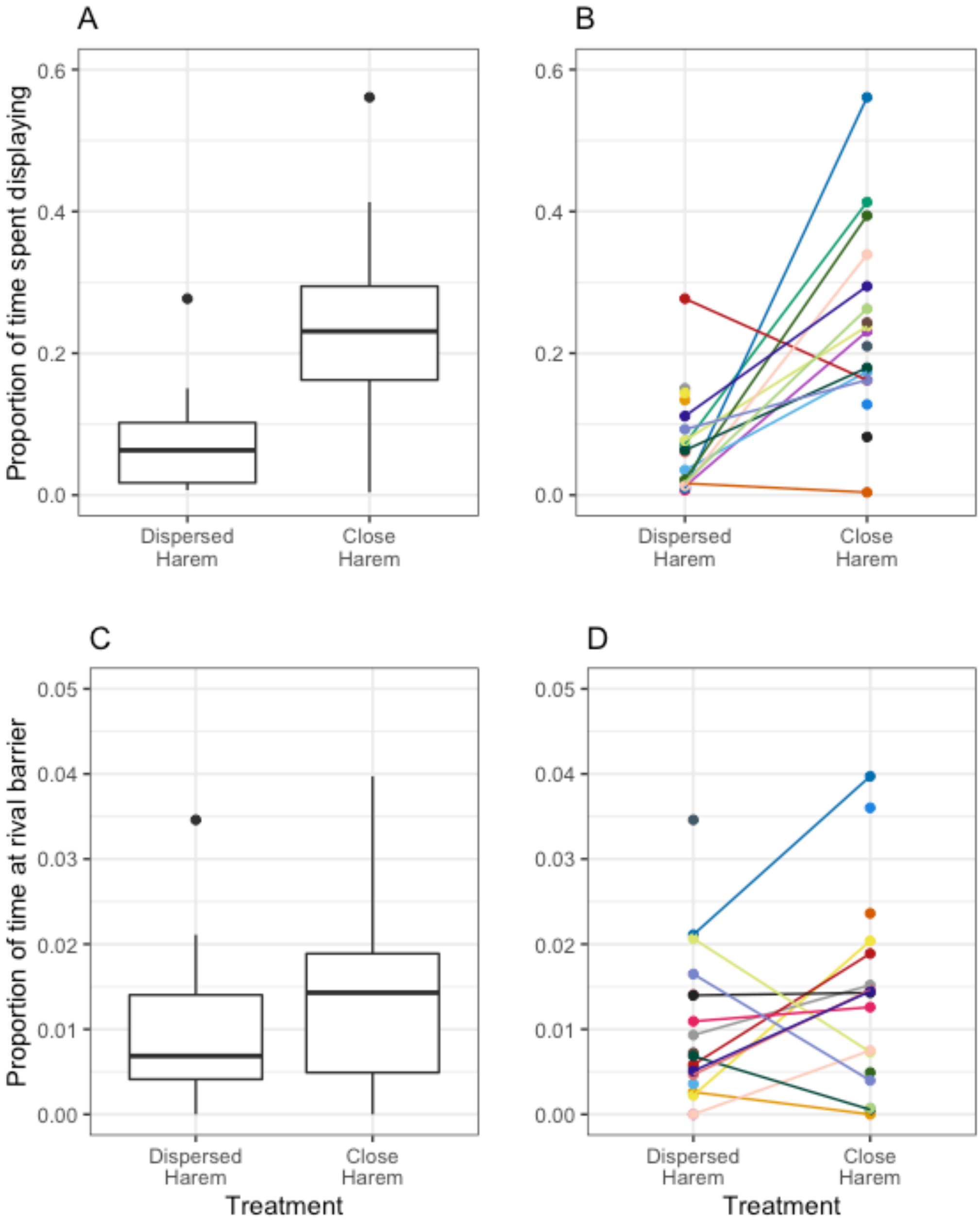

Figure 4.3. The effect of harem treatment on the proportion of time that females spent displaying to the rival female (A and B) and in contact with the barrier between female territories (C and D). A and C are boxplots showing the median and interquartile ranges for broods in each treatment after controlling for brood stage. Black points indicate outliers. B and D show female means with each female represented by a different colour.

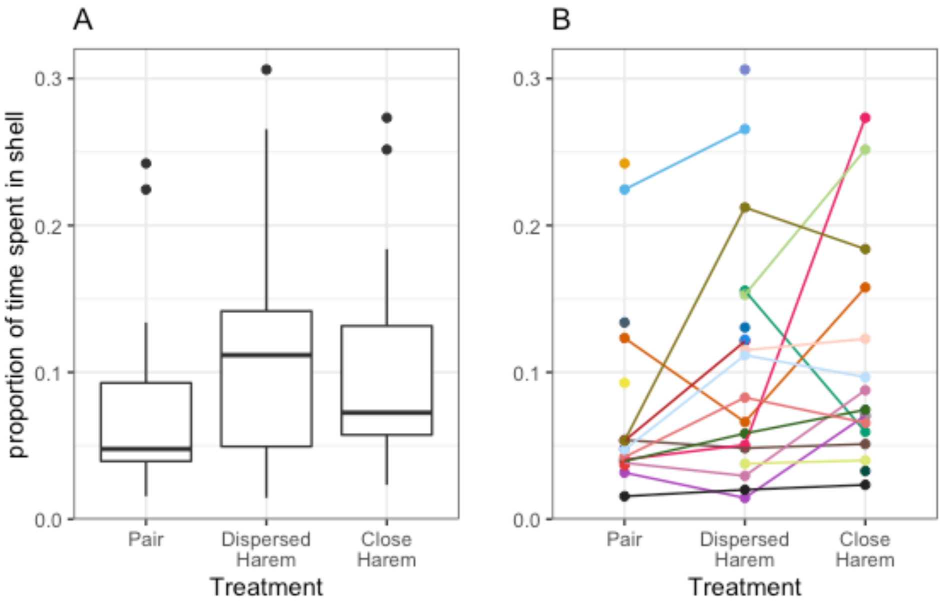

Figure 4.4. The effect of treatment on the proportion of time that females spent in their shell during the egg stage. The single outlier of 1514 seconds (0.84) has been removed. A is a boxplot showing the median and interquartile range for broods in each treatment with black points denoting group outliers. B shows female means with each female represented by a different colour.

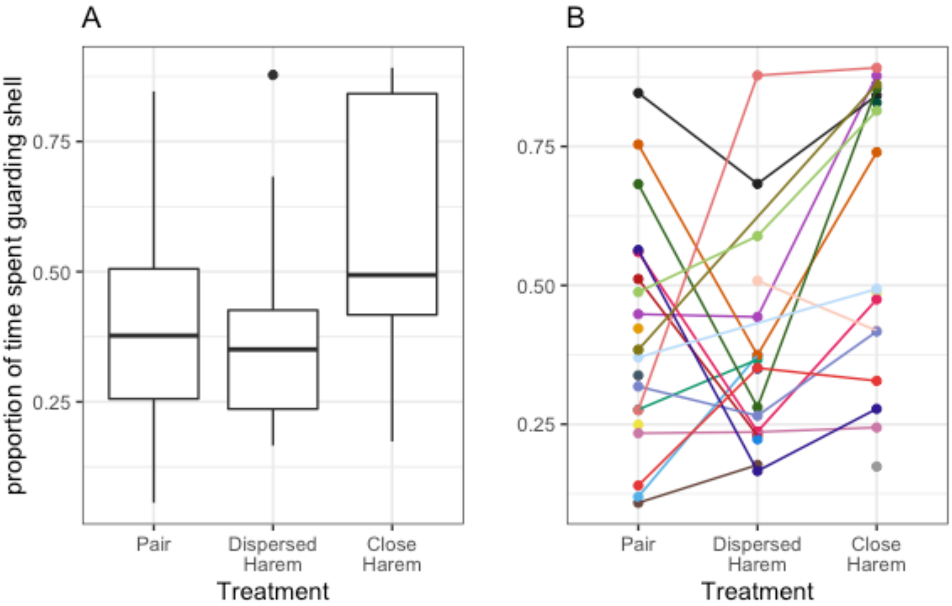

Figure 4.5. The effect of treatment on the proportion of time that females spent guarding their shells across the brood cycle. A is a boxplot showing the median and interquartile range of average values for each brood in each treatment after controlling for brood stage. Black points denote group outliers. C shows female means with each female represented by a different colour.

4.3.3 The effect of competition on hatching success

There was a significant effect of competitive treatment on hatching success (GLMM, $\chi^2 = 76.28$, df = 2, $p < 0.0001$; N = 53; Figure 4.6b). In Close Harems, 35 ± 24 % of eggs hatched on average, which was significantly lower than in both Dispersed Harems (58 ± 27%; estimate ± SE =-1.25 ± 0.16, Z = -8.037, $p < 0.001$) and pairs (59 ± 25%; estimate ± SE = -0.307 ± 0.10, Z = -3.217, p = 0.0039). There was no difference in hatching success between Dispersed Harems and Pairs (estimate ± SE = -0.019 ± 0.16, Z = -0.122, p = 0.9028). Hatching success was significantly lower in round 2 of the experiment than round 1 (estimate ± SE = -0.65 ± 0.32, Z = -2.008, p = 0.0447). There were no differences in hatching success between populations ($\chi^2 = 5.62$, df = 5, p = 0.3455) and no effect of female weight on hatching success (estimate ± SE = 0.926 ± 1.60, Z = 1.036, p = 0.563; N = 34). There was no effect of time spent in shell on hatching success (estimate ± SE = -0.181 ± 0.13, Z = -1.381, p = 0.1673). However, one outlier was identified for time spent in shell (1524s). When this was removed there was a significant negative correlation between time spent in shell and hatching success (estimate ± SE = -0.471 ± 0.19, Z = -2.448, p = 0.0144; N = 52; Figure 4.7c). In this model there was also no significant difference in hatching success between the two rounds (estimate ± SE = -0.529 ± 0.33, Z = -1.605, p = 0.1084; N = 26).

When restricting the analysis to successive broods produced in the harem treatments, I found no correlation between hatching success and either previous display time (GLMM, estimate ± SE = -0.284 ± 0.26, Z = -1.091, p = 0.2754; N = 23; Figure 4.7a) or current display time (estimate ± SE = -0.154 ± 0.27, Z = -0.575, p = 0.5651; Figure 4.7b). I also found no effect of brood interval on hatching success (estimate ± SE = 0.048 ± 0.07, Z = 0.643, p = 0.5202).

4.3.4 The effect of competition on brood size

I found no effect of treatment on brood size (LMM, $\chi^2 = 0.840$, df = 2, p = 0.657; N = 57, Figure 4.6a). Average brood size was 32 ± 11 eggs in Close Harems, 31 ± 9 eggs in Dispersed harems and 29 ± 11 eggs in Pairs. There were no differences in brood size between the two experiment rounds (estimate ± SE = 1.188 ± 2.99, df = 26, t = 0.397, p = 0.694). Brood size also did not correlate with brood number (estimate ± SE = 0.288 ± 0.44, df = 45, t = 0.654, p = 0.517) or female weight (estimate ± SE = 25.79 ± 14.51, df = 29, t = 1.777, p = 0.086; N = 38).

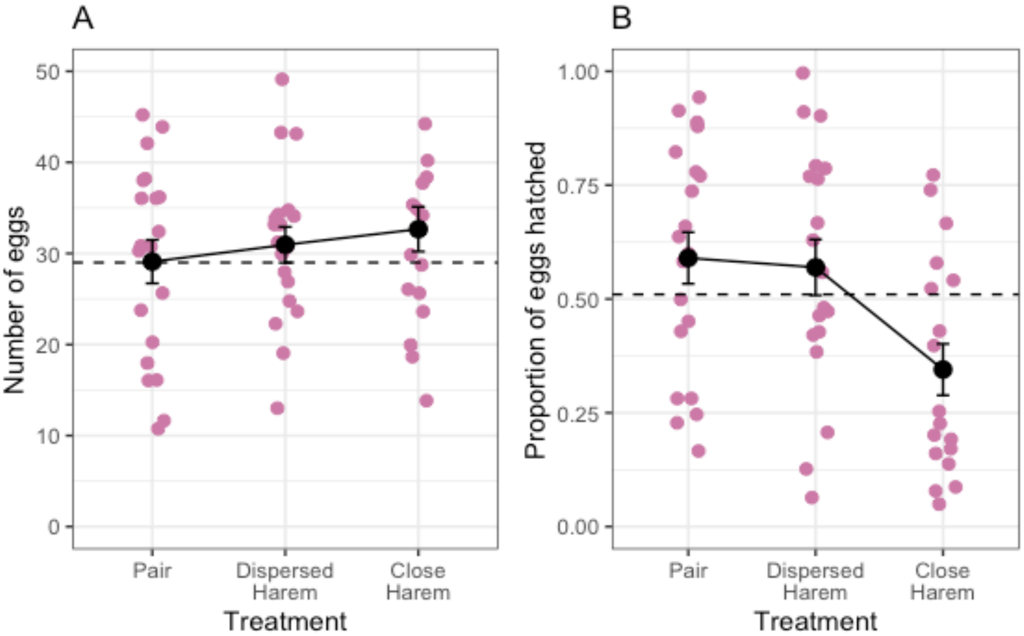

Figure 6. The effect of competition on A) hatching success and B) brood size. Black points represent the mean (± se) of the second brood in each competitive treatment while coloured points are values for each brood. The dotted line indicates the mean value of the whole sample.

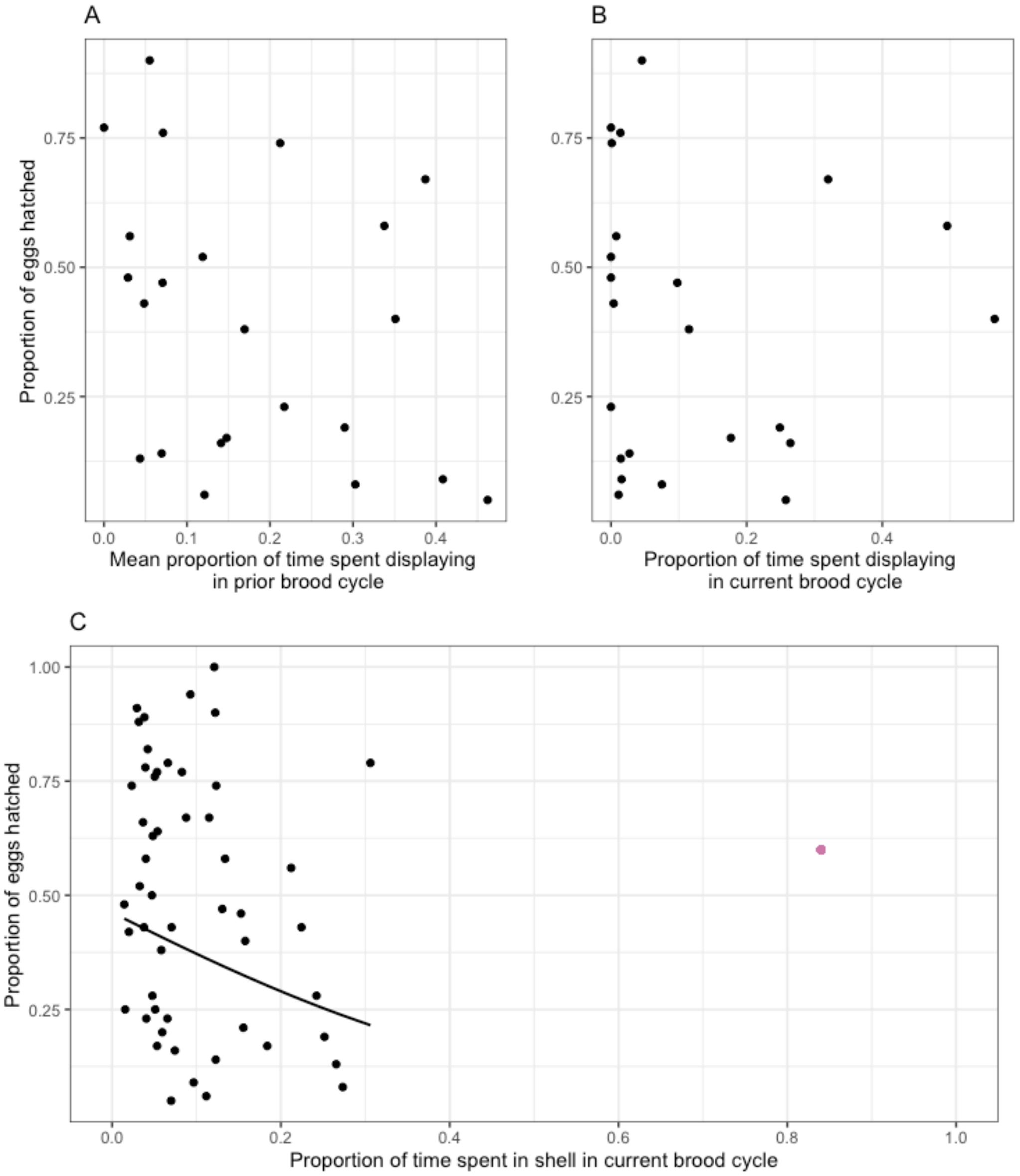

Figure 7. Correlation between hatching success and A) the mean proportion of time that females spent displaying during the previous brood cycle (harems only). B) the proportion of time that females spent displaying during the egg stage of the current brood cycle (harems only) and C) the proportion of time that females spent in their shells during the egg stage (all treatments). Each point represents one brood cycle. The coloured point indicates the single outlier (1514s) and the regression line is estimated from the GLMM with this outlier excluded.

