

**Reproductive behaviour
in the male poeciliid fish
*Brachyrhaphis episcopi***

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Abstract

Ecology has a major impact on the evolution of reproductive traits. The extent of ecological selection on adaptive traits such as reproductive behaviour has been powerfully demonstrated using cross-species comparisons. Such comparisons typically assume that behaviour is invariant within a species but there is increasing evidence that within a species, reproductive behaviours show substantial geographical and temporal variation. I have examined variation in the reproductive behaviour of the Panamanian bishop *Brachyrhaphis episcopi*, a tropical poeciliid fish. Firstly, I explored male mate choice, which is an understudied but rapidly growing area of research. I found that even in this promiscuous species, males were choosy about the females that they mated with. Males showed mating preferences for unfamiliar females and for larger females, preferences that could increase the number of offspring sired. Male mate choice showed some degree of plasticity both seasonally and on a much shorter time-scale, which may reflect fluctuations in the costs and benefits of being choosy. Secondly, I examined between and within population variation in reproductive behaviour to find out whether ecological factors driving variation in closely-related species could be more generally applied. Field observations revealed that, despite large inter-population variation in predation pressure, there was little inter-population variation in male reproductive activity or behavioural repertoire. Instead, male reproductive behaviour showed significant variation within populations, which may be linked to local operational sex ratio. Reproductive success in this species is likely to be linked to male-male competition rather than female choice. Thirdly, I examined variation in individual reproductive investment by males. I looked at both reproductive tactics and other traits associated with mating success, such as testes mass. I found relationships between male size and mating behaviour, but no size-based differences in relative testes investment.

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Declaration

The research reported in this thesis is work that I have devised, run, analysed and interpreted. However, I feel it is important to recognise that a number of people have helped to shape and direct my work, and collect data. In recognition of this, I have written chapters 2 to 6 using plural authorship and each is written as a stand-alone paper, so some repetition of information is inevitable.

My co-authors are my supervisors, Dr. Victoria Braithwaite and Dr. Nick Colegrave, and Dr. Culum Brown, Chris Howard and Adel Heenan who have helped me collect data and work in the field. I am the first author for each of the papers, as detailed at the beginning of each chapter. Chapter 2 is published in *Animal Behaviour* (see Appendix 2) and the remaining chapters are written in paper format but have yet to be submitted.

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1. Introduction

1.1 Variation in reproductive behaviour

Behaviours associated with reproduction, such as mate competition, mate choice, individual mating success and parental care, are fundamental determinants of an organism's fitness and consequently key components describing animal breeding systems (Emlen & Oring, 1977). Breeding systems vary among and within species (Vehrencamp & Bradbury, 1984), and understanding the reasons for this variation in a framework encompassing ecology and life-history has been the focus of much research in modern behavioural ecology (reviewed in Reynolds, 1996).

There can be no doubt that ecology affects the evolution of breeding systems (Emlen & Oring, 1977). Cross-species comparisons have been employed to infer selective causes of adaptive variation in reproductive behaviour (e.g. Arnold & Owens, 2002), and have had a major impact on evolutionary biology (Fisher & Owens, 2004). Comparative approaches necessarily treat reproductive behaviours within a species as invariant, however, there is increasing evidence that reproductive behaviour varies greatly within species (Foster, 1999; Verrell, 1999; Saarikettu et al., 2005).

Population comparisons, which can also be used to infer causes of variation, have some advantages over comparisons made among higher taxonomic units: divergence is more recent, covarying traits may be fewer, and as populations are more likely to reside in areas where differences evolved, the chance of correctly identifying the selective cause of variation is increased (Foster & Endler, 1999a). Among-population variation in mating behaviour and sexual selection may also be associated with speciation or lack of divergence (West-Eberhard, 1983).

In addition to understanding causal factors of population variation, the study of variation in mating behaviour within species is of importance in its own right. It

allows us to understand the effects of environmental, intrinsic and social factors on an individual's behaviour or mating tactic and supplies a crucial wealth of knowledge for diverse areas of research. Variation in the strength and direction of mate choice, for example, has important implications for models of sexual selection (Jennions & Petrie, 1997).

Factors that may elicit within-species variation in mating behaviour fall into three areas (Partridge & Green, 1985; Magurran, 1993); environmental effects (e.g. climate, food availability, availability of breeding sites, predation risk, sex ratio, population density), phenotypic effects (e.g. age, size, sex, condition, genetically determined behaviour) and social effects (e.g. frequency-dependent mating tactics, socially-facilitated alteration or suppression of behaviour, co-operation with others). Below I discuss how these factors can lead to variation in reproductive behaviour among populations, among individuals and within individuals. As the effects are not necessarily exclusive, I discuss how different factors may interact. I also briefly outline possible non-adaptive explanations for within-species variation. My review is not exhaustive. Rather, I aim to illustrate some of the major environmental, phenotypic and social determinants of within-species variation in reproductive behaviours that facilitate gamete union.

1.2 Environmental effects

1.2.1 General environmental effects

Environmental factors that affect reproductive behaviour include physical factors such as climate, weather and availability of breeding sites. However, I use the term environment more generally here to include biological factors, such as food availability, predation risk, availability of mates and population density, as well as physical factors. Indeed, for the purpose of this review, environmental factors can be considered as sources of personal information that an individual obtains directly from its surroundings (Dall et al., 2005).

Divergent selective pressures in different environments can lead to different behavioural optima. Mating behaviour, in particular, may be affected in at least two general ways. Firstly, signals used in animal communication may vary among populations from different environments, as different qualities and components of signals may be transmitted more efficiently in habitats that have different physical properties. Signal evolution may also be modified by biological properties of the environment, such as predation pressure or competition with conspecifics (see examples below). If signal evolution is coupled with receiver evolution, populations in different environments may diverge in mate-recognition systems (reviewed in Verrell, 1999).

Secondly, variation in selective pressures among environments may lead to variation in the strength of sexual selection. Habitats may vary in environmental factors such as food, mates or breeding sites; these are all critical resources for reproduction over which individuals may compete. If these resources can be economically defended, some individuals (typically males) may prevent or hinder other individuals of the same sex from mating, thus facilitating increased reproductive skew and a greater opportunity for sexual selection (Emlen & Oring, 1977; reviewed in Reynolds, 1996). In turn, reproductive skew may be weakened by alternative mating tactics, such as sneaky mating attempts, female mimicry or co-operation with resource-holding males (reviewed in Taborsky, 2001). Under different levels of sexual selection, individuals may exhibit variation in frequencies and repertoire of reproductive behaviours.

Thus, temporal and spatial variation in the environment can affect signal transmission and sexual selection, and may play an important role in explaining adaptive variation in mating behaviour among populations and over time. Here I focus on three aspects of the environment that appear to be particularly important: operational sex ratio, predation pressure and habitat quality.

1.2.2 Operational sex ratio

Competition for mates plays an important role in explaining variation in breeding systems both among and within species. Simply examining the ratio of mature males and females in a population, however, is not a reliable predictor of competition as not all mature individuals within a population are ready to mate at any given time. A better measure of competition for mates is the operational sex ratio (OSR), which looks at the ratio of males and females that are ready to mate at a given time.

Alternatively, the ratio can be calculated from the proportion of time that each sex is ready to mate (reviewed in Kvarnemo & Ahnesjö, 1996). I will begin by discussing the role that OSR plays in determining intrasexual competition and mate choice, and illustrate how temporal variation in OSR can lead to a shift in sex roles within a population. As OSR may thus influence the potential for sexual selection, I also consider whether variation in OSR can affect the frequencies of alternative mating tactics within a population.

As the proportion of sexually-active males to fertilisable females becomes more male-biased, theory predicts that male competition for mates will increase (Emlen & Oring, 1977). Numerous studies agree with these predictions. For example, male-male competition for females increases as OSR becomes more male-biased among natural populations of soapberry bugs *Jadera haematoloma* (Carroll & Salamon, 1995) and within a population of adders *Vipera berus* (Madsen & Shine, 1993). Similar results were found in laboratory experiments in the spider mite, *Tetranychus urticae* (Enders, 1993) and the sand goby *Pomatoschistus minutus* (Kvarnemo et al., 1995). In addition to the increase in male-male competition in these studies, there was also an increase in reproductive skew as large males obtained proportionally more matings or eggs than other males at male-biased OSRs.

However, it is not always straightforward to predict how OSR will affect male-male competition. As OSR becomes more male-biased, the costs of defending resources associated with reproduction may increase, perhaps making resources economically undefendable. Accordingly, at male-biased sex ratios, large male European bitterling

Rhodeus sericeus are unable to defend territories and monopolise spawnings with females. Male-male aggression and variance in male mating success is therefore highest at equal OSR (Mills & Reynolds, 2003; also see Reichard et al., 2005). High costs of competition at extremely male-biased OSR may prevent males from attempting to mate altogether if an opportunity exists to mate under relatively competitor-free situations (e.g. deer mole salamander *Ambystoma talpoideum*, Verrell & Krenz, 1998).

Theory also predicts that as the number of potential mates increases, individuals may become more choosy about whom they mate with (Emlen & Oring, 1977). This may arise because the benefits of being choosy increase when there are more individuals to choose from because the cost of rejecting mates decreases (Hubbell & Johnson, 1987; Crowley et al., 1991) or perhaps because an individual can compare a greater range of potential mates (e.g. Balmford, 1991). So, in addition to the effects on intrasexual competition, OSR may also be an important determinant of the strength and direction of mating preferences in a population. For example, female guppies *Poecilia reticulata* have a stronger preference for males with more orange colour when OSR becomes more male-biased (Jirotkul, 1999). In laboratory studies of the role-reversed pipefish *Syngnathus typhle* (Berglund, 1994), males preferentially mate with large females when the sex ratio is female-biased but mate at random in a male-biased sex ratio. It is worth noting at this point that the effects of OSR on the strength of mate choice are similar to the effects of density: individuals become more choosy as density increases (e.g. Palokangas et al., 1992; Enders, 1993; Berglund, 1995), presumably because the costs of missed mating opportunities are reduced at high densities (Crowley et al., 1991).

Temporal variation in OSR may be sufficient to elicit changes in sex roles within a population: females may compete for males, which in turn may be choosy about their choice of mates (Kvarnemo & Ahnesjö, 1996; Kokko & Monaghan, 2001). This has been demonstrated in the two-spotted goby *Gobiusculus flavascens*, where the number of sexually-active males decreases over the course of the breeding season,

creating a shift from male-male competition and courtship of females to female-female competition and courtship of males (Forsgren et al., 2004).

Finally, high costs of competition under male-biased OSRs may cause males to use alternative mating tactics that avoid direct competition with other males (Gross, 1996; Taborsky, 1998; Taborsky, 2001). Studies to date suggest that male mating tactics show plasticity in responses to the OSR of the rearing environment, and also to short-term variation in OSR during adulthood. Responses include trade-offs between mating and interfering with other males (e.g. guppy *P. reticulata*, Jirotkul, 1999), courtship and sneaky matings (e.g. common goby *Pomatoschistus microps*, Borg et al., 2002) or scramble competition and contest competition/guarding (e.g. thrips *Elaphrothrips tuberculatus*, Crespi, 1988; hemipteran *Parastrachia murinus*, Tsukamoto et al., 1994; gray mouse lemur *Microcebus murinus*, Eberle & Kappeler, 2004).

1.2.3 Predation pressure

Predation is a major selective force acting on numerous traits, such as life-histories (reviewed in Abrams & Rowe, 1996), physical characteristics (e.g. Endler, 1980) and behaviours (reviewed in Lima & Dill, 1990). Reproductive behaviours are no exception: as many aspects of reproduction entail a cost of increased predation risk (reviewed in Magnhagen, 1991), individuals are expected to alter their mating behaviour under increased predation risk (reviewed in Sih, 1994). Additionally, individuals may trade-off between behaviours associated with avoiding predation and other tasks (Magurran, 1993). Firstly I examine individual plasticity in response to immediate predation risk, and discuss whether predation may select for differences in population norms of mating behaviour (Foster & Endler, 1999b). I then consider how predation may affect the strength of sexual selection through its effects on mate choice, intrasexual competition and alternative reproductive tactics.

Individual plasticity and population norms

Predation typically causes a decrease in mating behaviour: in the presence of a predator, individuals may reduce mating activity or sexual displays (e.g. Sih, 1988; Sih et al., 1990; Fuller & Berglund, 1996; Candolin, 1997; Jones et al., 2002), decrease frequency of advertisement calls or cease calling altogether (Tuttle et al., 1982; Tuttle & Ryan, 1982), increase latency to approach mates (Cooper, 1999), reduce harassment of females (Sih & Krupa, 1992) or reduce likelihood of sperm transfer (Uzendoski et al., 1993).

There is plenty of evidence that individuals alter mating behaviour in response to immediate predation risk, but studies showing that disparate predation risk among populations can select for differences in population norms of mating behaviour are surprisingly rare. Perhaps the only species in which among-population variation has been extensively studied is the guppy *Poecilia reticulata*, populations of which co-exist with and without piscivorous Piscean predators. Several studies have found population differences in reproductive behaviour, but not all studies find the same patterns (reviewed in Houde, 1997). Some studies found greater rates of courtships by males in low compared to high predation populations (Luyten & Liley, 1985; Houde & Cassidy, unpublished data cited in Houde, 1997) but others found the opposite trend (Farr, 1975; Magurran & Seghers, 1990 (predator absent)) or no population differences at all (Magurran & Seghers, 1990 (predator present); Shaw et al., 1994). The patterns of coercive mating, however, are more consistent: higher rates of coercive matings occur in high than low predation populations (Luyten & Liley, 1985; Magurran & Seghers, 1990; Magurran & Seghers, 1994a; with the exception of Shaw et al., 1994). Plasticity in mating behaviour in response to variable predation risk, however, is only found in guppy populations that co-exist with predators, not in those that are predator-free (Magurran & Seghers, 1990). The plasticity of the high-predation populations may play some role in explaining discrepancies among studies that have attempted to compare population mean norms (Houde, 1997).

Population variation in courtship and male-male aggression in three-spined sticklebacks *Gasterosteus aculeatus* may also be attributable to disparate predation pressure. In comparison to males from low predation populations, males from high predation populations are less aggressive to other males and also have reduced courtship activity (Peeke & Morgan, 2000). A further example of among-population variation in mating behaviour and predation pressure can be found in the amphipod *Hyallela azteca*. Strong (1973) compared amplexus duration of pairs from populations with varying risks of predation from Piscean predators. Amplexus, which may increase the risk of predation, was of longer duration in predator-free populations than in populations that co-existed with predators. Additionally, when cultured in the laboratory, population differences in amplexus duration remained suggesting that populations had genetically diverged for this mating behaviour.

Interestingly, the effects of phonotactic parasitoid flies (which locate insect prey through male mating calls) on mating behaviour may show parallels with the effects of predation. Zuk et al. (1993) studied the calls of male field crickets *Teleogryllus oceanicus* from populations that exist both with and without parasitoid flies. They found that, compared to parasitoid-free populations, males from the parasitoid-present population had shorter calls and were less likely to call at the times of day when the parasitoids were active, a result consistent with the selective pressure of parasitoids on advertisement calls. However, as the study was correlational, an alternative explanation is that infection with parasitoids affects the calls of crickets: parasitized crickets may have shorter calls and call at different times of the day compared to uninfected crickets. As such, the population differences in behaviour reported by Zuk et al. (1993) could be a constraint as a result of infection rather than an adaptive response to parasitism risk. Adaptive responses against parasitoid flies have also been proposed to account for seasonal variation in the calls of field crickets of the *Gryllus* genus (Higgins & Waugaman, 2004).

Sexual selection

Predation pressure may affect three components of sexual selection: mate choice, intrasexual competition and success of alternative mating tactics. Firstly, mate choice may increase the risk of predation through mate searching activity or proximity to sexual displays or signals (Forsgren, 1992). If mate choice becomes sufficiently risky under predation risk so that the costs of being choosy are greater than the benefits, theory predicts that individuals should become less choosy (Crowley et al., 1991). In a number of species, mate choice certainly weakens or alters in the presence of a predator (Forsgren, 1992; Berglund, 1993; Godin & Briggs, 1996; Johnson & Basolo, 2003), although it is possible that a reduction or shift in mating preferences may also occur because individuals are unable to make informed choices under the risk of predation or because individuals have a reduced sexual motivation (Forsgren, 1992).

Secondly, competitive interactions may increase the risk of predation (Jakobsson et al., 1995), and so the intensity or the type of male-male competition should decrease in the presence of predators (Sih, 1994). There is evidence in support of this prediction in fish (Brick, 1998; Kelly & Godin, 2001) and in mice (Roberts et al., 2001).

Thirdly, bourgeois tactics, where males defend territories or mates, or display to females using visual, acoustic or chemical signals (*sensu* Taborsky, 2001), have been associated with an increased risk of predation (Magnhagen, 1991; also see Gwynne, 1989). In comparison to bourgeois males, parasitic males (sneakers or female mimics) are expected to be under less risk of predation and so the frequencies of males exhibiting parasitic tactics should be more common when predation risk increases (e.g. Sih, 1994; Godin, 1995; Magnhagen, 1995). Initial studies with guppies *P. reticulata* found support for this risk-sensitive hypothesis: males attempted more sneaky matings and fewer courtships in the presence of a predator (Endler, 1987; Magurran & Seghers, 1990; Magurran & Nowak, 1991; Godin, 1995). Recent work, however, shows that males may not respond directly to increased

predation risk (as suggested by Magurran & Nowak, 1991; also see Godin, 1995). Rather, it seems that females are responding to the presence of predators by increasing anti-predator behaviour and that males are responding to the change in female behaviour rather than predation risk itself, perhaps because males exploit a reduced ability of females to avoid coercive mating attempts (Evans et al., 2002).

1.2.4 Habitat quality

One further aspect of the environment, habitat quality, appears to play an important role in explaining within-species variation in mating behaviour. Below, I illustrate how habitat quality may affect signal-receiver evolution, as signals may vary depending on the transmission properties of the habitat. I then discuss how habitat quality may also affect sexual selection, either directly by altering the economic defendability of resources critical to reproduction, or indirectly through altering OSR and the costs and benefits of mate choice.

Signal evolution

The efficient transmission of visual and acoustic signals depends on the physical properties of the habitat. Divergent selection in different habitats can lead to population-specific variation in signal quality or character (Verrell, 1999). Habitat density, in particular, may affect the transmission of acoustic signals used in mate attraction or intrasexual competition. For example, in the cricket frog *Acris crepitans*, calls of forest males have greater transmission efficiency compared to males from open habitats as a result of environmental selection for reduced attenuation of the call in forests (Ryan et al., 1990). Accordingly, female frogs show local mate preference based on male calls (Ryan & Wilczynski, 1988) and the auditory receptors of forest females are better at filtering out environmental noise compared to females from open habitats (Witte et al., 2005). Many bird calls also show population variation, which has been attributed to selection on call properties in different habitats (e.g. Slabbekoorn & Smith, 2002).

Like acoustic signals, visual signals may vary between populations in response to selection for more efficient signals (e.g. Reimchen, 1989; Endler & Houde, 1995; Endler & Théry, 1996; Boughman, 2001). For example, the sigmoid courtship display of the guppy *P. reticulata* requires good water clarity for efficient transmission to females. In populations from turbulent waters, male guppies attempt very few displays and instead resort to coercive matings that do not require visual cues (Luyten & Liley, 1985).

Sexual selection

Habitat quality can affect the costs of resource defence, the costs and benefits of mate choice and the costs of competition. As we have seen above, these aspects of behaviour can affect reproductive skew and the strength of sexual selection. I begin by discussing the effects of habitat quality on breeding systems and levels of polygyny. I then focus on variation in mate choice criteria in different habitats, and finally illustrate how habitat quality can affect OSR and levels of intrasexual competition.

Habitat quality may affect the ability of individuals to monopolise access to mates, and consequently affect the extent of polygyny in a breeding system (Emlen & Oring, 1977). The spatial distribution of food, for example, can influence the costs of resource defence. In dunnocks, *Prunella modularis*, dense clumped food resources lead to a polygynous breeding system, whereas sparse resources are associated with monogamy (Davies & Lundberg, 1984). Habitats with good food availability reduce the requirement for off-territory foraging, which consequently improves male ability to mate guard and in red-winged blackbirds *Agelaius phoeniceus* (Westneat, 1994). Habitat density also affects mate guarding: in dense habitats, male yellow-breasted chat *Icteria virens* find it difficult to guard females, which increases the risk of extra-pair copulations and may affect the opportunity for sexual selection (Mays & Ritchison, 2004).

In addition to its effects on breeding systems, habitat quality can affect mate choice criteria. For example, a seasonal decrease in water flow leads to temporary confinement in some guppy (*P. reticulata*) habitats: males from these confined habitats show a mating preference for unfamiliar females, which is likely to be advantageous to males as it promotes mating with as many different females as possible (Kelley et al., 1999). Female common gobies *Pomatoschistus microps* prefer males with elaborate nests under normal levels of dissolved oxygen, but when dissolved oxygen levels are reduced, females show no preference for elaborate nests either because assessment is too costly under these conditions or females use different assessment criteria (Jones & Reynolds, 1999). A further interesting possibility is that the genetic benefits of mate choice may change in different environments (reviewed in Qvarnström, 2001). For example, it has been shown in two strains of predatory soil mite (*Hypoaspis aculeifer*) that hybrid growth depends on diet composition. The two strains are adapted to different prey species and females select mates on the basis of their diet even if this means mating with a male from the different strain. Lesna and Sabelis (1999) proposed that by making this selection, females are ensuring their offspring have optimum genotypes for the environment they are likely to experience.

Variation in habitat quality has a further important effect on mating behaviour: it can directly determine OSR. Studies of the Mormon cricket *Anabrus simplex* (Gwynne, 1993) and the katydid *Kawanphila nartee* (Simmons & Bailey, 1990; Gwynne et al., 1998) are perhaps the most elegant demonstrations of how habitat quality affects OSR, and consequently leads to reversal of typical sex roles. Males of both species produce nutritious nuptial gifts that females eat during insemination. When diet is poor, fewer males can produce nuptial gifts. The OSR becomes more female-biased and in turn, typical sex roles are reversed. It is not clear whether food availability affects OSR in species without nuptial gifts but it is likely if the effects of food deprivation are more pronounced in one sex. Similarly to the availability of food, access to nest or breeding sites can also affect OSR. This is because a reduction in the availability of nests affects the proportions of each sex that can mate at a given time. Variation in nest site availability and therefore OSR can affect mating activity,

mate choice and intrasexual competition (e.g. Forsgren et al., 1996; Borg et al., 2002; Lehtonen & Lindstrom, 2004) and may explain temporal shifts in sex roles in the common goby *P. microps* (Borg et al., 2002), and among-population variation in sex roles of the blennid fish *Salaria pavo* (Almada et al., 1995).

Scarcity of nest sites or breeding substrate may also facilitate an increase in the frequency of alternative mating tactics in a population. There is good evidence for this in the cichlid *Lamprologus callipterus* (reviewed in Taborsky, 2001) and in the black goby *Gobius niger* (Mazzoldi & Rasotto, 2002). It is assumed that increases in observed frequencies of alternative mating tactics necessarily lead to a decrease in reproductive skew, but this may not always be the case. For example, despite variation in nest site availability and intrasexual competition between two populations of sand goby *P. minutus* (Forsgren et al., 1996), paternity analysis revealed no differences in success rates of sneaky matings between populations (Jones et al., 2001).

1.3 Phenotypic effects

Whilst considering the effects of the environment on mating behaviour, I have largely ignored the actual phenotype of the individual. However, it is of course the case that individuals within a population will differ markedly in many aspects of their phenotype, which in turn affect the reproductive decisions that an individual makes. Phenotypic effects may arise as a result of some intrinsic character of an individual, such as age, size, condition or genotype, and may be modified by interactions with social or environmental factors (e.g. Enders, 1993; Reynolds et al., 1993; Bisazza & Marin, 1995; Rodd & Sokolowski, 1995). As phenotypes are not necessarily constant over time, individuals may adopt different behaviours at different time points. Thus, in addition to explaining variation among individuals, intrinsic effects can also lead to temporal variation in an individuals' behaviour. There is evidence for phenotypic effects on alternative mating tactics and mate choice, which I discuss below.

Not all individuals within a species use the same techniques to increase their reproductive success (Krebs & Davies, 1987). In many mating systems, males defend resources or mates, or invest in traits that attract females (Andersson & Iwasa, 1996) but other males use sneak tactics to obtain matings, for example, by mimicking females, rather than competing for or courting females directly (e.g. Taborsky, 2001). The reproductive strategy that an individual uses is a genetically based rule that determines what tactic the individual will follow (Gross, 1996). Strategies may be determined by genetic polymorphisms (e.g. Ryan et al., 1992; Lank et al., 1995) although conditional strategies, where the alternative reproductive tactic (ART) adopted by an individual depends on some measure of its status or condition, are much more common (Gross, 1996). Theoretically, it is also possible for tactics within a population to be determined by both genetic polymorphisms and condition (a mixed strategy; Plaistow et al., 2004). Choice of tactics under a conditional strategy may be determined by size (e.g. Danforth, 1991; Ruchon et al., 1995; Emlen, 1997a; Aubin-Horth & Dodson, 2004), age (e.g. Slagsvold & Saetre, 1991; Brockmann & Penn, 1992; de Fraipont et al., 1993; Eggert & Guyétant, 2003; Eberle & Kappeler, 2004), attractiveness to the opposite sex (Kempnaers et al., 1992) and condition (e.g. Eadie & Fryxell, 1992; Eggert & Guyétant, 2003; Aubin-Horth & Dodson, 2004; Neff et al., 2004).

Optimal mate choice, as well as mating tactics, may depend on an individual's own quality or phenotype, particularly where both sexes exhibit some degree of choosiness (Parker, 1983). Evidence in the sailfin molly *Poecilia latipinna* (Ptacek & Travis, 1997) and the two-spotted goby *Gobiusculus flavicens* (Amundsen & Forsgren, 2003) suggests that smaller males are less choosy than larger males, presumably because smaller males have fewer mating opportunities and thus a greater cost to rejecting potential mates. Optimal mate choice may also depend on an individual's genotype (reviewed in Tregenza & Wedell, 2000) and mate choice may be exercised to avoid inbreeding (e.g. Berger & Cunningham, 1987; Foerster et al., 2003), optimise genes involved in pathogen resistance (e.g. Landry et al., 2001;

Aeschlimann et al., 2003) or to promote genetic compatibility (e.g. Ryan & Altmann, 2001).

1.4 Social effects

Mating behaviours not only vary as a result of environmental and phenotypic factors, but may also be moderated by the behaviours of others. This could include suppression or alteration of mating behaviour as a direct consequence of aggression from others (e.g. Emlen, 1997b), and to this extent, some effects of intrasexual competition already discussed could be considered as social effects. Here, however, I focus on social effects that are more subtle, highlighting how socially-acquired information may affect mate choice and mating tactics.

Firstly, an individual may gain information about the quality of mates in an environment by observing the mate choice decisions of others. Copying may be advantageous as it could reduce sampling costs or allow a female to rapidly obtain information about potential mates (reviewed in Dall et al., 2005). A number of studies have demonstrated that females do indeed copy the mate choice of other females (reviewed in Pruett-Jones, 1992; Jennions & Petrie, 1997). However, there are likely to be costs to mate-choice copying, such as a risk of mating with a sperm depleted male (Nakatsuru & Kramer, 1982) or an increased risk of predation (Brooks, 1999). Additionally, the information acquired from others may not be reliable (Dall et al., 2005). If mate-choice copying is common then information on potential mates may cascade through a population and, within a population, among-individual variation in mating preferences may decrease. However, mate-choice copying may lead to variation in criteria used in mate choice among-populations, if individuals at the start of the cascade differ in their choice of mates between populations (Godin et al., 2005).

Secondly, an individual's choice of mating tactic may be determined not just by phenotypic effects already described, but also by the tactics of rivals. As the fitness

of the chosen tactic may depend on the relative competitive abilities of others in the population, the status of the individual within the population could determine which tactic it should adopt to attain the highest possible fitness. Gross (1996) proposed a model of status-dependent selection, where individuals incorporate information about their own status and the status of others in the population to decide which tactic to use. To date, evidence for status-dependent selection is scant, although support for a number of key predictions have been shown in the beetle *Onthophagus taurus* (Hunt & Simmons, 2001).

1.5 Interactions among factors

So far, I have considered environmental, phenotypic and social effects independently but in reality this is almost certainly not the case. In different environments, it is unlikely that individuals of different phenotypes will experience equal costs and benefits regarding resource defence, mate choice, competition etc. For example, small male spider mites *Tetranychus urticae* appear to be affected by the degree of male-male competition, and become less selective about females as OSR becomes more male biased. Large males, however, remained selective regardless of OSR (Enders, 1993).

Further plasticity in mating behaviour is illustrated by interactions with predation pressure. We have seen that individuals alter their mating behaviour in response to predation, but not all individuals are expected to respond in the same way. For example, large male guppies *P. reticulata* perform fewer courtship displays under high predation risk compared to small males (Reynolds, 1993) and male mice *Mus musculus* that scent mark at high frequencies show a greater reduction in scent-marking frequency under predation risk compared to low-frequency marking males (Roberts et al., 2001). These results may arise because large male guppies and high-marking mice are at greater risk of predation. However, the current and future probabilities of reproduction may also affect risk-taking under predation pressure and perhaps large male guppies and high-marking mice expect higher future reproductive

success. Certainly, the trade-off between current and future reproductive probabilities explains why older male gobies *Gobius niger* and older male sticklebacks *Gasterosteus aculeatus* were more willing to take risks than younger individuals (Magnhagen, 1990; Candolin, 1998; Candolin & Voigt, 2003a).