4.3.5 The effect of competition on brood failure

There was a significant effect of treatment on the probability of the second brood failing (GLMM, χ^2 = 6.387, df = 2, p = 0.041; N = 57). Brood failure was more common in Dispersed Harems than Close Harems (9/19 vs 2/18 females; estimate ± SE = 2.961 ± 1.30, Z = 2.282 p = 0.0225) and showed a trend towards being more common in Dispersed harems than in Pairs (9/19 vs 3/20 females; estimate ± SE = 1.791 ± 0.93, Z = 1.935 p = 0.0529). There was no difference between Pairs and Close Harems (3/20 vs 2/18 females; estimate ± SE = 1.169 ± 1.24, Z = 0.942 p = 0.3464).

4.3.6 The effect of competition on female condition and inter-brood interval

I found no differences in Fulton's condition factor between the three treatments (LM, $F_{2,20}$ = 0.241, p = 0.788). In Pairs, Dispersed Harems and Close Harems, inter-brood intervals were, on average, 8 ± 8, 6 ± 10 and 7 ± 9 days respectively. There was a significant effect of treatment on inter-brood interval (χ^2 = 14.63, df = 2, p = 0.0007). Intervals were higher in pairs than in both Dispersed Harems (estimate ± SE = 0.5350 ± 0.23, Z = 2.382, p = 0.0172) and Close Harems (estimate ± SE = 0.6067 ± 0.17, Z = 3.586, p = 0.0003). There was no effect of female weight on inter-brood interval (estimate ± SE = 1.264 ± 1.22, Z = 1.039, p = 0.2988), but the interval decreased with increasing brood number (estimate ± SE = -0.127 ± 0.06, Z = -2.278, p = 0.023). However, there were seven significant outliers identified (17-46 days). When these were removed, there were no differences in inter-brood interval between treatments (χ^2 = 2.27, df = 2, p = 0.3221).

4.4 Discussion

The reproductive consequences of competitive investment in females have not previously been tested using experimental manipulations of the competitive environment. I investigated the reproductive costs to females of breeding in three competitive environments and of investing in competition with other females in their breeding group. I found that female *L. ocellatus* suffered a reduction in hatching success when breeding in the most intensely competitive environment, where their territory was close to the rival female's territory, compared to the two less competitive environments where there was a greater distance between territories or the absence of a rival. Females spent significantly more time in aggressive behaviour in these Close Harems indicating that competitive investment is likely to have negative consequences for hatching success in this species. In contrast I found no effects of competitive environment on brood size.

The negative effect of competition on hatching success was only observed in Close Harems. As distances between females of 25cm have not been reported in the wild, this result demonstrates the importance of manipulations that inflate competition for revealing reproductive trade-offs. The greater distances observed between females in natural harems may be a consequence of the reproductive costs to females of closer proximity demonstrated here. Females may avoid attempting to nest at such close proximity to other females to reduce the need for such high investment in competition. Harems with females in close proximity may also be unstable if the risk of expulsion by the other female is high. Previous work has shown that when females compete for shells 25cm apart, in the absence of a male, both females are rarely able to settle (Brandtmann et al. 1999).

In this experiment females were fed regularly and their maintained condition could have prevented the detection of a trade-off, particularly in Dispersed Harems. However, the large variation in hatching success across treatments and the clear decline in Close Harems indicates that females were experiencing constraints on their investment in reproduction. Although this experiment was primarily designed to assess differences in hatching success between successful broods, I also found that females in Dispersed Harems were more likely to have a failed brood. This might suggest that females breeding in harems at greater distances also experience reproductive costs but further experiments assessing reproductive output between treatments are needed to confirm this.

While females may prefer to nest away from one-another, they do not have total control over where they can settle. Group composition will be partly determined by the natural distribution and density of shells on the sand bed, as neither males or females are capable of moving shells. Males are also likely to play an important role in determining settling distances. Males bury shells that are unused and only uncover them when a female is ready to spawn, exerting control over which shells in their territory are occupied by females (Bills 1997). Since male reproductive success also depends on how many eggs hatch, negative effects of female competition on hatching success may influence male behaviour and settlement decisions. Males have been shown to interfere with female contests which greatly reduces the number of aggressive interactions between females (Walter and Trillmich 1994). In my experiment, the reduction in hatching success experienced by females in Close Harems results in each female producing only half the number of surviving offspring as a female breeding in a Pair. In contrast, the combined offspring number produced by females in Dispersed Harems is double that produced by pair-breeding females. While in this experiment the male was kept separate from the females and could not prevent aggressive interactions, in the wild male peacekeeping may reduce the negative effects of aggression on hatching success observed in females breeding in close proximity. However, if males can achieve greater reproductive success when females breed further apart, without the need for investment in peacekeeping, maintaining harems at close distances has potential costs. Further experiments are needed to investigate whether females avoid settling close to other females and/or whether males control female settlement as a way to limit the negative consequences of female competition, as well as assessing the influence of natural shell densities.

Although females did not show a reduction in display behaviour over time, heightened aggression, measured as time in contact with the barrier to the rival's territory, reduced between successive brood cycles in Close Harems. Despite some evidence of habituation to the rival, treatment order was randomised between females and this effect is therefore very unlikely to be responsible for the differences in aggression observed between the harem treatments. In addition, only data from the second brood cycle of each treatment was analysed in recognition that moving females between treatments could cause initial changes to behaviour, including changes in aggressive behaviour as females re-establish territory boundaries.

Although my results demonstrate that breeding under high levels of competition can have detrimental effects on reproductive output, the mechanism behind this trade-off remains elusive. By expending greater energy on competitive behaviour, females may lose body condition leading to a reduced ability to produce viable eggs. Negative effects of female condition on egg quality have been found in fish (Ouellet et al. 2001), birds (Hanssen et al. 2002) and insects (Ianora and Poulet 1993). In addition, if females feel more threatened by competitors they may spend more time defending their shells and less time foraging. I found no differences in female condition at the end of the brood cycle across any of the three treatments, but females in Close Harems did spend more time guarding their shells (Figure 4.5). Alternatively, females may cannibalise some eggs from their brood to maintain their condition. Although filial egg cannibalism by fathers can affect condition status in fish (e.g. Marconato and Bisazza 1988; Lindström and Sargent 1997; Manica 2004), this effect has not been investigated in females. In this study, I often observed eggs disappearing before hatching, indicating that females were either consuming eggs or removing them from the shell.

Energetic trade-offs can lead to increases in the interval between broods. Increased inter-spawning intervals have been observed in fish in response to greater care investment (Balshine-Earn 1995; Grüter and Taborsky 2004), and increases in stress (Mileva et al. 2011) but the effects of investment in competition have not previously been explored. In *L. ocellatus*, I found that females had shorter intervals between spawns when they were in a harem and there was no effect of female weight which does not support condition-related effects. However, other aspects of the competitive environment may influence inter-spawning intervals. In particular, females in harems are at constant risk of being evicted by other resident females and therefore it may benefit them to produce broods closer together while they still have control of a shell. I also found that intervals were significantly shorter between later broods. Females were both larger and older when they produced later broods, and shorter intervals may indicate that females were investing more in current reproduction by allocating more resources to reproduction than somatic growth (Kozłowski and Teriokhin 1999). Alternatively, decreasing intervals could be a result of greater familiarity between males and females during later brood cycles leading to earlier spawning. Familiarity between pairs has been shown to positively influence reproduction in many species ("mate familiarity effect"; Black and Hulme 1996). For example, in zebra finches (*Taeniopygia guttata*), pairs with stronger and more stable relationships were faster to initiate reproduction (Maldonado-Chaparro et al. 2021).

Besides the physical condition of the mother, competition-driven maternal effects could be influencing hatching success. Stress in mothers can lead to corticosteroids entering eggs and affecting offspring viability (Mccormick 1998; Hayward and Wingfield 2004; Warner et al. 2009; MacLeod et al. 2018). For example, in barn swallows (*H. rustica*), eggs with higher concentrations of corticosterone have lower hatchability and produce lower quality fledglings (Saino et al. 2005). Similarly, androgens linked to aggression, such as testosterone, can be passed to offspring via the egg (Schwabl 1993; Bentz et al. 2016). Higher yolk testosterone levels have been shown to have positive effects on offspring quality in some birds (Schwabl 1996; Lipar and Ketterson 2000; Bentz et al. 2013) but negative or null effects in others (Sockman and Schwabl 2000; Andersson et al. 2004). Finally, the distribution to eggs of micronutrients essential for healthy development, such as antioxidants and amino acids, may be reduced by mothers under high competition (Harrison et al. 2011; Reading et al. 2018). Investigations into hormonal and nutritional changes in females during competition may help us to identify a mechanism for the observed effects of breeding under high competition on hatching success.

Since healthy sperm are also essential for the formation of a viable embryo, the condition of males, as well as females, may be an important factor affecting hatching success. In this experiment, harem males were kept the same across treatments (except in one case where the male was swapped due to illness). Genetic differences in fertility or sperm quality between males would, therefore, not have influenced observed differences in hatching success between treatments. However, males often intervene in female contests (Walter and Trillmich 1994) and although in this study males and females were physically separated, males could still observe aggressive interactions between females. Male responses to female aggression, such as increased investment in aggressive displays in Close Harems, along with any associated physiological effects, may have caused changes in male condition between treatments. As in females, stress effects and nutritional deficiencies can reduce gamete quality in male fish with consequences for reproductive success (Bobe and Labbé 2010; Valdebenito et al. 2015). For example, in zebrafish (*Danio rerio*), males with lower quality sperm have reduced fertilisation rates and produce offspring with a higher percentage of embryo malformations (Riesco et al. 2019). Assessing changes in the behaviour and condition of males between treatments was beyond the scope of this study, but future studies should consider how female competition in harems impacts breeding males and the potential effects on reproduction.

In addition to the indirect effects of parental physiology on offspring, parental care may also be affected by changes in competition and aggressive investment. For example, dark-eyed junco females (*Junco hyemalis*) treated with testosterone reduced their time brooding and their intensity of nest defence (O'Neal et al. 2008). Female *L. ocellatus* spend more time inside their shells during the egg and larval stages than after the fry become free-swimming, implying that females are providing care inside the shell. From personal observations, females within shells appear to check or clean eggs using their mouths and their movement in and out of the shell entrance is likely to assist with water flow keeping eggs and larvae oxygenated and free of infection. However, I found that hatching success negatively correlated with the amount of time that females spent in their shell (Figure 4.7c) and that females spent less time in their shells when in pairs than harems (Figure 4.4). Whether female behaviour while inside the shell is reducing hatching success or whether females spend more time in their shells because their eggs are failing for other reasons is unclear.

If fertilised eggs are failing prior to hatching, identifying the developmental stage at which this occurs can provide information about the cause of embryo death (Hemmings and Birkhead 2016). Initial investigations into the causes of egg failure in *L. ocellatus* have found that, when the eggs of females breeding in close proximity fail, this occurs early in development, prior to segmentation (P. Jedryszek and J. P. Green, unpublished data). In this study, although each pair had already produced a viable brood, it is not clear whether eggs failed in second broods because they were unfertilised, or they were fertilised but not viable. To determine the mechanism behind a reduction in hatching success with greater social stress, it will be important to establish whether egg failure is a result of reduced fertility in females or males, or a result of embryo mortality (Assersohn et al. 2021). However, this early failure suggests that physiological effects of competition on mothers are more likely to explain low hatching success than a reduction in parental care.

The experiment was performed in two rounds that were separated by the first pandemic lockdown from March to June 2020 and hatching success was found to be significantly lower during round 2. I took care to control experimental conditions between the two rounds but there were two factors that differed between the two periods and may have influenced hatching success. First, there were differences in the populations and generations of both males and females. Fish from the first generations of populations A-D were used during round 1 while in round 2 I used second generation fish from populations B and C and fish from newly acquired populations E-F. Natural variation in the reproductive quality of populations could therefore have led to differences in reproductive output (Griffith et al. 2017). However, I found no effect of population on hatching success. In addition to genetic differences between females, the feeding conditions had been changed between rounds. Before the start of round 2, the amount of frozen food in the diet had been reduced from 5 times to 1 time per week. This decision was the result of an ongoing issue with illness (exopthalmos) which was affecting the stock population. I attempted to reduce the risk of my fish developing this condition by reducing the richness of their diet but this may have also affected reproduction and egg viability through reducing the condition of males or females or limiting some nutrients essential for successful breeding, as discussed above. Despite these differences between the two rounds I could control for any effects by including the round number as a fixed effect in all models of reproductive output, and I was still able to detect a significant effect of competitive environment.

In contrast to the reduction in hatching success under high competition, competitive treatment had no effect on brood size. Despite large variation in brood size, I found no significant predictors of this variation among the variables that I recorded for each brood. As well as offspring number, female investment in competition may have consequences for offspring quality which will ultimately influence their probability of survival. In tree swallows (*Tachycineta bicolor*), Rosvall (2011) similarly found no correlation between female aggression and brood size but higher female aggression had a negative effect on offspring size. In *L. ocellatus,* as eggs and larvae are confined to the shell it was not possible to record aspects of quality, such as weight, and once they become free-swimming they feed independently, leading to rapid divergences in size within a brood. Although free-swimming fry are independent feeders, females invest in defending the brood against predators and therefore still have a direct influence on their survival rates. In Chapter 5, I investigate how female investment in competition is affected by the presence of a brood predator to further understand the costs and benefits of harem breeding.

Females showed the highest levels of aggression when they had free-swimming larvae, a result consistent with that found by Walter and Trillmich (1994). This is in contrast to many other species with maternal care, where female competitive behaviour is strongest prior to the initiation of parental care (Liker and Székely 1997; Bentz et al. 2019; Diniz et al. 2019). For example, in the South American cichlid *Cichlasoma dimerus*, females were more aggressive pre-spawning than when they were caring for a brood (Tubert et al. 2012). This is also observed in biparental males and is thought to be driven by a trade-off between aggression and parental care (Wingfield et al. 1990). If the main purpose of intrasexual aggression is to secure a breeding site or a mate, the need for aggression is likely to decline once brood care has commenced. However, in species where threats to the nesting territory continue beyond territory establishment, females may need to continue investing in aggression despite the potential negative effects on parental care (Robart and Sinervo 2018; Bose et al. 2021). Female *L. ocellatus* breed continuously and asynchronously, and the likelihood of intrusion by other members of the harem and external females is not related to a resident's breeding status. As a result, reductions in aggression during brood care could put females at increased risk of eviction and brood loss.

In addition, rival females may pose a direct threat to a female's offspring through infanticide, which may explain persistent or increased aggression during parental care. (Wolff and Peterson 1998; Sinn et al. 2008). Female *L. ocellatus* are capable of cannibalising fry and I observed females attempting to pounce on fry through the barrier. Offspring continue to use the shelter of the shell, even after they become free-swimming, but when they are away from the shell they do not shoal, instead moving around independently keeping close to the sand. As a result, they are much less defendable and females may need to invest more in keeping rival females from entering the territory. Similar increases in female aggression during care have been observed in the closely related cichlid *Neoloamprologus multifasciatus*, which is linked to the potential risk posed by other females to offspring (Bose et al. 2021). This suggests that female aggression may play a role in brood defence as well as against female competitors.

Whether female aggression is correlated across these two contexts will be investigated in Chapter 5.

In summary, my results show that breeding in close proximity to other females, where intrasexual competition is heightened, generates intrinsic costs for females, resulting in reduced hatching success. This provides evidence for a trade-off between intrasexual competition and reproductive output in females, which supports the findings of other manipulation experiments that have demonstrated this trade-off in females (Morales et al. 2009; Doutrelant et al. 2012). In *L. ocellatus*, increased investment in competitive behaviour may lower hatching success through reductions in egg viability or increases in filial egg cannibalism. However, further studies are needed to investigate these mechanisms and determine the proximate causes of this trade-off.

Chapter 5

The influence of brood predators and male care on female-female competition in Lamprologus ocellatus

Rebecca Goldberg, Jonathan Green

Author contributions: Jonathan Green and I conceived of the study. Jonathan Green and I designed the laboratory experiment. I designed and created the model predator system. I collected and analysed the data. I wrote the chapter and incorporated comments from Jonathan Green.

Abstract

While offspring depend on protection against predation from their mothers, successful reproduction for females often also depends on competing with other females for limited resources, and investment in competition may negatively affect their ability to provide effective care. The consequences for offspring survival of a trade-off between investment in intrasexual competition and in brood defence are likely to depend on the nature of the resource that females compete for. While obtaining some resources will not directly improve offspring safety, competition for male contributions to defence may reduce the effects on offspring survival of reduced investments in defence by mothers. Female *Lamprologus ocellatus* breed in empty snail shells in small harems and simultaneously experience threats from rival females and threats to their offspring from brood predators.