How are bourgeois and parasitic males affected by the risk of predation? Magnhagen (1995) examined the effects of predation risk on nest defence and sneaky mating attempts in male common gobies *Pomatoschistus microps*. Both nest owners (bourgeois males) and sneaks (parasitic males) reduced their activity in the presence of a predator: nest owners were less likely to attack approaching males and sneak males were less likely to attempt sneaky matings. Although the study does not examine whether predation risk leads to changes in the frequencies of males performing tactics, it suggests that parasitic males as well as bourgeois males perceive a risk of predation.

Different habitats are also associated with variation in predation risk. Three-spined sticklebacks *G. aculeatus*, for example, experience increased predation risk in open compared to vegetated habitats. In the presence of predators, males in vegetated habitats reduce courtship less than males in open sites, presumably because the extra cover in vegetated sites offsets an increased risk of predation from courtship (Candolin & Voigt, 1998). Male guppies *P. reticulata* reduce their sexual behaviour under bright light conditions, which are associated with increased predation risk (Endler, 1987; Reynolds et al., 1993).

1.6 Non-adaptive explanations for variation

Above, I have discussed adaptive explanations for within-species variation in mating behaviour. Variation, however, may also be non-adaptive, as a result of correlated responses, founder events and genetic drift, and I briefly outline these below.

Correlated responses

Mating behaviour may be correlated with some other trait on which selection is acting. This may occur because of pleiotropy (e.g. genes that determine the trait under selection also determine other traits, such as mating behaviour; Lande, 1980), or because of selection for a trait that in turn affects mating behaviour (e.g. body size, Ryan & Wilczynski, 1988). Thus, selection on one trait may constrain the evolution of adaptive behaviour. An example of a correlated response that has recently aroused much interest is a behavioural syndrome, where an individual or population exhibits consistent correlated behaviours in two or more situations. Behavioural syndromes may constrain behaviours so that they exhibit limited plasticity and appear non-adaptive. However, different environments may select for a different suite of behavioural correlations, leading to adaptive differences in behavioural syndromes among populations (reviewed in Sih et al., 2004).

Founder events and genetic drift

Another factor that may contribute to or even explain variation among populations is the founder event and subsequent genetic drift (Verrell, 1999). For example, the advertisement calls of the túngara frog *Physalaemus pustulosus* differ between two major allozyme groups, which are thought to represent multiple invasions from South America and Panama. However, geographically close populations have more similar calls, regardless of allozyme similarity, which may be evidence of convergent evolution (Ryan et al., 1996). Founder events may also affect traits that are culturally inherited, which could lead to stochastic population differentiation if mating behaviour has a learnt component (e.g. Baker & Jenkins, 1987) or is copied (Godin et al., 2005).

1.7 Summary

Many aspects of mating behaviours, including intrasexual competition, mate choice and mating tactics, show variation within species. In addition to intrinsic effects, mating behaviour shows plasticity in response to environmental variability in a diverse range of taxa. The importance of social effects on mating behaviour is also becoming more apparent in a number of recent studies.

There is a surprising paucity of studies that consider population variation in mating behaviour and sexual selection, despite its promise as a fruitful and important field of research (Foster, 1999). Work on the Trinidadian guppy, *P. reticulata*, illustrates the productivity of population comparisons (Houde, 1997). Whilst the guppy is an important model system for examining many behavioural and evolutionary traits, particularly with regard to the effects of predation, we do not know how far such effects generalise to other species.

In this thesis, I have examined within-species variation in a system (see below) that in many ways resembles that of the Trinidadian guppy (Brown & Braithwaite, 2004). In particular, I have focused on male mate choice (which is widespread in fishes and insects; Sargent et al., 1986; Bonduriansky, 2001) and male mating tactics, making comparisons among populations, among individuals and over time in an attempt to infer selective causes of adaptive variation.

1.8 Ecology and biology of *Brachyrhaphis episcopi*

The Panamanian bishop *Brachyrhaphis episcopi* (Pisces: Poeciliidae, also known as *Brachyrhaphis episcopi*) is a small livebearing fish found in rainforest streams in central Panama. I studied populations of *B. episcopi* in multiple streams in the Parque Nacional Soberania (fig. 1.1), where there is diverse predation pressure from piscivorous fish (Jennions & Telford, 2002; Brown & Braithwaite, 2004).

Table 1.1 Relative percentages of fish fauna found in each stream

River	Location	<i>B. episcopi</i>	Predators	Others
Rio Agua Salud (AS)	Above falls	99	0	1
	Below falls	55	45	0
Rio Macho (RM)	Above falls	79	0	21
	Below falls	37	54	9
Rio Limbo (RL)	Above falls	86	2.5	11.5
	Below falls	5	80	15
Quebrada Juan Grande (QJG)	Above falls	99	1	0
	Below falls	16	78	6

Reproduced from Brown & Braithwaite (2004)

The streams flow over an escarpment, creating waterfalls that mark a shift in fish communities. In the headwaters of streams, *B. episcopi* is the predominant species and co-exists with small numbers of least killifish *Rivulus brunneus* (Angermeier & Karr, 1983). Whilst *R. brunneus* may prey upon juvenile *B. episcopi*, it is incapable of eating adults. In the midwaters of streams, *B. episcopi* are found with a suite of larger predatory fish including cichlid and characid species (table 1.1), all of which are known to include other fish such as *B. episcopi* in their diet (Angermeier & Karr, 1983; Kramer & Bryant, 1995). Thus, there is a high predator density below the falls in comparison to above the falls.

Populations with high and low densities of predators show variation in body size and life histories (Jennions & Telford, 2002), cognitive abilities (Brown & Braithwaite, 2005), stress responses (Brown et al., 2005a), boldness-shyness traits (Brown & Braithwaite, 2004; Brown et al., 2005b) and cerebral lateralisation (Brown et al., 2004), all of which are consistent with changes in predation risk.

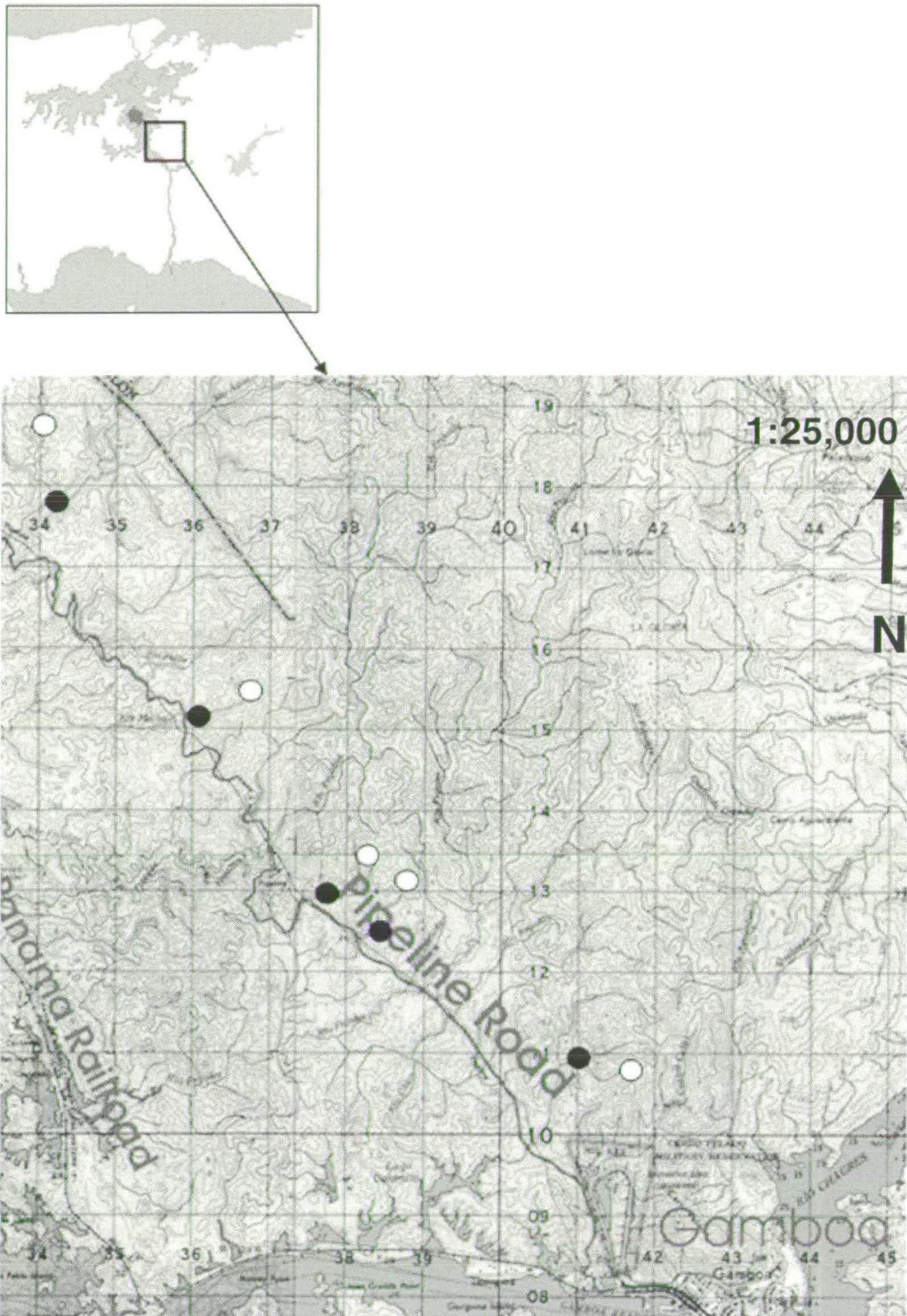


Figure 1.1 Location of study area, Parque Nacional Soberania, in the Republic of Panama: High (●) and low (○) predator density sites in streams sampled during this study (from North to South): Rio Agua Salud, Rio Macho, Rio Limbo 2, Rio Limbo 1, Quebrada Juan Grande.

Little is known about the reproductive behaviour of *B. episcopi*, but preliminary laboratory observations showed that males used both courtship displays and coercive thrusts when attempting to mate with females (Appendix 1; see table 1.2 for descriptions of behaviours). Coercive mating attempts have previously been reported before for this species (Farr, 1989), but courtships in natural populations have not. Females are rarely receptive to males, but when they are, they adopt a head-down posture and move forward very slowly, allowing the male to mate (pers. obs.).

Table 1.2 Descriptions of male mating behaviours in *B. episcopi*

Male behaviour	Description
Association with female	Male orientated towards and within one body length of female
Thrust	Coercive mating attempt, where male darts rapidly underneath female
Courtship	Male displays side-on with erect fins to female with at least one turn
Display towards female	Male displays side-on with erect fins to female but does not turn
Gonoporal nuzzle	Males places mouth on female gonopore
Gonopodium jerk	Male swings gonopodium forwards and arches body

Like other poeciliid fish, fertilization is internal: males transfer sperm to females by means of a modified anal fin, the gonopodium, and females can store sperm for several months. Females do not develop more than one brood at a time (Turner, 1937). Females give birth to live young (clutch size ranges from 1 to 40: Turner, 1938) and after birth, there is no subsequent parental care.

Mature males do not possess any elaborate sexual ornaments but are smaller than females (males: 15-36mm standard length; females: 20-50mm standard length (fig. 1.2). Upon maturity, male growth rate is negligible, but females continue to grow (Snelson, 1989). A small hook can be seen on the snout of mature males, but the function of the hook is not known.

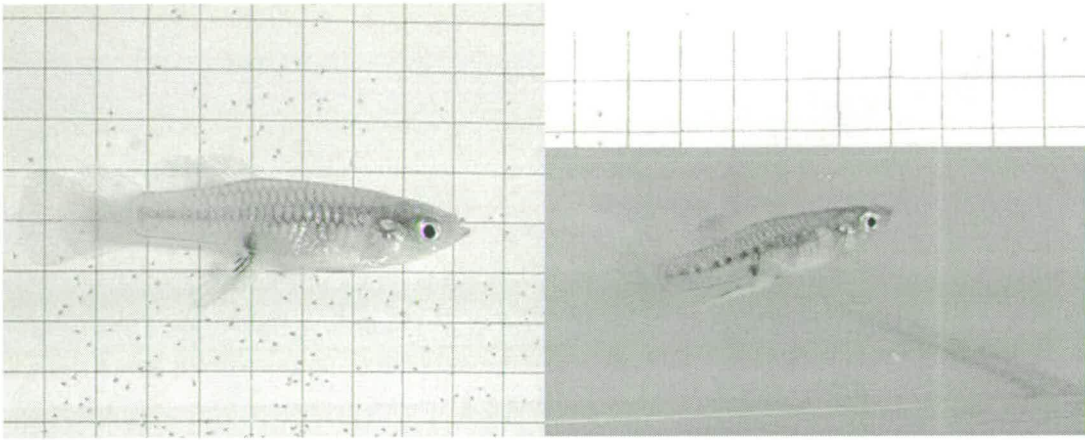


Figure 1.2 Mature female (left) and mature male (right) *B. episcopi*. Photographs taken against 5mm grid for scale.

Both male and female *B. episcopi* are highly aggressive. Large females defend profitable foraging spots in streams, and large males attempt to defend these areas from other males (H. Simcox, pers. obs). Like most poeciliid fishes, the adult sex ratio is female-biased but shows some variation within and among populations (Jennions & Kelly, 2002).

1.9 Aims

In this thesis, I present a series of laboratory and field experiments examining male mating behaviour and traits associated with reproduction in *B. episcopi*. I examine environmental, intrinsic and social factors that contribute to variation among populations, among individuals and temporal variation. Specifically, I look at:

1. Population variation in male mate choice and the effects of partner familiarity
2. Temporal variation in male mate choice *in situ* and the effects of female size and familiarity
3. Variation in male mating behaviour: the roles of predation pressure and OSR
4. Variation in male mating behaviour: the effect of male size under intrasexual competition
5. Variation in male traits associated with reproduction: testes allometry, gonopodium length and male melanic colouration

2. Context-dependent male mating preferences for unfamiliar females

Authors: Helen Simcox, Nick Colegrave, Adel Heenan,
Chris Howard & Victoria A. Braithwaite

2.1 Abstract

We used laboratory and field-based experiments to examine male mate choice in the promiscuous Panamanian bishop *Brachyrhaphis episcopi* (Poeciliidae). As the primary determinant of male fitness is often the number of matings he obtains, a polygynous male could increase his reproductive success by selectively mating with different females. To test this, males in both the laboratory and field were allowed to choose between a familiar (same tank or pool) and unfamiliar female (different tank or pool). We compared males from streams and seasonally occurring pools to see whether lack of access to new females in pools promotes male mate choice for unfamiliarity. In addition, we chose streams and pools both with and without predatory fish to examine the influence of predation risk. In both the laboratory and the field, males attempted more matings with unfamiliar than familiar females. Field experiments showed that courtship preferences differed between males from populations with and without predatory fish: males from populations with predators were choosy only when light levels were dim, whereas males from populations without predators were choosy when light levels were brighter. Males from both streams and pools discriminated between familiar and unfamiliar females, but there were no differences in mating preferences between males from each habitat. Although the reasons for a preference for unfamiliar females remain unclear, the plasticity of this behaviour is evident. Differences in male mate choice between populations with and without predators suggest that males may face a trade-off between the costs and benefits of being choosy.

2.2 Introduction

An individual's choice of mate or mates may have profound consequences for their subsequent fitness (1983) and thus has an important evolutionary role (Partridge & Halliday, 1984). As females usually invest more in each offspring, they tend to be more discriminating than males in their choice of mates (Bateman, 1948; Trivers, 1972). If there is variation in female quality, however, and the relative costs of being choosy are lower than the benefits, males should preferentially mate with females that will maximize their reproductive success (Parker, 1983). Male mate choice has been documented repeatedly, with selection for more colourful females (e.g. Amundsen et al., 1997; Amundsen & Forsgren, 2003), larger females (e.g. Ptacek & Travis, 1997; Herdman et al., 2004; but see Basolo, 2004) and genetic compatibility (e.g. Ryan & Altmann, 2001). A female-biased operational sex ratio may also promote choosiness in males (Hubbell & Johnson, 1987).

Even the most promiscuous male may be choosy. If male reproductive success increases with the number of female partners, a male should benefit from selectively mating with different females (Adler, 1978). Such a benefit is invoked to explain the Coolidge effect, where satiated polygamous males show a heightened sexual interest in new females (Dewsbury, 1981). A preference for new or unfamiliar females has also been shown in males that were not satiated, in lizards (*Holbrookia propinqua*, Cooper, 1985; *Anolis sagrei*, Tokarz, 1992; *A. carolinensis*, Orrell & Jensen, 2002), salamanders (*Desmognathus ochrophaeus*, Donovan & Verrell, 1991), wild horses (*Equus caballus*, Berger & Cunningham, 1987) and guppies (*Poecilia reticulata*, Kelley et al., 1999). Male mate choice for unfamiliar females would allow a polygynous male to mate with as many different females as possible, potentially enabling him to sire a greater number offspring. (Adler, 1978) It may also be a mechanism to avoid inbreeding, if familiar individuals are more likely to be related (Farr, 1977).

Male guppies, *P. reticulata*, show population variation in their mating preference for unfamiliar females (Kelley et al., 1999). Male guppies confined in seasonal pools

preferentially court unfamiliar over familiar females, but males from rivers show no preference. Kelley et al. (1999) suggested that males from rivers move between schools of females to maximize their mating opportunities and do not need to, or cannot, discriminate between familiar and unfamiliar females. Males trapped in pools, however, may benefit from identifying and mating with unfamiliar females.

Mate choice behaviour varies not only between populations, but also with environmental conditions (e.g. Endler & Théry, 1996; Houde, 1997). Predation risk, in particular, may affect mate choice: models predict that choosiness is reduced or absent as the costs of choice increase (Sutherland, 1985; Hubbell & Johnson, 1987; Crowley et al., 1991). In a number of fish species, female mate choice is absent or altered when a predator is present (e.g. Forsgren, 1992; Briggs et al., 1996; Godin & Briggs, 1996; Johnson & Basolo, 2003). Predation risk affects male mating behaviour (e.g. Endler, 1987; Magurran & Seghers, 1990; but see Evans et al., 2002) and may also affect male mate choice (Houde, 1997). A recent study supports the theory that male mate choice should change as the costs of choosing increase (Wong & Jennions, 2003), but the relationship between predation risk and male mate choice has been specifically addressed only in pipefish, which have a role-reversed mating system (Berglund, 1993).

We examined male mate choice in the Panamanian bishop *Brachyrhaphis episcopi* (Poeciliidae), a promiscuous live-bearing fish native to central Panama. In the wild, males occasionally court females, but the majority of attempted matings are coercive (Chapters 3 & 4). The operational sex ratio is highly skewed in favour of females, which defend profitable foraging areas within a stream. Larger males attempt to monopolize access to females through aggression towards smaller males (pers. obs.). Lack of paternal care combined with female sperm storage means that males could benefit from mating with as many different females as possible. We predicted that, if males could acquire familiarity with resident females, those males would preferentially mate with unfamiliar females.

We used both laboratory and field-based mate choice experiments to examine whether male *B. episcopi* prefer unfamiliar females, and how this choice varies between populations under different environmental conditions. We compared populations from streams and seasonally occurring pools, since this factor affects male mating preferences in guppies (Kelley et al., 1999). We also examined the possible influence of predation risk on male mate choice by selecting streams and pools with and without predatory fish. We estimated light levels in the field, because these may interact with factors such as predator density to affect male mating behaviour (e.g. Endler, 1987).

2.3 Methods

2.3.1 Study System

We sampled *B. episcopi* from five independent freshwater streams that run into the Panama Canal (Pipeline Road, Parque National Soberania, Panama (fig. 2.1). Each stream flows across an escarpment creating a waterfall, which acts as a barrier to the upstream movement of larger fish (Jennions & Telford, 2002). Above the falls, the fish community is dominated by *B. episcopi* and the killifish *Rivulus brunneus* (Cyprinodontidae). Although *R. brunneus* may prey on juvenile *B. episcopi*, it is incapable of eating adults (Angermeier & Karr, 1983). Below the falls is a suite of piscivorous fish that live in sympatry with *B. episcopi*, such as the tigerfish *Hoplias microlepis*, various cichlids and tetras (Brown & Braithwaite, 2004).

Brachyrhaphis episcopi inhabits the headwaters of streams and is replaced further downstream by two other poeciliid species, *Brachyrhaphis cascajalensis* (Mojica et al., 1997; Jennions & Telford, 2002) and *Neoheterandia tridentiger* (pers. obs.). This transition occurs upstream of any junctions where our study streams merge. We therefore consider each stream to be independent and treat sites with high and low predator densities within a stream as independent populations in our analyses (as

defined by Johnson & Belk, 2001; Jennions & Kelly, 2002; Jennions & Telford, 2002).

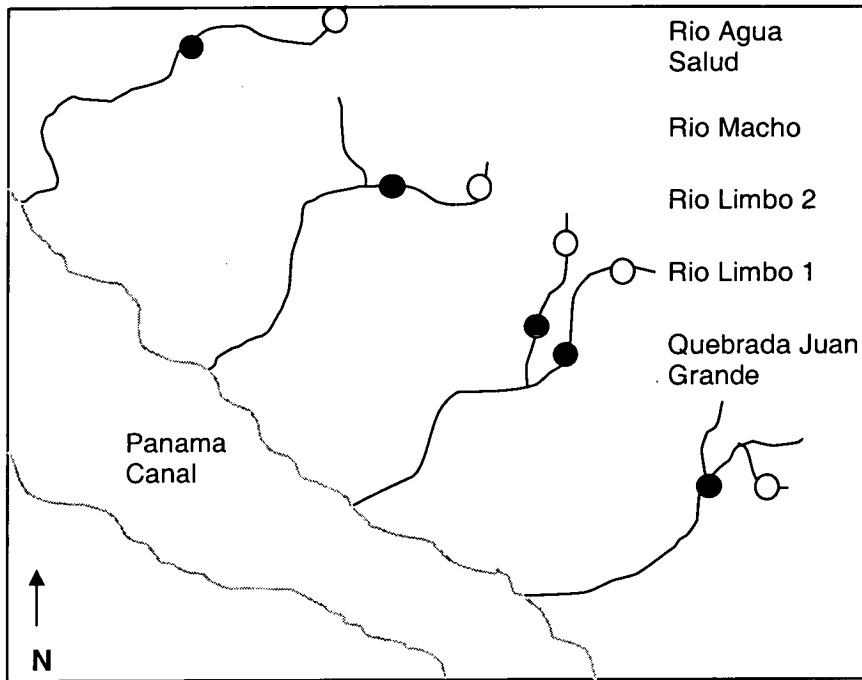


Figure 2.1 High (●) and low (○) predator density populations of *Brachycephalus episcopi* sampled from the Frijoles drainage basin, Parque Nacional Soberania, Panama.

2.3.2 Laboratory Experiment (June-August 2003)

Fish were collected from the wild with dip nets and were air-freighted to the University of Edinburgh under a permit from the Autoridad Nacional del Ambiente (ANAM) and an import permit from the Scottish Executive Environment and Rural Affairs Department. A total of 400 fish were transported in July 2002 and February 2003 as part of a larger study. All fish survived transport. Fish were housed for a minimum of 4 months in mixed-sex 90 litre tanks before testing. Between one and four males were housed with around 15 females from the same population, reflecting the highly skewed sex ratio found in the wild. Any offspring from the wild-caught fish were removed and housed separately to the adults. Tanks were maintained at 24-26°C on a 12:12 light: dark regime and fish received standard flake food daily.

We tested 20 males, but because males from each tank were not considered to be independent, we pooled data for each tank ($N = 11$). Three populations were in sites with a high predator density (Rio Agua Salud, $N = 2$; Rio Macho, $N = 2$; Quebrada Juan Grande, $N = 2$) and three in sites with a low predator density (Rio Agua Salud, $N = 2$; Rio Macho, $N = 2$; Rio Limbo 1, $N = 1$). Sampling was not equal for all populations as a result of differing sex ratios in the holding tanks before the experiment. We recorded the standard lengths of all fish (mean \pm SD; males: $25.3 \pm 2.90\text{mm}$; familiar females: $35.9 \pm 4.36\text{mm}$; unfamiliar females: $36.2 \pm 4.90\text{mm}$) and no fish was used more than once in the experiment.

We ran two tests to examine male mate choice between a 'familiar' female (same tank) and 'unfamiliar' female (different tank, same population). The first was a dichotomous choice test where a female was contained at either end of a tank, and the relative time that a male spent in the 'choice zone' next to each female was used to infer mating preference. This was followed by an open tank test, where the male could interact freely with both females.

We did not know the reproductive status of females, but around 10% of females were probably receptive to males (based on a 3-day receptive period in a 30-day reproductive cycle in other Poeciliids, e.g. Houde, 1997). There is no reason to suspect that, on average, familiar and unfamiliar females differed in receptive state. As females store sperm, we do not know whether familiar females had successfully reproduced with test males before testing, but it seems unlikely that copulation with the familiar female had not occurred during the 4-month pre-experimental period.

We assembled the test arena inside a 90 litre tank (fig. 2.2), which was masked by white fabric. The back and sides of the arena were made from grey plastic, and we made observations via a video camera through the clear plastic front. The arena was divided into three compartments separated by grey and clear plastic removable screens. The tank was lit from above by a 15W fluorescent lamp, which had an overall colour output close to that of natural sunlight including components in the ultraviolet (Arcadia, Croyden, U.K.). As UV light affects mate choice in other

poeciliids (Smith et al., 2002), we used clear plastic that did not block its transmission.

With all partitions in place, we placed a male into a clear tube in the centre compartment, a familiar female into one side compartment and a size-matched unfamiliar female into the other (sides assigned at random). We allowed the fish to settle for 10 minutes before we lifted the opaque screens and for a further 3 minutes before the trial began. We lifted the clear tube to release the male and, for 10 minutes, we recorded the time that he spent in each choice zone. For a trial to be valid, the male had to visit both choice zones. To minimize the handling stress to the fish, we did not repeat the trial with females positioned on opposite sides. Over all trials males showed no side preference (paired t-test: $T_{19} = 0.83$, $P = 0.416$).

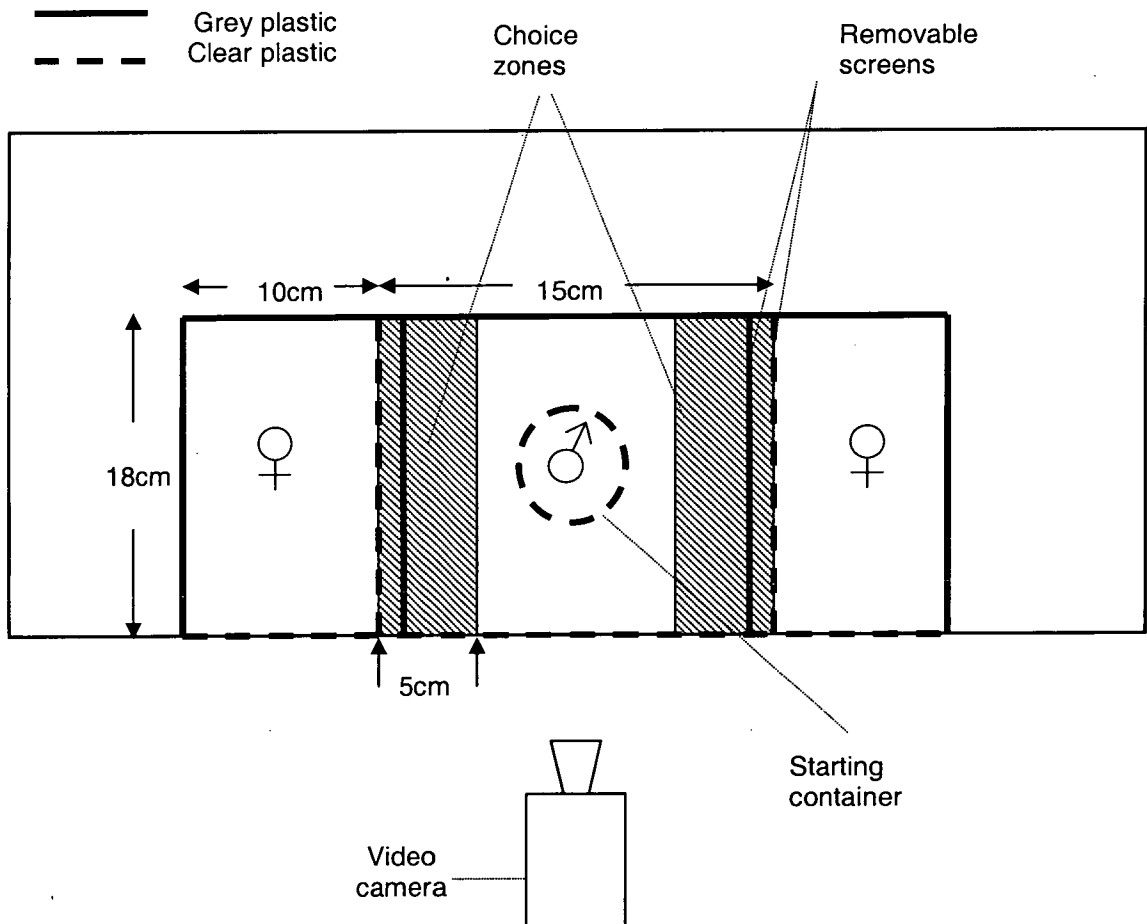


Figure 2.2 Schematic diagram of view above the holding tank containing the test arena used for the laboratory experiment (water height 26cm).

Immediately after the dichotomous choice trial, we lifted the clear screens allowing all three fish access to the whole arena. By replaying the video recording, both the familiar and unfamiliar female acted as focal females for the purposes of data collection. For 10 minutes, we recorded (i) association time, where the male was oriented towards and within one (female) body length of the focal female (time spent attempting thrusts and courting was included in this measure), (ii) number of thrusts (coercive mating attempts) towards the focal female, where the male darted quickly underneath the female, (iii) number of courtship attempts, where the male displayed with extended fins and gonopodium in front of the female. Courtship attempts included at least one turn. Each attempt was scored as one courtship until the display stopped (female or male swam away or male attempted to mate). We also counted (iv) number of aggressive acts (nips or chases) by the focal female towards the male, as an indication of female receptivity (Reynolds & Gross, 1992). A male may avoid nips or short chases by increasing the distance between himself and the female. Prolonged chase sequences, however, may indicate that the male is unable to avoid aggression from the female. Thus, we stopped any trials where the male was continuously chased for more than 10 seconds. Under this criterion, one trial was aborted after 8 minutes 2 seconds, although the male did not show any physical damage. All fish were returned to their holding tanks for use in future behavioural observations.

2.3.3 Field Experiment (January-April 2004)

To examine whether males discriminate between familiar and unfamiliar females in the wild, we tested fish, immediately after capture, in an artificial pool at the side of each stream. The artificial pool (diameter 1m, mean water height 15cm) was lined with small pebbles and filled with stream water.

We tested 53 males from six populations. Three populations occurred with high densities of predators (Rio Macho, N = 10; Rio Limbo 1, N = 8; Rio Limbo 2, N = 8) and three with low densities of predators (Rio Macho, N = 10; Rio Limbo 1, N = 8; Rio Limbo 2, N = 9). As a result of low discharge during the dry season, Rio Limbo

1 had dried up into a series of widely separated pools. Rio Macho and Rio Limbo 2, however, were still flowing and could be characterized by defined pool-riffle sequences (Angermeier & Karr, 1983). Each male came from a different pool, and we used no fish more than once.

Male mate choice was tested with a familiar female (same pool) and unfamiliar size-matched female from the same population (different pool, 20m-100m along the river). We captured fish with dip nets under an ANAM permit (SE/A-11-04) and returned them to their pool of capture after testing. We use the terms 'familiar' and 'unfamiliar' for consistency with the laboratory experiment, although we cannot be sure that fish caught in the same pool in Rio Macho or Rio Limbo 2 were familiar with each other. As with the laboratory experiment, we did not know the reproductive status of females, but there is no reason to suspect that familiar and unfamiliar females differed, on average, in receptive state. We measured all fish used (mean \pm SD; males: 22.6 ± 2.55 mm; familiar females: 30.8 ± 4.79 mm; unfamiliar females: 31.1 ± 4.97 mm).

We released a familiar and an unfamiliar female into the artificial pool and allowed them to acclimatize for 10 minutes. In pilot trials, females swam slowly around the pool and foraged within this time. After 10 minutes, we released a male into the pool at a point equidistant from both females. Males settled more quickly than females and began to show sexual interest in the females within 3-5 minutes. Behavioural recording began after the male had been in the pool for 5 minutes.

Two of us sat at opposite sides of the pool recording male mating behaviour towards the familiar or unfamiliar female. We alternated between females for subsequent trials. Females could be individually identified by small differences in size, shape or markings.

For 10 minutes, we recorded behaviours as detailed in the open tank laboratory experiment. We also counted displays, where a male positions himself with extended fins and gonopodium in front of a female but does not turn. We interpreted these

displays as the beginning of courtship attempts that were terminated by the female (swam away or was aggressive towards male) or by the male (swam away). During one trial, the display was part of an agonistic encounter, rather than courtship, and the counts were not included in our analyses.

Given the importance of light levels for guppy mating behaviour (Endler, 1987; Reynolds, 1993; Reynolds et al., 1993), we incorporated estimates of ambient light level into our analysis. We estimated the mean light levels on the pool during the trial as dim, dim/medium, medium/bright or bright. As a result of changes in cloud cover, light levels changed quickly during the observation period and across the area of the pool. Thus, it was not appropriate to take a single reading before or after the trial or at any one point over the pool, and we believe that our estimates, although not quantitative, provide a relative measure of mean light intensity differences between trials.

We found no significant differences in light levels between individual sites (Kruskal-Wallis test: $H_5 = 8.00$, $P = 0.156$) or by predator density (Kruskal-Wallis test: $H_1 < 0.01$, $P = 0.965$). Median light levels were 'dim/medium' in low predator density sites for both tributaries of the Rio Limbo and for the high predator density site in Rio Macho. At the other sites, median light levels were 'medium/bright'.

Irradiance measurements were made by Endler (1993) in March 1989 in a lowland tropical rainforest on Barro Colorado Island, a few km from our study area. In sunny conditions in large forest gaps, mean total light intensity (400-700nm) was $1195.8 \mu\text{mol.m}^{-2}.\text{s}^{-1}$ (Endler, 1993). In cloudy conditions, total light intensities are likely to be lower than this, although this is highly site specific, depending on the size of the canopy gap (Endler, 1993).

2.3.4 Data Analyses

We defined male mating preference as male behaviour towards the unfamiliar female minus behaviour towards the familiar female. Differences in female aggression were

calculated in the same way (score for unfamiliar female minus familiar female). Not all males attempted thrusts, courtships or displays, and we analysed variation in male mating preference using subsets of data that excluded these males. To examine whether our measures of mating preference were correlated with each other, we used the whole data set (including those males that did not court, display or thrust). For statistical analyses, we used Minitab (Minitab v.13, Minitab Inc., State College, PA, USA) and StatView (StatView v5.01, SAS Institute Inc., Cary, NC, USA). Where necessary, data were transformed to meet requirements of parametric analysis.

For the laboratory experiment, we standardized the dichotomous choice data by dividing male preference (time spent in unfamiliar choice zone minus time in familiar choice zone) by the total time spent in both choice zones. Data from the open tank trial that was aborted after just over 8 minutes was excluded from our analyses. We did not have enough data points to examine predator density, stream and fish size in one analysis, so we tested each variable separately.

For the field experiment, we used GLMs to examine male behavioural differences between stream (random factor), predator density and light levels. There were not enough cross-factor combinations to analyse the full three-way interaction. Male and mean female standard length, time of day and distance between familiar and unfamiliar pools were added as covariates to the maximal GLM.

2.4 Results

2.4.1 Laboratory Experiment

Males preferred unfamiliar to familiar females, but only in the open tank trial. Males spent more time associating with and attempted more thrusts with unfamiliar females (association time: paired t test: $t_9 = 2.76$, $P = 0.022$, mean difference \pm SE = 49.0 ± 17.7 s; thrusts: paired t test: $t_8 = 3.03$, $P = 0.016$, mean difference \pm SE = 2.9 ± 1.0 thrusts, fig. 2.3). For one tank, the test male did not attempt any thrusts with either

female. There was no significant difference in the number of courtships towards unfamiliar and familiar females (Paired t test: $t_9 = 1.71$, $P = 0.122$, mean difference \pm SE = 1.7 ± 1.0 courtships). In the dichotomous choice trial, where there was no physical access among fish, males showed no preference for either female (Paired t test: $t_{10} = -0.70$, $P = 0.499$).

Association preference and courtship preference were significantly positively correlated, but thrust preference was not correlated with either of these (table 2.1). Although preferred females were sometimes more aggressive to males (table 2.1, thrust preference), there were no differences in the number of aggressive acts received by males from familiar and unfamiliar females (Paired t test: $t_9 = 0.49$, $P = 0.633$).

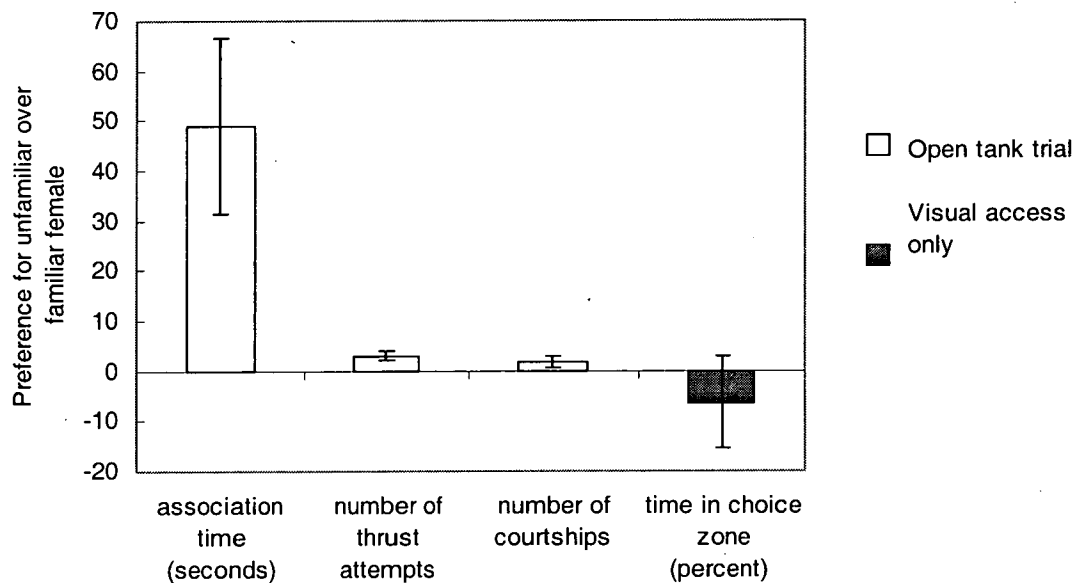


Figure 2.3 Mean \pm SE male mating preferences for familiar over unfamiliar females in the laboratory. Choice zone preference was not standardised (see methods) for ease of presentation.

There were no differences in our measures of male mate choice between males from high and low predator density populations (Association time: Two sample t test: $t_8 = 1.00$, $P = 0.349$; thrust attempts: Mann Whitney U test: $W = 31.5$, $N_1 = 6$, $N_2 = 3$, $P = 0.792$; courtship attempts: Two sample t test: $t_8 = 1.41$, $P = 0.197$) or from different streams (association time: Kruskal-Wallis test: $H_3 = 2.98$, $P = 0.394$; thrust attempts:

Kruskal-Wallis test: $H_3 = 2.37$, $P = 0.500$; courtship attempts: Kruskal-Wallis test: $H_3 = 1.82$, $P = 0.610$), however, sample sizes for each stream were very low. Male mate choice was not correlated with male standard length or difference in female standard lengths, but there was a marginally non-significant positive correlation between courtship preference and female size difference (table 2.1).

Table 2.1 Pearson correlations between measures of male mating preference, female aggression and standard lengths of fish in the laboratory (N=10)

Behaviours	Association preference		Thrust preference		Courtship preference	
	R_s	P	R_s	P	R_s	P
Thrust preference	0.576	0.081	-	-	-	-
Courtship preference	0.969	<0.001	0.527	0.117	-	-
Aggression difference	0.589	0.073	0.796	0.009	0.532	0.113
Male size	0.281	0.431	0.582	0.077	0.193	0.592
Female size difference	0.584	0.076	0.381	0.277	0.617	0.057

2.4.2 Field Experiment

All 53 males spent some time associating with each female: 41 of these males attempted at least one thrust, 29 males displayed and 22 males courted at least once. Three of our measures of male mate choice (association time, thrusts and courtships) were significantly and positively correlated with each other, but not with display preference (table 2.2).

Table 2.2 Spearman rank correlations between measures of male mating preference in the field (N=52)

Ranked behaviours	Association preference		Thrust preference		Courtship preference	
	R_s	P	R_s	P	R_s	P
Thrust preference	0.531	<0.001	-	-	-	-
Courtship preference	0.325	0.018	0.570	<0.001	-	-
Display preference	-0.011	0.936	0.010	0.943	0.132	0.348

Males spent more time associating with unfamiliar than familiar females but only in bright light levels (mean difference \pm SE = 52.4 \pm 14.4 s). In light levels dimmer than this, males showed no preference between females (GLM: $F_{3,49} = 3.27$, $P = 0.029$). There were significant complex interactions involving stream of origin, predator density, light, time of day, mean female size and male size. However, after we removed interactions with size (which may be part of overall population differences) the remaining interactions were not significant.

Male courtship preference varied with light levels, but there was a significant interaction with predator density (GLM: $F_{3,14} = 4.41$, $P = 0.022$; fig. 2.4). Males from low predator density populations courted unfamiliar females more than familiar females only in bright light (mean difference \pm SE = 4.0 \pm 1.9 courtships) and medium/bright light (mean difference \pm SE = 1.3 \pm 0.8 courtships). In light levels dimmer than this, males showed no preference between females. Conversely, males from high predator density populations preferentially courted unfamiliar females only in dim light levels (mean difference \pm SE = 4.0 \pm 1.9 courtships). In light levels brighter than this, males showed no preference. The total number of courtships by males towards both females did not vary with light levels (GLM: $F_{3,49} = 0.90$, $P = 0.449$).

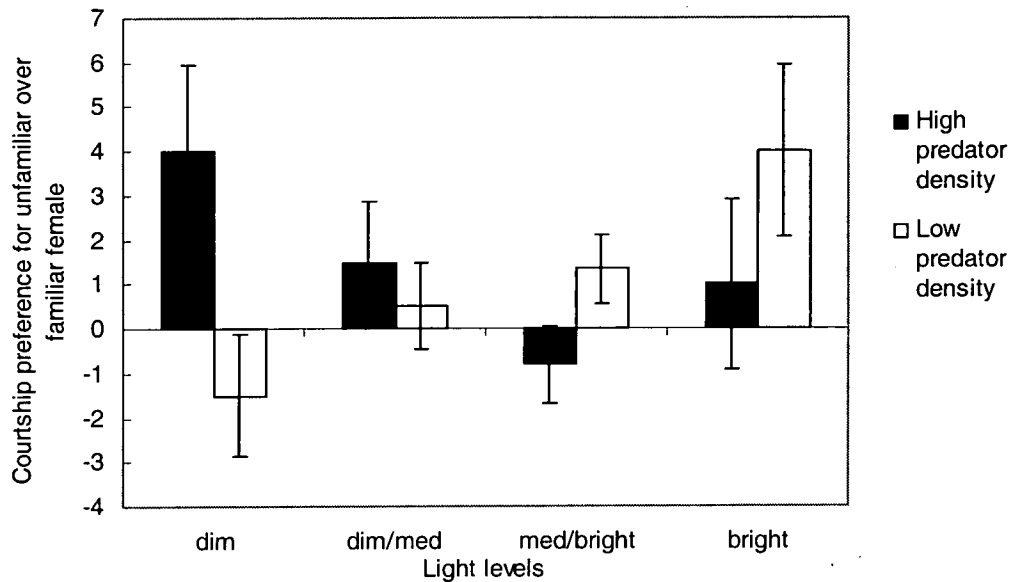


Figure 2.4 Least square mean \pm SE courtship preference for unfamiliar over familiar females in the field.

Display preferences also varied, with a significant interaction between predator density and light levels (GLM: $F_{3,13} = 4.89$, $P = 0.017$), but the results differed to those observed for courtship preferences. In dim light levels, males from low and high predator density populations displayed more to unfamiliar than familiar females (mean difference \pm SE = 2.7 ± 0.8 and 3.2 ± 1.2 displays, respectively), but in intermediate light levels, males showed no preference for either female. In bright light levels, males from high predator density populations displayed more to unfamiliar females (mean difference \pm SE = 4.5 ± 0.8 displays) but males from low predator density populations displayed more to familiar females (mean difference \pm SE = -12.0 ± 1.5 displays).

Male display preferences also varied, with an interaction between stream and light levels (GLM: $F_{6,13} = 7.09$, $P = 0.002$). In dim light levels, males from Rio Limbo 2 displayed more to unfamiliar females (mean difference \pm SE = 9.3 ± 1.6 displays) but males from Rio Macho and Rio Limbo 1 showed no preferences. In intermediate light levels, males from all three rivers showed no preference for either female. In bright light levels, males from Rio Macho displayed more to unfamiliar than familiar females (mean difference \pm SE = 8.8 ± 0.9 displays) but males from Rio Limbo 1 and

2 displayed more to familiar females (mean difference \pm SE = -7.8 ± 1.3 and -12.3 ± 1.7 displays respectively). The total number of displays by males towards both females did not vary with light levels (GLM: $F_{3,49} = 1.67$, $P = 0.187$).

Males showed no preferences between females when attempting thrusts, irrespective of stream of origin, predator density, light levels or any interactions between these factors (light levels: GLM: $F_{3,32} = 0.38$, $P = 0.766$; predator density: $F_{3,35} = 0.22$, $P = 0.638$; stream: $F_{2,38} = 0.75$, $P = 0.478$). We found no relationships between courtship or thrust preferences and male standard length, mean female standard length, time of day, distance between familiar and unfamiliar pools and differences in female aggression. In addition, familiar and unfamiliar females did not differ in the number of aggressive acts towards the male (Wilcoxon signed-ranks test: $T = 311.5$, $N = 53$, $P = 0.217$).

2.5 Discussion

We found that male *B. episcopi* preferentially mated with unfamiliar females, in both the laboratory and the field. In the open tank laboratory experiment, all males appeared to prefer unfamiliar females, but with visual access only, males showed no mating preference. In the field, patterns of male mate choice were more complex than in the laboratory and depended on context. Males from high and low predator density populations showed variation in courtship and display preferences under increasing light levels. Association preferences also varied with light levels, but there were no population differences in this behaviour. In the laboratory, males attempted more thrusts with unfamiliar females, but this preference was not repeated in the field. Our results highlight the importance of testing animal behaviour in both laboratory and field-based experiments.

Populations with high and low predator density showed striking differences in male courtship preferences as light levels changed in the field. Males from high predator density populations preferentially courted unfamiliar females but only when light

levels were dim. In brighter light, males showed no courtship preference. Conversely, males from low predator density populations preferentially courted unfamiliar females in brighter light levels, but this preference decreased in dimmer light levels. Given the number of potential interactions in this study, a type I error is possible. There are, however, good biological reasons to suspect that these patterns are real. Guppies experience an increased risk of predation in bright light (Endler, 1987); we suggest that the risk is similar for *B. episcopi*. Certainly, predation pressure affects life-history variables (Jennions & Telford, 2002), boldness (Brown & Braithwaite, 2004; Brown et al., 2005b), cognitive abilities (Brown & Braithwaite, 2005) and cerebral lateralization (Brown et al., 2004) in this species, so there is ample cause to suspect it also influences mate choice. If so, the response of the high predator density population is consistent with an evolutionary trade-off between the benefits and costs of being choosy (Fuller & Berglund, 1996).

Predation pressure offers an additional explanation for the results reported by Kelley et al. (1999). Both river populations, where males did not discriminate between females, came from sites with high predator densities. In contrast, the pool populations, where males were choosy, came from a site with low predator density and a laboratory stock bred from a site with high predator density (J.L. Kelley pers. com.). As responses to predators may decline in laboratory-reared fish (Kelley & Magurran, 2003), these results are consistent with the idea that males are choosy only when the risk of predation is low.

It is less clear why courtship preferences in males from low predator density populations varied with light levels. Long & Rosenqvist (1998) showed that male guppies from low predator density populations courted females at greater distances in bright light than in dim light. Male courtship may be more efficient in bright light conditions, and, in the absence of predators, males from low-predation populations are free to use this efficiency to their advantage. In turn, doing so may select for more conspicuous colour patterns in *B. episcopi* from low predator density areas, as seen in guppies (e.g. Endler, 1978).

We found no evidence that overall light level differences covaried with predator regimes, supporting the idea that a common factor such as predator density may explain population differences. Other variables may correlate with fish predator density, however, including ecological (e.g. resource competition), physical (e.g. elevation, stream width) and biological (e.g. stress responses, sex ratio, differential cue use) factors. One or a combination of these factors could interact with light levels to create the observed patterns of male mate choice. Further experiments are required to test the role of predation, perhaps by conducting open tank mate choice experiments with wild-caught fish, while manipulating the risk of predation.

Unlike the study on guppies by Kelley et al. (1999), we found no differences in mating preferences between males from pools and males from rivers: males from both habitats discriminated between familiar and unfamiliar females. This result suggests that all males acquire familiarity with individuals in the same pool, and use this information during mate choice decisions. Why might *B. episcopi* differ to guppies? Male guppies move between schools of females (Griffiths & Magurran, 1998), but perhaps male *B. episcopi* are much more sedentary. Mark-release-recapture data suggest that *B. episcopi* move relatively little over a 3-week period (C. Brown, F. Jones, N. Brydges & V. A. Braithwaite, unpublished data); females defend profitable foraging areas and males defend access to females (H. Simcox, pers. obs.). This sedentary behaviour could promote the acquisition of familiarity in streams as well as pools and explain why males from both habitats showed a preference for unfamiliar females.

We expected males from pools to show more interest than males from streams in unfamiliar females, but perhaps the pools had not been isolated for sufficient time for us to detect this difference. In guppies, familiarity (indicated by schooling preferences) takes 12 days to develop (Griffiths & Magurran, 1997; Croft et al., 2004a). We tested pools 2-4 weeks after isolation, which should have been enough time to acquire familiarity, but the strength of individual recognition may increase further after this time. The development of familiarity may also be species-specific and depend on the context in which it is used (Ward & Hart, 2003).

The numbers of adult *B. episcopi* in our sample pools (mean = 11.8, range = 4-29) were not noticeably different to those tested by Kelley et al. (1999) (range = 11-24). Our small group sizes could promote familiarity, but do not appear to explain differences between our results and those of Kelley et al.

It would also be interesting to know how male status affects male mate choice, as optimal choice may depend on the male's own quality or phenotype (Parker, 1983). Evidence in the two-spotted goby *Gobiusculus flavescens* (Amundsen & Forsgren, 2003) and in the sailfin molly *Poecilia latipinna* (Ptacek & Travis, 1997) shows that smaller males are less choosy than larger males, presumably because smaller males usually have fewer mating opportunities and thus a greater cost to being choosy. Although we found no relationship between male size and choosiness, we do not know the relative size and dominance status of each of our males within their home pool. More extensive mark-release-recapture studies are required, particularly examining differences in site fidelity between the sexes and between dominant and subordinate males. If combined with a measure of social networks (Croft et al., 2004b), this approach could be a powerful way to measure inter- and intrapopulation variation in familiarity before testing mate choice.

Kelley et al. (1999) found that only confined male guppies preferentially courted unfamiliar females, presumably as a mechanism to increase the number of different mating partners. In contrast, we found that both confined and non-confined male *B. episcopi* could choose on the basis of familiarity, and we suggest that males in this species may be confined to a site by their behaviour even if they are not physically confined. Thus, males could benefit from mating with as many different partners as possible to increase their reproductive success (Adler, 1978). We consider below other possible explanations that could be driving a preference for new unfamiliar females.

A mating preference for unfamiliar individuals may evolve to promote outbreeding, as well as to increase the number of partners (Farr, 1977). Mate choice is thought to

be cheaper than dispersal as a way of avoiding inbreeding (Blouin & Blouin, 1988). We do not know whether *B. episcopi* are inbred or suffer inbreeding depression; fine-scale resolution of genetic relatedness would be required to examine this.

Alternatively, male preference for unfamiliar females may result from a sensory bias for novelty that has arisen from some purpose other than mate choice (Hughes et al., 1999). *B. episcopi* are opportunistic omnivores (Angermeier & Karr, 1983), so an attraction to novelty may be an adaptation for competitive foraging rather than optimal mate choice.

We used aggression from the female to the male as an indication of her unwillingness to mate. Familiar and unfamiliar females did not differ in how aggressive they were to the male, suggesting that our results were affected by male mate choice rather than female choice. We cannot be sure, however, that our results are not confounded by female mating preferences. Females rarely approached males and usually responded to male association by swimming away, but responses to males might have been more subtle than we could observe. Female poeciliid fish also prefer to mate with unfamiliar or novel males (Farr, 1977; Hughes et al., 1999; McLaughlin & Bruce, 2001), and responsiveness to ambient light spectrum and predation risk are not inconsistent with female guppy mating behaviour (Evans et al., 2002; Gamble et al., 2003).

A further potential indication of female receptivity might be gained by examining the number of displays. We interpreted displays as failed courtships that may result from female aggression as well as a decision by the male not to continue courtship. Display preference was not correlated with any other male behaviour and we suggest that it may not be a reliable indicator of male mating preference. Furthermore, displays are frequently observed during agonistic encounters between females and between males (H. Simcox, pers. obs.) and may be primarily involved in appeasing aggression or establishing dominant-subordinate relations rather than courtship. Hence, we do not attempt to explain why male display preferences varied with light

level, stream and predator density. We did not collect data on why courtships were terminated and collecting this information would be useful in future studies.

Finally, we consider the cues that males use to discriminate between females. Olfactory cues are thought to be important in recognition and mate choice in other fish (e.g. Crow & Liley, 1979; McLennan & Ryan, 1997) and the failure of our dichotomous choice test to predict mating preferences suggests that visual cues alone are not sufficient for recognition. The dichotomous choice test might not have been suitable to elicit a mating response, however, because males did not court females through the clear partition. Further investigation of cue use in recognition in *B. episcopi* is required, with emphasis on potential population variation.

To conclude, we found that *B. episcopi* can discriminate between mates on the basis of familiarity and we suggest that this ability represents an intrinsic preference to mate with unfamiliar females. Although the reasons for this preference remain unclear, the plasticity of this response is evident and may represent a cost-benefit trade-off in male mate choice.

2.6 Acknowledgements

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3. Seasonal variation in male mate choice

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Nick Colegrave & Victoria A. Braithwaite

3.1 Abstract

Mate choice decisions are not necessarily invariant, and there is a growing body of evidence that they vary in different environmental and ecological conditions. Changes in the quality of the opposite sex, operational sex ratio or costs of being choosy, for example, may elicit temporal or spatial variation in mate choice. We examined seasonal male mate choice in the poeciliid fish *Brachyrhaphis episcopi*, performing *in situ* tests in the wet and dry season. We measured male choice for two female traits: size and familiarity. Males exhibited a mating preference for larger females, a preference that was much stronger in the dry season than the wet. This increased preference for larger females in the dry season might be explained if the fecundity of larger females is less affected by a reduction in food supply compared to smaller females, thus males benefit from choosing more fecund females when variation in female quality is large. To investigate this hypothesis, we re-analysed published data on brood size in this species and found that, when controlling for female size, variance in brood size was highest when rainfall was low. However, there were not sufficient published data to determine whether fecundities of different-sized females might be disproportionately affected by changes in food availability. Our field investigations revealed an effect of familiarity only in one analysis, as part of a complex interaction with female size and predator density, and we are not able to conclude whether familiarity played a significant role in male mate choice in this experiment. We also looked for differences among high and low predator density populations, as theory predicts that males should be less choosy when the risk of predation is high. Male reproductive behaviour, but not necessarily mate choice, varied among high and low predator density populations. The results support the idea that mate choice decisions are context-dependent. We highlight the

need for further work in natural populations examining how mate choice decisions interact with environmental variables, despite the complexities that can arise.

3.2 Introduction

Understanding how and why mate choice evolves is a central topic in evolutionary biology and has important implications for models of sexual selection (Andersson & Iwasa, 1996). Females are usually the choosier gender, as they typically invest more in a breeding attempt than males (Bateman, 1948; Trivers, 1972). Given sufficient variation in female quality (Parker, 1983) or shifts in costs of breeding (Kokko & Monaghan, 2001), however, theory predicts that males may also be choosy. Empirical studies support these predictions (Bonduriansky, 2001). Males exhibit mate choice for larger females, presumably because they are more fecund (e.g. Gwynne, 1981; Sargent et al., 1986; Herdman et al., 2004), or use another indicator of fecundity, such as presence or absence of offspring (Craig et al., 2002). Other examples include male mate choice for more colourful females (Amundsen & Forsgren, 2001), females that are genetically compatible (Ryan & Altmann, 2001) or unfamiliar females (e.g. Cooper, 1985; Donovan & Verrell, 1991; Kelly et al., 1999; Simcox et al., 2005; Chapter 2 this thesis).

Mate choice, as with other animal behaviours, is unlikely to be invariant and may vary with environmental and ecological contexts (Qvarnström, 2001). The benefits and costs of mate choice may change in different environments, leading to fluctuations in both the strength and direction of mate choice (e.g. Lesna & Sabelis, 1999). Similarly, shifts in the quality or availability of the opposite sex during the breeding season may lead to temporal changes in mate choice. Choosiness decreases as the costs (e.g. intrasexual competition, search time) of mating with a high 'quality' partner increase. For example, in the two-spotted goby *Gobiusculus flavescens*, males cease courting as the breeding season progresses. At this stage, females begin to court and compete for males as a result of a shift in the operational sex ratio. (Forsgren et al., 2004). A decrease in the number of sexually-active males, when diet

is poor, can also lead to increased female courtship of males in the Mormon cricket, *Anabrus simplex* (Gwynne, 1993).

We examined male mate choice in the Panamanian bishop *Brachyrhaphis episcopi* (Poeciliidae), a tropical freshwater fish found in small forest streams in central Panama. A previous study of *B. episcopi* showed that males exhibit mate choice for unfamiliar females (Simcox et al., 2005; Chapter 2 this thesis), which may enable males to mate with as many different females as possible (Adler, 1978). We expected that males would also show a mating preference for larger females, as female size is positively correlated with fecundity (Jennions & Telford, 2002) and plays a role in male mate choice in a number of closely related species (*B. rhabdophora*, Basolo, 2004; *Poecilia reticulata*, Herdman et al., 2004; *P. latipinna*, Ptacek & Travis, 1997).