It is not clear whether males contribute to brood defence but females have been found to compete for breeding positions that are closest to the male. In this chapter I first investigate whether female investment in competition is constrained by investment in brood defence by assessing the responses of breeding females to a rival in the presence and absence of a brood predator. I then investigate whether male contributions to defence may be a benefit of female competition by testing whether males respond differently to a predator in the presence of a brood I find that females reduce their vigilance and aggression towards same-sex rivals in the presence of a predator. I also find that males show more aggression towards predators when guarding a brood than when no brood or female is present. These results suggest that, although females experience a trade-off between brood defence and intrasexual competition, the effects of this trade-off on offspring survival may be reduced by male contributions to defence.

5.1 Introduction

The defence of broods against predation is a key component of parental care for many animals with important consequences for offspring survival (Montgomerie and Weatherhead 1988). As well as investing in parental care, mothers may benefit from investing in intrasexual competition for reproductive resources that will also contribute to the success of their current or future offspring (Clutton-brock and Huchard 2013). However, investing in competition with same-sex rivals may reduce the resources available for brood defence or decrease vigilance towards predators with detrimental consequences for offspring survival (Hess et al. 2016; Ota 2018). This trade-off is, therefore, likely to have an important influence on the costs and benefits to females of investing in competition, but the interaction between parental care and competition in females is complex.

Successful reproduction in females can depend on a range of different resources that vary among species. The extent to which a reduction in brood defence resulting from increased investment in competition impacts offspring survival will likely vary depending on the type of resource that females compete for. In particular, some resources, such as male parental care or offspring shelters, may directly decrease the risk of predation on offspring. Obtaining these resources could reduce the costs of competition to offspring survival associated with decreased female vigilance and defence. In contrast, if females compete for resources that do not reduce offspring predation, such as mates or nest sites, investment in competition may come at a cost to offspring defence. Knowledge of the resources that drive competition between females is therefore important for understanding how this trade-off manifests and the consequences for the evolution of competitive traits.

As well as constraints imposed on trait expression by resource trade-offs, behavioural traits may be phenotypically correlated, restricting the plasticity of responses to different ecological contexts. This may result in an individual exhibiting suboptimal behaviour in some contexts which would increase the costs associated with all correlated traits. For example, in the spider, *Agelenopsis aperta*, aggressive females are effective competitors but can be overly aggressive hunters, killing more prey than they can consume, resulting in unnecessary energy expenditure (Riechert and Hedrick 1993; Maupin and Riechert 2001). Alternatively, if competitive traits can benefit individuals in multiple contexts, this can reduce the conflict between investments and behaviours may become functionally integrated (Bergmüller and Taborsky 2007). For instance, aggression may assist individuals during resource contests and during brood predator defence (Cain et al. 2011; Clutton-Brock and Huchard 2013).

In females, correlations in aggression may be beneficial if those that engage in more contests have reduced predator vigilance, necessitating more aggressive responses to intruding brood predators when they are detected (Hess et al. 2016). However, if females compete for resources that influence brood predation risk, maintaining plasticity in aggression between contexts may be more beneficial. For instance, if females that are more aggressive competitors obtain more male assistance, they could benefit from reducing aggression towards brood predators and conserving their resources for other reproductive demands. In this case, females that are less competitive would lack male assistance and consequently benefit from displaying high aggression towards brood predators. Examining how traits are correlated across the contexts of resource competition and brood defence can contribute to our understanding of the costs and benefits of competition for females. Combining this with knowledge of the resources that drive competition will help us to determine whether female competitive traits are likely to be constrained or enhanced by phenotypic associations between traits.

The shell-dwelling cichlid *Lamprologus ocellatus* is facultatively polygynous and females compete with one another for access to shells in which to breed and for membership within a harem (Brandtmann et al. 1999). Female investment in competitive behaviour was shown in Chapter 4 to cause reduced hatching success, but this experiment excluded the influence of brood predation on offspring survival. If females that invest heavily in competition are unable to appropriately respond to threats, due to reduced vigilance, depleted resources, or correlations between traits restricting plasticity, this would increase the risk of brood predation leading to further costs to offspring survival. Intrasexual aggression in males of this species has already been shown to reduce vigilance towards predators (Ota 2018). Alternatively, if investment in competitive aggression improves responses to predators, or results in greater brood defence from the male, the costs of competition for offspring survival would be reduced. Males are larger than females and defend their territories aggressively from both conspecific and heterospecific intruders that would threaten eggs and fry. Females have been shown to compete for shells that are closer to the male's primary shell where they may benefit from direct brood defence from the male or incidental defence as a biproduct of territorial defence, but this has not yet been demonstrated (Brandtmann et al. 1999).

Here I present the results of a laboratory study into the effect of brood predation on female competition. I first exposed guarding females to a model brood predator to assess how female investment in competition changes with and without the presence of a brood predator and determine whether brood defence constrains investment in intrasexual competition. I then exposed males to the same model to assess whether males respond differently to a predator when their female partner and brood are present compared to when they are alone to determine whether males assist with brood defence.

5.2 Methods

5.2.1 Experimental set-up

The experimental set-up is shown in Figure 5.1. Two experimental tanks measuring 90 x 80 x 30cm (90 litres) were divided into three compartments by inserting two barriers made from a 49 x 25cm straight piece of opaque plastic and two 25 x 25cm pieces of transparent plastic attached to each end at a 160° angle. This created a diamond shaped central compartment for the model brood predator. This shape was used so that during exposure to the predator the rival female would not be able to see into the predator compartment. Opaque plastic inserts were used to cover the transparent sections of these barriers allowing the view from the side compartments into the central compartment to be concealed or exposed. Additional barriers were inserted to the two side compartments dividing each into three smaller compartments: a long section along the outer edge of the tank for the male and two sections between the central and male compartment for the focal and rival females. The male and female compartments were connected by removable transparent ‘doors’.

Twenty-five breeding pairs of *L. ocellatus* were established in home tanks, and once their brood had hatched, they were moved into one of the side compartments of the experimental tanks. The number of hatched larvae was recorded and the standard length of females was measured to the nearest 0.1mm using a calliper while females were in a net held up against the wall of the tank. Pairs were formed randomly with respect to size and population, while (in all but one case) avoiding pairing males and females from the same population. A rival female from a different population and home tank to the focal female was added between 5 and 7 days before the trial. Because populations were housed separately and were sourced from different breeders, by pairing females from different populations I could exclude any potential effects of familiarity or relatedness on female-female aggression.

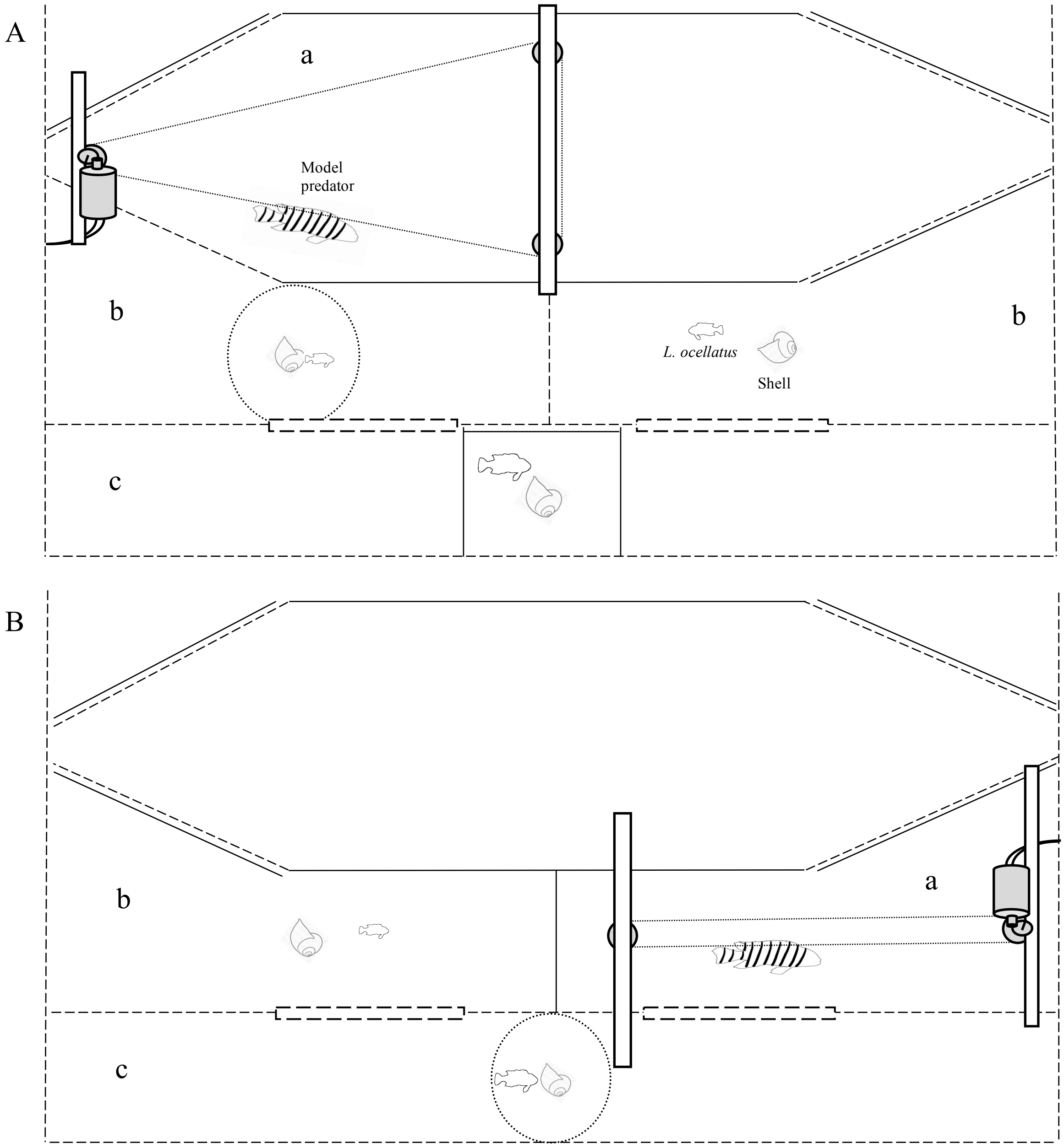

Figure 5.1. Experimental set up for A) female trials and B) male trials, showing the central predator compartment and one of the side compartments. Dashed lines indicate transparent barriers whilt solid lines are opaque barriers. a – compartment for model predator with motorized pulley system above. b – compartments for female *L. ocellatus* and their shells. c – compartment for male *L. ocellatus* and shell. The dotted circles denote the area around the shell where the focal fish was classed as guarding their shell (~10cm diameter for females and ~7 cm diameter for males). In the space to the right of the circle focal females were classed as towards the rival and focal males as towards the predator. In the space to the left of the circle, focal females were classed as away from the rival or focal males as away from the predator.

5.2.2 Model brood predator

The brood predator used in this study was a model of a *Lepidiolamprologus* cichlid, a group that is known to threaten the fry of *L. ocellatus* but poses no risk to adults (Bills 1997). To make the model, I made a silicone mould (Polycraft GP-3481-F RTV Silicone Rubber) from a dead female *Lepidiolamprologus hecqui* specimen, from which I could then make polyurethane resin casts (Polycraft SG2000 Casting Resin) that were realistic replicas of the original fish. Well exposed, high resolution photographs were taken of a different *L. hecqui* specimen using an Olympus OM-D EM10 mirrorless camera. These were printed and then glued to each side of the three-dimensional cast. The cast was then coated in transparent epoxy resin (Polycraft Translux D150 Epoxy Resin) creating a coloured, water-proof replica (Figure 5.2). The model measured 75mm (total length).

I attached a transparent piece of plastic (150mm x 36mm) to the model and connected this to a piece of fishing line that ran around the top of the tank above the water. This line was wrapped around a set of pulleys, one of which was attached to an automated DC motor (PC Control Ltd.). For the female trials, three pulleys were used so that the predator moved in a triangular trajectory around one half of the central compartment. For the male trials, two pulleys were used so that the predator moved back and forth in the compartment. To mimic more realistic behaviour, the movement of the model was interspersed with periods where the model was motionless.

The responses of males and females to the model predator were consistent with those expected for a brood predator threat. Females responded by positioning themselves between the predator and the shell while displaying and were also observed swimming rapidly at the predator as it approached and making contact with the barrier between their compartments. Males were also observed displaying aggressively as the model approached and making contact with the barrier. Similar responses were observed in wild populations with both sexes displaying to and chasing approaching *L. meeli* intruders (Chapter 6)

Figure 5.2. Model predator. Images of A) live *Lepidiolamprologus sp.* specimen (male) and B) *Lepidiolamprologus sp.* replica used in the predator exposure experiment.

5.2.3 Female trial procedure

Female trials were run on one day between Day 14 and Day 17 post-spawning when the offspring were free-swimming. At least two days prior to the trial the opaque insert blocking the view between the predator and the focal female compartments was removed to allow focal females to become acclimatised to this new space. Immediately prior to the trial, three opaque barriers were placed around the male's shell to prevent the male from viewing the predator. The motorised system was set up at the start of the trial. To record the response of the focal female to the rival female in the absence of predation threat, females were first filmed for 30 minutes using three cameras (GoPro Hero 7 White), two positioned at the sides of the two female compartments and one positioned directly above (No Predator Treatment). Following this, the model predator was added to the central compartment, the cameras were restarted and the motor started. To record the response of the focal female to the rival female in the presence of a predator, females were then filmed for a further 30 minutes (Predator Treatment). Following completion of the trial, the model predator was removed, the opaque insert was replaced and the rival female and her shell were removed. The door between the rival female and the male's compartment was opened to allow the male to explore the area of his territory that would later contain the predator.

The No Predator treatment always preceded the Predator treatment to avoid any influence of after-effects following exposure to the model predator on behaviour in the No Predator treatment (Lima 1998). Habituation to the rival over a trial lasting 1 hour is not expected. Evidence from Chapter 4 shows that females housed for multiple months with the same rival continue to display high levels of aggressive behaviour. In this experiment, rival females were added at least 5 days prior to the trial and any initial effects on aggression stimulated by a change in social group composition will not have influenced the trial. To provide further evidence that habituation to the rival female will not have influenced female behaviour across the two treatments I compared aggressive behaviour towards the rival between the first and last five minutes of the No Predator treatment for each trial (details in 5.2.6).

5.2.4 Male trial procedure

Male trials were run on two days between Day 15 and Day 19 post-spawning and at least one day after the female trial. On the first day, before initiating the trial, the door between the rival female and male compartments was closed and the transparent barrier between the female compartments was replaced with an opaque barrier to prevent the female from viewing the predator. Two of the cameras were positioned at the top and on the predator side of the tank, the predator sequence was started and male behaviour was filmed for 15 minutes to record male responses to the predator in the presence of a female and brood (Female Treatment). The model predator was then removed and the female and her offspring were moved out of the experimental tank. The door between the pair female and the male compartment was opened to allow the male to explore this area of his territory that no longer contained a brood. On the second day of the trial, the door between the compartments was closed again and the male was exposed to the predator for a further 15 minutes to record male responses the predator in the absence of a female and brood (No Female Treatment). The procedure for both female and male trials is summarised in Figure 5.3.

Treatment order was kept the same for each trial. To run the No Female treatment first would require testing males prior to breeding and, since the timing of breeding is variable and unpredictable, it would not have been possible to standardise the time period between the two treatments. By running the No Female treatment second, I could control the breeding experience of males in this treatment across trials and could run both treatments 1-2 days apart. It is possible that changes in the behaviour of males towards the predator in the No Female treatment are a result of habituation to the model predator rather than due to the absence of a brood. However, exposures to the model were short and separated by at least a day and are likely to be viewed by males as distinct intrusions. Under natural conditions, males experience territory intrusions from *L. meeli* adults regularly (field data from Chapter 6; mean ± SD intrusions per hour by *L. meeli* = 18 ± 16) and habituation to these intruders is unlikely to be beneficial as they pose a constant threat to offspring. In wild groups, repeated intrusions by the same *L. meeli* individuals from neighbouring territories were met with consistent high aggression from males and females (pers. obs). To provide further evidence that habituation to the predator model is unlikely to have influenced male behaviour across the two treatments I compared aggressive behaviour towards the predator between the first and last five minutes of the Female treatment for each trial (details in 5.2.6).