B. episcopi experiences seasonal habitat changes that may lead to temporal differences in the strength of male mating preferences. For example, increased competition for limited prey during low rainfall might lead to greater variance in female fecundity and thus stronger male mate choice for larger females at this time. Low rainfall occurs in the dry season (mid-December to early May) and during this period general insectivores, like *B. episcopi*, experience a reduction in food abundance (Zaret & Rand, 1971; Angermeier & Karr, 1983; Chapman & Kramer, 1991). Although there is a marked decrease in rainfall and water flow in the dry season (Turner, 1938; Zaret & Rand, 1971), variation in light and temperature throughout the year is thought to be relatively slight (Turner, 1938; Kramer, 1978).

Predator density varies greatly among *B. episcopi* populations (Jennions & Telford, 2002) and is an additional factor that must be considered. There is some evidence that male *B. episcopi* from high predator density populations become less choosy when the risk of predation increases (Simcox et al., 2005; Chapter 2 this thesis) as predicted by theory (Sutherland, 1985; Hubbell & Johnson, 1987; Crowley et al., 1991). To date, tests of mate choice in this species have been carried out in aquaria or confined artificial pools. We do not yet know whether the effects of predator density, or even familiarity, on male mate choice can be detected in field tests.

We used two approaches to address these different issues. To look for at how female fecundity varied between the wet and the dry season, we re-examined data published by Turner (1938). Turner (1938) found no seasonal variation in reproduction in *B. episcopi*, but he did not control for female size in his analysis. To examine male mate choice in *B. episcopi*, we carried out *in situ* tests in the wet and the dry seasons, and asked three different questions. Firstly, do male mating preferences show seasonal variation? If variance in female fecundity is greater in the dry season than in the wet season, males should show a stronger preference for larger females in the dry season. Secondly, how does predator density affect male mate choice? We expected that males from low predator density sites should be choosier than males from high predator density sites. Thirdly, is there any evidence for male mate choice based on familiarity or female size when tested in the natural habitat? We tested the effects of familiarity on male mate choice *in situ*.

3.3 Methods

3.3.1 Re-examination of Turner's (1938) data

Turner (1938) studied a population of *B. episcopi* from Barro Colorado Island (BCI), Panama during the period June 1936 – April 1937. He presented data on the monthly mean brood size in gravid females of different standard lengths (25mm to 48mm in 1mm size classes). We performed a regression of mean brood size (\log_{10} transformed) against female standard length and calculated monthly mean and variance of the residuals from this analysis.

To examine our prediction that variance in brood size would be greater when rainfall was low, we calculated total monthly rainfall using a data set provided by the Meteorological and Hydrological Branch of the Panama Canal Authority for BCI from June 1936 – April 1937. We tested whether the mean and variance of the monthly residuals were correlated with total rainfall for the corresponding month.

3.3.2 Experimental test

We examined male mate choice in *B. episcopi* in a tributary of the Rio Limbo during the wet (October 2003) and dry (January 2004) seasons. Rio Limbo is one of a series of independent streams in the Parque Nacional Soberania, Panama, that drain into the Panama Canal. Rio Limbo is about 12km E of Turner's (1938) study population on BCI. Within a stream, the habitat of *B. episcopi* can be divided into areas of high and low predator density (Jennions & Telford, 2002). In the upper reaches of the stream, *B. episcopi* co-exists with the killifish *Rivulus brunneus*. In the middle reaches, separated from the upper reaches by a waterfall barrier, a suite of larger piscivorous fish are found together with *B. episcopi* (Brown & Braithwaite, 2004; Brown & Braithwaite, 2005). Thus, there is a high density of potential predators of *B. episcopi* below the waterfall in comparison to above the waterfall.

Using dip nets, we collected up to 10 females from each of five pairs of sites along the stream (Autoridad Nacional del Ambiente permits SE/A-55-03 and SE/A-11-04). Each pair of sites was located approximately 30m apart. One site from each pair was chosen as the 'release' site, where we released females individually and recorded male behaviour towards them. 'Familiar' females were caught from the release site; 'unfamiliar' females were caught at the second site from each pair. Two pairs of sites were low predator density sites, three pairs were high predator density sites. The same sites were paired together in both seasons, with the exception of one low predator density release site, which was paired with a different unfamiliar site in the dry season as a result of low numbers of fish.

Brachyrhaphis episcopi appears to show a high degree of philopatry, with little movement occurring between pools within the streams. A mark-recapture study conducted over a three week period during the wet season found that only one of 80 tagged fish was recaptured away from the original site of capture (at a distance of 10m upstream of its original tagging location) despite heavy rainfall and associated flash flooding. None of the marked fish were located outside the marking area and total recapture rate was 89% (C. Brown, F. C. Jones, N. Brydges and V. A.

Braithwaite unpublished data). Thus, unfamiliar females were unlikely to be familiar to either the release site or the fish within it.

After capture, familiar and unfamiliar females were temporarily housed in two buckets containing 15L of stream water and a number of leaves and stones for cover. Prior to release, we recorded the standard length of each female, and placed her in a dark water-filled container to settle. Two minutes later, she was transferred to a dark plastic shelter (8cm wide, 10cm long, 19cm high) placed in shallow water at the edge of the release site. A trapdoor (3cm wide, 5cm high) was located on one side of the shelter, which could be opened using a pulley system (described in Brown et al., 2005b). After one minute, we opened the door and allowed the female to swim out. When the female had moved 15cm from the shelter, we began recording male mating behaviour towards her.

We recorded i) association time, defined as a male orientated towards and within one (female) body length of the female; ii) number of thrusts (coercive mating attempts) towards the female, where a male darted quickly underneath the female; iii) number of courtships, where a male performs at least one turn in front of the female with his fins and gonopodium extended. Each attempt was scored as one courtship until the display stopped (female or male swam away or male attempted to mate). We observed the female for five minutes, unless she swam out of sight within this time.

All females were released using the same protocol, alternately releasing familiar or unfamiliar females. Of 153 fish caught and released, only 120 fish that were observed for at least 1 minute were included in analyses (table 3.1).

Table 3.1 Numbers of familiar and unfamiliar females released at each site with observation times between one and five minutes

Predator density (release site)	Wet season		Dry season	
	Familiar	Unfamiliar	Familiar	Unfamiliar
Low (1)	7	6	7	5
Low (2)	6	5	6	8
High (1)	9	7	6	7
High (2)	6	7	5	7
High (3)	6	4	3	3

3.3.3 Statistical analyses

Not all females had males associating, attempting thrusts or courtships towards them. Firstly, we examined which females were or were not recipients of measured behaviours, using binary response variables. We used S-Plus (S-Plus 6 Professional Edition, 2001, Insightful Corp., Seattle, WA) to fit generalised linear models (GLM) with binomial error structures. The maximal model examined full interactions among season, familiarity, predator density and female size. Significance was estimated by examining changes in deviance caused by removal of a term from the model and non-significant terms were removed in a step-wise deletion (Crawley, 2002). All terms in the minimal model were significant at $P < 0.05$ and main effects were kept in the model if their interaction terms were significant. Since responses for familiar and unfamiliar females within each release site were not strictly independent of each other, we also fitted generalised linear mixed models, with site within predator density included as a random factor. However, the results were unaffected and site did not explain significant deviance. Consequently we present only the results of the GLMs.

Secondly, we examined variation in the amounts of male behaviour received by females, excluding those females that were not recipients of the behaviour in

question. We converted association time to a percentage of observation time and thrust data to number per minute to control for differences in observation time. To check that rates of behaviour did not depend on observation time, we found no correlations with observation time and percentage association time (Pearson's correlation: $r_{87} = -0.117$, $P = 0.280$) or thrusting rate (Pearson's correlation: $r_{23} = -0.318$, $P = 0.122$). To meet the assumptions of normality and homogeneity of variance, percentage of association time and rate of thrusting were \log_{10} transformed. We carried out analyses using general linear models in Minitab (Minitab Release 14.1, 2003, Minitab Inc., State College, PA). The maximal model examined interactions among season, familiarity, female size and predator density, with site nested as a random factor within predator density. For association time, we also included interactions with site (nested within predator density) in the maximal model, but for thrust rate, we were unable to fit interactions with site due to lack of replicate observations.

3.4 Results

3.4.1 Re-examination of Turner's (1938) data

Mean brood size was significantly positively related to female size (Regression: $F_{1,127} = 261.98$, $P < 0.001$). Using the residuals from the regression, we found a significant negative correlation between the variance in residual brood size and total rainfall for each month (Pearson's correlation: $r_9 = -0.666$, $P = 0.025$, fig. 3.1). There was no relationship between the mean and variance of residual brood size (Pearson's correlation: $r_9 = 0.079$, $P = 0.817$) nor between mean residual brood size and total rainfall for each month (Pearson's correlation: $r_9 = -0.426$, $P = 0.191$). However, there were peaks in residual mean brood size; one in January and one in June (fig. 3.2).

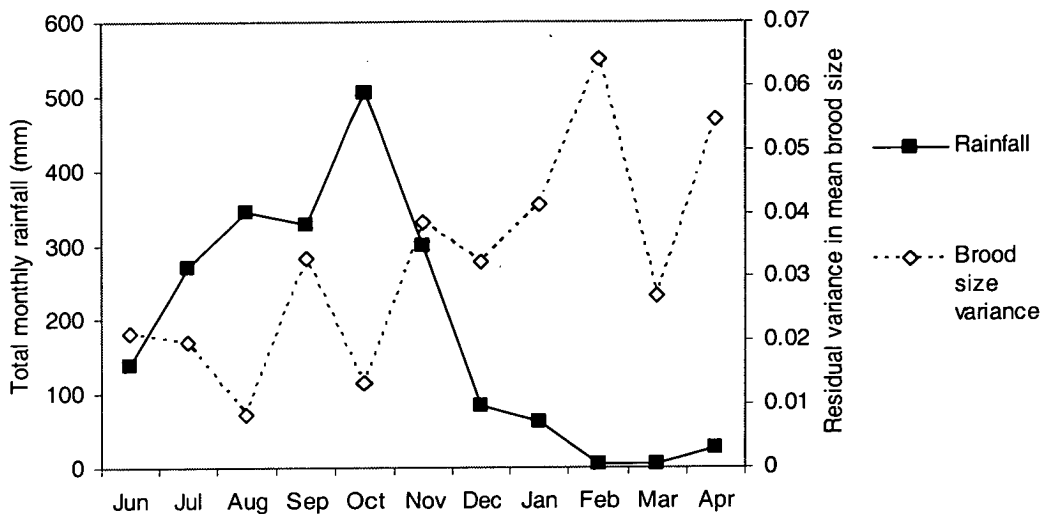


Figure 3.1 Monthly rainfall and residual variance in mean brood size for June 1936-April 1937. Residuals were calculated from a regression of mean brood size (\log_{10} transformed) against female standard length.

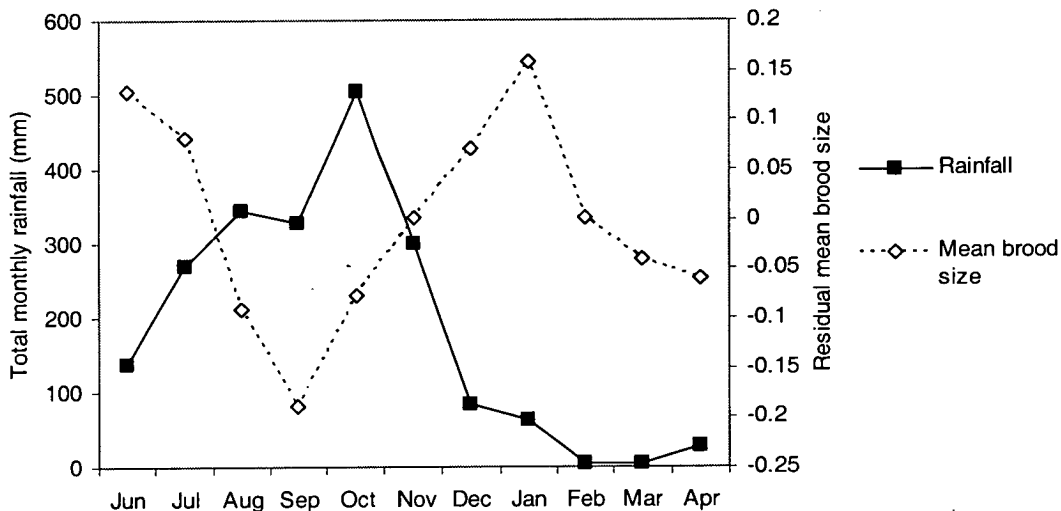


Figure 3.2 Monthly rainfall and residual mean brood size for June 1936-April 1937. Residuals were calculated from a regression of mean brood size (\log_{10} transformed) against female standard length.

3.4.2 Experimental test

In our field experiment, males associated with 87 of the 120 females released, performed thrusts towards 25 females and courted 4 females. As counts of courtship were extremely low, we do not include them in any further analyses.

Factors affecting the probability that females were recipients of male mating behaviours

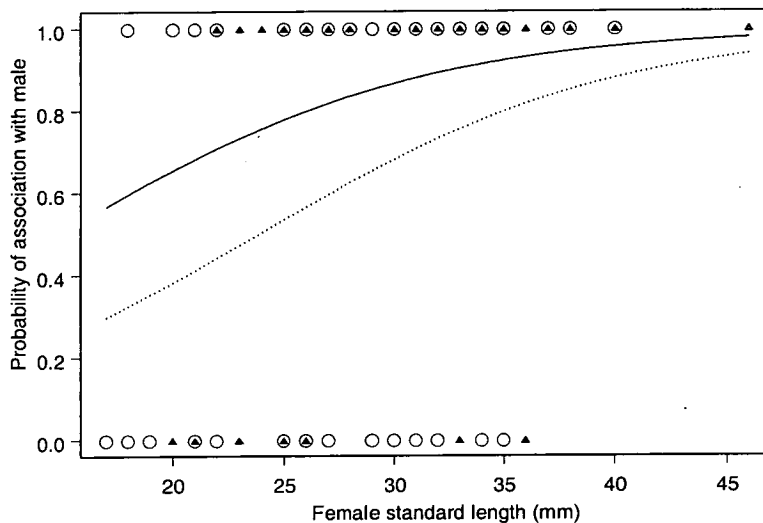


Figure 3.3 Effects of season and female size on the probability that females had males associating with them during the observation period. Open circles and dashed line represent the dry season; solid triangles and solid line represent the wet season. Lines show the predicted probabilities from a generalised linear model with a binomial error structure.

The probability of females having males associating with them was affected by season and female size. Females were more likely to have males associating with them in the wet season than the dry season (GLM: $F_{1,117} = 6.04$, $P = 0.015$, fig. 3.3) and larger females were more likely than smaller females to have males associating with them (GLM: $F_{1,117} = 8.95$, $P = 0.003$, fig. 3.3). The probability of males associating with the test female did not differ between familiar and unfamiliar females (GLM: $F_{1,115} < 0.01$, $P = 0.990$) nor between females in high and low predator density sites (GLM: $F_{1,116} = 1.14$, $P = 0.289$).

Season and female size also affected the probability that females received one or more thrusts from males, but here the interaction between size and season was significant (GLM: $F_{1,116} = 4.59$, $P = 0.034$). Female size had a strong effect in the dry season: larger females were more likely than smaller females to receive one or more thrusts. The effect of female size was positive, but weak, in the wet season (fig. 3.4). The probability of receiving one or more thrusts from males did not differ between familiar and unfamiliar females (GLM: $F_{1,115} = 0.15$, $P = 0.701$) nor between females in high and low predator density sites (GLM: $F_{1,114} < 0.01$, $P = 0.978$).

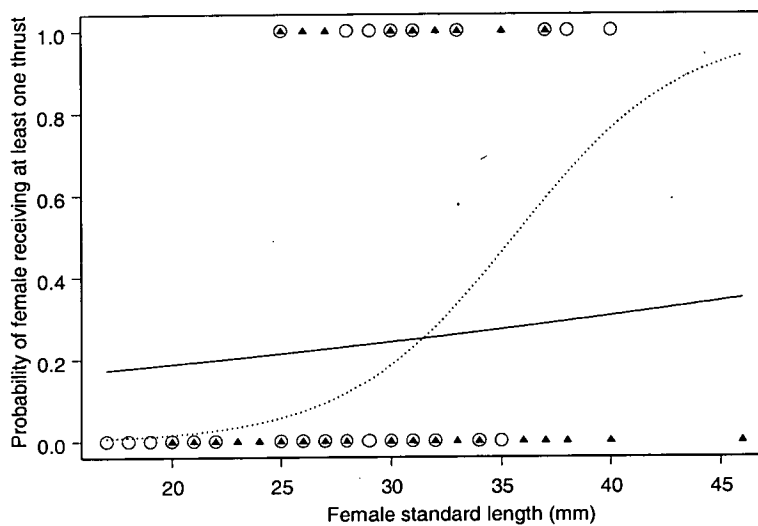


Figure 3.4 Effects of season and female size on the probability that females received one or more thrusts during the observation period. Open circles and dashed line represent the dry season; solid triangles and solid line represent the wet season. Lines show the predicted probabilities from a generalised linear model with a binomial error structure.

Factors affecting variation in male mating behaviours

The percentage of time that females spent with males associating with them was affected by female size, season and predator density. There was a significant interaction between season and female size: larger females had greater association times than smaller females but only in the dry season (GLM: $F_{1,79} = 6.48$, $P = 0.013$, fig. 3.5). Females from high and low predator density sites differed in the percentage of time spent with males associating with them (GLM: $F_{1,3} = 24.67$, $P = 0.011$). In

high predator density sites, females had a mean \pm SE association time of $23.1 \pm 0.17\%$. In comparison, females in low predator density sites had a mean \pm SE association time of $7.8 \pm 0.17\%$. There was no difference in male association times between familiar and unfamiliar females (GLM: $F_{1,78} = 0.04$, $P = 0.839$).

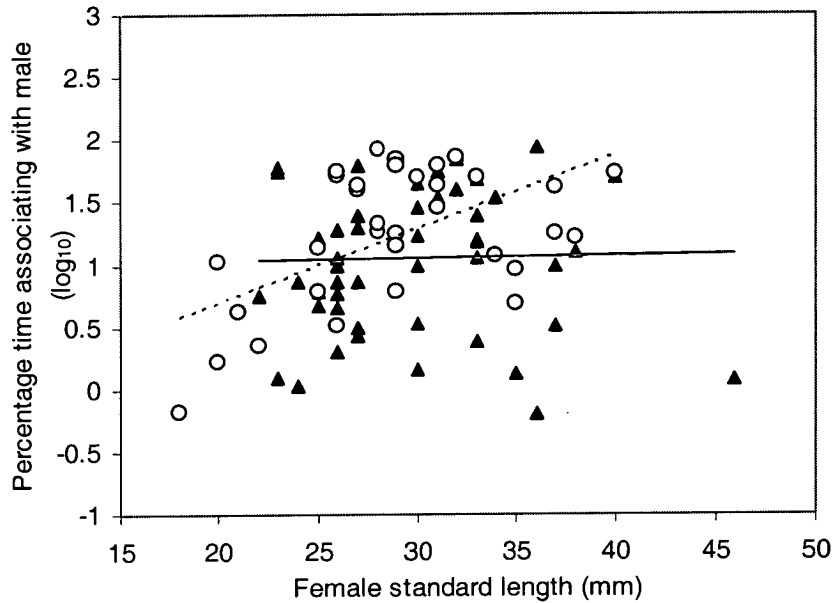


Figure 3.5 Effects of season and female size on percentage association time by males with females. Data points show \log_{10} transformed percentage association time with linear regression lines. Open circles and dashed line represent the dry season; solid triangles and solid line represent the wet season.

The rate of thrusting varied with a complex significant interaction among familiarity, predator density and female size (GLM: $F_{1,14} = 7.05$, $P = 0.019$, fig. 3.6). In high predator density sites, there was a positive relationship between thrusting rate and female size for familiar females, but in low predator density sites, this relationship was negative. Variation in thrusting rates received by familiar females, whilst statistically significant, was small: observed thrusting rates ranged from 0.3/min to 1.2/min in high predator density sites and 0.2/min to 2.0/min in low predator density sites. For unfamiliar females in both high and low predator density sites, there was no significant relationship between thrusting rate and female size.

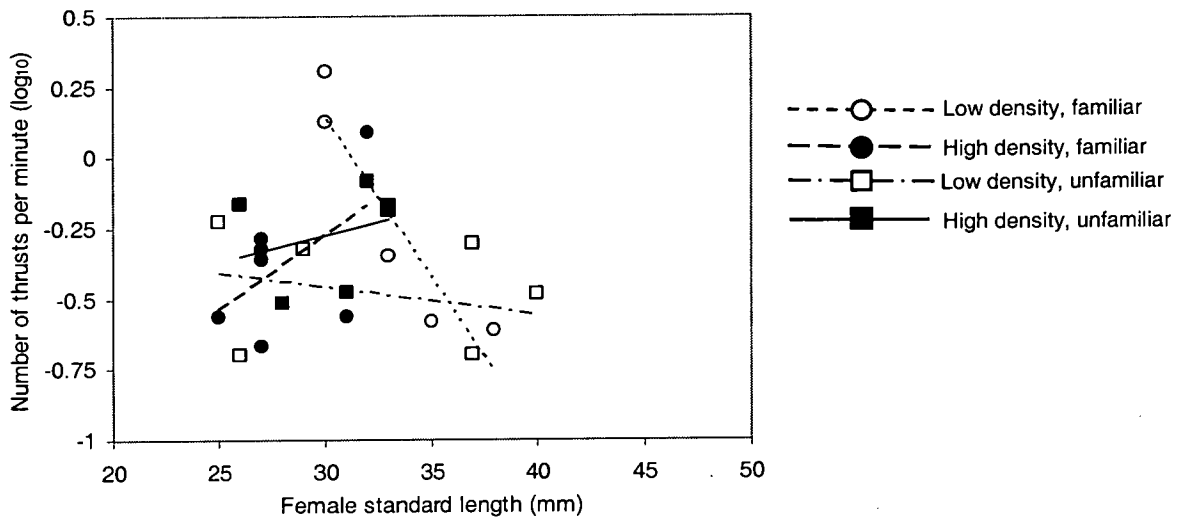


Figure 3.6 Effects of female size, familiarity and predator density on rate of thrusting. Points show \log_{10} transformed numbers of thrusts per min fitted with linear regression lines.

3.5 Discussion

3.5.1 Seasonal variation in male mate choice

We examined seasonal variation in male mating preferences in *B. episcopi* and suggest that variation in female fecundity may lead to variation in the strength of male mate choice for large females. Re-analysis of Turner's (1938) early study on monthly mean brood size in this species showed that when female size was controlled for, residual variance in mean brood size increased as monthly rainfall decreased. We then made *in situ* observations of seasonal changes in male mate choice in a population of *B. episcopi*. We measured male mate choice based on female size and familiarity in regions of high and low predator density. Our behavioural measures of male mate choice show a preference for larger females, which was much stronger in the dry season than the wet season.

Our results suggest that male *B. episcopi* show mating preferences for larger females in the wild. Given that fecundity, reproductive allotment (dry embryo weight divided by the sum of dry mother and embryo weight) and brood size increase with female

size in this species (Jennions & Telford, 2002), males selecting large females are likely to have greater reproductive success.

Why might male mating preference for larger females be stronger in the wet than the dry season? One possibility is that the fecundities of different-sized females are disproportionately affected by a change in food availability. Decreased rainfall leads to a decrease in food abundance (Zaret & Rand, 1971) but larger females are least likely to be affected by a reduction in food supply as they defend the most profitable foraging spots within a stream (H. Simcox and C. Brown, pers. obs.). Smaller females, however, are not always able to defend a foraging area, and so may be most affected by a reduction in food supply. Food supply and fecundity are positively linked in other fish species (e.g. Scott 1962, cited in Reznick & Yang, 1993; Reznick, 1983). Thus, larger females may be able to maintain high fecundity year-round but fecundity of small females is likely to be lowest when food is in short supply (dry season), increasing the potential benefits for males if they do select larger and comparatively more fecund females at this time (e.g. Gwynne, 1993). Accordingly, male mate choice for larger females was greater in the dry than the wet season in two of our behavioural measures of male mate choice, as evidenced by steeper gradients against female size (figs. 3.4 & 3.5).

Re-analysis of Turner's (1938) data shows that, when female body size is controlled for, variance in brood size increases when rainfall decreases. However, this does not provide evidence for the hypothesis that smaller females have lower fecundity in the dry than wet season. In fact, Turner's data suggest that in the dry season, female size is a less-good predictor of fecundity than in the wet season, so perhaps males should be less selective for large females in the dry season. As Turner only presented mean brood size of females in each size class for a selection of months, we cannot explicitly test the above hypothesis using his data.

If fecundity and food supply are positively linked, it is not clear why we found no relationship between residual mean brood size and monthly rainfall in Turner's (1938) data. In fact, we found peaks in residual mean brood size shortly after the

beginning of the wet and dry seasons (June and January). Additionally, a recent study found that reproductive allotment in this species was greater in the wet than the dry season (M. D. Jennions, B. B. M. Wong, C. Donnelly and A. Cowling, unpublished data). It may not be a simple task to infer selective causes of seasonal shifts in reproduction, as female reproductive allotment is likely to be affected by both contemporary and past environmental conditions (Reznick & Yang, 1993) and factors such as juvenile competition, predator community or breeding site availability (Kramer, 1978). For example, reproduction could be timed to coincide with reduced water flow, which may reduce fry mortality associated with dislodgement during flash flooding during the wet season. Certainly we do not know exactly how rainfall, food abundance and fecundity are linked, and further examination of seasonal variation in fecundity of *B. episcopi* is required. It also remains to be shown whether Turner's (1938) data are applicable in our study population. Seasonal variation may differ in sites that are geographically close as a result of drainage patterns and surrounding vegetation and in some tropical forests, insect abundance in riparian habitats may be more abundant in the dry season (Janzen, 1973).

Changes in the availability, rather than the quality, of females may also explain the change in male choosiness between the two seasons. Population composition, such as density, operational sex ratio or age structure may change over time (e.g. Pettersson et al., 2004), and such characteristics of a population may affect the strength of sexual selection. In the role-reversed pipefish *Syngnathus typhle* (Berglund, 1994), for example, males preferentially mate with large females when the sex ratio is female-biased but mate at random in a male-biased sex ratio. Thus, males may have a mating preference for larger females throughout the year but this choice is exhibited more strongly at certain times due to changes in mate availability or competition.

We found one significant negative relationship with female size as part of a complex interaction among familiarity, predator density and female size: small familiar females received more thrusts than large familiar females in low predator density sites. We cannot suggest any plausible biological explanation for this interaction and therefore consider that it may be a type 1 error.

3.5.2 Predator density

Predator density is known to affect life-history strategies (Jennions & Telford, 2002), boldness-shyness personality traits (Brown & Braithwaite, 2004; Brown et al., 2005b), cognitive abilities (Brown & Braithwaite, 2005), stress responses (Brown et al., 2005a) and the extent of cerebral lateralisation (Brown et al., 2004) in *B. episcopi*. It may also play a role in male mate choice (Simcox et al., 2005; Chapter 2 this thesis). Here we found only one piece of weak evidence that males in high predator density sites were less choosy than males in low predator density sites. In low predator density sites, the relationship with thrust rate and familiar female size was much stronger (steeper slope) than the corresponding relationship in high predator density sites. We do not believe that this is convincing evidence for a role of predator density on male mate choice in this study.

It is likely, however, that predation risk will affect reproductive behaviour in *B. episcopi* as it does in guppies *P. reticulata* (reviewed in Houde, 1997). We found that female *B. episcopi* had longer association times with males in high than low predator density sites. Similar results were also found in guppies, where males in high predation-risk populations spent more time following females than in low risk populations (Magurran & Seghers, 1994a). Thus, there is a pressing need to examine the role of predation risk and mate choice in detail in *B. episcopi*, with replication in multiple populations.

3.5.3 Familiarity

Unlike previous work in aquaria and artificial pools (Simcox et al., 2005; Chapter 2 this thesis) we found little effect of familiarity on male mating preferences in this study. Familiar and unfamiliar females differed only in the rate of thrusts they received from males, but the direction of the preference was not consistent between high and low predator density sites. In low predator density sites, familiar females received a greater rate of thrusts than unfamiliar females, but the difference between

familiar and unfamiliar females decreased as female size increased. In high predator density sites, male mating preferences for unfamiliar females also varied with female size, but the actual differences in thrust rates are small and may not be biologically important. As discussed above, we feel that the complex interaction among familiarity, predator density and female size may be Type 1 error.

We suggest some caution must be taken when interpreting the effect of familiarity from the current study. Firstly, a field-based study, carried out subsequent to this experiment, showed that light levels affect the strength of male mating preferences for unfamiliar females: males from high predator density sites showed a preference for unfamiliar females only in dim light, whereas males from low predator density sites showed this preference only in bright light (Simcox et al., 2005; Chapter 2 this thesis). At the time the current data were collected, light levels were not known to affect male mating preferences in this species, thus any effect of familiarity is hard to interpret. Secondly, the experimental protocol may have subjected familiar and unfamiliar females to different experiences. As this species is relatively philopatric, we assume that familiar females were released in a site that they knew, whereas unfamiliar females were not familiar with the release site or the fish in it. This could lead to differences in activity levels or differences in the females' ability to avoid males, which could confound results. Although familiar and unfamiliar females do not differ in boldness levels when leaving the start box (C. Brown, F. C. Jones, H. Simcox and V. A. Braithwaite, unpublished data), it is plausible that behavioural differences arise after this point.