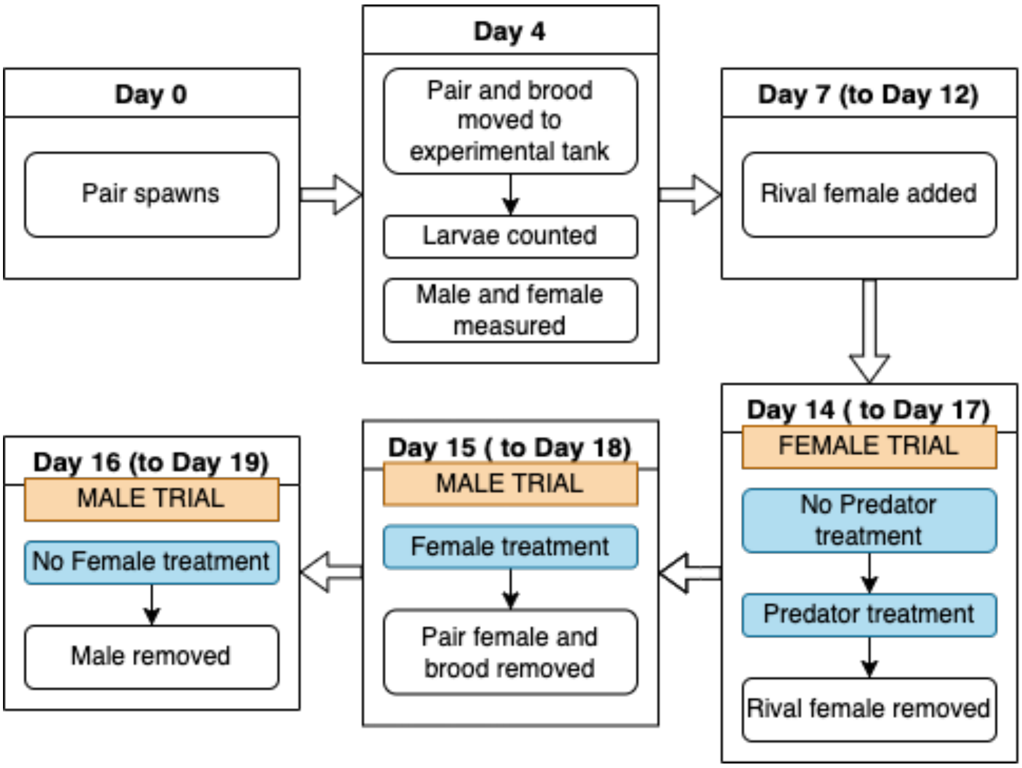

Figure 5.3. Schematic representation of the procedure and timings for the predator experiment. In panel headings, unbracketed days indicate the earliest day that each step could take place, bracketed days indicate the last day that each step could take place. For each trial, the possible day range for each step depended on which day the previous step was completed, as described in 5.2.3 and 5.2.4

5.2.5 Video analysis

Videos were analysed using the Behavioural Observation Research Interactive Software ('BORIS'; Friard and Gamba 2016). For the female trials, females were considered to be guarding their shells if they were within two body lengths of their shell. Displaying in this area was coded as ‘undirected display’. Otherwise the female was either closer to the rival than the predator (‘towards rival’) and displaying in this region was considered as ‘displaying near the rival’, or closer to the predator than the rival (‘away from rival’) and displaying here was coded as ‘displaying near the predator’. To quantify the effect of predator presence on vigilance by focal females to the approaches of rival females, for each occasion that the rival female moved towards the focal female I recorded whether the focal female responded to her approach by also moving towards the rival when previously she had been away or guarding her shell. Finally, I recorded the incidence number and duration that focal females were in contact with the barrier to the rival and the predator compartments.

For the male trials, males were considered to be guarding their shells if they were within one body length of their shell. This shorter distance was used so that when they were in contact with the predator barrier they were not considered to be guarding. Displaying while guarding was coded as ‘undirected display’. If the male was away from his shell he was either adjacent to the predator barrier (‘towards predator’) or adjacent to the female barrier (‘away from predator’), displaying in these regions was coded as ‘display to predator’ and ‘interaction with female’ respectively. I also recorded the incidence number and duration that males were in contact with the barrier to the predator compartment.

5.2.6 Statistical analysis

Statistical analyses were run in R version 4.0.4. (R Core Team 2021). Unless specified, for all analyses I used two-tailed statistical tests in which $\alpha = 0.05$. I ran linear mixed models (LMM) using the packages lme4 (Bates et al. 2015), lmerTest (Kuznetsova et al. 2017) and glmmTMB (Brooks et al. 2017). Each focal male and female was assigned a unique identifier and, unless specified, all models included a random effect for either female ID (female trials) or male ID (male trials) as in each trial the focal male or female experienced both treatments. Residual analysis was performed using diagnostic plots for LMMs and non-parametric overdispersion tests for generalised LMMs (GLMM) in the DHARMa package (Hartig 2021). Variables that did not initially meet the assumptions of normality of residuals were square-root transformed (female trials: total display time, display to rival time, time towards rival). Several variables were characterised by a high proportion of zeros (female trials: display to predator time; male trials: display to predator time). These variables were modelled using zero-inflated negative binomial models in the glmmTMB package which solved overdispersion issues (Brooks et al. 2017). The inclusion of additional fixed effects was based on comparing the fit of models with and without the variable using likelihood ratio tests.

1) Female trials

I compared how females focus their aggressive behaviour in the presence and absence of a predator using three LMMs. The response variables were: display time near the predator, display time near the rival and total display time. Treatment was the main predictor. In each model I included three additional predictors: (1) brood size, which determines brood value and can therefore influence parental investment decisions (Montgomerie and Weatherhead 1988); (2) female standard length, as size can contribute to resource holding potential and influence contest decisions (Arnott and Elwood 2009), and (3) rival display time, as focal female responses were likely to be influenced by aggressive behaviour exhibited by the rival.

To test whether focal females responded less to the approaches of rival females during the Predator treatment I used a binomial GLMM to compare the probability of responding in the Predator and No Predator treatments. This model also included rival display time, brood size and female length as fixed effects. In three trials, the rival female never approached the focal female's territory during one of the treatments. These three trials were therefore omitted from this analysis. To investigate whether females invest more in aggression towards the rival or the predator when both threats were present I used a Wilcoxon signed-rank test.

I investigated whether female display behaviour was correlated within females across the contexts of female competition and brood defence using two linear models (random effect for female ID removed). In the first model I set time displaying near the rival in the No Predator treatment as the response variable, and time displaying near the predator during the Predator treatment and time displaying near the rival during the Predator treatment as the predictors. In the second model I focused only on the Predator treatment and set time displaying near the predator (log-transformed) as the response variable and time displaying near the rival as the predictor. In both models I also controlled for brood size and female length as both of these factors may influence investment decisions.

Finally, I investigated whether habituation to the rival female across the trial could be influencing the effect of treatment on aggression. I tested whether display time and barrier contact time decreased between the first and last five minutes of the No Predator treatment using two, one-tailed Wilcoxon signed-rank tests.

In one trial, the focal female did not display to either threat during the predator treatment and remained guarding her shell for the duration of the film. In a second trial, the focal female spent the total time towards the rival during the predator treatment and the possibility that she never observed the predator model could not be ruled out. These two trials were, therefore, omitted from all analyses. Total sample size was thus 23 trials.

2) Male trials

Trials were run on 23 males. To compare differences in male aggression towards the predator in the presence and absence of a brood I fitted two models. I set either time displaying near the predator (GLMM with zero-inflated negative binomial error distribution) or number of contacts with the predator barrier (GLMM with Poisson error distribution) as the response variable, and treatment and male size as the predictors. To compare differences in the amount of time that males spent guarding their shell between treatments, I fitted a LMM with time spent guarding as the response and treatment and male size as the two predictors. Lastly, I tested whether habituation to the brood predator may be influencing the effect of treatment on male aggression by comparing display times between the first and last five minutes of the Female treatment using a one-tailed Wilcoxon signed-rank test.

5.2.7 Ethics statement

This laboratory experiment was conducted under project licence no. PA6DCCB23. I ran all trials under personal licence no. I84783471.

5.3 Results

All averages are presented as mean ± standard deviation. Behaviour times are seconds out of a 30-minute recording.

5.3.1 The effect of predator exposure on female behaviour

There was no difference in the total amount of time that females spent in aggressive displays between the No Predator and Predator treatments (470 ± 352s vs. 534 ± 464s; estimate ± SE = 1.59 ± 2.02, df = 27.49, t = 0.788, p = 0.4375, N = 23). Females spent more time in aggressive displays when they had larger broods (estimate ± SE = 0.812 ± 0.24 df = 23.38, t = 3.45, p = 0.0021, N = 23), and there was a trend for larger females to display more (estimate ± SE = 1.391 ± 0.69 df = 23.10, t = 2.025, p = 0.0546, N = 23).

During exposure to the predator, females spent less time directing displays towards the rival female than during the No Predator treatment (142 ± 166s vs. 278 ± 169s; estimate ± SE = -4.30 ± 1.04, df = 25.12, t = -4.14, p = 0.0003, N = 23; Figure 5.5c and 5.5d). Females spent less time towards the rival and responded to fewer of the rival's approaches during the Predator treatment. Females spent on average 264 ± 224s in the area of the tank towards the rival's compartment and responded to only 47 ± 33% of approaches by the rival towards their territory during the Predator treatment compared to 541 ± 262s and 62 ± 21% of approaches during the No Predator treatment (towards rival: estimate ± SE = -7.25 ± 1.71, df= 23.66, t = -4.24, p = 0.0003, N = 23; approach response: estimate ± SE = -0.55 ± 0.23, z = -2.38, p = 0.017, N = 20; Figure 5.6). At the same time, females spent more time displaying near the predator's compartment when the predator was present versus absent (223 ± 323s vs. 45 ± 110s; estimate ± SE = 0.92 ± 0.25, z = 3.65, p = 0.0003, N = 23; Figure 5.5a and 5.5b). Finally, there was no difference in the amount of time that females invested in displays towards the predator and towards the rival during the Predator treatment (Wilcoxon signed-rank test: V = 125 p = 0.975, N=23).

I did not find evidence that females became habituated to the rival during a trial. Females showed no decline in the amount of time spent in aggressive displays (Wilcoxon signed-rank test: V = 34.5, p = 0.9987) or at the barrier (Wilcoxon signed-rank test: V = 41, p = 0.9859) between the beginning and end of the No Predator treatment.

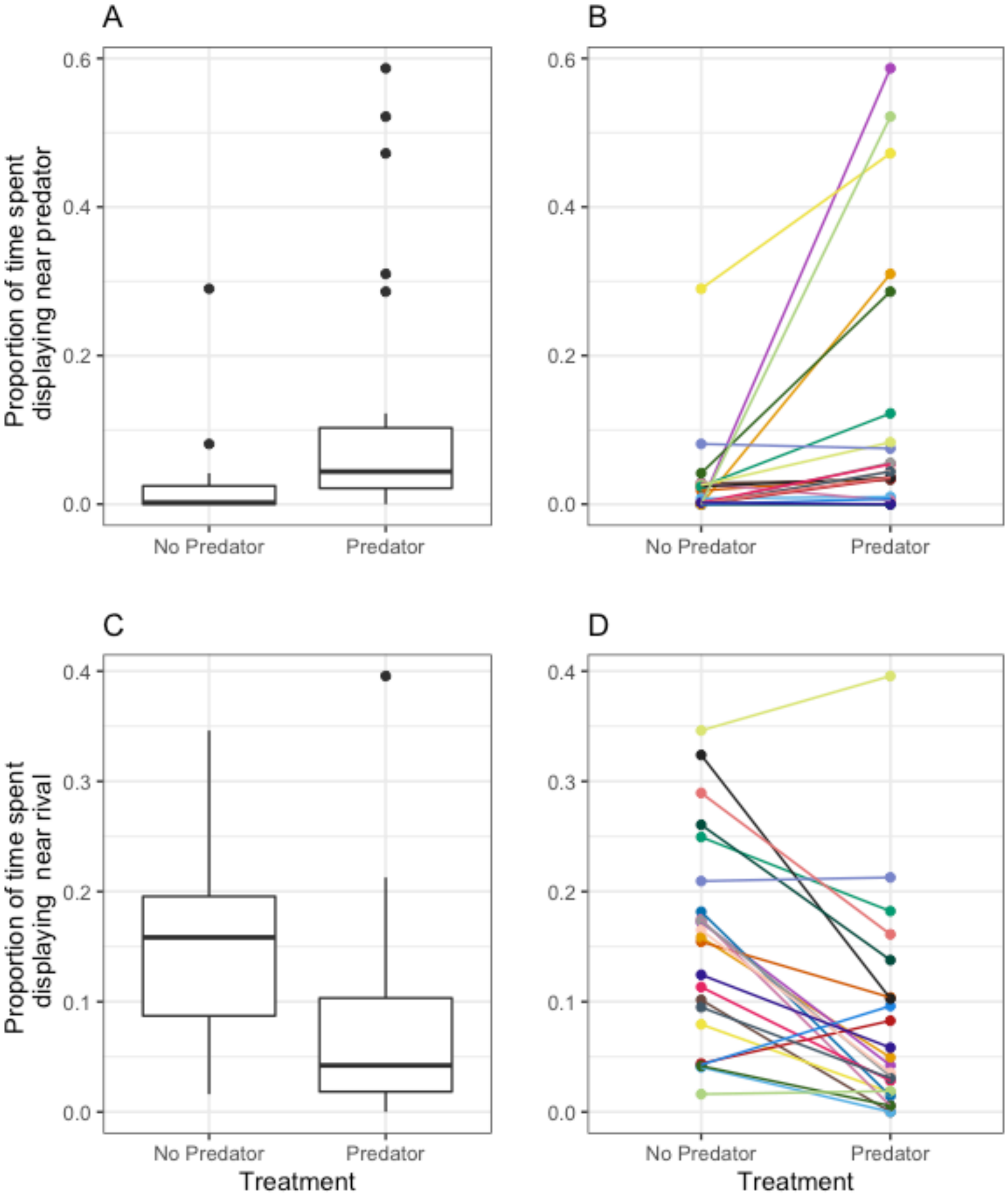

Figure 5.5. The effect of treatment on the proportion of time that females spent displaying in the compartments near the predator (A and B) and near the rival (C and D). A and C are boxplots showing the median and interquartile ranges. Black points indicate outliers. B and D show female means with each female represented by a different colour.

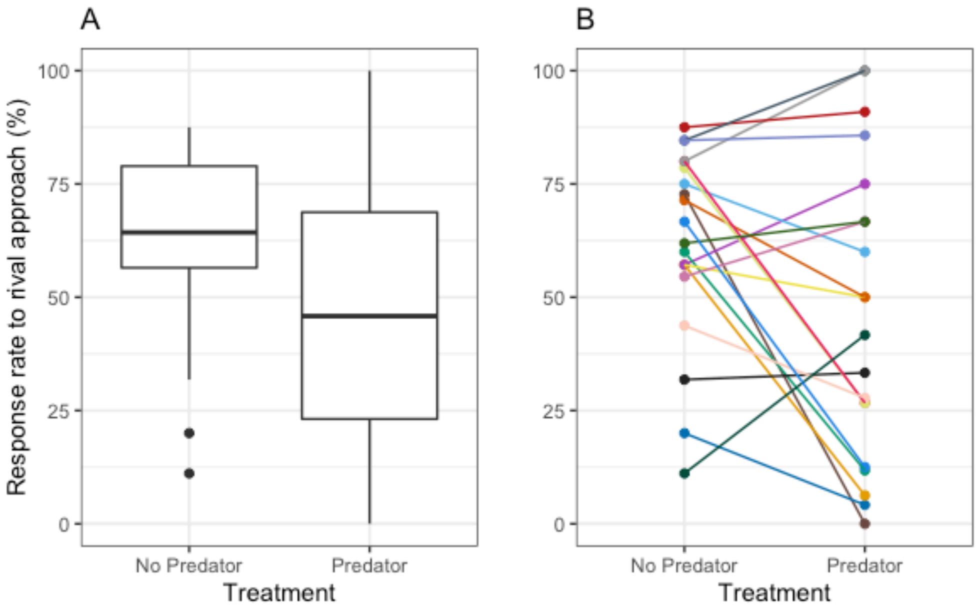

Figure 5.6. The effect of treatment on responses by focal females to approaches from the rival. A) boxplots show the median and interquartile ranges for each treatment with black points representing outliers. B) Points are female means with each female represented by a different colour.

5.3.2 Female aggression across contexts

I found no correlation between the amount of time that focal females spent displaying to the rival female during the No Predator treatment and the amount of time that females spent displaying to the predator during the Predator treatment (estimate ± SE = -0.07 ± 0.08, t = -0.82, p = 0.424, N = 23; Figure 5.7a). I also found no correlation between display time to the rival and display time to the predator during the Predator treatment (estimate ± SE = -0.003 ± 0.003, t = -0.95, p = 0.354, N = 23; Figure 5.7c). However, the time that females spent displaying to the rival was positively correlated between the two treatments (estimate ± SE = 0.60 ± 0.21, t = 2.89, p = 0.0098, N = 23; Figure 5.7b).

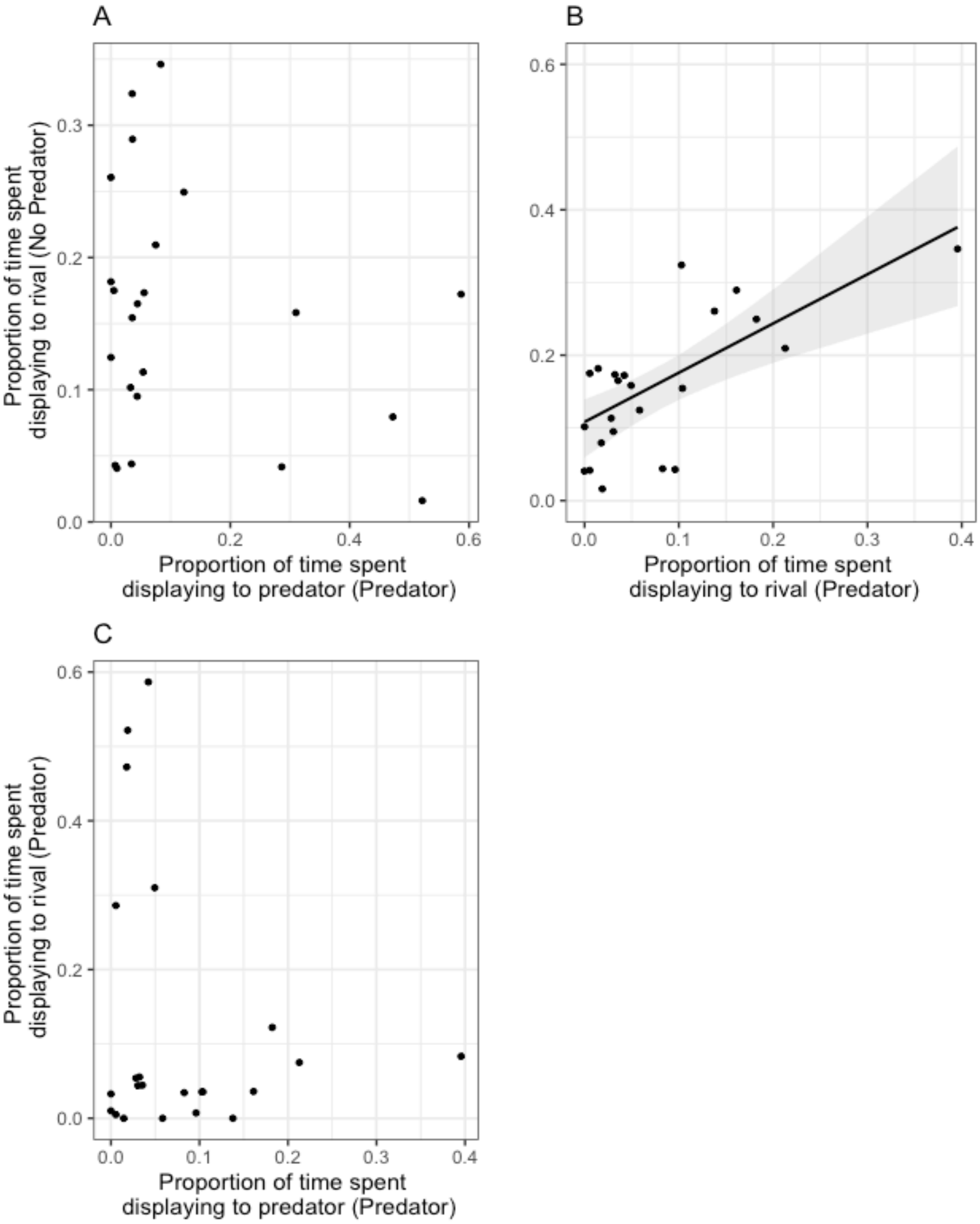

Figure 5.7. Female aggression across contexts. Correlation between A) time spent displaying to the rival (No Predator treatment) and time spent displaying to the predator (Predator treatment) B) time spent displaying to the rival (No Predator treatment) and time spent displaying to the rival (Predator treatment). Regression line and 95% confidence intervals (shaded bar) predicted from the linear model. C) time spent displaying to the rival (Predator treatment) and time spent displaying to the predator (Predator treatment).

5.3.3 The effect of predator exposure on male behaviour

Males spent more time displaying to the predator when the female and her brood were present compared to after they had been removed (Female: 68 ± 95s vs. No Female: 29 ± 51s; estimate ± SE = 1.051 ± 0.37, z = 2.849, p = 0.0044, N = 22; Figure 5.8a). However, males did not interact more often with the predator barrier during the Female treatment (estimate ± SE = -0.19 ± 0.19, z = -0.961, p = 0.337, N = 22; Figure 5.8b). There was a trend for larger males to spend more time displaying to the predator (estimate ± SE = 0.206 ± 0.11, z = 1.876, p = 0.0607) but there was no effect of male size on the number of barrier contacts (estimate ± SE = 0.006 ± 0.17, z = 0.036, p = 0.971). Males moved away from their shell more during the No Female treatment, spending on average 529 ± 231s guarding their shell, compared to 629 ± 232s when the female was present (estimate ± SE = 99.82 ± 28.35, df = 22.00, t = 3.52, p = 0.002, N = 22). There was no decrease in aggressive displays towards the model predator between the beginning and end of the Female treatment (Wilcoxon signed-rank test: V = 104, p = 0.1005).

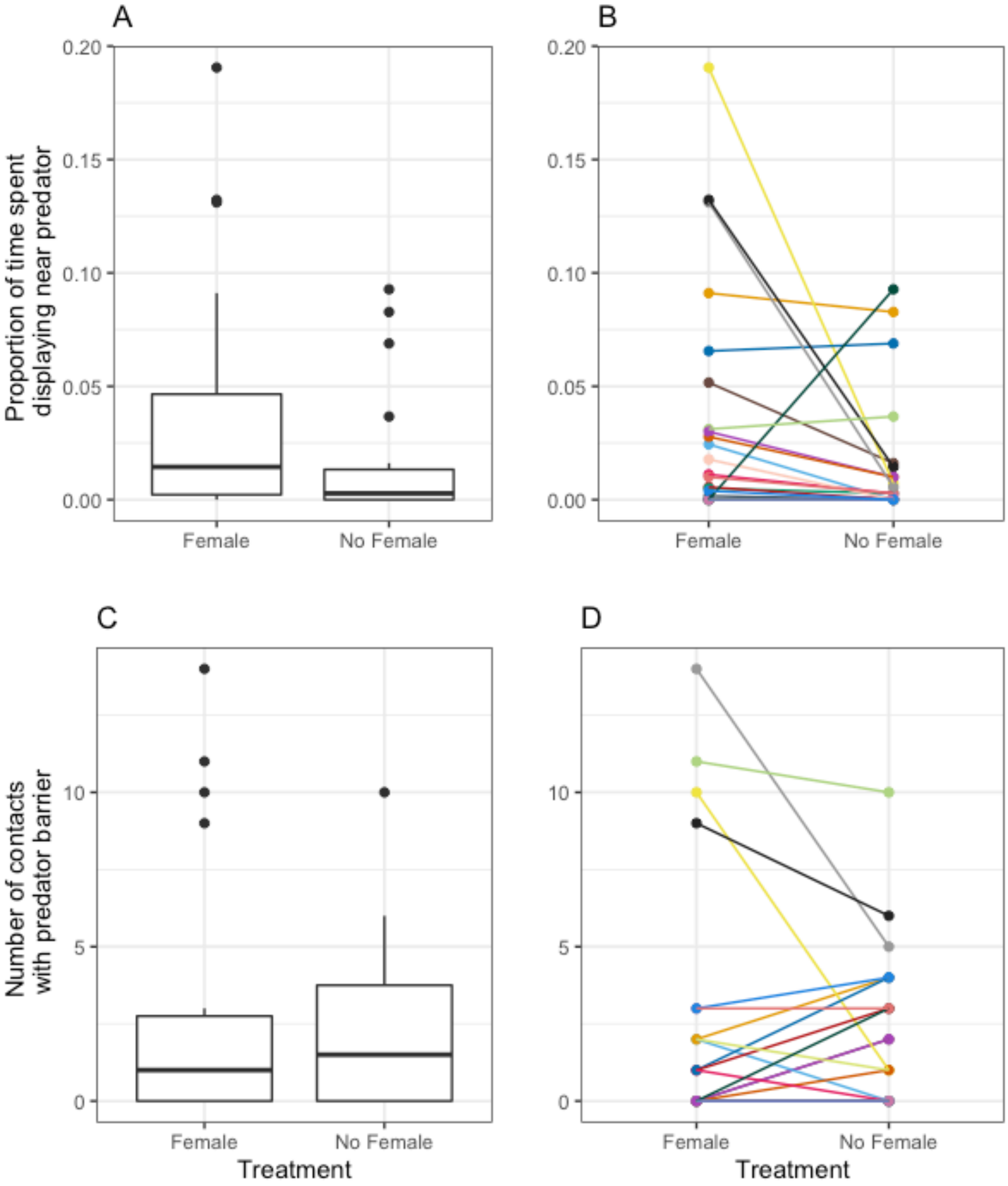

Figure 5.8. The effect of treatment on the proportion of time that males spent displaying in the compartment near the predator (A and B) and the number of times males contacted the predator barrier (C and D). A and C are boxplots showing the median and interquartile ranges with points representing outliers and B and D show male means with each male represented by a different colour.

5.4 Discussion

Although females can acquire important resources through intrasexual competition, when caring for vulnerable offspring, investment in brood defence may reduce the effectiveness of their responses to intrasexual competitors. This may occur due to resource trade-offs or correlations in aggressive traits reducing the plasticity of responses between different contexts. In addition, the nature of the contested resource will influence the trade-off between competition and brood defence, since some resources will directly improve the protection of offspring while others will enhance female fitness via different routes. In this study I show that female *Lamprologus ocellatus* experience a temporal trade-off where the presence of a predator reduces the time that females spend in intrasexual aggression and reduces vigilance to rival approaches. However, I find no correlation between responses to a rival and to a predator, suggesting that the expression of predator aggression does not constrain or enhance competitive aggression. I found that males responded more aggressively to predators when guarding a brood and this result, combined with previous results showing that females compete for breeding positions closest to the male (Brandtmann et al. 1999), suggest that increased male assistance with brood defence may be an important benefit of female competition in this species.

In Chapter 4 I showed that female investment in competitive behaviour can be costly for females by reducing hatching success. To develop a clearer understanding of the costs and benefits for females of investing in competition we must consider all aspects of reproduction, including parental defence. One hypothesis for the evolution of costly competitive behaviours is that they can be integrated with traits that benefit individuals across contexts (Sih et al. 2012). Correlations in aggression towards conspecific intruders and towards predators have been found in several species (Huntingford 1976; Duckworth 2006; Cain et al. 2011), including in subordinates of the related cichlid *Neolamprologus pulcher* (Bergmüller and Taborsky 2007; Witsenburg et al. 2010). I found no evidence that female *L. ocellatus* that are more aggressive towards rival females are also more aggressive towards brood predators, indicating that the costs of competitive investment would not be offset by greater brood defence.

These results are consistent with those found in yellow-bellied marmots, where both social aggression and defensive aggression show consistency between situations but aggression is not correlated between these two contexts (Blumstein et al. 2013). In *L. ocellatus*, I also found that aggression towards same-sex rivals was correlated between situations (Predator vs No Predator) indicating that there may be consistent individual differences in intrasexual aggression and that some individuals are more competitive than others. The lack of a correlation between competitive and defensive aggression however, suggests that competitive phenotypes are not linked to caring phenotypes and that there is plasticity in female responses to different threats which may be beneficial, especially in environments where females face a high rate of intrusions from different species (Satoh et al. 2021).

Although aggression was not correlated across contexts, the amount of time that females invested in total aggressive displays remained constant with and without the presence of a predator, resulting in reduced engagement in competitive interactions during predator exposure. This may indicate that the budget that females allocate to aggressive behaviours is fixed. Although not directly tested here, this also suggests that investment in competitive interactions may reduce female responses to brood predators which may represent an additional cost to females of breeding in harems. Engagement in intrasexual contests has been shown to affect predator vigilance in several species, including male *L. ocellatus* (Dunn et al. 2004; Hess et al. 2016; Ota 2018). In this experiment I did not compare the responses of females to a competitor and a predator separately, which may have prevented me from detecting a correlation between different contexts. However, breeding females are unlikely to experience these threats in isolation and, therefore, correlated aggression would only be beneficial if heightened responses to both threats could occur simultaneously, and we would expect an increase in total aggressive behaviour when both threats are present.

When exposed to both a predator and a rival simultaneously, there was no difference in the amount of time that females spent displaying to each of these intruders. This might suggest that females considered both intruders to have a similar threat level. Species have been shown to adjust their aggression in response to the perceived degree of threat from different intruder species (Sowersby et al. 2018; Satoh et al. 2021). Female *L. ocellatus* increase their aggression towards conspecific females when their brood becomes free-swimming (Walter & Trillmich 1994; Chapter 4). This may be due to the increased predation threat posed by rival females on free-swimming larvae, or the need for females to secure shells that can be used by their dispersing offspring. Juveniles have been observed using the male's shell as well as other shells in the territory as shelters (pers. obs.). Therefore, the threat of predation to offspring from both the female and the heterospecific brood predator may drive similar responses in females. In addition, the brood predator used in this experiment was modelled on a species that also competes with *L. ocellatus* for shells. Similar results were found in *Neolamprologus pulcher*, where female breeders showed similar defensive responses to a fry predator and a female conspecific when they were presented together (Desjardins et al. 2008).

However, if females were viewing rival females as posing the same threat as an intruding brood predator, we would expect to see a correlation between rival and predator display. Four females in my experiment also showed no aggression towards the predator but stayed low to the substrate at the entrance to their shell facing the predator compartment. This behaviour was never observed when only the rival female was present. In addition, females respond more aggressively towards *L. ocellatus* than *L. meeli* intruders in the wild (Chapter 6).

Factors besides species differences in threat level are likely to influence female aggressive responses. Size differences between females and intruders will affect both the likelihood of successful deterrence and the risk posed directly to females (Montgomerie and Weatherhead 1988; Arnott and Elwood 2009). In this study, both larger females and larger males spent more time in aggressive displays. Similar results were shown in *Neoloamprologus savoryi* where contributions to predator defence correlated with helper size (Josi et al. 2020), while in *N. pulcher,* responses were found to be related to intruder type but not to size differences between residents and intruders (Desjardins et al. 2008). In addition, familiarity between residents and intruders can influence aggression (Temeles 1994; Christensen and Radford 2018). In natural populations, *L. meeli* territories often closely border female *L. ocellatus* territories but intrusions by both species are likely to have be a combination of neighbours and floaters (pers. obs.). Finally, the age and size of the brood will influence both its value and its vulnerability (Montgomerie and Weatherhead 1988). Increases in parental care in response to offspring value are well documented in animals (e.g. Tilgar and Kikas 2009; Ward et al. 2009; Thünken et al. 2010), including fish (see Chapter 2). In this experiment, females were more aggressive when they had larger broods. Further studies are therefore needed to disentangle these effects and to understand how females make decisions about conspecific and heterospecific threats.

Although previous studies have shown that females prefer to settle close to males, and dispersing fry have been observed to use the males shell (Brandtmann et al. 1999; pers. obs.), the contribution of males to brood defence had not previously been tested. Here, I show that males increase their aggressiveness towards a predator intruder when defending a territory containing a female and her brood. Male care may be an important benefit of female competitive behaviour in this species and may explain why the most competitive females did not also invest more in defence. My finding that males change their response to a brood predator when they have a brood also provides evidence that male assistance with predator defence is not simply a by-product of territory defence, as has been observed in other cichlids such as *Variabilichromis moori.* (Zimmermann et al. 2021) and *N. savoryi* (Josi et al. 2020). To further investigate whether female competition may be driven by male contributions to care in this species, in Chapter 6 I use field data to test whether females receive more assistance with territory intrusions from males when they are nesting closer to the male.

The results presented here demonstrate that female *L. ocellatus* are likely to face a cost to brood defence by investing in competition, through reduced vigilance and time and energy constraints. However, if more competitive females receive greater assistance from the male in brood defence, this may reduce the costs of competition and independently select for intrasexual aggression that is uncorrelated with defensive behaviour.

Chapter 6

Male brood defence as a benefit of female competition in Lamprologus ocellatus

Rebecca Goldberg, Alex Jordan, Jonathan Green

Author contributions: I conceived of the study. Jonathan Green, Alex Jordan and I designed the study. Alex Jordan facilitated the collection of field data. I collected and analysed the data. I wrote the chapter and incorporated comments from Jonathan Green.

Abstract

Females have been found to compete for a variety of reproductive resources. Knowledge of the resources that drive female competition is important for our understanding of competitive trait evolution and the consequences of female competition for the evolution of mating systems. In the polygynous cichlid *Lamprologus ocellatus*, females compete for access to breeding shells and for positions close to the male, but the benefits of this competition are not clear. In this field study, I show that females receive less help from the male with intrusions from brood predators when they breed further from the male, and that females in secondary positions in a harem (further from the male) receive less help than females in monogamous pairs or primary harem positions. I also find evidence that this contribution by males may allow females to reduce their own investment in defence. This demonstrates that male care may be an important resource driving competition between females for breeding positions.

6.1 Introduction

While intrasexual competition in males is usually driven by limited mating opportunities, the resources that improve reproductive success and drive intrasexual competition in females vary between species (Stockley and Bro-Jørgensen 2011). Knowledge of the contested resource is important for our understanding of the benefits of female-female competition and can help us to interpret differences in patterns of competitive trait expression (Joseph A. Tobias et al. 2012; Stockley and Campbell 2013). The nature of the resource is also likely to influence female distributions, group formation and breeding behaviour, with consequences for the evolution of mating systems (Clutton-brock and Huchard 2013). For instance, competition for food in cooperative and communal breeders can influence group size and structure through the eviction of subordinates or their offspring by dominant females when food is limited (Woodroffe and MacDonald 1995; Clutton-Brock et al. 2001; Wong et al. 2008). In polygynous systems where males contribute to care, competition for male care has been demonstrated to explain the exclusion of females from groups (Sandell 1998), increased distances between female territories (Slagsvold et al. 1992) and reduced breeding synchrony within groups (Kempenaers 1995).