3.5.4 Female mate choice

It is possible that female mating preferences may have influenced male behaviour. Measures of aggression from females to males may be used to indicate female receptivity (Reynolds & Gross, 1992; Simcox et al., 2005; Chapter 2 this thesis), but we observed little, if any, aggression in this experiment despite its prevalence in captivity. We also observed no female co-operation or willingness to mate in this study, although it is occasionally observed in the field (H. Simcox, pers. obs.).

Females responded to male mating attempts by swimming away and all attempted matings observed here were coercive. Thus, we saw no evidence for female mate choice in this study. Determining whether female *B. episcopi* can control the paternity of their offspring will help to explore whether male coercion can undermine female choice. The relationship between female choice and male mating behaviour has important evolutionary implications as it may affect the strength of sexual selection (Endler, 1983; Magurran, 2001) and mediate population differentiation (Magurran, 1998).

3.5.5 Summary

Temporal and spatial variation in mate choice can help us to infer selective causes of mate choice (Foster, 1999). Male *B. episcopi* showed a mating preference for large females and this preference was much stronger in the dry season than the wet season. The observed pattern might be explained if a reduction in food supply in the dry season leads to a greater decrease in fecundity of smaller females than larger females. If variation in female quality is large, males may benefit from choosing larger and therefore more fecund females. However, when female size was controlled for, we found that variance in female fecundity increased during periods of low rainfall, suggesting that female size may not be as good a predictor of fecundity in the dry season than the wet season. Seasonal changes in population composition or density of potential mates may also affect the strength of male mate choice. Further investigation into seasonal variation in population composition and female fecundity is now required.

As the present study reminds us, several variables appear to contribute to mate choice decisions and the relationship among these variables may be complex. In *B. episcopi*, for example, predator density and familiarity are likely to play a role in mate choice but we have yet to demonstrate this *in situ*. It will be challenging to perform reliable tests in the field, but the importance of testing in natural populations has been recently highlighted by Mitchell and colleagues (2004), amongst others, despite the potential complexity of the results that they can yield.

3.6 Acknowledgements

We thank Adel Heenan for help collecting data in the field and Daniel Nussey for assistance with generalised linear mixed-effects models. We are also grateful to the Smithsonian Tropical Research Institute (STRI) for provision of facilities in Panama and STRI's Environmental Science Program (ESP) for allowing us to access to precipitation data that was originally collected by the Panama Canal Authority. We present a modified version of the precipitation data and not the original data or documentation distributed by the ESP.

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4. *In situ* observations of mating behaviours: effects of predator density and operational sex ratio

Authors: Helen Simcox, Nick Colegrave,
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4.1 Abstract

Predation has an impact on many aspects of prey traits. Accordingly, populations of the poeciliid fish *Brachyrhaphis episcopi* show variation in life histories, body size, stress responses, boldness-shyness traits and other behaviours that correspond with differential predation pressure. Male reproductive behaviours should also vary between populations with contrasting predator densities and in particular we expected males in high predator density populations to attempt more coercive thrusts than males in low predator density populations. We carried out *in situ* observations of 6 natural populations of *B. episcopi*, 3 of which occurred with a high density of predators and 3 with low. The reproductive behaviour of focal males and focal females did not differ significantly between high and low predator density populations. There was one exception to this: in one stream, we observed that coercive thrusts were received by focal females in more pools in the high predator density population than in pools in low predator density population. Within a population, we found no relationships between local predator number and any measured behaviour. In fact, within-population variation in behaviour appeared to be determined by operational sex ratio. As the sex ratio became more female-biased, males spent more time associating with females and were involved in fewer aggressive acts with other males. We concluded that variation in predation pressure does not result in consistent differences in male reproductive behaviours either between or within populations in this species. At this stage, we cannot rule out a role of predator pressure on reproductive behaviours without greater understanding about how temporal variation in predation risk may affect behaviour at the population and individual level.

4.2 Introduction

One of the many costs of reproduction is an increase in predation risk (reviewed in Lima & Dill, 1990; Magnhagen, 1991). Physical displays, vocalizations or sexual ornaments that increase an individual's reproductive success may make an animal more susceptible to predation (e.g. Tuttle & Ryan, 1981; Belwood & Morris, 1987). Animals may respond to the presence of predators on an evolutionary timescale, for example, by evolving smaller or less colourful traits (Endler, 1980). Many mating behaviours show plasticity in the short term, decreasing or altering in response to the current predation risk (e.g. Tuttle et al., 1982; Sih et al., 1990; Berglund, 1993; Magnhagen, 1995; Fuller & Berglund, 1996; Candolin, 1997), although predation pressure is not expected to affect all sexual behaviours equally (Candolin, 1997).

It is clear that individuals alter mating behaviour in response to immediate predation risk, but studies showing that disparate predation risk among populations can select for differences in population norms of mating behaviour are surprisingly rare (but see Strong, 1973; Peeke & Morgan, 2000). Perhaps the only species in which the effects of predation risk on population variation in mating behaviour have been extensively studied is the Trinidadian guppy *Poecilia reticulata* (Poeciliidae). Males exhibit two mating tactics: courtship, which requires female co-operation for successful insemination, and coercive thrusts, which occur in the absence of co-operation (Baerends et al., 1955; Liley, 1966). The frequencies of these behaviours vary with predation risk, but not all studies report the same patterns (reviewed in Houde, 1997). Field and laboratory studies by Luyten & Liley (1985) and Houde & Cassidy (unpublished data cited in Houde, 1997) found greater rates of courtships by males in low compared to high predation populations but the opposite patterns were reported from field observations by Farr (1975). In agreement with Farr (1975), laboratory studies by Magurran & Seghers (1990) found that males from high predation populations courted at a higher frequency in the absence of a predator, but found no population differences when a predator was present. Furthermore, Shaw et al. (1994) found no courtship differences among low and high predation populations. The

patterns of coercive mating are more consistent: higher rates of coercive matings occur in high than low predation populations (Luyten & Liley, 1985; Magurran & Seghers, 1990; Magurran & Seghers, 1994a; with the exception of Shaw et al., 1994).

Male guppies, however, do not respond directly to changes in predation risk (Evans et al., 2002). In the presence of predators, females engage in more anti-predator behaviour and devote less time to avoiding male harassment (Magurran & Nowak, 1991). Males exploit this shift in behaviour and attempt more thrusts, which females are less able to avoid (Magurran & Nowak, 1991; Evans et al., 2002). Whilst the effects of predation on mating behaviour have been extensively studied in the guppy, how much these effects generalize to other similar fish species is not well known. Thus we examined how differences in predation pressure affect the reproductive behavior of the Panamanian bishop *Brachyrhaphis episcopi* (Poeciliidae), the ecology and behaviour of which in many ways resembles that of the guppy.

Brachyrhaphis episcopi experience diverse predation pressure from piscivorous fish (Jennions & Telford, 2002; Brown & Braithwaite, 2004). Populations with high and low densities of predators show variation in body size and life histories (Jennions & Telford, 2002), cognitive abilities (Brown & Braithwaite, 2005), stress responses (Brown et al., 2005a), boldness-shyness traits (Brown & Braithwaite, 2004; Brown et al., 2005b), and cerebral lateralisation (Brown et al., 2004), all of which are consistent with changes in predation risk. Therefore, predation pressure is highly likely to affect reproductive behaviour in *B. episcopi* (Simcox et al., 2005; Chapter 2 this thesis). Whilst there is some evidence from studies in artificial habitats that predation pressure may affect male reproductive behaviour (Simcox et al., 2005; Chapter 2 this thesis), little is known about variation in reproductive behaviour of this species in their natural habitat.

We looked at variation in mating behaviour at two scales: among and within populations. Firstly, we compared populations with high and low densities of predators where differences may have led to the evolution of different behavioural

optima. Secondly, we looked at predator density in the local environment within populations, as reproductive behaviour may show plasticity or very fine-scale local adaptation in response to predation risk (Lima & Bednekoff, 1999). Like guppies, male *B. episcopi* exhibit both courtship and thrusts (Simcox et al., 2005; Chapter 2 this thesis), although courtship is less common in the field (Chapter 3 this thesis). It is difficult to make predictions about how courtship frequency should vary in response to predation because patterns in guppies are inconsistent (see above). However, we can predict that higher densities of predators will be associated with greater rates of thrust mating.

In the presence of predators, male-male competition may decrease (e.g. Kelly & Godin, 2001), perhaps because females become less choosy when predation risk is high (Kelly & Godin, 2001) or because fighting incurs increased predation risk (Jakobsson et al., 1995). There is no evidence to date for female mate choice in *B. episcopi* and male mating success is more likely to be determined by male-male competition. Large males defend areas where females forage and will chase other males away (H. Simcox, pers. obs.). In the absence of female choice, local operational sex ratio (OSR) may be a more important determinant of levels of male-male competition and additionally may cause changes in male mating tactics (e.g. Jirotkul, 1999; Evans & Magurran, 1999).

We made non-manipulative *in situ* observations of six natural populations of *B. episcopi* to explore three aspects of male reproductive behaviour. Firstly, do populations that differ in predation risk consistently differ in reproductive behaviour? We compared populations that occur with high and low densities of predatory fish and looked within populations to see whether reproductive behaviour varied with predator number in the local environment. Thirdly, we examined the role of OSR in reproductive behaviour by looking for within-population relationships. We also asked whether local OSR was a better predictor of reproductive behaviours than local predator number.

4.3 Methods

4.3.1 Experimental design

We observed the behaviour of six natural populations of *B. episcopi* in the Parque National Soberania, Republic of Panama. Observations took place between 0900 and 1600 during February-April 2004.

We sampled three streams that drain into the Panama Canal: Rio Macho (RM), Rio Limbo (RL) and Quebrada Juan Grande (QJG). Each stream flows across an escarpment, creating a waterfall that marks a shift in the fish community. Below the waterfall ('downstream'), *B. episcopi* are found with a high density of predatory fish that include other fish such as *B. episcopi* in their diet (Angermeier & Karr, 1983). Above the waterfall ('upstream'), there is a very low density of predatory fish. *B. episcopi*, the predominant species, is found with small numbers of killifish *Rivulus brunneus* that are not capable of eating adult *B. episcopi* (Angermeier & Karr, 1983). As there is little upstream/downstream movement of fish (C. Brown, F. C. Jones, N. Brydges & V. A. Braithwaite, unpublished data), we consider upstream and downstream fish in each stream to represent separate populations (Johnson & Belk, 2001; Jennions & Kelly, 2002; Jennions & Telford, 2002).

We made observations in 16 pools (8 upstream and 8 downstream) in each stream. To enable *in situ* observations, pools were not chosen at random: we selected pools that were relatively shallow with little overhanging vegetation and slow moving water. We visually surveyed the fish community in each pool, noting the number and sex of *B. episcopi* and the numbers of predatory fish (local predator number; table 4.1).

In each pool, we recorded the behaviour of between 5 and 10 adult fish, making observations on a total of 319 fish (table 4.1). Some pools contained small numbers of *B. episcopi*, so we sampled selectively to ensure we did not record the behaviour of any fish more than once. In pools with small numbers of fish, we were able to

identify fish individually by size, sex, territory and colour and could ensure we did not record the behaviour of any fish more than once. In pools with greater numbers of fish, we made observations of two or three fish from each size class to minimise our chances of re-sampling the same fish. In one pool, we only collected female data.

We recorded the sex of the focal fish. Mature female *B. episcopi* are usually larger and have a rounder belly than males, and can also be identified when they are recipients of male reproductive behaviours (see below). Mature males are rarer as the sex ratio is female biased, but can be identified by a combination of features: male reproductive behaviours (see below) or presence of a gonopodium (modified anal fin) that is visible when extended or swung forward. Additionally, males may have a distinctive gold hue (common for larger males), prominent melanic body markings and a smaller, narrower body shape than females.

We noted the relative size of the focal fish on a five point scale which indicated whether the fish was smaller, similar or larger in size compared to other fish of the same sex in the pool. For a three-minute period, we recorded the number of foraging events and the number of aggressive acts towards and from conspecifics. If known, the sex of the conspecific was noted. For focal females, we recorded association time, number of attempted thrusts and number of courtships received from males. For focal males, we recorded association time, number of attempted thrusts and number of courtships towards females. For definitions of these behaviours, see table 4.2.

For 190 observations, we estimated average light levels on a four-point scale from dim to bright as light has been shown to affect male mate choice in this species (Simcox et al., 2005; Chapter 2 this thesis) and may affect overall levels of behaviour (Endler, 1987; Reynolds et al., 1993).

Table 4.1 Mean number (*percentage*) contribution of *Brachyrhaphis episcopi*, *Rivulus brunneus*, family Characidae and family Cichlidae to the fish fauna observed after visual surveys in 8 pools in each population. It was not possible to sex most small *B. episcopi* that may or may not have been sexually mature: these individuals are classed as unknown sex. Also shown are the numbers of focal males and focal females in each population.

Population		Fish fauna: mean number (<i>percentage</i>)									Number of focal fish	
		<i>Brachyrhaphis episcopi</i>			<i>Rivulus brunneus</i>		Characidae	Cichlidae	<i>Brachyrhaphis episcopi</i>			
		Adult females	Adult males	Unknown sex (including juveniles)					Females	Males		
RL	Upstream	7.0 (42.1)	3.4 (21.8)	6.3 (35.3)	0.1 (0.8)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)		34	18	
RM	Upstream	5.8 (52.2)	2.9 (24.6)	3.0 (21.1)	0.1 (2.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)		28	22	
QJG	Upstream	18.5 (50.4)	2.3 (6.8)	13.0 (36.4)	2.0 (5.5)	0.4 (1.0)	0.0 (0.0)	0.0 (0.0)		39	18	
RL	Downstream	8.9 (37.0)	4.8 (18.9)	6.5 (23.5)	0.3 (1.2)	5.9 (16.2)	1.1 (3.2)			30	24	
RM	Downstream	8.6 (27.9)	4.5 (14.7)	3.0 (10.2)	0.4 (1.0)	17.8 (46.2)	0.0 (0.0)			34	24	
QJG	Downstream	4.5 (31.3)	3.5 (24.2)	2.3 (13.4)	0.1 (0.6)	6.3 (29.1)	0.4 (1.3)			28	20	

Table 4.2 Definitions and notes on measured behaviours

Behaviour	Definition	Notes
Foraging event	Bites at food item on the surface of the water, in the water column or on substrate	More reliably quantified as a series of discrete events rather than time spent foraging
Aggressive act	Nips, lunges or chases to or from conspecific	
Association time	Length of time male within one body length and orientated towards female	Usually escalates to a chase and often precedes a thrust attempt
Attempted thrust	Coercive mating attempt, where male darts rapidly underneath female	
Courtship	Male displays side-on with erect fins to female with at least one turn	

4.3.2 Statistical methods

Since the sexes are likely to differ in their reproductive behaviours, leading to complex statistical interactions that are not the focus of this study, we chose to analyze association time and attempted thrusts for males and females separately. However, aggression given by the focal fish, aggression received by the focal fish and foraging were analysed for both sexes together as we wished to make comparisons between the sexes. To examine the levels of aggression that males received from females, we summed the number of aggressive acts given by focal females to males and received by focal males to females. Similarly, to examine the levels of aggression between males (male-male competition), we summed the number of aggressive acts that a focal male gave to or received from another male.

We investigated variation in behaviours using general linear models (GLMs). In the maximal models, we fitted stream, upstream/downstream, and relative size as factors and all two and three way interactions among these factors where possible. For analyses of both sexes together, we also included a main effect of sex, and

interactions with sex where possible, in maximal models. As we aimed to generalize about differences between upstream and downstream sites, we fitted stream as a random factor. We also considered observations made within each pool to be non-independent and fitted pool as a random factor nested within stream and location.

We did not have relative size data for 20 fish: these fish were only included in analyses when size was found to be a non-significant factor. We examined the role of light levels in a subset of data (N = 190), using the model described above plus light as a factor and any interactions with light that our data would allow.

In two populations (RL upstream and QJG upstream), no focal females received thrusts: lack of variation in these populations meant that the number of thrusts received by females could not be analyzed using GLMs. Instead, we counted the number of pools in each population where thrust attempts received by focal females were and were not observed; we then used a Fisher's Exact Test to see whether this was independent of upstream/downstream within each river.

As female *B. episcopi* can store sperm, we considered all mature females as well as all mature males to be ready for mating; thus, for each pool, we calculated OSR as number of adult females per adult male. To examine the effects of OSR on behaviour, we calculated mean frequencies of behaviours for each pool and used this variable as the response. We fitted OSR, upstream/downstream, stream (as a random factor), and all interactions using general linear models. Stream and upstream/downstream were retained in all models to ensure that the relationship between sex ratio and the response behaviour was fitted independently for each population. As there was no variation in the mean thrust number received by focal females in upstream pools in RL and QJG, the only stream in which we were able to examine the effects of OSR in both populations within a stream was RM. We fitted upstream/downstream, OSR and an upstream/downstream interaction with OSR to data from RM.

To examine the effects of local predator number, we analysed mean behaviour in each pool. As only three upstream pools contained any predatory fish, we looked at the effects of local predator number only in downstream pools. We fitted stream as a random factor to all models, plus local predator number, OSR and their interactions with stream.

For all parametric analyses detailed above, data were transformed where necessary to ensure that the residuals from the models met the assumptions of normality and homogeneity of variance. Analyses were carried out with Minitab (Minitab Release 14.1, 2003, Minitab Inc., State College, PA), which approximates denominator degrees of freedom for some F ratios in unbalanced mixed models. We have reported these approximate degrees of freedom, which are not always integers, in our results.

4.4 Results

4.4.1 *Fish communities*

No predatory fish were observed in upstream pools in RL and RM, and one characin fish was observed in three of eight upstream pools in QJG, but predatory fish were observed in 6, 7 and 8 downstream pools in RL, RM and QJG respectively (details of fauna in table 4.1). Predatory fish were thus more likely to be observed in downstream than upstream pools (Fishers exact test: RL: $P = 0.007$, RM: $P = 0.001$, QJG: $P = 0.026$), which supports population differences in predation pressure demonstrated by previous extensive surveys of the same streams (Brown & Braithwaite, 2004; table 1.1 this thesis).

Upstream populations tended to have a more female-biased OSR than downstream populations (Friedman test: $S = 3$; d.f. = 1, $P = 0.083$; estimated median number of females per male: upstream = 2.24, downstream = 1.96). In downstream populations, there was no relationship between OSR and local predator number (Spearman's Rank Correlation $N = 8$: RL: $R_s = -0.235$, $P = 0.575$; RM: $R_s = 0.195$, P

= 0.643; QJG: $R_s = 0.444$, $P = 0.271$); we could not examine this relationship in upstream populations because of lack of variation in predator number.

4.4.2 Reproductive behaviours

The proportion of time spent associating with females did not differ between upstream and downstream populations (by focal males: GLM: $F_{1,2.01} = 0.09$, $P = 0.789$; received by focal females: GLM: $F_{1,2.00} = 4.36$, $P = 0.172$). The mean proportion of time that focal males spent associating with females increased as the OSR became more female-biased (GLM: $F_{1,40.00} = 8.44$, $P = 0.006$), with strongest relationship in RM and the weakest in RL (stream and OSR interaction: GLM: $F_{2,40.00} = 5.84$, $P = 0.006$; fig. 4.1). Although focal males spent more time associating with females as OSR increased, mean association time received by focal females tended to decrease as OSR increased, presumably because any increase in male behaviour was diluted among more females (GLM: $F_{1,43.00} = 3.93$, $P = 0.054$). Mean proportion of association time in downstream pools was not affected by local predator number (by focal males: GLM: $F_{1,18.00} = 0.87$, $P = 0.362$; received by focal females: $F_{1,20.00} = 2.10$, $P = 0.163$).

Table 4.3 Numbers of pools in which at least one focal female received an attempted thrust from a male.

Stream	Upstream		Downstream	
	Thrusts received	Thrusts not received	Thrusts received	Thrusts not received
RL	0	8	3	5
RM	3	5	2	8
QJG	0	8	5	3

The number of attempted thrusts by focal males did not differ between upstream and downstream populations (GLM: $F_{1,2.01} = 0.10$, $P = 0.787$), although thrust number varied between pools (GLM: $F_{41,79.00} = 1.76$, $P = 0.016$). We observed thrusts towards focal females in a greater number of downstream than upstream pools in QJG but not in RL or RM (Fisher's Exact Test: QJG: $P = 0.026$; RL: $P = 0.200$; RM:

$P > 0.99$; table 4.3). During an average 3 minute observation period in QJG, focal females in downstream pools received 0.61 thrusts compared to 0 received in upstream pools.

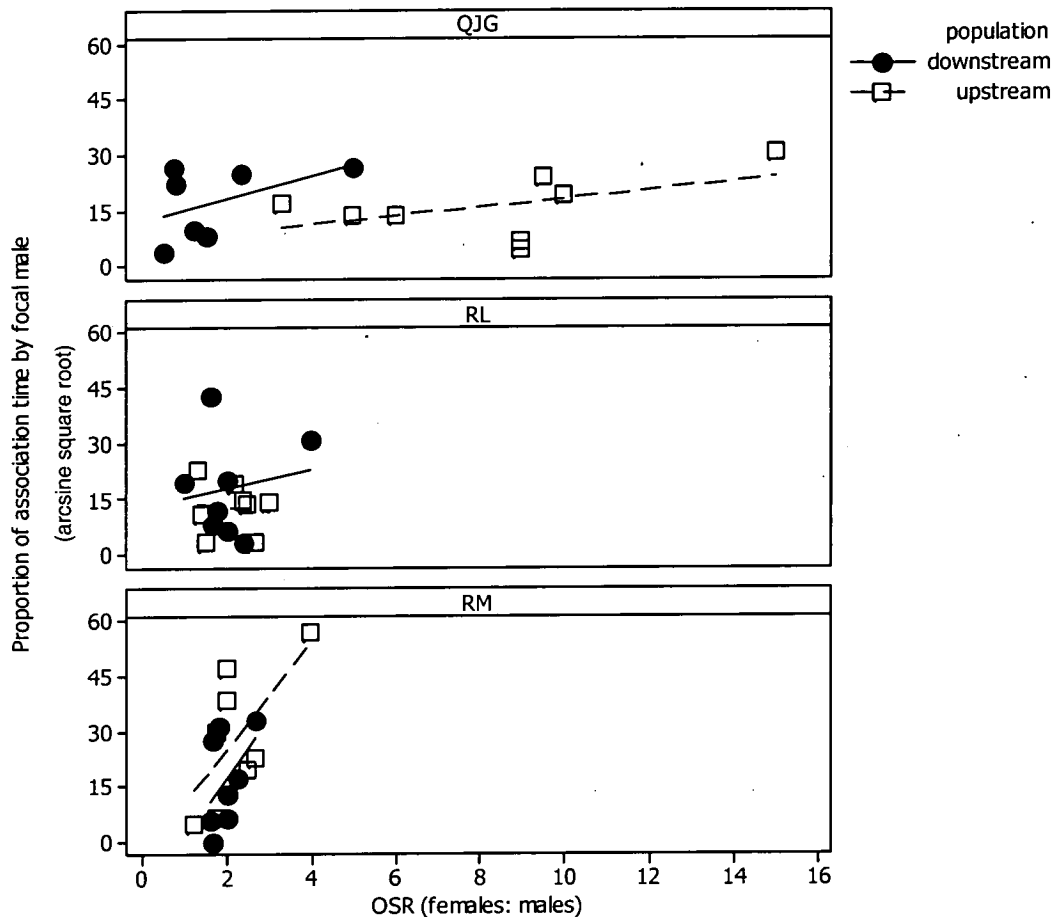


Figure 4.1 Mean proportion of time focal male spent associating with females against operational sex ratio in each pool for all six populations (arcsine square root transformed).

Mean numbers of thrust attempts in a pool were not affected by OSR (by focal males: GLM: $F_{1,42.00} = 0.53$, $P = 0.470$; received by focal females in RM: $F_{1,13.00} = 1.31$, $P = 0.273$) nor by local predator number (focal males: GLM: $F_{1,18.00} = 0.11$, $P = 0.745$; focal females: GLM: $F_{1,19.00} < 0.01$, $P = 0.954$).

We observed seven focal males courting females and two focal females being courted by males so were not able to analyse this behaviour. Seven of the nine

courtships observed occurred in QJG. There were no effects of light or relative fish size on any reproductive behaviours (light: $P > 0.11$; size: $P > 0.21$).

4.4.3 Aggression

Males performed and received more aggressive acts than females (GLM: $F_{1,246.00} = 8.85$, $P = 0.003$ and $F_{1,226.00} = 91.67$, $P < 0.001$ respectively; fig. 4.2). Small fish carried out fewer aggressive acts than larger fish (GLM: $F_{4,246.00} = 5.69$, $P < 0.001$) and smaller fish generally received more aggressive acts than larger fish (GLM: $F_{4,10.67} = 7.33$, $P = 0.004$), although there were some differences among populations (significant interaction among stream, upstream/downstream and size: GLM: $F_{8,226.00} = 1.99$, $P = 0.048$).

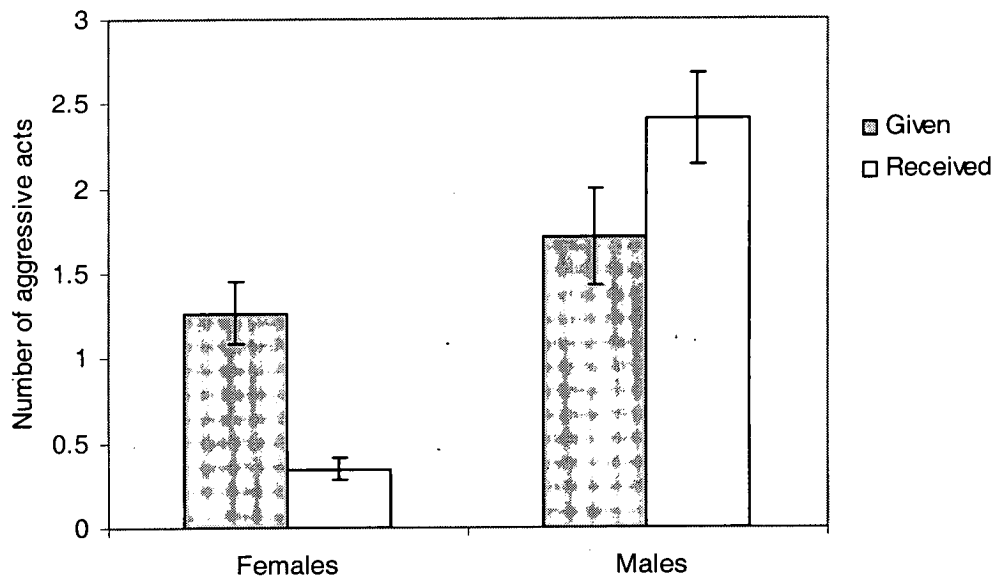


Figure 4.2 Mean \pm SE number of aggressive acts given and received by females and males.

There were no effects of OSR or local predator number on the mean number of aggressive acts from females to males, nor were there differences between upstream and downstream populations (GLM: OSR: $F_{1,43.00} = 1.66$, $P = 2.05$; local predator number: $F_{1,19.00} = 1.31$, $P = 0.267$; upstream/downstream: $F_{1,2.01} = 1.16$, $P = 0.393$). Populations differed in numbers of aggressive acts between males (stream and upstream/downstream interaction: GLM: $F_{2,45.98} = 5.26$, $P = 0.009$): male-male

aggression was higher downstream than upstream in RL and RM, but the reverse was found in QJG. When these population differences were controlled for, we found that male-male aggression varied with OSR but the relationship varied between streams (stream and OSR interaction: GLM: $F_{2,38.00} = 6.14$, $P = 0.005$; fig. 4.3): in RL and RM, the mean number of aggressive acts between males declined as the OSR became more female-biased, but there was no relationship with OSR in QJG pools.

There were no effects of light on the aggressive behaviours discussed above ($P > 0.15$).

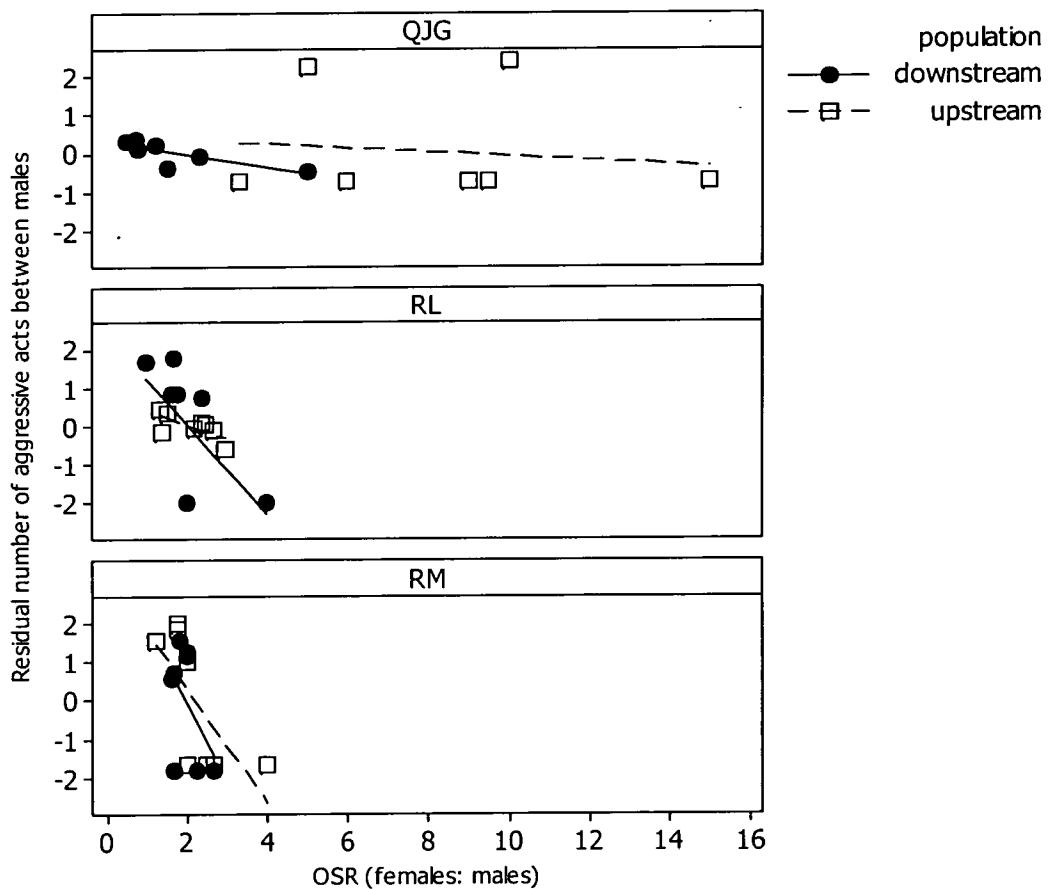


Figure 4.3 Residual mean numbers of aggressive acts between males against operational sex ratio in each pool for all six populations. Residuals were from a model that accounted for population variation by fitting stream, upstream/downstream and an interaction among these two factors.