In the polygynous shell-dwelling cichlid *Lamprologus ocellatus,* females compete aggressively for access to breeding shells. In particular, females have been shown to prefer shells positioned closer to the male and more competitive females are more likely to settle in these shells (Brandtmann et al. 1999). The benefits afforded to females that secure these positions are not clear, but preference for proximity to the male suggests that male contributions to care may be important. Although males are not observed to enter female shells to provide care, males aggressively defend the territory from conspecific and heterospecific intruders, including those that would pose a threat to eggs and juveniles (Bills 1997).

In Chapter 5, I showed that male *L. ocellatus* responded more aggressively to a brood predator in the presence of a brood, suggesting that males may contribute to brood protection by deterring predators. In this field study I test the hypothesis that male assistance with brood defence drives female competition for breeding positions within a harem. If more competitive females benefit from greater contributions to care by males, females are expected to receive more help with brood predator intrusions when they are breeding monogamously rather than polygynous and when they are breeding in positions closer to the male.

6.2 Methods

6.2.1 Field sites and group sampling

Fieldwork took place in October 2021 at two sites along the southern coast of Lake Tanganyika: Isanga bay (8° 39'04"S, 31° 11'44"E) and Chezi village (hereafter, Chezi; 8° 46'46"S, 31° 00'2l"E). At each site, a 30m transect line was set at a depth of 11m and groups were sampled within 3m either side of this line resulting in a depth range of 8.9-11.7m. An additional group, at a depth of 15m, was sampled from an unmarked area of Isanga Bay prior to transect set-up. In total, 10 groups were mapped; 4 at Isanga bay and 6 at Chezi (Figure 6.1).

Groups and the position of individual shells were marked with small numbered stones and distances between occupied shells were measured using a measuring tape. Males and females were initially identified by size and the presence of a white rimmed dorsal fin in females. Initial 10-minute observations confirmed the sex of individuals and membership within harems by observing interactions between males and settled females. Observations also determined whether females were guarding free-swimming fry in their shell.

To investigate total intrusion rates by species that were a threat to fry and to quantify male contributions to defence, I filmed 4 females at Isanga bay and 6 females at Chezi. Two cameras (GoPro Hero 5 Black) in underwater casings were mounted on a single tripod. These were positioned at least 60cm away from the female, facing towards the female's shell with the male's shell further in front. Cameras were left for between 35 minutes and 4 hours before being collected.

Figure 6.1. Images from videos at six of the ten groups showing the distances between the focal female's shell (circled in white) and the males shell (circled in black). Labels on each panel show the group identifier indicating whether the group was part of the Isanga bay (IB) or Chezi village (CHZ) population and the distances in cm between the male's shell and the female's shell.

6.2.2 Video analysis

I analysed the first 30 minutes of each video. During video observations, females experienced intrusions from two categories of potential brood predators. *Lepidiolamprologus meeli* and *Lamprologus ocellatus* (conspecifics) are substrate feeders that predate on small invertebrates and juvenile fish (Bills 1997; Martinez et al. 2018) and were observed either alone or in small groups of up to four individuals. These species are also shell competitors and therefore pose a risk to offspring in the shell as well as to free-swimming fry. *Ectodus descampsii*, *Enantiopus melanogenys, Grammatotria lemairii* and *Xenotilapia sp.* forage on small organisms by sifting through the sand (Huber et al. 1997; Martinez et al. 2018). They do not hunt free-swimming fry but are likely to predate on them if they encounter them on the sand. These species are not shell-dwellers and are generally much larger then both *L. ocellatus* and *L. meeli*. They were predominantly observed passing through the territory in large groups but were also occasionally observed alone.

I focused my analysis on intrusions by *L. ocellatus* and *L. meeli* since these species are most likely to elicit a defensive response from females and the laboratory model was based on a *Lepidiolamprologus sp.* allowing for comparisons between the two studies. I recorded the number of intrusions as the total number of fish that entered the screen that were identifiable as one of these two species. For each intrusion I recorded the species ID, whether the female responded to the intruder by displaying or chasing, and whether the male assisted with the intrusion by displaying or chasing. Although cameras were always at the same height, a certain amount of error will be present in the measurement of intrusion rates due to differences in the view captured by cameras positioned at different angles. Despite this error, the majority of intrusions were close to the female's shell and likely to have been captured by all views, and responses by males and females could be accurately observed. Improvements to the video analysis methods used here are currently being developed which will enable me to standardise the distance at which a fish can be considered as intruding on the female's territory.

6.2.3 Statistical analysis

To compare intrusion rates between the two sites, and whether rates were affected by male proximity and response rate, I calculated the total number of intrusions experienced by each female and fitted a linear model. I set intrusion number as the response variable and site (Isanga bay or Chezi), distance between the male and female, and the proportion of intrusions that received male help as fixed effects. I also initially compared intrusion rates between pairs and harems to test whether the presence of other females reduces intrusions. As there was no difference in intrusion rates between pairs and harems, this variable was not included in the final model with the other three variables.

To investigate whether the likelihood of females receiving help with intrusions depended on their breeding position I fitted two GLMs. For each recording I calculated how many intrusions the male helped with, and set this as the binary response variable in both models. In the first model I set the distance between the male and female as the main predictor. In the second model the main predictor variable was a three-level factor indicating whether females were breeding monogamously, in a harem and closest to the male (primary) or in a harem and furthest from the male (secondary). As I did not sample from any groups with more than two females these were the only levels. Intrusion number were also included as a fixed effect in both models. Finally, to investigate variation in female responses to intrusions I fitted a GLMM with binomial error distribution. The probability of a female responding to an intruder was set as the response variable, and intruder species (*L. ocellatus* or *L. meeli*), whether the male assisted (Yes or No), and whether the female was guarding fry (Yes or No) were included as fixed effects. This model also included recording ID as a random effect as multiple intrusions were analysed from each video.

6.2.4 Ethics statement

Fieldwork was conducted with the permission of the Fisheries Department of Zambia under a study permit issued by the government of Zambia (SP264880) and was also approved by the Animal Welfare & Ethics Review Board in the University of Oxford's Department of Zoology. The study species is listed "Least Concern" on the IUCN Red List of Threatened Species.

6.3 Results

Data on group structure were collected from 9 *L. ocellatus* groups comprising a male plus one (n = 4), two (n= 4) or three (n = 1) females. 5/13 females were observed to have free-swimming fry; four females belonged to a two-female harem, one female was in a pair. Male primary shells were on average 96 ± 35cm away from the closest female and 165 ± 45cm from the second female while females were on average 140 ± 71cm from one another.

Females experienced on average 7 ± 5 intrusions by *L. ocellatus* and 9 ± 8 intrusions from *L. meeli* in 30 minutes and received help from the male during 52 ± 29% of intrusions. Total intrusion rates did not differ between harems and pairs (F = 0.4993, p = 0.5114; N = 10; Figure 6.2a). Intrusion rates showed a trend towards being higher at Isanga bay than at Chezi (estimate ± SE = 7.982 ± 3.29, t = 2.429, p = 0.0513; Figure 6.2b). Intrusion rates were negatively correlated with the distance between the male and the female (estimate ± SE = -0.148 ± 0.05, t = -3.229, p = 0.01644; Figure 6.2c) and the proportion of intrusions receiving male help (estimate ± SE = -21.587 ± 7.22, t = -2.990, p = 0.0243; Figure 6.2d).

The amount of help that females received from males with brood defence depended on their breeding position. The proportion of intrusions receiving male help decreased with the distance between male and female shells (estimate ± SE = -0.020 ± 0.007, z = -2.819, p = 0.0048; Figure 6.3a). In addition, secondary females received significantly less help than both primary (estimate ± SE = -1.51 ± 0.648, z = -2.331, p = 0.0198) and monogamous females (estimate ± SE = -1.973 ± 0.713, z = -2.766, p = 0.0057), but there was no difference between primary and monogamous females (estimate ± SE = -0.463 ± 0.512, z = -0.903, p = 0.3665; Figure 6.3b).

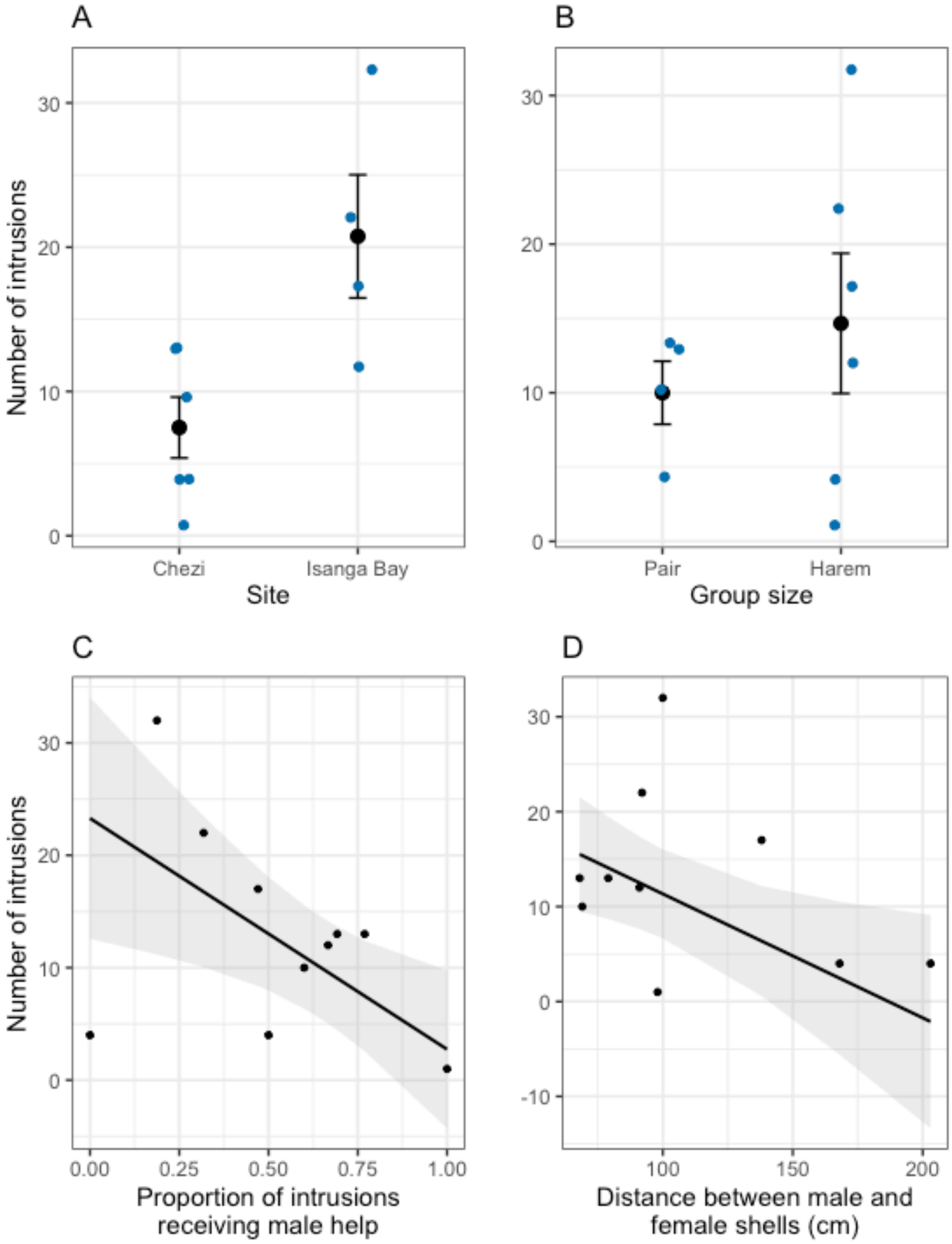

Figure 6.2. Intrusion rates: at the two sites (A); in pairs and harems (B); correlated with the proportion of intrusions that received male help; and correlated with the distance between male and female shells. In A and B black points and lines indicate means ± se with coloured points representing each female. In C and D each point is one female and regression line and confidence intervals (shaded bar) are predicted from the linear model.

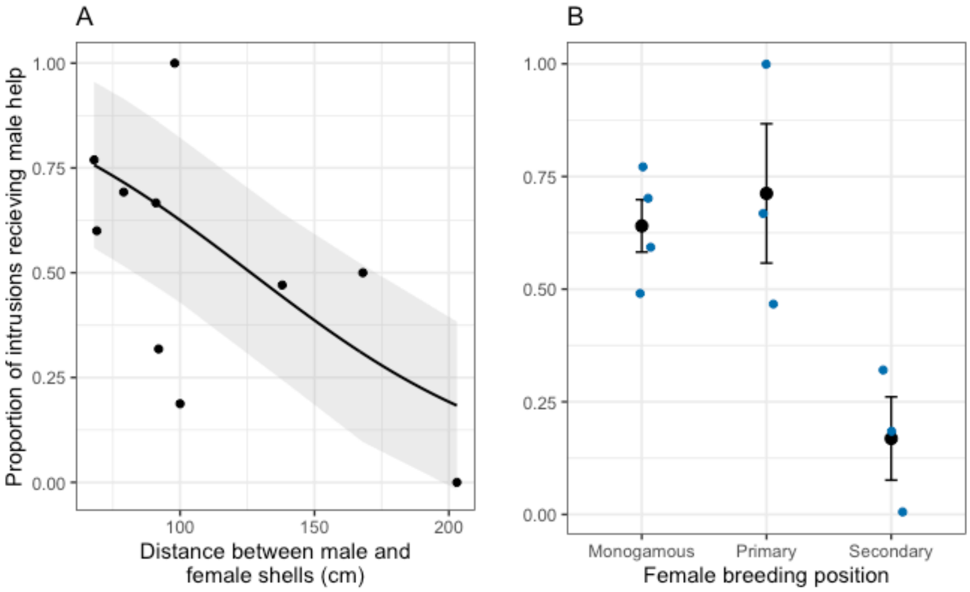

Figure 6.3. Male assistance with brood defence. A) The relationship between the proportion of intrusions receiving male help and the distance from the focal female to the male. Each point represents one female. Regression line predicted from the generalised linear model. B) Differences in the proportion of intrusions receiving male help between females in different breeding positions. Black points and lines indicate means ± se with coloured points representing each female.

Females responded differently to the two categories of intruders. Females were more likely to respond by displaying or chasing *L. ocellatus* than *L. meeli* intruders (estimate ± SE = 1.361 ± 0.543, z = 2.508, p = 0.0121; Figure 6.4a and 6.4b). Females were also less likely to respond to intrusions when they received male assistance (estimate ± SE = -1.079 ± 0.437, z = -2.467, p = 0.0136; Figure 6.4c and 6.4d). I found no effect of the presence of fry on female responses (estimate ± SE = 0.8331 ± 0.6018, z = 1.384, p = 0.1663; Figure 6.4e) but two of the 4 females with fry experienced the two lowest intrusion rates (5 and 1 intrusions).

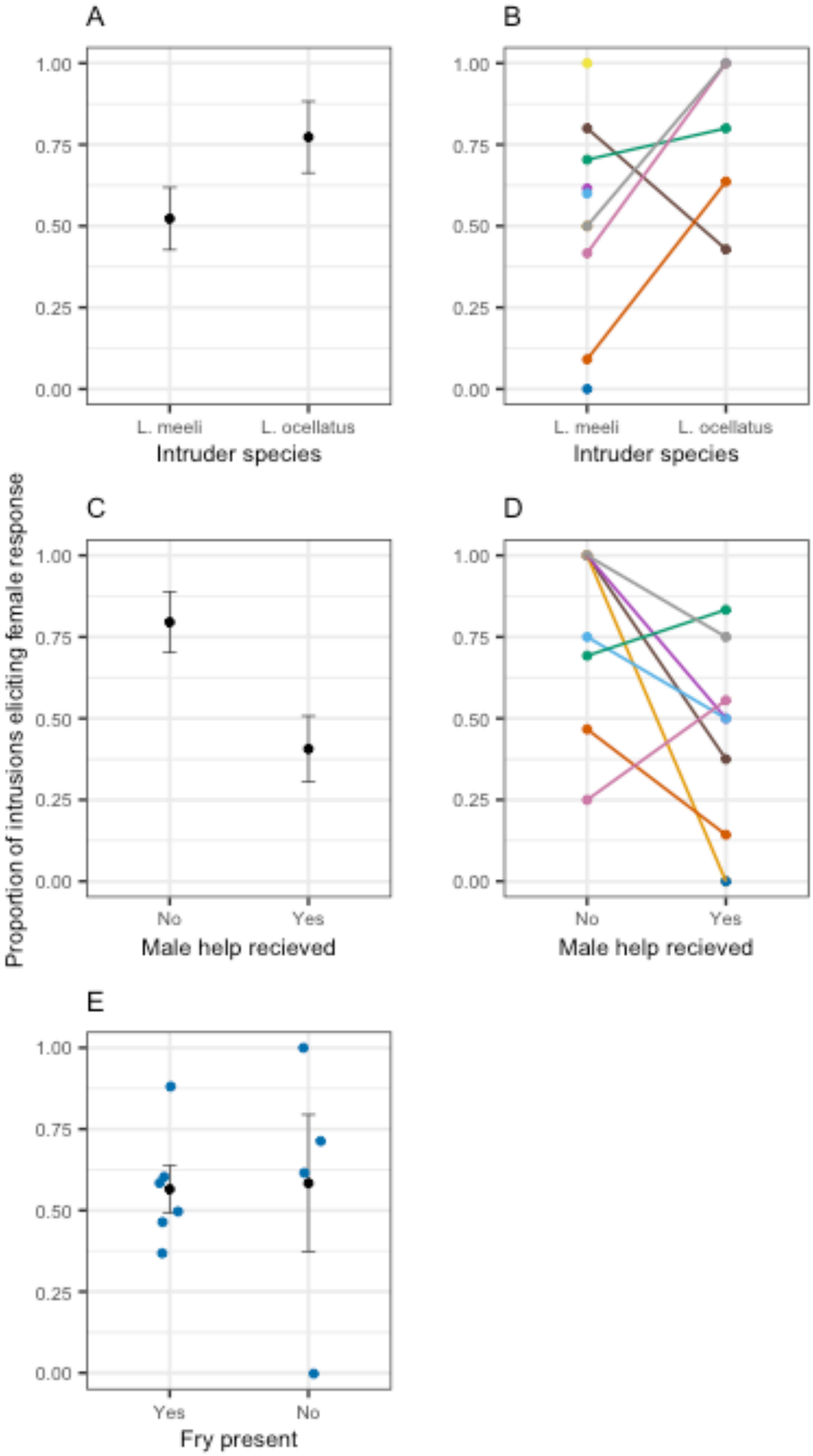

Figure 6.4. Female response to intrusions: at the two sites (A and B); when they did or didn't receive male help (C and D); and when they did or didn't have fry at the shell (E). In A, C and E black points and lines indicate means ± se, E includes a coloured point for each female. In B and D points show proportions for each female represented by a different colour.

6.4 Discussion

Female *L. ocellatus* aggressively compete for membership within a harem and for positions close to the male. This study demonstrates that male contributions to offspring defence may be a limited resource driving competition in females. Males assisted with more brood predator intrusions when females were nesting closer to their shell and secondary females received less help than both primary and monogamous females. This assistance may increase the survival of the female's brood or reduce the amount that females need to invest in defence with benefits for their future reproduction. These results are consistent with those found in numerous other polygynous species (e.g. Summers 1990; Smith et al. 1994; Huk and Winkel 2006; Desjardins, Fitzpatrick, et al. 2008). For example, in pied flycatchers (*Ficedula hypoleuca*), monogamous females receive more help with nestling provisioning from the male than polygynous females, but in polygynous groups, males provide more care to the primary than the secondary female's brood (Huk and Winkel 2006).

Although I have shown that males assist females with predator deterrence, whether this results in fitness benefits for females still needs to be tested. For example, in *N. pulcher*, although polygynous males provide less care than monogamous males, greater male care was not shown to improve short-term offspring or female survival and is therefore unlikely to be an important driver of competition between females (Desjardins, Fitzpatrick, et al. 2008; Jungwirth et al. 2016). Male assistance may benefit females by directly increasing brood survival or by reducing the costs of parental care for females (Kempenaers 1995). Here, I show that female *L. ocellatus* were less likely to engage with intruders when there was assistance from the male, which may suggest that females can reduce their investment in defence when they breed closer to the male. However, further studies are needed to measure the fitness consequences of male assistance.

Although both *L. meeli* and *L. ocellatus* intruders are a threat to their offspring, females were more likely to respond to conspecifics. This may indicate that females view *L. ocellatus* as a greater threat to their offspring or that they represent a greater threat to females overall due the additional risk of territory take-overs. In particular, female *L. ocellatus* intruders may have been more likely to instigate an interaction with the resident to compete for her shell. Alternatively, since *L. meeli* are the larger species, it may be riskier for females to engage with them, perhaps indicating an additional benefit of male assistance.

As well as increased assistance with intrusions, there may be other benefits to females of nesting in shells closer to the male. When fry disperse from their natal shell they will seek alternative shelters and have been observed using the male's shell (Brandtmann et al. 1999; pers. obs.). Proximity to the male's shell may, therefore, improve the survival of dispersing fry or enable them to disperse earlier allowing females to breed again sooner. When closer to the male, female's may also benefit from more interactions with their partner leading to greater familiarity and reduced aggression which may improve breeding success (Black and Hulme 1996; Maldonado-Chaparro et al. 2021). However, it is expected to be in the interest of the male to maintain bonds with all females and males regularly patrol their whole territory and visit each resident female (pers. obs.). Finally, during courtship and spawning, pairs are distracted and exposed, potentially increasing their risk of predation (Magnhagen 1991). Long distances between male and female shells are likely to further increase this risk, since females must approach males to initiate courting and spawning occurs in the female's shell. Changes to courtship and mating behaviour in response to predation risk have been documented in numerous species (e.g. Godin 1995; Candolin 1997; Acharya and McNeil 1998; Koga et al. 1998). By breeding closer to the male, females may benefit from safer, less disrupted mating. A better understanding of how breeding positions affect females across the whole brood cycle would help us to determine whether these alternative benefits may influence competition between females.

Male contributions to care have been shown to be an important predictor of female competition in a number of other species (Slagsvold et al. 1992; Kempenaers 1995; Sandell 1998; Wong et al. 2008), and competition could be expected to increase when there is an increased risk of brood predation, since male defence will be more valuable. However, additional breeding females could also help to reduce brood predation, if the presence of additional broods creates a dilution effect, or if females help to deter predators near the territory. For example, in the greater ani (*Crotophaga major*), communal nesting is costly, except under high predation, when larger groups experience reduced nest predation due to better defence (Riehl 2011). *L. ocellatus* groups show variation in size and structure. Groups can consist of one male plus one to four females and the distances between females range from less than 0.5m (Brandtmann et al. 1999) to greater than 2m (pers. obs.). The factors that cause this variation are unknown, but the risk of brood predation is likely to be an important ecological factor due to its potential influence on the trade-off between competition and brood defence. In this study I found no difference in the number of brood predator intrusions experienced by harems and pairs which may suggest that brood predation risk does not have a large influence on harem size. Pilot data from the field which further investigates the relationship between brood predation threat and harem structure are presented in Appendix B.

These results provide support for the hypothesis that intrasexual aggression in female *L. ocellatus* is driven by competition for male care. By excluding additional females from the harem and controlling breeding shells that are close to the male's shell, females can obtain more assistance with brood defence.

Appendix B

Supplementary material for Chapter 6: The influence of brood predation threat on harem structure

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B.1 Background

While female investment in intrasexual competition for reproductive resources is expected to be constrained by investment in parental care, we know little about how this relationship varies under different social and ecological conditions. When brood predators are present, investing in competition with conspecifics may come at the expense of increased offspring mortality. Therefore, the risk of brood predation will be an important ecological factor influencing the strength of a trade-off between care and competition. In polygynous harems, a male defends a territory with several breeding females and may or may not contribute to parental care, including predator defence. How brood predation threat influences competitive investment will depend on whether male assistance with defence is a contested resource, as well as how other females in the group affect brood predation risk. For instance, if the presence of conspecifics dilutes the risk of predation to a brood, or if they directly contribute to predator vigilance and defence, these contributions may reduce the benefits of competition, leading to larger group sizes under high predation (Marino 2010; Schädelin et al. 2012; Groenewoud et al. 2016).

In contrast, if larger groups reduce the safety of offspring, for example, by attracting more predators or diluting male care, the competitive exclusion of group members may be promoted. In polygynous groups, females may benefit from the presence or increased proximity of additional breeding females that help to deter or distract intruding brood predators (Riehl 2011). Alternatively, when males contribute to care, the exclusion of additional females can prevent a reduction in paternal help (Kempenaers 1995; Desjardins, Fitzpatrick, et al. 2008). Female-female competition has been shown to play an important role in determining the size and composition of social groups (Slagsvold and Lifjeld 1994; Eens and Pinxten 1995; Sandell 1998; Wong et al. 2008), yet how brood predation threat interacts with female competition to influence social structure has not been investigated.

In Chapter 5, I showed that the responses of female *Lamprologus ocellatus* to same-sex rivals are constrained by investment in brood defence, but that competition can increase male contributions to brood defence. Under high predation, females may therefore reduce investment in competition so that they can be more vigilant and aggressive towards brood predators which may result in larger groups or shorter distances between females. Alternatively, females may increase aggression towards other females under high predation to secure care from the male, leading to reversed effects on group size and/or spatial structure. In this pilot experiment I collected data on group size, distances between group members and proximity to brood predator territories to investigate the role of brood predation threat in shaping group structure in *L. ocellatus*.

B.2 Methods

The groups used in this study were the same groups sampled in Chapter 5 from two transects set up at two sites on the Southern cost of Lake Tanganyika: Isanga Bay (8° 39'04"S, 31° 11'44"E) and Chezi village (Hereafter, Chezi; 8° 46'46"S, 31° 00'2l"E). At both sites, *L. ocellatus* shared the sand bed with *Lepidiolamprologus meeli*. This species is also an obligate shell-dweller that competes with *L. ocellatus* for shells and predates on their young. *L. meeli* are generally monogamous and groups consist of the breeding pair and juveniles, but harems with up to five females have also been observed (Sunobe and Munehara 2003). At my sites, *L. meeli* breeding territories are characterised by shallow craters dug into the sand that contain several shells (Figure B.1). As these territories are small, *L. meeli* forage outside of their territories and are likely to intrude on the larger territories of neighbouring *L. ocellatus* (Bills 1997; Martinez et al. 2018). I therefore used the presence and proximity of *L. meeli* territories as a measure of brood predator threat. In 9 *L. ocellatus* groups I measured the distance from each male and each female shell to the nearest *L. meeli* territory and counted the number of *L. meeli* territories within two metres of any member of the group. The greatest distance observed between a male and one of his females was 168cm so *L. meeli* territories within 2m of an occupied shell would be closely bordering the territory of the group.

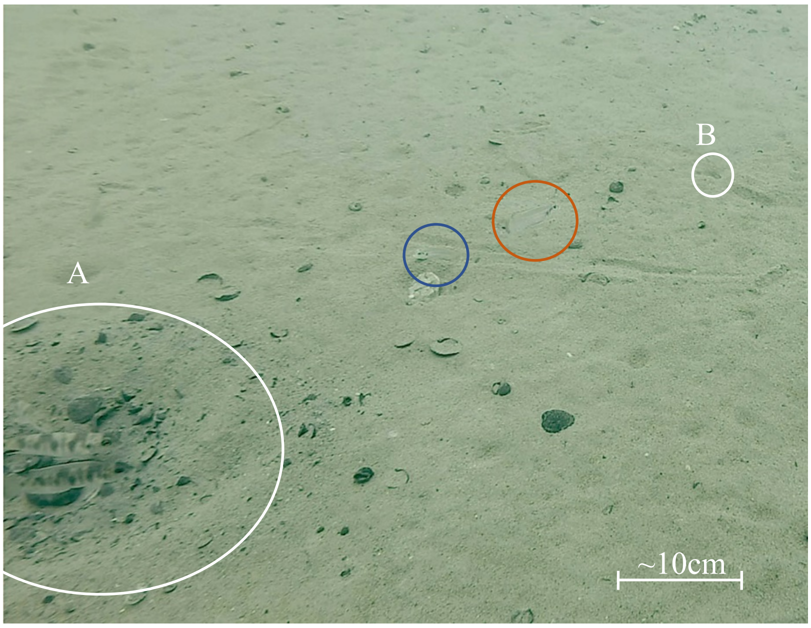

Figure B.1. *Lepidiolamprologus meeli* territory neighbouring group four at Isanga Bay. A = *L. meeli* breeding territory with three individuals present. B = *L. ocellatus* female shell. The resident *L. ocellatus* female is circled in blue and the resident male is circled in orange.

Statistical analyses were run in R version 4.0.4 (R Core Team 2021). Linear regression models were fitted using the package lme4 (Bates et al. 2015). Due to observed differences in intrusions rates between Isanga bay and Chezi (Chapter 5), site was included as a fixed effect in all models. To compare whether males or females were closer to *L. meeli* territories I fitted a linear mixed model. I set the distance between the *L. ocellatus* shell and the *L. meeli* territory as the response variable with sex as the predictor variable. I included a random effect for group as I sampled males and females from the same groups. To investigate whether the distance between the male and his closest female was correlated with his distance to the closest *L. meeli* territory I used a linear model with male to female distance as the response and male to *L. meeli* territory distance as the predictor. To investigate whether predation threat influenced the number of females in the group I fitted an ordinal logistic regression model using the MASS package (Venables and Ripley 2002). I set number of females as the response and the distance between the male and the nearest *L. meeli* territory and the number of *L. meeli* territories within two metres of a resident's shell as predictor variables. Reported results from this model are t-values and confidence intervals. P-values are not reported due to bias in predictions with the small sample size presented here.

B.3 Preliminary results

Males were on average 207 ± 84cm (mean ± sd) from the closest *L. meeli* territory while females were on average 165 ± 90cm from the closest *L. meeli* territory. The distance to the closest *L. meeli* territory did not differ between males and females (estimate ± SE = 25.489 ± 25.52, t = 0.999, p = 0.3435; Figure B.2). Groups had between zero and three *L. meeli* territories bordering their territories (within 2m of a resident's shell).

I found no relationship between the distance from the male to the closest *L. meeli* territory and the distance from the male to the closest female (estimate ± SE = 0.033 ± 0.21, t = 0.159, p = 0.8788; Figure B.3). There was also no difference in male to female distance between the two sites (estimate ± SE = 4.079 ± 35.29, t = 0.116, p = 0.9118). I found no effect of predator presence on the size of harems: there was no relationship between the number of females in a harem and either the number of *L. meeli* territories within 2m (estimate ± SE = 5.765 ± 6.82, t = 0.8458, CI = -7.59 to 19.12; Figure B.5a) or the distance between the male and the closest *L. meeli* territory (estimate ± SE = 0.082 ± 0.078, t =

1.05, CI = -0.07 to 0.24; Figure B.4). However, I found that larger groups were more common at Isanga Bay than at Chezi (estimate ± SE = 20.89 ± 0.02, t = 860.59, CI = -20.84 to 20.94; Figure B.5b).

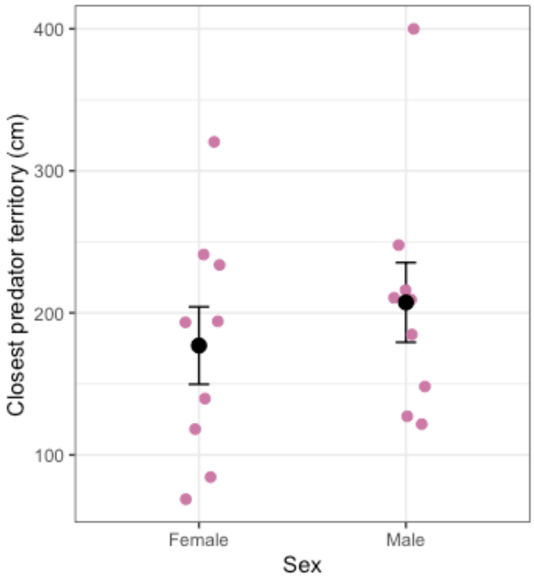

Figure B.2. The difference in distance to the closest *L. meeli* territory between males and females. Black points are means ± SE and coloured points represent individual fish.

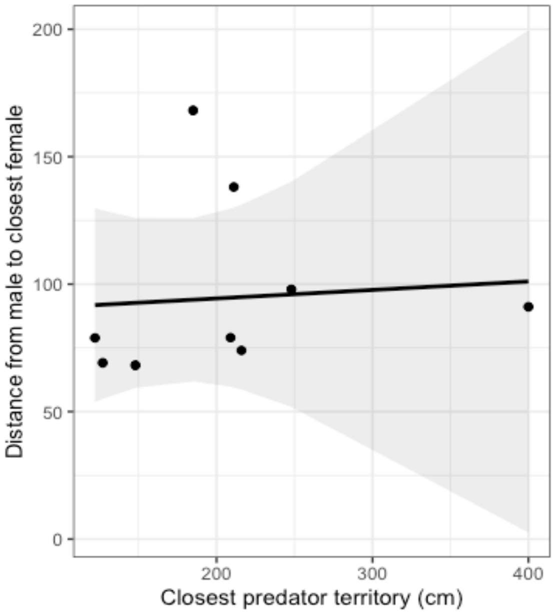

Figure B.3. The relationship between the distance from the male to the closest *L. meeli* territory and the distance from the male to the closest female. Points indicate one group and the line and confidence intervals (shaded bar) are predicted from the linear model.