4.4.4 Foraging

The number of foraging events did not differ between upstream and downstream populations nor between males and females (GLM: upstream/downstream: $F_{1,2.03} = 0.11$, $P = 0.768$; sex: $F_{1,238.00} = 1.77$, $P = 0.185$), but there was a significant interaction between stream and relative fish size (GLM: $F_{8,239.00} = 3.17$, $P = 0.002$; fig. 4.4). Light levels did not affect the number of foraging events (GLM: $F_{3,149.00} = 1.14$, $P = 0.335$).

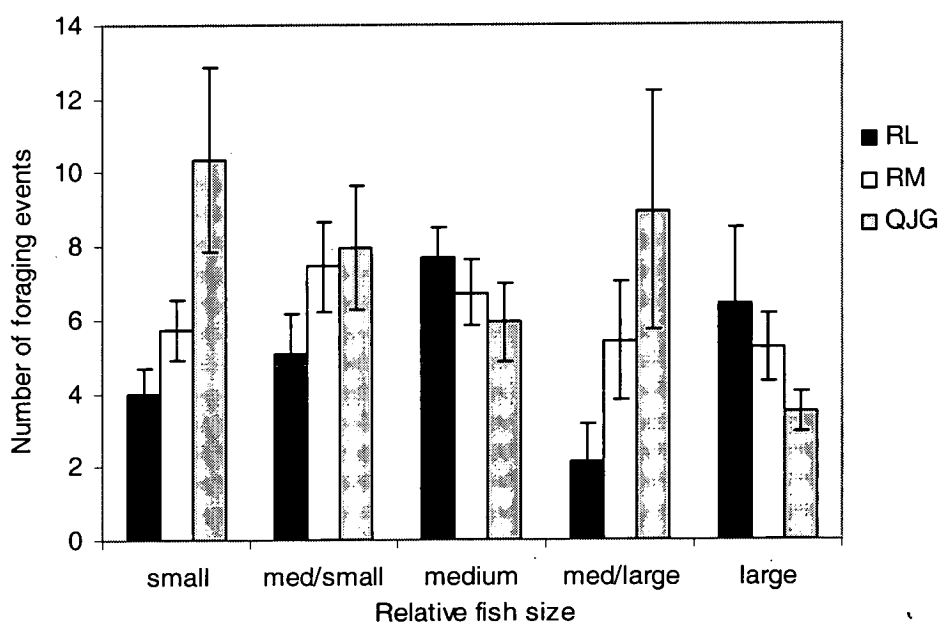


Figure 4.4 Mean \pm SE number of foraging events by fish of different relative size classes in each stream. The relationship between relative fish size and number of foraging events varied in each stream.

4.4.5 Relationships amongst behaviours

Females that received more attention from males (greater proportion of association time or more attempted thrusts) had a lower number of foraging events. These females also carried out more aggressive acts towards males (table 4.4)

Male reproductive behaviours were positively correlated with each other and males that spent more time associating with females also attempted more thrusts. Males that spent a greater proportion of time associating with females or attempted more thrusts

had a lower number of foraging events, and also received more aggressive acts from females. Males that performed more reproductive behaviours did not receive more aggressive acts from other males (table 4.4). However, in pools with higher mean numbers of aggressive acts between males, there was a trend for mean association time to decline (Pearson's correlation: $r_{47} = -0.272$, $P = 0.064$).

Table 4.4 Pearson's correlations between behaviours for females (N=193) and males (N=126).

Sex		Association time		Thrusts	
		R	P	R	P
Females	Thrusts	0.490	< 0.001	-	-
	Aggression to males	0.433	< 0.001	0.208	0.004
	Foraging	-0.181	0.012	-0.188	0.009
Males	Thrusts	0.646	< 0.001	-	-
	Aggression from females	0.406	< 0.001	0.297	0.001
	Aggression from males	-0.013	0.881	-0.097	0.280
	Foraging	-0.407	< 0.001	-0.199	0.025

4.5 Discussion

We studied the reproductive behaviours of *B. episcopi* in six populations that varied in predator density between upstream and downstream populations. We found no differences between upstream and downstream populations in reproductive behaviour (association time and thrusts) of focal males. Within a population, males spent more time associating with females as the OSR became more female-biased. In two out of three streams, the number of aggressive acts between males declined as the OSR became more female-biased. In the one of these streams, we observed attempted thrusts on focal females in more pools in the downstream population than the upstream population despite our finding that focal males did not behave differently. Neither OSR nor local predator density explained within-population variation in numbers of thrusts received by females.

Male reproductive behaviour was surprisingly unresponsive to population variation in predator density, yet other studies in this species strongly suggest that males show plasticity in response to fluctuations of environmental and ecological factors (Simcox et al., 2005; Chapters 2 & 3 this thesis). It seems unlikely that there really is no effect of predation pressure on male reproductive behaviour given the effects of predation on several other aspects of the biology of *B. episcopi* (see above). Whilst similarities with the Trinidadian guppy system led us to predict population differences in male behaviour, not all comparisons between guppy populations yielded significant results (reviewed in Houde, 1997). Indeed, our results resemble those of Shaw et al. (1994), who found that male behaviours differed most among streams and not between high and low predation populations. As Magurran & Seghers (1990) demonstrated, estimates of population differences depend on the presence or absence of a predator, and in the current study, predators were present in nearly every high predation site.

Perhaps then the difference between populations resides in plasticity or fine-scale adaptation to local predation pressure. However, local predator number had no effect on any of our measured behaviours. Additionally, we found no effect of light levels on any behaviours, although it is likely that increased light is associated with an increased risk of predation (Endler, 1987; Reynolds et al., 1993; Simcox et al., 2005; Chapter 2 this thesis). Perhaps we require greater knowledge about temporal variation in predation risk (Sih et al., 2000). Whether predation risk in downstream populations is low, with pulses of high risk, or chronic could have crucial impacts for predicting the effects of predation on reproductive and other behaviour (Lima & Bednekoff, 1999). As reproductive behaviours of focal males showed significant variation between pools in the present study, we may need to consider temporal variation in predation and other potential factors such as multi-species interactions (Sih, 1994), predator diet and predator hunger levels (Smith & Belk, 2001) in future studies.

Competition may be costly in the presence of a predator (Jakobsson et al., 1995), and so we expected male-male competition to be lower in high predator density populations (e.g. Kelly & Godin, 2001). Our predictions were met in only one stream

(QJG), and we found the reverse in the other two streams (RL and RM). Certainly, there are no consistent effects of predator pressure on male-male competition. However, OSR has been shown to affect intrasexual competition in a number of species: typically, competitive interactions among males decrease with increasing proportions of females (e.g. Enders, 1993; Madsen & Shine, 1993; Carroll & Salamon, 1995; Kvarnemo et al., 1995). Interestingly, in two streams in the present study (RL and RM), numbers of aggressive acts between males decreased as OSR became more female-biased. We might also expect that if males are spending less time in aggressive or agonistic encounters, they would spend more time trying to mate and indeed, within a pool, there was a trend for association time to increase as male-male aggression decreased. Furthermore, as the sex ratio became more female-biased, males spent increasingly more time associating with females. Thus, there is good correlational evidence that males may respond behaviourally to changes in OSR, altering relative amounts of direct and indirect competition with other males by switching between male-male aggression and male-female interactions. Whilst we controlled for OSR and predation pressure in the present study, these factors may interact and it will be necessary to make empirical controls to tease apart and understand any interactions.

The only mating behaviour that might be attributed to predator density was number of thrust attempts received by females, which was higher downstream than upstream in Quebrada Juan Grande. Whilst the result is consistent with population differences in guppies (Luyten & Liley, 1985; Magurran & Seghers, 1990; Magurran & Seghers, 1994a), it is hardly convincing evidence of a general effect of predation, which if real, would be expected in more than one stream. A more likely explanation is that population differences are just an artefact of differences in operational sex ratio: in upstream populations, the sex ratio tends to be more female-biased so perhaps male behaviours are simply diluted among females. We were not able to examine the relationship between OSR and thrusts received by females in QJG or RL but in RM, where there was sufficient variation to examine this, we found no relationship between OSR and thrusts received by focal females. Regardless of the cause, population differences in coercive rates of mating may lead to differences in sperm

competition. As downstream females are likely to have been inseminated by more males than upstream females, downstream males may invest in greater sperm production and have relatively larger testes than upstream males (e.g. Stockley et al., 1997). However, whether the slight differences in rates of coercive matings are sufficient to elicit population differentiation in male testes size in QJG is doubtful.

We were unable to examine among and within population variation in courtship frequency as courtship was rarely observed. In artificial pools and tanks, male courtship is observed more frequently than *in situ* studies (*in situ*: this study; Chapter 3 this thesis; artificial environments: Simcox et al., 2005; Chapter 2 this thesis). It seems unlikely that courtship is missed *in situ* by observers as it is a conspicuous behaviour. Female escape behaviour may be hindered in an enclosed, artificial pool or tank and perhaps the male interprets this for sexual interest. In guppies too, laboratory and field experiments can yield different results (reviewed in Houde, 1997). It may be important to understand discrepancies between experimental set-ups for future studies on reproductive behaviour of *B. episcopi*.

Foraging costs resulting from male harassment have been reported in mosquitofish *Gambusia holbrooki* (Pilastro et al., 2003), guppies *P. reticulata* (Magurran & Seghers, 1994b) and sailfin mollies *P. latipinna* (Schlupp et al., 2001). In agreement, we found that number of foraging events declined as association time and thrust attempts increased, although foraging effort or the quality of food items may have varied. Thus, it seems that male reproductive behaviour may have costs for males and for females in the form of reduced foraging success.

In a wide range of taxa, size is an important determinant of mating behaviour (e.g. Ryan & Causey, 1989; Danforth, 1991; Bisazza & Pilastro, 1997; Emlen, 1997a). Although the present study found no size-based differences in male behaviour nor increased reproductive behaviour towards larger females (see Chapter 3 this thesis), we do not place much emphasis on these results as our estimate of size was crude. Also, note that very small males are difficult to identify *in situ* and may have been missed from our observations. Perhaps the difficulty with obtaining a useful estimate

of size is why it has not been considered during *in situ* observations on guppies *P. reticulata* (Luyten & Liley, 1985; Endler, 1987; Magurran & Seghers, 1994a). However, we now know that responses to predation in guppies *P. reticulata* (Reynolds et al., 1993) and boldness-shyness traits in *B. episcopi* (Brown & Braithwaite, 2004; Brown et al., 2005b) are size-dependent, so it would be unwise to rule out size effects in the present study. Whilst relative size is an important predictor of mating success in sailfin mollies *P. latipinna* (Farr et al., 1986), there is no reason to suppose that this is the case in *B. episcopi* and male behaviours may vary with absolute rather than relative size. Unfortunately, we could not test these relationships in the present study.

In summary, we found little evidence for population differences in reproductive behaviour in *B. episcopi*, suggesting that population differences in predation pressure do not consistently affect male reproductive behaviour. Additionally, there were no relationships between any measured behaviour and local predator number. Given the effects of predation on many other aspects of this species' biology, the result is surprising. Effects of predation can be complex: temporal variation in predation risk and effects of multi-species interactions may have a greater impact on reproductive behaviour than simply the presence or absence of potential predators (Sih, 1994). Although males do not appear to be sensitive to predator pressure, their behaviour did vary with OSR: as the sex ratio became more female-biased, males spent more time associating with females and were involved in fewer aggressive acts with other males. There are marked differences in courtship frequency between artificial and laboratory experiments, however, suggesting that results from laboratory studies should be verified with field tests whenever possible.

4.6 Acknowledgements

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5. The effects of male size and competitor size on male reproductive behaviour

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5.1 Abstract

Male reproductive behaviour often varies quantitatively and qualitatively with male size, but may also change depending on the level of intrasexual competition. Thus where male reproductive success depends largely on male-male competition, it is essential to examine size-based reproductive behaviours of males in the presence of other males. We tested pairs of male Panamanian bishop (*Brachyrhaphis episcopi*; Pisces: Poeciliidae) from 4 populations to see how mating behaviours were affected by (i) focal male size and (ii) whether the focal male was smaller or larger than the rival male. Despite high levels of aggression from large males to small males in each pair, small males were not completely excluded from attempting to mate with females. In three out of four populations, small males spent increasingly more time associating and attempting thrust matings with females as small male size increased. In contrast, the same behaviours decreased or showed no relationship with the size of the large male. Potential reproductive opportunity was lowest for the smallest males, but for intermediate-sized males, even if they were the smaller of a pair of males, reproductive opportunity was at least equal to that of the largest males. We cannot determine with certainty whether the reproductive success of males of any sizes were hindered by competition in the present study. There were effects of absolute rival male size, however, on both aggression and thrust attempts: rival male size tended to affect the behaviour of the large focal males more than the small focal males, but this was not consistent among populations. Thrust attempts were only important when focal male size was controlled for, suggesting absolute rival male size *per se* does not affect any male mating behaviours.

5.2 Introduction

One major cause of variation in intensity of sexual selection between populations and between species is the ability of some individuals to restrict the access of other individuals to potential mates (Emlen & Oring, 1977). In numerous taxa, males prevent other males from mating by defending females or defending critical resources required for reproduction, which results in skewed reproductive success between males (Partridge & Halliday, 1984). Determinants of male success in male-male competition may be based on social rank (Estep et al., 1988; Bruce & White, 1995), fighting ability (Lailvaux et al., 2004), colour (de Fraipont et al., 1993; Tarof et al., 2005) or male size (e.g. Berven, 1981; Johnson, 1982).

Large males are more likely to win in male-male competition, giving them a reproductive advantage over small males (e.g. Morris et al., 1992; Hutchings et al., 1999; Forstmeier, 2002; Hagelin, 2002; Jenssen et al., 2005; Magellan et al., 2005; Reichard et al., 2005). The reproductive success of small males may increase through the use of genetically-determined or condition-dependent alternative reproductive tactics (Gross, 1996; Taborsky, 2001). Thus male size is often associated with quantitative or qualitative variation in reproductive behaviour (e.g. Ryan & Causey, 1989; Zimmerer & Kallman, 1989; Morris et al., 1992; Candolin & Voigt, 2003b; Pilastro et al., 2003; Magellan et al., 2005).

There are many examples of males altering reproductive behaviour in response to competition. For example, male barn swallows *Hirundo rustica* alter characteristics of their song as potential male competition increases (Galeotti et al., 1997) and male Trinidadian guppies *Poecilia reticulata* (Poeciliidae) alter their reproductive tactics depending on operational sex ratio (Jirotkul, 1999) or coloration of the competing male (Farr et al., 1986). In the European bitterling *Rhodeus sericeus*, dominant males are not able to defend territories in high densities of males. An increase in alternative reproductive behaviours and a decrease in courtship rate allows smaller males the opportunity to obtain a greater number of sperm releases, reducing the opportunity for sexual selection (Mills & Reynolds, 2003).

The effects of competition may vary with the size of the competitor (reviewed in Riechert, 1998). In a similar-sized pair of male mosquitofish *Gambusia holbrooki* (Poeciliidae), the smaller male receives high levels of aggression from the larger male and consequently his sexual activity is severely reduced. In a different-sized pair of males, the smaller male receives far less aggression from the larger male and remains sexually active (McPeck, 1992). Similarly, in the swordtail *Xiphophorus nigrensis* (Poeciliidae), the sexual activity of small males continues when paired with large males, but intermediate-sized males cease all sneak-chases and perform fewer displays (Zimmerer & Kallman, 1989). This probably occurs through direct interference, as large male swordtails try to prevent rivals from approaching females particularly when the rival male is also large (Morris et al., 1992). It appears that male-male competition may be greater in similar-sized pairs than disparate-sized pairs, possibly because similar-sized pairs are more evenly matched so dominance can only be established through direct competition. Certainly, the mating success of smaller males is more severely affected by competition when paired with a similar-sized male rather than a disparate-sized male in both *X. nigrensis* and *G. holbrooki*.

The effects of competition are not consistent across all poeciliid fish. The behaviour of small and large male sailfin mollies *Poecilia latipinna* are little affected by the presence of other males regardless of their relative size, but intermediate-sized males reduce their sexual behaviour under intrasexual competition, particularly from large males (Travis & Woodward, 1989). In the guppy *P. reticulata*, male-male competition is thought to be less important than female choice in determining male reproductive success, although there may be a role for interference competition rather than overt aggression (e.g. Kodric-Brown, 1993; Bruce & White, 1995). Darwell (1989, cited in Houde, 1997) found that male guppies performed a higher rate of displays as male size, relative to the size of the competing male, increased and a reduced number of displays as the absolute size of the competitor increased. However, Magellan et al. (2005) found that the behavioural response by male guppies to competition was independent of male size. It is possible that differences in experimental design contributed to inconsistencies among results as a number of

these studies examined competition in the presence of multiple rival males (Travis & Woodward, 1989; Bruce & White, 1995; Magellan et al., 2005).

Given the importance of intrasexual competitive ability in determining male reproductive success in many poeciliid species (e.g. Zimmerer & Kallman, 1989; McPeck, 1992), it is crucial to examine how reproductive behaviours vary under male-male competition. We examined the effects of absolute male size and rival male size on reproductive behaviour in the male Panamanian bishop, *Brachyrhaphis episcopi*, a tropical freshwater poeciliid fish. Like swordtails and mosquitofish, male *B. episcopi* exhibit overt aggression towards other males: in the field, large males attempt to control access to areas where females forage by chasing smaller males that frequently approach these areas (Chapter 4). Males perform courtship displays but coercive thrust attempts are more common (Chapter 4). In the absence of competition, courtships increase with male body length (Appendix 1). Male behaviours vary with operational sex ratio in the field (Chapter 4), but little is known about the relationship between male size and reproductive behaviours in a competitive situation.

As large males are more aggressive than small males and small males tend to be recipients of male aggression (Chapter 4), we expected large males to dominate access to females. Consequently, within a pair of males, we expected the small male to spend less time associating with females and carry out fewer courtships than the large male. We also examined whether the small male had a greater thrust rate than the large male, which may be a behavioural response to increase potential reproductive success under competition. We looked to see whether the relationship between male size and reproductive behaviour varied depending on whether the male was paired with a larger or smaller male, which would suggest that the effect of male competition on behaviour was dependent on the relative size of the male. Finally, when controlling for focal male size, we looked for a relationship between male behaviour and the size of the rival male. As aggression between males is likely to be higher in similar-sized pairs of males than between pairs of males disparate in size (Riechert, 1998), we were particularly expecting that small males would be less

aggressive to rivals and large males would be more aggressive as the size of the rival male increased.

5.3 Methods

5.3.1 Experimental design

We sampled 4 populations of *Brachyrhaphis episcopi* in the Parque Nacional Soberania, Panama (February- March 2004). Two populations were from Rio Macho and two from Rio Limbo. The streams cross an escarpment that creates waterfalls. Within each stream, we sampled a population from above (upstream) and below (downstream) the waterfalls. Downstream populations co-exist with a suite of larger predatory fish that include smaller fish such as *B. episcopi* in their diet (Angermeier & Karr, 1983; Kramer & Bryant, 1995). In contrast, upstream populations of *B. episcopi* co-exist only with small numbers of killifish *Rivulus brunneus* that are not capable of eating adult *B. episcopi* (Angermeier & Karr, 1983).

We sampled 39 pairs of males: 10 pairs from Macho upstream, Macho downstream and Limbo downstream and 9 pairs from Limbo upstream. We collected each pair of males and three females from a pool or small riffle using hand-nets, choosing males that were as disparate in size as possible (1mm to 8mm difference in standard length; mean size \pm SD: large males = 24.1 ± 2.64 mm; small males = 20.1 ± 1.64 mm). The five fish used in a trial came from the same riffle or pool and we did not sample any pool or riffle more than once. Adult sex ratios are female-biased (0.5 to 15 females per male; chapter 4) so the sex ratio (0.67 females per male) in our trials represented a high level of competition.

Since hierarchies are likely to be an important component of competition in this species, we tested fish immediately after capture to maintain any dominant-subordinate relationships. We transferred females into an artificial pool (1m in diameter) set up at the side of the stream. The pool was lined with small rocks and

pebbles, and filled to a depth of 15cm with stream water. Meanwhile, we transferred males to individual containers. We allowed females to settle for 10 minutes: during this time period, females usually began to forage. We then released the males into the pool from opposite sides. The fish were left for a further 5 minutes, during which time males usually began to exhibit mating behaviours.

We made two 10-minute observations of both males; the first began 5 minutes after releasing the males into the pool and second began 90 minutes after the first had ended (105 minutes after males were introduced). We recorded (i) time spent associating with females, defined as time spent within one body length and orientated towards the females; (ii) number of thrust attempts, where the male darts underneath the female and attempts to mate; (iii) number of courtships, where the male performs a display with his fins extended and including at least one turn in-front of the female; (iv) number of aggressive acts (nips, chases or lunges) directed towards the rival male.

At the end of the second observation period, we measured the size (standard length) of each fish to the nearest mm and returned the fish to their site of capture.

5.3.2 Statistical analyses

As a full analysis of the effects of focal and rival male size would be complex, we carried out two stages of analyses to aid with interpretation. Firstly, we examined the effects of focal male size, taking account whether the rival male was larger or smaller male but ignoring the actual size of the rival male. However, it is possible that the size difference between the focal and rival male could also be important, so we carried out a second analysis with rival male size in the model as well.

Data for large and small males within an observation were not independent. Thus, in each population, we randomly assigned each pair of males into one of two equally-sized (or nearly equal) groups. For one group, we used data from the first observation (after 5 minutes) for the large male and data from the second observation (after 105

minutes) for the small male. In the second group, we used first observation data from the small male and second observation data from the large male. The gap of 90 minutes between observations should be sufficient time for the behaviour of a male in the second observation to be independent of his behaviour in the first observation. In support of this, male guppies *P. reticulata* resume sexual behaviour within an hour of successful copulation (Houde, 1997, p39).

For all analyses described below, data were transformed to ensure that the residuals from models met the assumptions of homogeneity of variance and normality. Proportion of association time was arcsine square-root transformed and all count data (numbers of thrusts, courtships and aggressive acts to rivals) were square-root transformed.

Effects of focal male size

We examined the relationship between focal male size and male behaviour using behavioural data from randomly chosen observations described above. We fitted male role (large or small), focal male size, stream and upstream/downstream and all possible interactions among these factors to a general linear model (GLM) in Minitab (Minitab 14.1, 2003, Minitab Inc., Six Sigma Academy International). Males within a pair were not independent so we fitted pair as a nested random factor within stream and upstream/downstream. Stream was also fitted as a random factor as we aimed to generalise about behaviours in all streams. We also fitted observation period (5 min or 105 min) as a factor. This enabled us to examine how male behaviours were affected by focal male size and male role. We were particularly interested in interactions between focal male size and male role, which would suggest that effects of focal male size on behaviour depended on whether the male was the large or small male in the pair.

Effects of rival male size

We then examined how rival male size, in addition to focal male size, affected male behaviours. We used linear mixed-effects models in S Plus (S-Plus 6 Professional Edition, 2001, Insightful Corp., Seattle, WA), fitting interactions among stream, upstream/downstream, male role and focal male size and interactions among stream, upstream/downstream, male role and rival male size. We also fitted observation period (5 min or 105 min) as a factor. As data for the large and small male in a pair were non-independent, we fitted pair as a random factor. We compared the changes in deviance between maximum likelihood (ML) models with different fixed effects structures. Main effects or their higher-order interactions that caused a significant increase in deviance when deleted from the model were retained; we present the likelihood ratio statistics (L. ratio) and P values associated with retention or removal of terms from the model. ML estimates do not take into account the degrees of freedom used up by the fixed effects when calculating the variance components, however, so we re-estimated ANOVA statistics for the minimal model using restricted maximum likelihood estimation (REML) (Crawley, 2002).

For behaviours that were affected by both focal and rival male size, we examined whether the effects of rival male size were independent of focal male size. We re-ran the analysis but excluded focal male size and any interactions with focal male size from the models.

5.4 Results

Relationships among male and female sizes

The size difference between males correlated with the size of the large male but not the small male, suggesting that the size difference between pairs of males was mainly due to increased size of the large male (Pearson's correlations: size difference and large male size: $r_{37} = 0.785$, $P < 0.001$; size difference and small male size: $r_{37} = 0.087$,

$P = 0.599$. Mean female size was not correlated with absolute size of large or small males, nor male size difference ($P > 0.4$).

Effects of focal male size

Proportion of time associating with females was affected by an interaction among male role, focal male size, stream and upstream/downstream, indicating that the relationship between association time and focal male size depended on both the role of the male in the trial and the population of origin ($F_{1,27} = 4.30$, $P = 0.048$; fig. 5.1). For the large male, actual size had little effect on association time, with potentially a slight negative relationship in some populations. In contrast, the actual size of the small male seemed to have a much more dramatic effect on the behaviour of the small male. The direction of the relationship varied between populations, however, with positive effects of small male size in three populations but strongly negative effects in the fourth population (Macho upstream). Association time did not differ between the first and second observation periods ($F_{1,26} = 2.03$, $P = 0.166$).

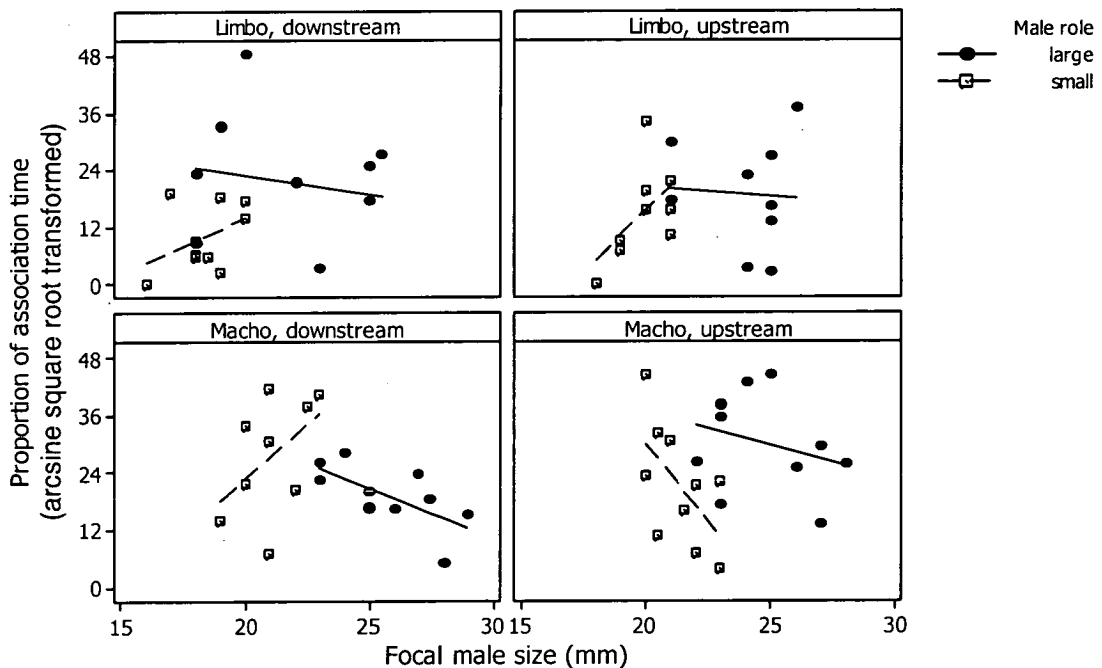


Figure 5.1 Proportion of time associating with females against focal male size by large and small males in each population. Figure shows arcsine square root transformed data and linear regression lines.

A similar pattern was seen for thrust attempts, with the number of thrust attempts affected by an interaction among male role, focal male size, stream and upstream/downstream ($F_{1,26} = 4.74$, $P = 0.039$; fig. 5.2). Thrust attempts were more frequent in the first than second observation. ($F_{1,26} = 9.19$, $P = 0.005$). Controlling for differences between observation periods, thrust attempts by the large male varied little with focal male size. For the small male, however, thrust attempts increased with focal male size in three populations and decreased in the fourth (Macho upstream), which mirrors the relationship between small male size and association time reported above.

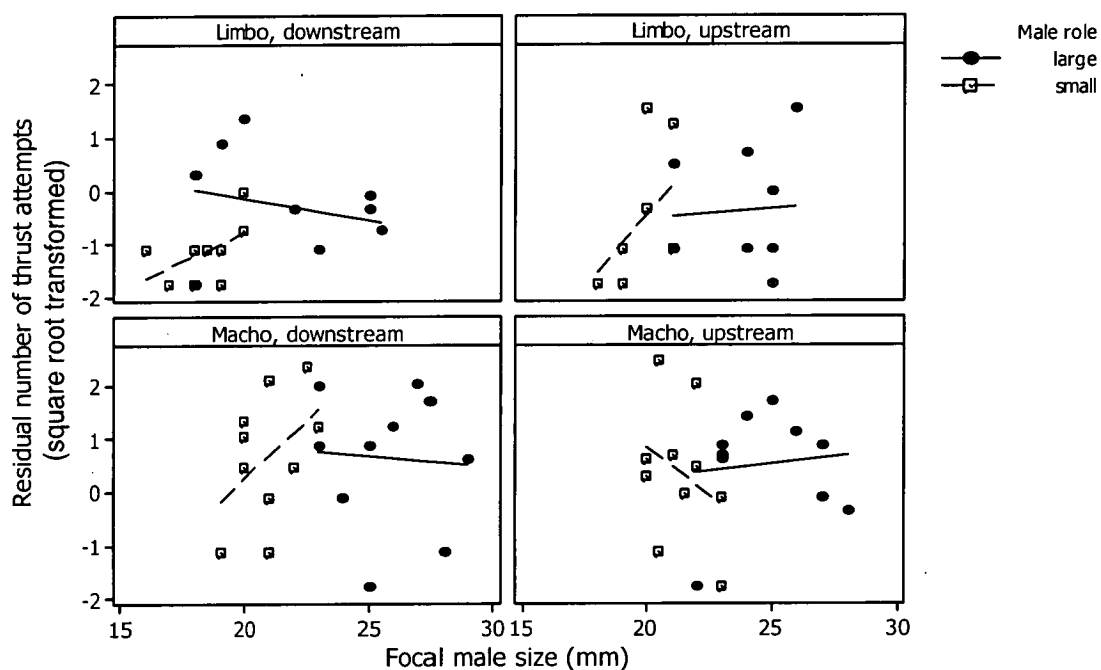


Figure 5.2 Residual number of thrust attempts, after controlling for differences between first and second observation periods, against focal male size by large and small males in each population. Figure shows residual square root transformed data and linear regression lines.

Males carried out a mean \pm SE of 0.46 ± 0.15 courtships during the observation period, but there were no effects of male role or focal male size on courtship number (male role: $F_{1,38} = 0.00$, $P = 0.977$; male size: $F_{1,37} = 0.00$, $P = 0.994$; interaction: $F_{1,36} = 0.80$, $P = 0.378$). Courtship also did not vary between the first and second observation period ($F_{1,30} = 0.00$, $P = 0.953$) nor were there any population differences in courtship ($P > 0.25$).

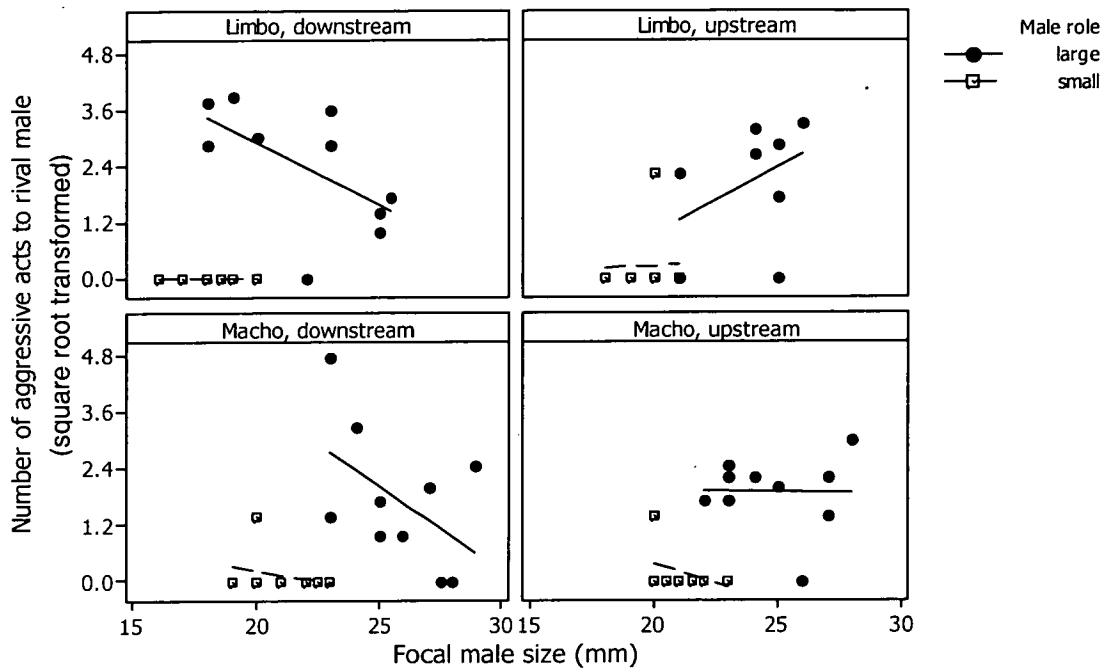


Figure 5.3 Residual number of thrust attempts, after controlling for differences between first and second observation periods, against focal male size by large and small males in each population. Figure shows residual square root transformed data.

The number of aggressive acts by focal males to rivals varied with several first order interactions (fig. 5.3), but surprisingly there was no interaction between male role and focal male size ($F_{1,31} = 0.71$, $P = 0.407$). Male role was important, however, and the large male was always more aggressive than the small male. Indeed, the small male rarely directed any aggression at all towards the large male, although the difference in aggression between the large and small male varied between populations (stream and male role interaction: $F_{1,33} = 4.80$, $P = 0.036$; upstream/downstream and male role interaction: $F_{1,33} = 13.25$, $P = 0.001$; fig. 5.3). Focal male size was also important, but again, the relationship varied between populations (stream and focal male size interaction: $F_{1,33} = 6.37$, $P = 0.017$; upstream/downstream and focal male size interaction: $F_{1,33} = 12.32$, $P = 0.001$; fig. 5.3). In the two downstream populations, aggression seemed to have a negative relationship with focal male size, but relationships were positive or absent in upstream populations.

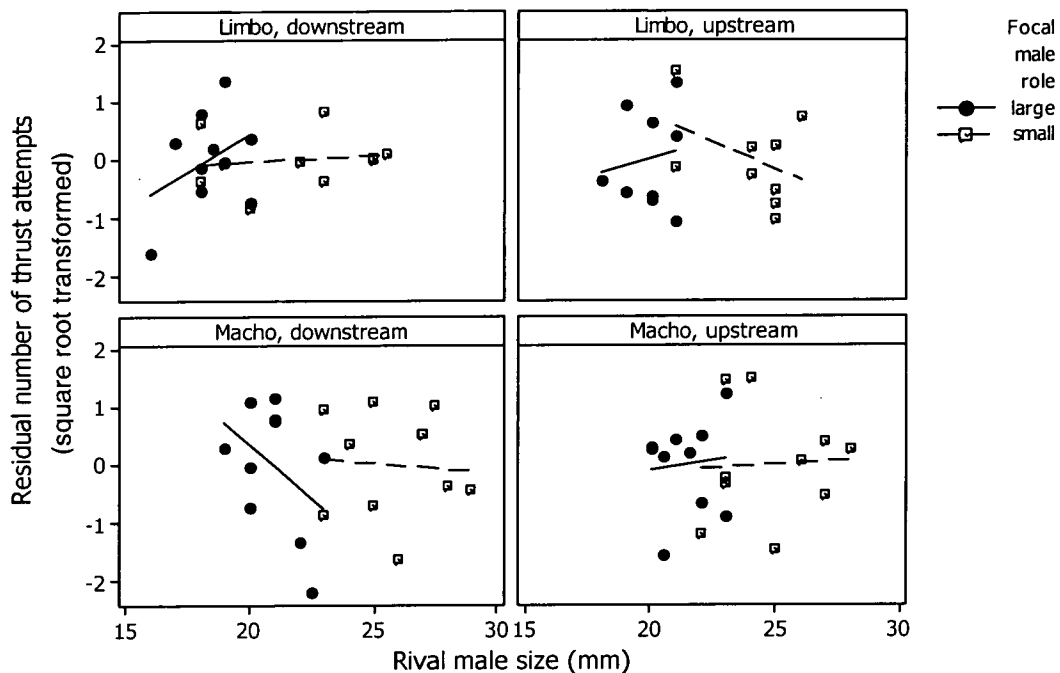
Effects of rival male size

Figure 5.4 Residual number of thrust attempts, after controlling for focal male size and interactions with focal male size, against rival male size for large and small males from each population. Figure shows residual square root transformed data and linear regression lines.

Rival male size explained significant amounts of variation in the number of thrust attempts, as indicated by significant increases in deviance caused by the removal of interactions between rival male size, male role and stream (L. ratio = 4.12, $P = 0.042$) and between rival male size, stream and upstream/downstream (L. ratio = 3.85, $P = 0.050$) from models that controlled for focal male size. Despite marginally significant increases in deviance, the interactions were not significant in ANOVA tests using REML ($F_{1,20} = 3.14$, $P = 0.092$ and $F_{1,20} = 3.14$, $P = 0.092$ respectively) so should be treated with caution. This caution is supported by visual examination of data, which shows little effect of rival male size on thrust attempts (fig. 5.4) except perhaps for negative relationships for the small male in one population (Limbo upstream) and for the large male in another population (Macho downstream). Without controlling for focal male size, there were no effects of rival male size on thrust attempts (L. ratio = 0.17, $P = 0.682$). Thus, any effects of rival male size on thrust attempts are only

important when taking into account the size of the focal male (i.e. the relative size of males is what matters).

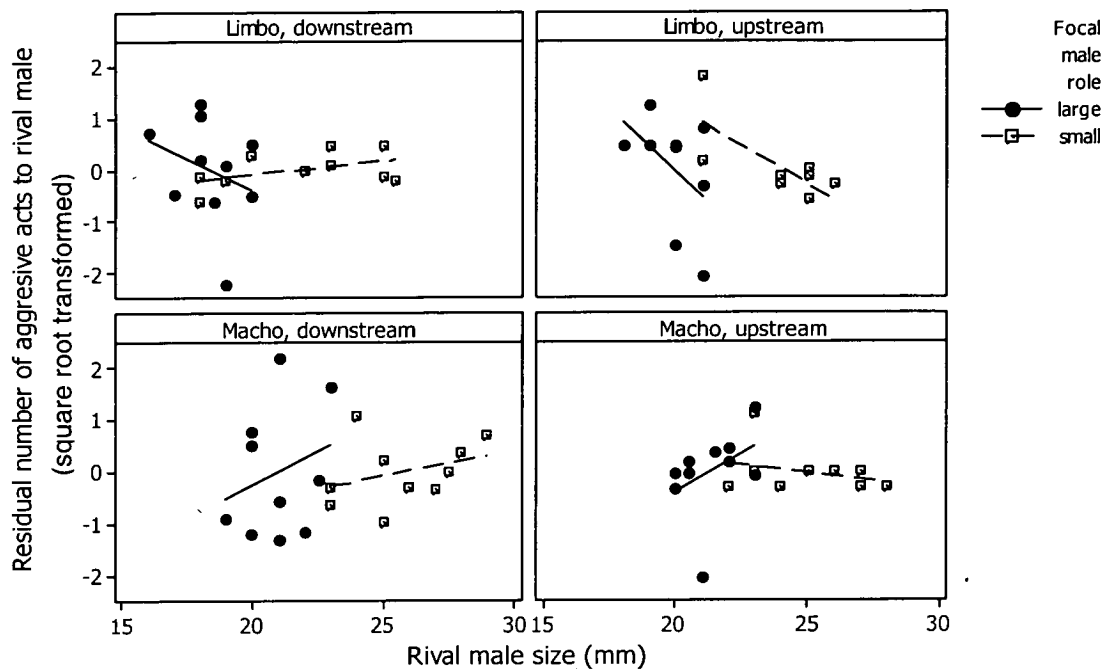


Figure 5.5 Residual number of aggressive acts to rivals (square root transformed), after controlling for focal male size and interactions with focal male size, against rival male size for large and small males from each population. Figure shows residual square root transformed data and linear regression lines.

The number of aggressive acts to rival males was affected by rival male size, when focal male size was controlled for (rival male size, male role, and stream interaction: L. ratio = 7.41, $P = 0.007$; $F_{1,27} = 4.64$, $P = 0.040$; rival male size and upstream/downstream interaction: L. ratio = 6.32, $P = 0.012$; $F_{1,27} = 8.37$, $P = 0.007$; fig. 5.5). As the small male rarely exhibited any aggression at all, his level of aggression was little affected by the size of the rival male. Aggression from the large male, however, was affected by rival male size but also varied among streams: the number of aggressive acts increased with rival male size in Macho populations but decreased in Limbo. In fact, the effects of rival male size on aggression to rivals appear to be remarkably similar, regardless of whether focal male size is controlled for or not (rival male size, male role and stream interaction not controlling for focal male size: L. ratio = 3.94, $P = 0.047$; $F_{1,33} = 4.02$, $P = 0.053$). The small male was

unaffected by rival male size, but aggression carried out by the large male increased with rival male size in Macho and decreased in Limbo.

We found no effects of rival male size on association time or courtship (L. ratio = 0.903; $P = 0.342$; L. ratio = 0.117, $P = 0.732$).

5.5 Discussion

We tested the reproductive behaviours of pairs of males of disparate sizes to examine how male size related to reproductive behaviour in a competitive situation. The large male in the pair was more aggressive than the small male; despite this, small males were not excluded from attempting to mate with females. With the exception of one of the four populations (Macho upstream), small males spent increasingly more time associating and attempting thrust matings with females as small male size increased. In contrast, time spent associating with females and attempted thrusts decreased or showed no relationship with large male size.

How do the potential reproductive successes of small and large males compare? For very small males (less than 20mm), both association time and thrust attempts were low. But for larger males (greater than 20mm) in the small male role, association times and thrust attempts were comparable to those found for similarly-sized males in the large role and may even have exceeded those of the largest males (greater than 27mm). These patterns suggests that very large males do not have a reproductive advantage under intrasexual competition and that intermediate-sized males, even if they are the smaller of a pair of males, have potential reproductive success that is at least equal to the largest males. We do not know why the behaviours of small males in Macho upstream differed from the other populations, but we did not test any males smaller than 20mm in this population, which, in other populations, had the lowest levels of reproductive behaviours.

The present study enables us to examine how male size affects reproductive behaviour in the presence of other males, but we cannot conclude whether behaviours vary with and without male-male competition as all males were tested with another male present. If male behaviour was not affected by the presence of a competitor, our results suggest that the relationship between male size and behaviour is non-linear, perhaps asymptotic or even quadratic. With little size overlap between males in the large and small role, we cannot rule out the possibility of a non-linear relationship between male size and male behaviour, but there is no evidence of non-linear relationships from previous observations of male behaviour in the absence of competition (Appendix 1). If there is a positive relationship between male size and behaviour, then the reproductive behaviours of very large males must decrease more than smaller males in the presence of competition. Alternatively, if there is a null or negative relationship between male size and behaviour in the absence of competition, as seen in other poeciliid species (Bisazza & Marin, 1995; Bisazza & Pilastro, 1997), small males may be worst affected by intrasexual competition. The present study, however, did not address this issue directly.

Although in most populations, very small males had lower reproductive behaviours than larger males, we do not know how these behaviours related to insemination or fertilization success. Small males may invest proportionally more into sperm production, or may have a greater proportion of successful mating attempts than large males. This may lead to a small male size advantage, which has been proposed to explain the maintenance of reversed sexual size dimorphism in poeciliid species with significant inter-male competition (e.g. Bisazza, 1993; Pilastro et al., 1997; Pilastro et al., 2003).

Surprisingly, there was no consistent among-population relationship between numbers of aggressive acts towards rival males with focal male size. As the size difference between pairs of males increased primarily with the size of the large male, we expected aggressive acts by the large male to decrease with focal male size. We found a negative relationship between aggression and size of large focal males only in downstream populations. The effects of rival male size were also inconsistent

among populations. We expected that, as rival male size increased, small males should be less aggressive but we found that small males were little affected by the absolute size of the rival male. We also expected that, as rival male size increased, large males should be more aggressive, but this positive relationship was only found in Macho populations. Aggression was almost completely uni-directional (from the large to the small male) and so presumably is primarily determined by whether the focal male is larger or smaller than his rival rather than the relative size difference. It is also possible that the unfamiliar test arena reduced competition between males. In a stream, large males appear to defend the areas where females forage (H. Simcox, pers. obs.) but the absence of a recognised foraging area in the artificial environment may hinder resource-defence by large males.

When we controlled for focal male size, we found no effects of rival male size on courtship or association time, but we did find an effect on the number of thrust attempts from the focal to the rival male. Rival male size seemed to affect the large male more than the small male, but there was little consistency among populations. Unlike the results obtained from comparing ML models, the ANOVA results of REML models did not provide support for an effect of rival male size on thrust attempts and we are cautious about trying to interpret this result further. We can conclude, however, that without controlling for focal male size, rival male size does not affect male mating behaviours towards females.

Courtship was not affected by focal male size or rival male size, nor did courtship frequency differ between populations. The frequency of courtship was lower compared to previous studies in artificial enclosures (Simcox et al., 2005; Chapter 2 this thesis; Appendix 1) and perhaps the absence of intrasexual competition plays a role in explaining elevated courtship rates in previous studies compared to *in situ* studies. There is evidence that male behaviour varies with the operational sex ratio (OSR) in the field (Chapter 4), but we do not know whether males respond to short-term fluctuations in OSR. In sand gobies *Pomatoschistus minutus*, males respond behaviourally to changes in OSR within 48 hours (Kvarnemo et al., 1995) but male guppy behaviour remains fixed for at least 24 hours after changes to OSR (Evans &

Magurran, 1999). In species where male-male competition may be an important determinant in male reproductive success, plasticity in response to fluctuating OSR could certainly be advantageous.

Size-assortative mating could be important in determining the relative mating success of differently sized males, but we did not consider this in the present study. Size-assortative mating is seen in the congener *B. rhabdophora* (Basolo, 2004), and the size-based distribution of males and females within the natural habitat may promote size-assortative male mating preferences in *B. episcopi* (Chapter 4). Female mate choice could also affect male behaviour. Female *B. episcopi* show little active mate choice (H. Simcox, pers. obs.) but this has not been explicitly studied to date. If females have a mating preference for larger males (e.g. McPeck, 1992; Reynolds & Gross, 1992; Magellan et al., 2005), very large males would likely have the highest association times with females, but we found no support for this.

By comparing behaviours of males across a range of sizes, we were able to examine how continuous changes in male size relate to changes in male behaviour. Other authors have also used continuous distribution of sizes to investigate competitive ability (e.g. Hughes, 1985; Bisazza & Marin, 1995; Johnsson et al., 1999; Morris et al., 2005). However, we could not directly address the effects of intrasexual competition on the relationship between male size and behaviour. Alternative approaches, such as repeated observations on focal males paired with a similar-sized or a different-sized male in each observation or observations on focal males in the presence or absence of a competitor, could be more informative. Comparisons between size classes of males are appropriate for species whose size at maturity is determined by allelic variation at a single locus (e.g. *X. nigrensis*: Zimmerer & Kallman, 1989; Morris et al., 1992) but we do not yet know how size at maturity is determined in *B. episcopi*. Even in studies of species with a continuous distribution of male size, males may be partitioned into separate size classes (Travis & Woodward, 1989; Pettersson et al., 1996; Pilastro et al., 2003), which may enable examination of the effects of both absolute and relative male size effects on male-male competition.

Reynolds et al. (1993) found that the reproductive behaviour of large and small male guppies *Poecilia reticulata* are affected differently by variation in light levels. Although mating preferences of *B. episcopi* are affected by light levels (Simcox et al., 2005; Chapter 2 this thesis), there is no evidence that overall levels of mating activity varies between males of different sizes under different light conditions. Even though we cannot rule out the possibility that large and small males are affected differently by variation in light levels, the fact that we did not collect data on light levels in the present study does not invalidate the results. Rather, the study represents the average response of males over all light levels.

We are not able to generalise too far about the effects of male size on male reproductive behaviours as we found large behavioural variations among streams. Rio Macho, Rio Limbo and other streams in the Parque National Soberania differ in reproductive behaviours (Chapter 4), male mate choice (Simcox et al., 2005; Chapter 2 this thesis; Brown & Braithwaite, 2005), cerebral lateralization (Brown et al., 2005b) and stress responses (Mojica et al., 1997) and genetic sequences from populations in nearby streams differ by up to 3%. Whether differences are driven by ecological or random factors is not known. Inter-stream variation emphasizes the necessity of sampling multiple streams to uncover repeatable patterns in behavioural and other traits in *B. episcopi*.

5.6 Acknowledgements

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6. Individual variation in traits associated with male mating success

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6.1 Abstract

Relative investment into traits associated with reproduction is likely to have major impacts on fitness. The amounts invested into particular traits, however, may vary depending on the status or condition of an individual. We examined investment into testes mass, intromittent organ (gonopodium) length and colouration in two populations of Panamanian bishop, *Brachyrhaphis episcopi*, a tropical freshwater fish. As smaller males have fewer mating attempts than larger males, we hypothesised that smaller males might invest in proportionally larger testes than larger males, leading to a negative allometric relationship between testes mass and soma mass. We found no evidence to support this prediction, although we found some suggestion that gonopodium length might be a better predictor of testes mass than soma mass. Males in better condition had a greater number and intensity of melanic colouration; relationships between expression of sexually-selected traits and condition have been found in other species, thus examination of the function of markings in this species could be a profitable area for future research.

6.2 Introduction

Throughout its life, an individual must allocate finite resources between growth and maintenance of different body parts (Stearns, 1989; Lessells, 1991; Trumbo, 1999). Trade-offs may arise between investment into somatic growth, growth of reproductive traits and tissue maintenance, and thus patterns of investment may have crucial implications for fitness (Lindström et al., 2005). One method of quantifying

investment into a trait is to look at the allometric relationship between relative trait size and body size (Tomkins & Simmons, 2002).

Investment into physical traits associated with reproduction can have a major impact on an individual's mating success. For example, males may invest in larger or more colourful ornaments, which are preferred by females, or in larger armaments or body size, which are often advantageous in intrasexual competition (Andersson & Iwasa, 1996). Competition with rivals may also occur after sperm release; thus to promote paternity under risk of sperm competition, males may increase the number of sperm released (Parker, 1982). As testes size largely determines sperm production in teleost fish (Billard, 1986), mammals (Moller, 1989) and birds (Moller, 1988), relative testes investment is often considered a good predictor of sperm production.

A number of factors may affect the level of investment into physical traits associated with mating success within a population. Intrinsic factors such as age, size and condition can affect the expression of physical traits associated with mating success. For example, the red nuptial coloration expressed by breeding male sticklebacks *Gasterosteus aculeatus*, which is preferred by females (Milinski & Bakker, 1990; Braithwaite & Barber, 2000) and associated with success in interactions with other males (Baube, 1997), varies in intensity depending on condition and parasite infection (Milinski & Bakker, 1990). Some traits may also be heritable, adding a genetic component to allocation decisions (e.g. male size in crickets *Gryllus bimaculatus*, Simmons, 1987; area of orange colouration in male guppies *Poecilia reticulata* (Poeciliidae), Houde, 1992).

Investment into sexual ornaments may also be associated with specific reproductive tactics, as in many species not all individuals use the same behavioural tactics to obtain mates (reviewed in Gross, 1996). For example, large male dung beetles *Onthophagus acuminatus* develop horns and defend females but small male dung beetles sneak matings with females and do not develop horns (Emlen, 1997a).

Males that are less likely to copulate with females might also compensate by investing more into spermatogenesis, in order to increase their potential reproductive success under sperm competition (Parker, 1990; Gage et al., 1995). Thus, in some species, males use sneak tactics to obtain matings, for example, by mimicking females, rather than competing or courting females directly (reviewed in Taborsky, 1998). Small male grass gobies *Zosterisessor ophicephalus*, for example, sneak fertilizations whereas larger males that defend territories, court females and provide paternal care. Accordingly, smaller males have proportionally larger testes and more numerous sperm in ejaculates than larger males (Scaggiante et al., 1999; Scaggiante et al., 2004). Not all intra-specific studies, however, find a relationship between sperm competition and testes size (Birkhead et al., 1997; Peer et al., 2000) and indeed, the reverse pattern may also occur, where males with greater mating success have larger testes, presumably to ensure fertility (e.g. Arctic charr *Salvelinus alpinus*, Liljedal & Folstad, 2003; red deer *Cervus elaphus*, Malo et al., 2005).

Characteristics of male genitalia have been implicated in mating success in a number of insect species (reviewed in Hosken & Stockley, 2004), and may also be important in internally-fertilising fish (Regan, 1913; Kelly et al., 2000). One character in particular that may be important in internally-fertilising fish is the length of the intromittent organ, which transfers sperm to females. A long intromittent organ may be advantageous during coercive mating as it reduces the distance between the male and female. In support of this hypothesis, male guppies *P. reticulata* that attempt more coercive matings have longer intromittent organs (gonopodium) (Reynolds et al., 1993). Gonopodium length also shows geographic variation, being longer in males populations exposed to a high compared to low risk of predation possibly because males in high-risk populations rely more heavily on coercive matings (Kelly et al., 2000). A study into gonopodium length in another poeciliid fish *Brachyrhaphis episcopi* favoured a natural selection argument over sexual selection to explain variation in gonopodium length as allometric relationships were negative, unlike most sexually selected traits, which typically show positive allometry (Jennions & Kelly, 2002). However, little is known about the effects of sexual conflict on allometric relationships (reviewed in Hosken & Stockley, 2004), so it may not be

possible to rule out an effect of sexual selection on gonopodium length in *B. episcopi*. If gonopodium length is related to mating success, it may also be related to relative testes size.

We examined relative investment into traits associated with mating success in the Panamanian bishop, *B. episcopi* (Poeciliidae), a tropical fish that exhibits two male mating tactics. Males occasionally court females but the majority of matings are coercive thrusts (Chapters 3 & 4). Male size is an important component of mating success: larger males spend longer associating with females and attempt more matings than smaller males. Male size at maturity shows substantial variation (standard length ranges from 15mm-30mm, although Turner (1938) reported males of up to 36mm in length) that cannot be attributed to post-maturational growth, which is negligible in male poeciliid fish (Snelson, 1989). Like the congener *B. rhabdophora* (Reznick et al., 1993), male size may exhibit a bimodal distribution (Turner, 1938). Given that discrete alternative mating tactics could heavily influence patterns of relative testes investment, we first determined whether male standard length or weight had a bimodal distribution. Secondly, we examined how male size was related to testes investment. Small males do not appear to compensate for reduced access to females by performing more thrust attempts than large males (Chapter 4). Instead, subordinate males may invest in greater sperm production (Liljedal & Folstad, 2003). If this occurs, we predicted that small males, which are subordinate, would have proportionally larger testes than large males. Finally, we examined two further traits that may be associated with variation in reproductive success: gonopodium length and male colouration. As gonopodium length may be related to mating success, we asked whether gonopodium length could explain variation in relative testes mass. We then made a preliminary examination of the black melanic bars along the sides of *B. episcopi*, the function of which is unknown. We hypothesised that, if such markings are costly, males in better body condition would have a greater number or intensity of markings (e.g. Barber et al., 2000).

6.3 Methods

6.3.1 Data collection

We sampled male *B. episcopi* from two populations in Quebrada Juan Grande, a stream in the Parque National Soberania, Panama, in September 2003. The populations are separated by a large waterfall and there is little inter-population movement (C. Brown, F. Jones, N. Brydges & V. A. Braithwaite unpublished data). The upstream population above the waterfall co-exists with the killifish *Rivulus brunneus*, which is not capable of eating adult *B. episcopi* although it may prey upon juveniles (Angermeier & Karr, 1983). The downstream population below the waterfall co-exists with several species of large predator fish including characin and cichlid species that include fish such as *B. episcopi* in their diet (Angermeier & Karr, 1983; Kramer & Bryant, 1995). Thus the downstream population co-exists with a high density of predators in comparison to the upstream population. There are also differences in OSR of *B. episcopi*: the sex ratio was more female biased upstream than downstream (table 6.1), in contrast to Jennions & Kelly (2002).

Table 6.1 Mean number (*percentage*) of fish making up fauna upstream and downstream in Quebrada Juan Grande, September 2003 after seine-netting in four upstream sites and twelve downstream sites. Other fish comprised of catfish and other poeciliid species.

	<i>Adult B. episcopi</i>		<i>Rivulus</i>	<i>Characidae</i>	<i>Cichlidae</i>	<i>Others</i>
	males	females	<i>brunneus</i>			
Upstream	7.8 (24.6)	19.3 (61.1)	4.5 (14.3)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Downstream	1.8 (8.6)	2.6 (12.1)	0.2 (0.8)	13.2 (61.7)	0.9 (4.3)	2.7 (12.5)

With hand-nets, we caught 31 males from the upstream population and 28 males from the downstream population. We sacrificed fish immediately with MS-222 and took three measurements of their standard length to the nearest 0.1mm using callipers (fig. 6.1). Fish were fixed in Dietrich's solution (900ml distilled water, 450ml 95% ethanol, 150ml 40% formaldehyde, 30ml acetic acid) for 14 days and subsequently

stored in 50% ethanol. We transported fish back to the laboratory in Edinburgh, UK, and took further measurements in December 2003.