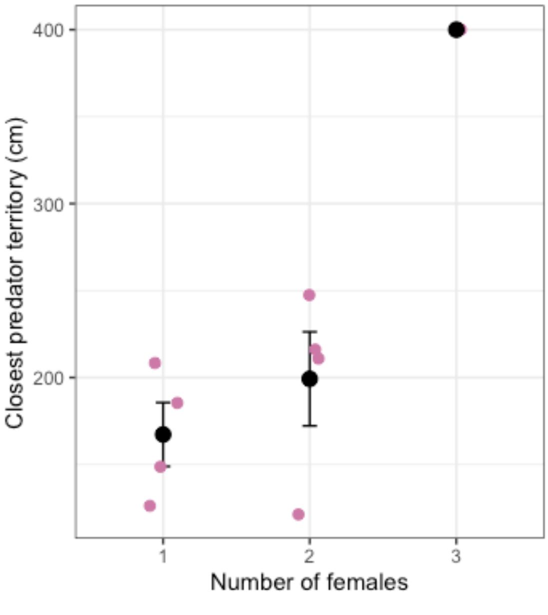

Figure B.4. Variation in harem size with the distance between the male and the closest *L. meeli* territory. Black points are means ± SE and coloured points represent each group.

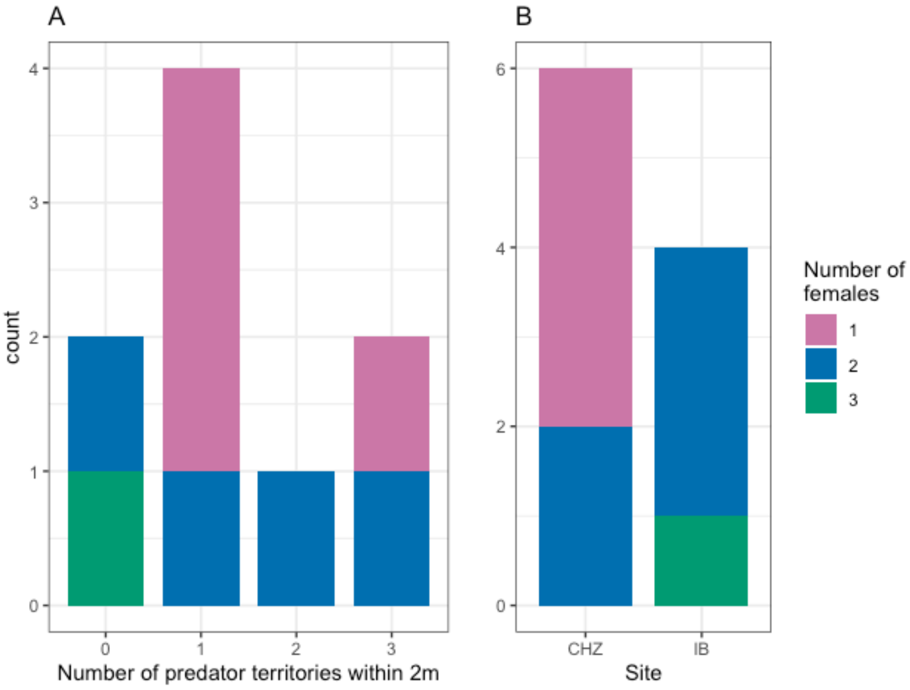

Figure B.5. Variation in harem size A) with the number of *L. meeli* territories within 2m, and B) between sites. y-axis counts indicate the number of groups.

B.4 Next steps

I did not find any effects of the threat from *L. meeli* on the composition of *L. ocellatus* groups. Although these results may suggest that predation threat does not have a significant impact on group structure, it is evident that a greater sample size is needed to fully investigate how *L. ocellatus* respond to changes in predation threat. In particular, a greater sample across harem sizes would help to determine any relationship between predation and the number of females in a group. From these data there is a trend for groups that are further from a *L. meeli* territory to contain more females, but this effect is predominantly being driven by the group with three females. In this study, while one- and two-female groups were evenly represented, I only sampled one group with three females.

Whether harems with more than two females are rare compared to smaller group sizes needs to be investigated, particularly as this group is associated with the largest distance to a *L. meeli* territory and the fewest territories within 2m (0 territories). In addition, with data from a greater number of harems I could also investigate how predator threat might influence distances between females within a group, not just between males and females, as tested here. Since distances between females can influence investment in competition, as shown in Chapter 4, this metric is likely to respond to differences in the magnitude of a trade-off between competition and brood defence.

In Chapter 5, I found that intrusion rates were different between Isanga Bay and Chezi. Differences in intrusion rates suggest that the density of predators across the sampled area at each site may be impacting group formation across groups within a population. In addition, here I found that larger group sizes were more common at Isanga bay than at Chezi. An alternative way to address this question would be to sample along multiple transects at each site and look at differences in population structure between sites. There are also numerous additional locations, besides those visited in this study, where *L. ocellatus* populations have been identified in the past (Bills 1997), but whether these populations still exist is not clear. This is under active study, alongside efforts to determine species composition at these sites. As information about these populations becomes available, the feasibility of repeating surveys of group structure and predation threat across populations will become clearer. With this approach, care would need to be taken to quantify additional factors at each site that may also have an important influence on group structure. These could include, species composition, shell density, food abundance, depth range and visibility. For example, although greater intrusion rates may signify a higher predation threat, they may also indicate that the density of *L. ocellatus* is higher at Isanga bay which may influence how many females choose to settle in harems.

This study demonstrates that *L. ocellatus* are a valuable system for exploring how ecological conditions can influence the costs and benefits of competition in females and offers an exciting opportunity to investigate how female competition can influence group structure in the wild. This work is needed to fully understand how selection shapes competitive traits in females and the consequences for the structure of social groups.

Chapter 7

General Discussion

7.1 Aims and main findings

Sexual selection theory predicts that, when one sex becomes limiting by investing in care, the other sex can maximise its reproductive success by investing in competition for mates (Trivers 1972; Clutton-Brock and Parker 1992). Since greater investment in sexually-selected traits reduces the resources that can be allocated to reproduction and care, and vice versa, a trade-off between competition and reproduction should select for one sex to be more competitive and the other to provide more care (Fromhage and Jennions 2016). However, in many species, this divergence between competitive and caring roles is not as clear as expected and individuals appear to invest heavily in both competition and reproduction (Ah-King and Ahnesjö 2013). Investigating how this trade-off operates in these species will help us to build a clearer picture of how reproductive investment in males and females can influence selection on competitive traits and the consequences for the evolution of mating systems and parental care. This thesis focused on investigating how competitive males can be selected to provide sole parental care and how reproduction in caring females is affected by female-female competition. To address these questions, I used a combination of phylogenetic meta-analyses across species and experimental and observational studies on a focal cichlid species.

In Chapter 2, I used a series of meta-analyses to show that paternal care in fish is more likely to be maintained by sexual selection than by direct benefits to offspring. Male care does not increase offspring survival but males that care for a brood are more attractive to females and therefore have higher mating success. Standard definitions of parental care specify that care traits should directly contribute to offspring fitness (Trivers 1972; Smiseth et al. 2012), but the care provided by male fish does not necessarily comply with this definition. In other taxa, paternal behaviours that appear to benefit offspring may also be maintained instead by mating benefits. For instance, the most common benefit of male care in mammals is a load-lightening effect that results in reduced lactation times and increased breeding frequency in females, and care is most common in species with stable mating pairs (West and Capellini 2016). When investigating the evolution of parental care strategies, it is therefore important to consider all the possible benefits of care for parents, not just those that benefit current offspring.

The outcomes of this study led to a further meta-analysis in Chapter 3. When assessing the costs of care for males, I collected data for the effect of care on male condition. However, it became apparent that this relationship was likely to be distorted in many species by males consuming some of their offspring while they were providing care, which could reduce any observed effects on condition. I therefore decided to investigate selection for filial egg cannibalism in males which would help us to determine whether this behaviour is a strategy by which males can limit the costs of care and maintain their condition for future investments (Manica 2002). My results showed that filial cannibalism by males is more common in species that show a greater degree of sexual dimorphism and are therefore likely to be under stronger sexual selection and experience greater competition for breeding territories. Nutritional contributions from eggs may support males in defending their breeding territories and caring for both current and future broods.

In these two chapters I demonstrate that female choice for caring males is a widespread behaviour with potential benefits for females. Stronger female preference for males with eggs was found in species where males provide more care for larger broods and where more males are partial brood cannibals. Females may, therefore, improve the survival of their offspring by choosing these males as the risk of brood abandonment is reduced and the risk of egg cannibalism is diluted. However, in Chapter 2 I also found that male care did not significantly increase short-term offspring survival, reducing the likelihood of female choice for direct care benefits. Instead, if providing care requires males to be of a certain quality or condition, the presence of a brood may act as an honest signal of male quality for females. Female mate choice is usually associated with selection for competitive traits in males that are unrelated to parental care, resulting in reduced parental investment by males (Andersson 1994; Firman et al. 2017). This case in fish demonstrates an important alternative outcome of female choice that goes against the assumption of a trade-off between care and competition.

In Chapter 4 I show that, in *L. ocellatus*, when females invest more in competition they suffer a cost to reproduction through reduced hatching success. This is likely to be the result of constraints on resource budgets affecting the reproductive condition of females, but the exact mechanism through which this cost manifests is not clear. Previous studies investigating the effects of female competition on reproduction have produced mixed results, but the few studies that have used manipulations of female investment have also demonstrated a trade-off between competition and reproduction in females (Morales et al. 2009; Doutrelant et al. 2012). While these two studies demonstrate how manipulations of reproduction and resource availability can influence investment in sexually-selected colour signals, my study represents the first attempt to manipulate the intensity of competition between females to alter investment into intrasexual aggression. These findings have important implications for our understanding of the evolution of competitive traits in females. Evidence of a trade-off indicates that high reproductive demands in females may constrain the evolution or expression of competitive traits. Reproductive costs in females may select for the plastic expression of costly behavioural traits, or for less exaggerated morphological traits than those often observed in males.

In Chapters 5 and 6 I find evidence that male contributions to care may drive female investment in competition in *L. ocellatus*, as males are more aggressive to predators in the presence of offspring and contribute more to predator intrusions when in close proximity to females. This highlights a potentially important benefit of female competition for shells close to the male which might outweigh some of the reproductive costs of aggression in females. In particular, although I find that more competitive females may allocate less time to brood defence, these females are likely to have greater assistance from the male, reducing the costs to offspring survival. Evidence for this benefit of competition indicates that aggression in females is likely to be responding to selection for increased competitive ability. This supports the increasing body of work demonstrating that competitive traits can assist females in acquiring resources that may increase their reproductive success (reviewed in: Tobias et al. 2012; Stockley and Campbell 2013).

Knowledge of the costs and benefits of competition in females is important for our understanding of trait evolution in females, but it also has wider implications for the structure of social groups and consequently, the evolution of mating systems. Through field observations presented in Appendix B, I identified that *L. ocellatus* could be a useful system for investigating how the interaction between female competition, brood predation and male care can influence the formation and structure of groups and the evolution of harem polygyny.

7.2 Future directions

7.2.1 What drives the evolution of partial brood cannibalism in males?

My results in Chapter 3 suggest that cannibalism provides energy for males that they can reallocate to territory defence. However, several studies have also demonstrated that egg cannibalism has a care function by protecting the brood from disease spread or oxygen deficiency (e.g. Payne et al. 2002; Klug et al. 2006; Bandoli 2016). Is partial brood cannibalism primarily selected through the energetic benefits it affords to males or the survival benefits it affords to offspring? If cannibalism provides a substantial energetic benefit, we would expect egg consumption to improve a male's condition (Gomagano and Kohda 2008). Similarly, we might expect that males in worse condition would cannibalise more (Manica 2004). Males would also be expected to cannibalise more when the demands of care are greater and therefore measuring relationships between care effort and filial egg cannibalism could also indicate how cannibalism is related to energy levels.

If partial brood cannibalism improves the condition of males, does this benefit males by improving survival of the current brood or increasing their future reproductive success? Since sexual size dimorphism is an indicator of the strength of sexual selection acting on males (Horne et al. 2020), the positive correlation between cannibalism and dimorphism found in Chapter 3 may suggest that more cannibalism occurs in males with greater potential mating rates and that cannibalism benefits males through increasing mating success. However, there are alternative explanations for this correlation that have not been excluded. To test the relationship between cannibalism and future reproduction more precisely more direct measures of mating opportunity are needed, such as the average number of lifetime breeding attempts by males. However, to calculate this metric, knowledge of three life history parameters is required: male breeding lifespan, the number of annual brood cycles, and the number of females contributing a clutch to each brood cycle. Obtaining this information requires detailed studies of annual breeding behaviour as well as longer-term studies that can track individuals across years to determine lifespan. At present, this comprehensive information is unavailable for the vast majority of teleosts, where long-term studies of wild populations are challenging to perform, and reliably calculating lifetime breeding opportunities is currently only possible for five species that also have cannibalism rates available.

Although the presence of filial cannibalism has been identified in numerous species, specific cannibalism rates have seldom been reported. In addition, further studies reporting life history data in fish and quantifying the costs and benefits of cannibalism for males and their offspring will be an important contribution that would enable further comparative studies to investigate the evolution of this unusual behaviour.

7.2.2 How does investment in competition reduce hatching success?

In Chapter 4 I demonstrate that females experience a reduction in hatching success when breeding under high competition, but there are multiple mechanisms through which investment in competition could result in fewer hatched eggs. In particular, prior investment in competitive behaviour can influence how resources are allocated to eggs (Ouellet et al. 2001; Hanssen et al. 2002), while investment in competition during the care period may reduce parental care and affect the development of otherwise healthy embryos (O'Neal et al. 2008). Distinguishing between these temporally different effects is important for understanding how changes to the competitive environment might influence different reproductive attempts. This is particularly important in species such as *L. ocellatus* where group size is dynamic and females are likely to experience different levels of competition across their breeding life. Studies that manipulate the availability of resources or examine egg quality and viability under different conditions would help to identify which specific resources may be responsible for this trade-off and the reasons for egg failure (Hemmings and Birkhead 2016). For example, manipulations of stress levels in barn swallows (*Hirundo rustica*) demonstrated how increases in corticosterone uptake by eggs can result in reduced hatching success when mothers are stressed (Saino et al. 2005). Initial results from *L. ocellatus* show that lower hatching success in females experiencing higher social stress is a result of egg failure early in development prior to segmentation (Jedryszek P and Green JP, unpublished data). This suggests that physiological effects are likely to be more important than changes to parental care but the exact mechanism has not yet been determined.

A better understanding of the proximate causes of reduced hatching success will also inform us about whether these effects will impact the quality of larvae that successfully hatch and how this may impact their continued survival and success. For instance, if egg failure is caused by hormonal deficiencies affecting the hatching process, these effects may not continue post-hatching when larvae are able to produce their own hormones (Brooks et al. 1997). Alternatively, if a reduction in certain nutrients affects development, leading to some embryos failing and others developing abnormally, this will have consequences for larval survival into independence. Determining the effects of competition on offspring quality, as well as initial egg survival, is necessary to fully assess the fitness consequences of competitive investment for females.

7.2.3 How do female competition and sexual conflict influence breeding group structure?

While females are likely to have the highest breeding success in a monogamous pair where they can monopolise male care, males can increase their reproductive success by attracting additional females, indicating that sexual conflict over polygyny is likely to influence social interactions between males and females (Slagsvold and Lifjeld 1994). In an attempt to maintain their monogamous position, females may interfere with male attempts to attract additional females through male distraction (Eens and Pinxten 1995) or direct aggression towards female intruders (Slagsvold et al. 1992; Sandell 1998). To prevent interference, males can increase their aggression towards primary females and disrupt female contests (Eens and Pinxten 1995; Schradin and Lamprecht 2000). In the laboratory, *L. ocellatus* males were shown to interrupt the majority of aggressive interactions between the females in their harems, significantly reducing the number of contests and increasing harem stability (Walter and Trillmich 1994). The outcomes of these interactions will determine how many females breed as part of the same group and the distances over which their territories are distributed. To understand how female competition and sexual conflict may drive the social and spatial structure of breeding groups in *L. ocellatus*, further studies are needed that can investigate the relative importance of both male and female behaviour in determining female membership of breeding groups, particularly in natural populations.

As discussed in Appendix B, the costs and benefits of female-female competition are likely to be influenced by ecological factors, such as predation risk, that may vary within and between populations. Such factors may also influence the ability of harem-holding males to regulate aggression among females. For instance, in high-density populations of *L. ocellatus*, males may need to invest more time in intrasexual contests with rival males and pay less attention to female disputes. Although studies in other species have demonstrated that female-female competition can limit the size of breeding groups and alter the distances between female territories (Slagsvold et al. 1992; Sandell 1998; Wong et al. 2008), it is not clear how these effects can vary under different ecological conditions. Future studies will need to consider the interaction between social and ecological factors to understand the broader consequences of female competition for the formation and stability of pairs and harems.

7.3 Concluding remarks

This thesis contributes to our understanding of how reproductive trade-offs can influence investment in competition, reproduction and parental care and the consequences of such trade-offs for the diversity of parental and mating strategies we observe across species. The finding that female choice can select for male care demonstrates that increasing sexual selection does not always lead to reduced care investment. To fully understand how parental care evolves we need to consider how care behaviours directly benefit parents as well as offspring. Experiments in *L. ocellatus* provide some of the first evidence for reproductive constraints on the expression of competitive traits in females. It is essential that we continue to recognise that intrasexual competition is an important selection pressure in females and that differences in the nature of competition between males and females, and the associated costs, may contribute to sex differences in the expression of competitive traits.

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