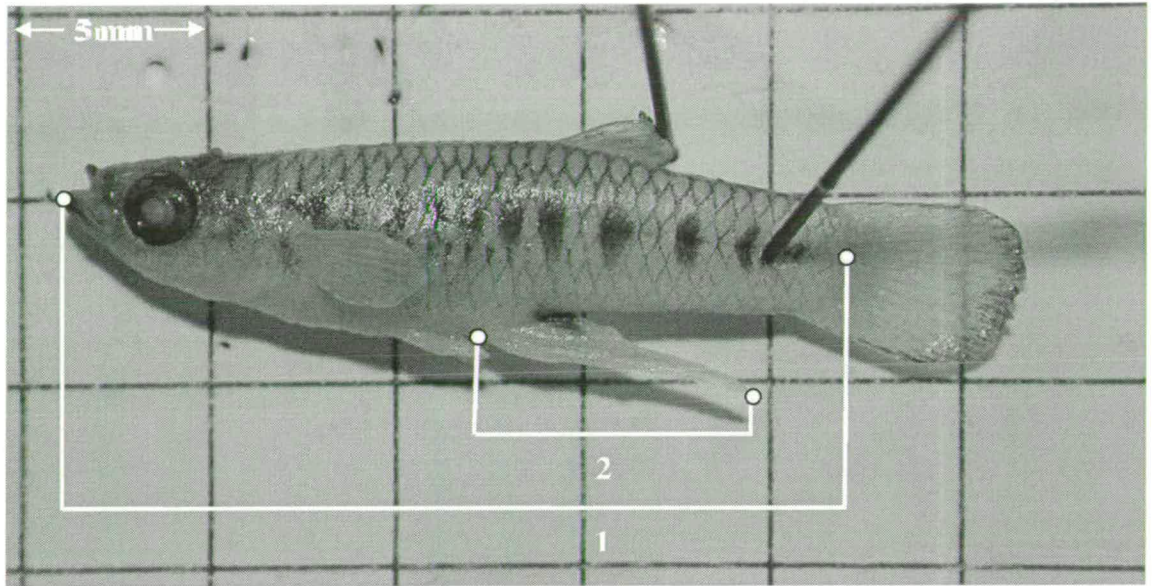


Figure 6.1 Specimen male showing measures of standard length (1) and gonopodium length (2). Black melanic bars are also visible along the side of the body.

To establish that all males were mature, we checked for the presence of a fully developed gonopodium (Rosen & Gordon, 1953). One upstream male did not have a fully developed gonopodium and was excluded from further study. Under magnification ($\times 12$), we measured the length of the gonopodium from the tip to the anterior base to the nearest 0.1mm using digital callipers (fig. 6.1)(Kelly et al., 2000).

We took a digital photograph of the left side of each preserved fish placed on 5mm graph paper for scale and illuminated from above with a halogen light. The photographs were presented in a randomized order to one observer (HS) with identity of the fish concealed. From the photographs, the observer counted the number of melanic bars visible along the flank of the fish, and noted whether the colour of the bars was high, medium or low intensity.

We blotted each fish on tissue paper to remove excess ethanol and recorded whole mass to the nearest 0.1mg. To prevent samples desiccating, we submerged fish in

50% ethanol during dissection. We removed the testes and recorded testes mass to the nearest 0.1mg, using a fine brush to absorb excess ethanol prior to weighing. Gonopodium length, whole mass and testes mass were measured five times.

6.3.2 Statistical methods

Unless described otherwise, all statistical analyses were carried out with Minitab (Minitab 14.1, 2003, Minitab Inc., Six Sigma Academy International).

Our measurements of standard length, gonopodium length, whole mass and testes mass were highly repeatable (Repeatabilities > 0.99; Lessells & Boag, 1987) and had low measurement errors (ME <0.01% to 0.54%; Yezerinac et al., 1992). We calculated mean values of the length and mass measurements and used these in further analysis. We examined the distributions of whole mass and standard length in each population for evidence of kurtosis, which if strongly negative can indicate a bimodal distribution (Sokal & Rohlf, 1969).

We calculated soma mass as testes mass subtracted from whole mass (Tomkins & Simmons, 2002). We used $[(\text{mass in g}/\text{standard length in mm}^a) \times 10^5]$ to calculate body condition factor, where a = slope of regression of \log_{10} mass (g) against \log_{10} standard length (mm) (Barber et al., 2001). For downstream males, $a = 0.9442$; for upstream males, $a = 0.9447$ (Reduced Major Axis (RMA) regression; Bohonak & van der Linde, 2004).

We used two methods to examine variation in testes investment. To examine relative testes investment to male size, we examined testes allometry using RMA regression of \log_{10} testes mass onto \log_{10} soma mass for each population (reviewed in Tomkins & Simmons, 2002). Allometric exponents (slope of the regression line) less than one indicate that smaller individuals have proportionally larger testes than larger individuals; exponents greater than one indicate that larger individuals have proportionally larger testes than smaller individuals. An exponent of one indicates that all individuals have testes of equal proportion (Tomkins & Simmons, 2002). We

examined the relationship between \log_{10} gonopodium length and \log_{10} standard length in the same way.

We then examined how variation in testes mass was affected by body condition, gonopodium length and population differences using a general linear model (GLM) that controlled for soma mass (recommended by Tomkins & Simmons, 2002). We fitted population, soma mass, body condition, gonopodium length and two-way interactions with population in the maximal model. To examine population differences in gonopodium length, we fitted a GLM with population, standard length and an interaction between population and standard length. We also examined factors that might be related to body condition by fitting population, intensity of bars, number of bars and two-way interactions with population into a GLM. In the above GLMs, all mass and length measurements were \log_{10} transformed. To estimate any population effect sizes of interest, we calculated and then back-transformed the 95% CI of the difference between the populations. However, the back-transformed values are estimates of the 95%CI for the ratio of medians of original scores, not the means (Ramsey & Schafer, 1997, p67-69).

6.4 Results

6.4.1 Male body size

Males from the upstream population were longer and heavier than males from the downstream population (standard length: GLM: $F_{1,56} = 11.91$, $P = 0.001$; mean \pm SD = 24.7 ± 3.0 mm vs 21.8 ± 3.2 mm; total mass: GLM: $F_{1,56} = 13.96$, $P < 0.001$; mean \pm SD = 343.1 ± 123.5 mg vs 230.5 ± 96.2 mg). Whilst the distributions of standard length and total mass showed negative kurtosis (standard length: upstream $g_2 = -0.71$, downstream $g_2 = -1.15$; mass: upstream $g_2 = -1.17$, downstream $g_2 = -1.07$; fig. 6.2), they did not significantly differ from normal (Anderson-darling; P values > 0.17). Thus, there was no evidence of a bimodal distribution of male mass or length.

Total mass, standard length, gonopodium length, soma mass and testes mass were all positively correlated with each other (Pearson's correlations: $0.499 \leq r_{56} \leq 1.0$; P values < 0.001). Body condition was weakly positively related to total mass and soma mass (Pearson's correlations: $r_{56} = 0.260$, $P = 0.048$; $r_{56} = 0.262$, $P = 0.047$) but not to any other morphometric measures ($P > 0.1$).

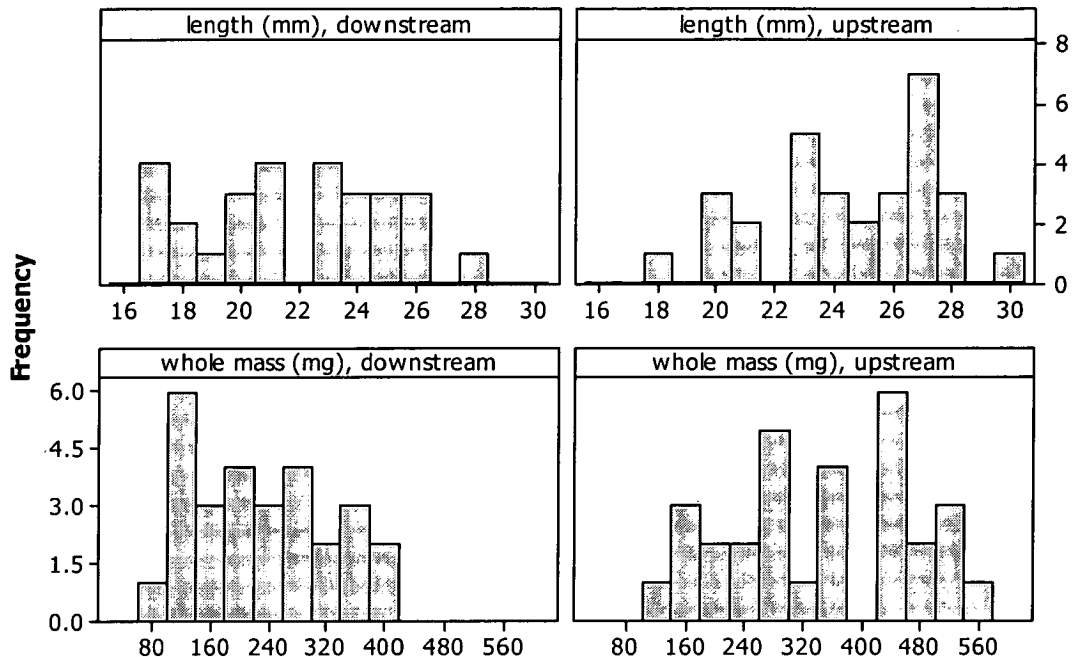


Figure 6.2 Standard lengths (mm) and whole mass (mg) of males sampled from upstream and downstream populations.

6.4.2 Testes investment

Initial examination of testes allometry highlighted two males (one from each population) with very small testes (0.08% and 0.19% of total body mass); for comparison, testes mass contributed a mean \pm SE of $1.1 \pm 0.04\%$ of total mass for upstream males (range: 0.6%-1.5%, $N = 29$) and a mean \pm SE of $1.4\% \pm 0.08\%$ of total mass for downstream males (range: 0.7% - 2.6%, $N = 27$). As the results of analyses differed with the inclusion and exclusion of the two males, we present results from both data sets below.

Firstly, we examined relative testes investment by males of different sizes using allometric slopes. The slope of the relationship between soma and testes mass did not differ from unity in either population (95%CI of RMA regression slopes included 1; table 6.2; fig. 6.3), regardless of whether we used the full or subset of data. Thus, smaller males did not have proportionally larger testes in comparison to larger males.

Table 6.2 RMA regression of \log_{10} testes mass (mg) on \log_{10} soma mass (mg) and \log_{10} gonopodium length (mm) on \log_{10} standard length (mm) for males from upstream (U) and downstream (D) populations.

Response	Pop'n	Slope	95% CI	Intercept	95% CI
	(N)	(SE)	slope	(SE)	intercept
Testes mass (all males)	D (28)	1.11 (0.19)	0.72, 1.51	-2.16 (0.45)	-3.08, -1.24
	U (30)	1.39 (0.23)	0.92, 1.86	-2.98 (0.57)	-4.16, -1.81
Testes mass (outliers removed)	D (27)	1.01 (0.14)	0.72, 1.30	-1.90 (0.32)	-2.56, -1.23
	U (29)	1.00 (0.09)	0.81, 1.19	-1.97 (0.23)	-2.43, -1.50
Gonopodium length	D (28)	0.94 (0.05)	0.84, 1.05	-0.39 (0.07)	-0.53, -0.25
	U (30)	0.95 (0.07)	0.80, 1.10	-0.40 (0.10)	-0.60, -0.19

Secondly, we examined other factors (gonopodium length, body condition factor, population) that might explain variation in testes mass. When we analysed the full data set, we found no population differences in testes mass (GLM: $F_{1,54} = 0.51$, $P = 0.478$) nor in the slope of the relationship between testes mass and soma mass, as indicated by a non-significant population and testes mass interaction (GLM: $F_{1,52} = 1.26$, $P = 0.267$). In fact, gonopodium length was better than soma weight at predicting testes mass (gonopodium length: GLM; $F_{1,54} = 13.02$, $P = 0.001$; soma mass: GLM: $F_{1,54} = 2.82$, $P = 0.099$). Males with a longer gonopodium had greater testes mass.

However, when we analysed the subset of data, we found different results. We found no relationship between gonopodium length and testes mass once data from the two males with very small testes were excluded (GLM: $F_{1,51} = 1.12$, $P = 0.296$). Soma weight was positively related to testes mass (GLM: $F_{1,53} = 94.26$, $P < 0.001$), and when soma weight was controlled for, there were marginally non-significant

differences in testes mass between populations (GLM: $F_{1,53} = 3.03$, $P = 0.088$). For a given soma mass, there was a trend for downstream males to have up to 1.3 times larger testes than upstream males (95%CI of ratio of downstream to upstream median testes size: 0.988, 1.323). The non-significant interaction between population and soma mass indicated that there was no significant slope heterogeneity between populations (GLM: $F_{1,50} = 2.08$, $P = 0.155$).

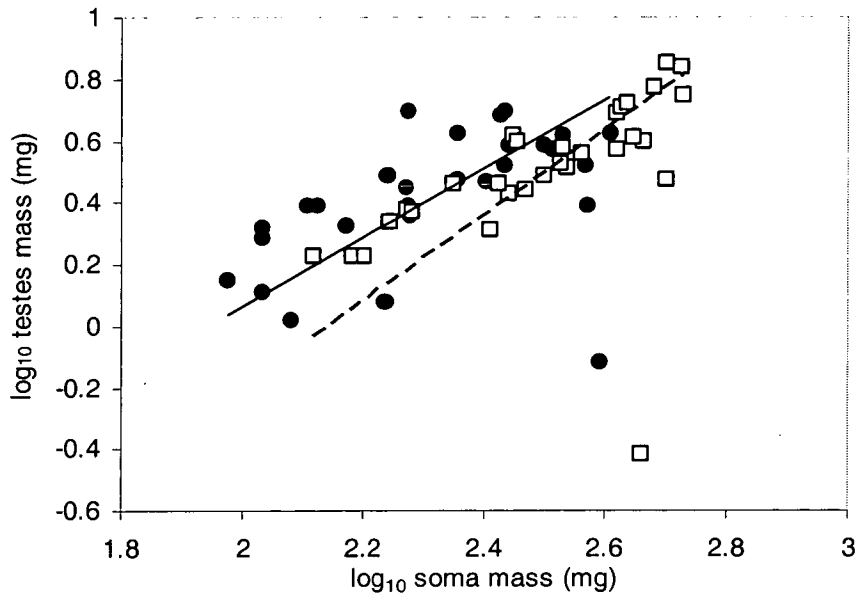


Figure 6.3 Data points and RMA regression lines of \log_{10} testes mass (mg) against \log_{10} soma mass (mg) for the full data set. Downstream males are represented by filled circles and solid line; upstream males are represented by open squares and dashed line.

We found no effects of body condition on testes mass with either data set (full data set: GLM: $F_{1,53} = 2.69$, $P = 0.107$; subset of data: GLM: $F_{1,52} = 2.80$, $P = 0.100$).

6.4.3 Gonopodium investment

Smaller males did not have proportionally longer gonopodia than larger males in either population (95%CI of RMA regression slopes included 1; table 6.2). There were no differences in gonopodium length between populations, when standard length was controlled for (GLM: $F_{1,55} = 1.07$, $P = 0.305$; estimated medians (from back transformed least square means): downstream vs upstream: 7.87mm vs 7.98mm).

Our measurements of gonopodium length were significantly greater than those reported by Jennions and Kelly (2002) for the same populations (One sample t-test: downstream against a mean of 6.37mm: $T_{27} = 7.56$, $P < 0.001$; upstream against a mean of 6.21mm: $T_{29} = 9.23$, $P < 0.001$). However, our CVs for gonopodium length (downstream 13.7%, upstream 11.7%) fell within the range reported by Jennions and Kelly (2002).

6.4.4 Male melanic markings and body conditions

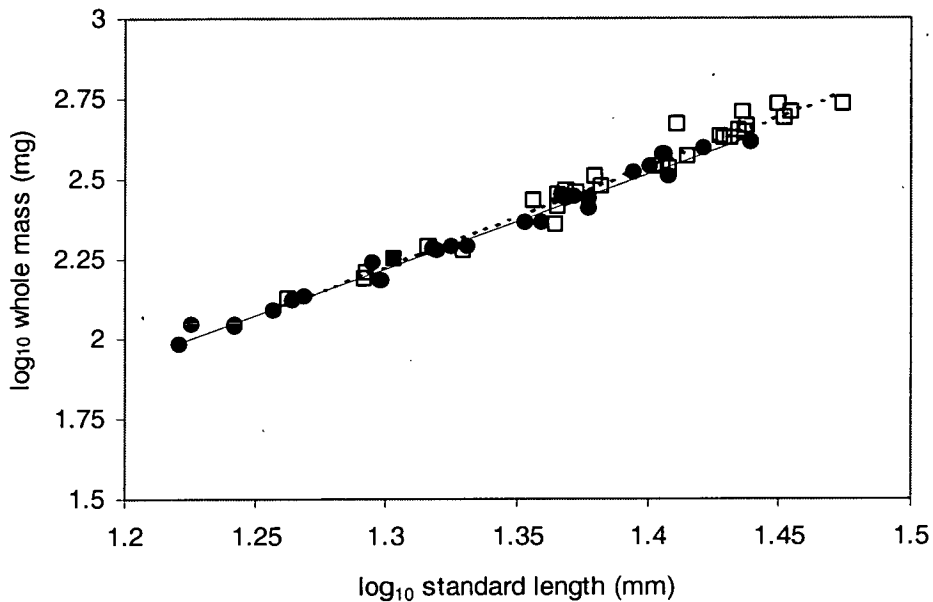


Figure 6.4 Data points and regression lines for \log_{10} whole mass (mg) against \log_{10} standard length (mm). Downstream males are represented by filled circles and solid line; upstream males are represented by open squares and dashed line.

We examined the relationship between whole mass and standard length to look at population variation in body condition, where greater mass for a given standard length indicates better body condition. For a given standard length, males from upstream populations tended to have a greater mass than males from downstream populations, but this result was marginally non-significant (GLM: $F_{1,55} = 3.73$, $P = 0.058$; fig. 6.4). An interaction among standard length and population was not significant (GLM: $F_{1,54} = 1.79$, $P = 0.187$), indicating no slope heterogeneity between populations.

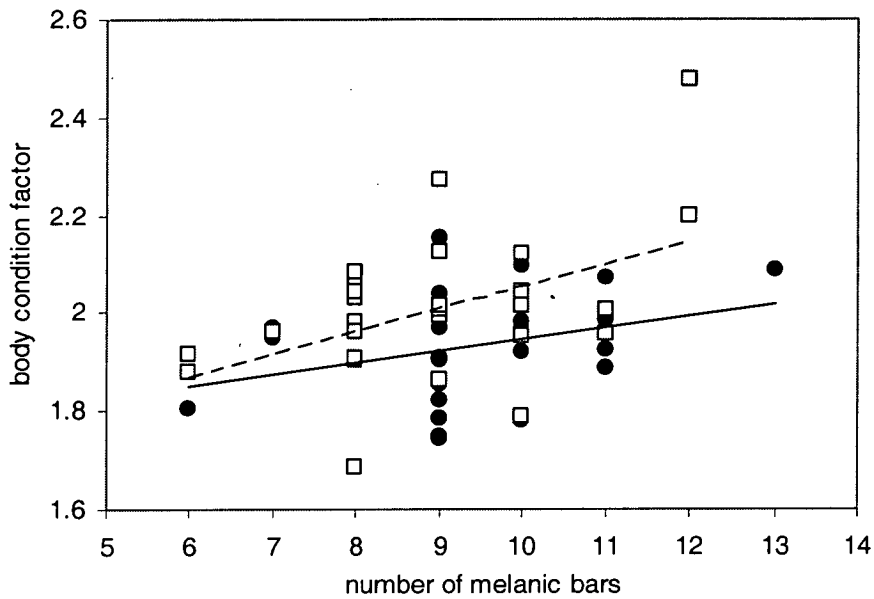


Figure 6.5 Relationship between number of melanic bars and body condition factor. Downstream males are represented by filled circles and solid line; upstream males are represented by open squares and dashed line.

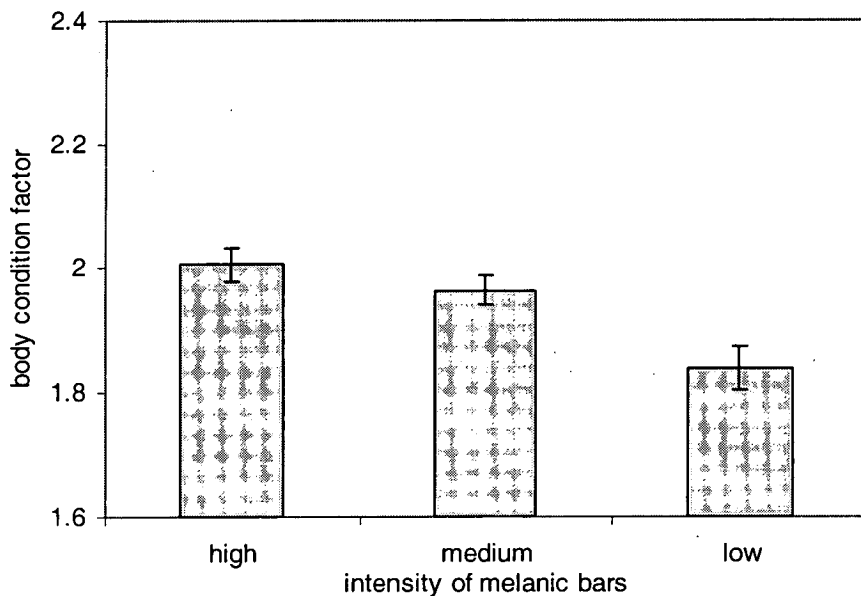


Figure 6.6 Least square mean body condition factor \pm SE for males with high, medium and low intensity melanic bars.

Males in better body condition had a greater number of melanic bars and more intensely-coloured bars (GLM: bar number: $F_{1,53} = 6.84$, $P = 0.012$; bar intensity: $F_{2,53} = 5.32$, $P = 0.008$; figs 6.5 & 6.6), however, the number and intensity of melanic bars varied between populations. Males in downstream populations had more bars

than males in upstream populations, controlling for body condition factor (GLM: $F_{1,55} = 4.29$, $P = 0.043$; least square means \pm SE: 9.57 ± 0.26 vs 8.80 ± 0.26 ; fig. 6.5). Males from the downstream population were more likely to have high-intensity bars, whereas males from the upstream population were more likely to have low-intensity bars (Chi-squared test: $\chi^2 = 14.4$, d.f. = 2, $P < 0.001$; table 6.3). Bar number was not significantly related to bar intensity or to standard length (GLM: bar intensity: $F_{2,52} = 1.01$, $P = 0.372$; standard length: $F_{1,54} = 1.43$, $P = 0.237$).

Table 6.3 Numbers of males with high, medium and low intensity bars for upstream and downstream populations (observed values in bold font; expected values in regular font; contributions to chi-squared test in italics). The intensity of bars was not independent of population ($\chi^2 = 14.4$, d.f. = 2, $P < 0.001$).

Population	Intensity of colour of bars			All
	High	Medium	Low	
downstream	16	11	1	28
	10.14	12.07	5.79	
	<i>3.390</i>	<i>0.095</i>	<i>3.966</i>	
upstream	5	14	11	30
	10.86	12.93	6.21	
	<i>3.164</i>	<i>0.088</i>	<i>3.701</i>	
Both	21	25	12	58

6.5 Discussion

Individuals of different size, status or quality may differ in relative investment into traits associated with reproduction. Here, we examined individual variation in testes investment, intromittent organ length and male colouration in two populations of male *B. episcopi*. We found no evidence that smaller males had proportionally larger testes nor proportionally larger gonopodia compared to larger males. Male body condition was not related to testes mass, but males in better condition had a greater number of melanic bars and more intensely-coloured bars than males in poorer

condition. We also found some population differences in melanic bars, and potentially in relative testes mass. We suggest possible explanations for variation between upstream and downstream populations below, but emphasize here that we cannot generalise to all up and downstream populations as we only sampled two populations.

6.5.1 Male body size

Turner (1938) observed that male *B. episcopi* had two tactics during sexual maturation: early maturation at a small size or delayed maturation at a large size. If true, maturation tactics do not appear to translate into bimodal size distributions of mature males as we found no evidence for a bimodal distribution in male standard length or whole body mass in either population. The results of the present study agreed with a study by Jennions & Telford (2002) that found unimodal distributions of log-transformed male standard lengths in 12 populations. We did not look at maturation time in the present study, but behavioural observations have shown little support for discrete size-based reproductive tactics in this species (Chapter 5).

We found population differences in male size: upstream males were longer and heavier than downstream males. Our results are consistent with patterns found in a larger study of the same species (Jennions & Kelly, 2002), and also patterns found in *B. rhabdophora* (Johnson & Belk, 2001) and *P. reticulata* (Reznick, 1989). These differences are likely to arise through the greater predation pressure experienced by downstream populations, which can select for smaller size at maturity (Reznick & Endler, 1982).

6.5.2 Testes investment

Smaller males did not invest proportionally more in testes mass than larger males. In combination with behavioural observations (Chapter 5), there is no evidence that smaller male *B. episcopi* attempt to increase their reproductive success either behaviourally or through increased sperm production. Of course, testes size may not

be related to sperm number in *B. episcopi*. In Arctic charr *Salvelinus alpinus*, for example, testes of subordinate males were relatively smaller than dominant males but had a higher relative density of sperm cells and higher relative sperm numbers (Liljedal & Folstad, 2003). There are other post-copulatory tactics that a male may use to increase his chances of paternity. In a number of fish species, males can vary ejaculate size in response to short-term changes in potential sperm competition risk (e.g. Pilastro et al., 2002; Evans et al., 2003a; Zbinden et al., 2003) or release substances that promote sperm longevity (e.g. Scaggiante et al., 1999). The sperm of more attractive males or smaller males may also be more fertile (e.g. guppy *P. reticulata*, Evans et al., 2003b). Furthermore, there is immense diversity in sperm size and morphology, particularly in internal fertilizers, that has been implicated in sperm competition (reviewed in Snook, 2005). We do not know whether any of the tactics listed above exist in *B. episcopi*.

We found two males with very small testes, although gonopodium morphology suggested that both males were mature. It is not clear why the testes were markedly smaller than other males, and perhaps we cannot justify the exclusion of these two males from the data. As the results depend on whether these two males were included or excluded from analyses, we discuss potential interpretations from each set of results, but firm conclusions cannot be made until we understand why these two males had such small testes.

When males with very small testes were excluded from the data, we found a trend for downstream males to have up to 1.3 times larger testes than upstream males for a given soma mass. Females in the downstream population receive between 1 and 2 thrusts more per hour than females in the upstream populations (Chapter 4), and it is possible that increased sperm competition downstream selects for proportionally large testes. However, it does seem unlikely that the slight population differences in coercive mating rates could lead to differences in testes mass. In two populations of guppies *P. reticulata* with rates of coercive matings that were much more disparate than in the present study, no differences in testes mass relative to body mass (gonadosomatic index) were found (Matthews et al., 1997; but see Tomkins &

Simmons, 2002 for discussion on the use of the gonosomatic index). In fact, only two inter-population studies that we are aware of found differences in testes mass that might be explained by differences in sperm competition (greenfinch *Carduelis chloris*, Merilä & Sheldon, 1999; dolphin *Stenella longirostris*, Perrin & Mesnick, 2003). Further inter-population comparisons of testes mass in *B. episcopi* will be required to determine whether population differences found in the present study are real, particularly as results were not upheld when all data were analysed.

In comparison to other poeciliids, the relative mean testes mass (as a percentage of body mass) of *B. episcopi* is noticeably smaller (*B. episcopi* 1.3% (present study); *P. reticulata*: 2.8%, (control group; Baatrup & Junge, 2001); *G. holbrooki*: 2.4% (uncontaminated groups in May; Toft et al., 2003)). Cross-species comparisons in other taxa suggest that relative testes mass is related to sperm competition (e.g. Byrne et al., 2002; Ramm et al., 2005; but see Kvarnemo & Simmons, 2004). Perhaps sperm competition in *B. episcopi* is not as important as in other poeciliids and a comparative study of testes size in the poeciliid fishes would now be timely.

6.5.3 Gonopodium length

In our analyses of the full data set, we found a stronger relationship between testes mass and gonopodium length than between testes and soma mass. Sexually-selected traits were positively associated with testes size in greenfinches *Carduelis chloris* (Merilä & Sheldon, 1999) and red deer *Cervus elaphus* (Malo et al., 2005), and it is therefore possible that gonopodium length could be an indicator of fertility. That male *B. episcopi* should benefit from advertising fertility seems unlikely. Perhaps males with longer gonopodium are preferred by females (e.g. Langerhans et al., 2005); preferred males obtain more matings and thus require greater sperm production. However, sexual selection may not act upon gonopodium length and perhaps testes mass has a tighter allometric relationship with gonopodium length than soma mass. Alternatively, measurement error may be greater than in soma mass than gonopodium length. This could arise as differences in gut content among

individuals at time of sacrifice might add noise to soma mass measurements, but would not affect gonopodium length.

Our measures of gonopodium length had a number of similarities with those of Jennions & Kelly (2002): they were highly repeatable with very low measurement error and had similar CVs but we found that allometric relationships between gonopodium length and body length did not differ from unity. Jennions & Kelly also reported that gonopodium length was longer in low compared to high predator density sites, but we found no population differences in gonopodium length. However, our measures of gonopodium length were significantly longer; we measured between the anterior of the joint between gonopodium/soma joint and the tip (Kelly et al., 2000) but Jennions & Kelly measured gonopodium length between the last horizontal base segment and the tip. The different techniques may explain discrepancies between our results and Jennions & Kelly.

Jennions & Kelly (2002) did not favour sexual selection to explain variation in gonopodium length of *B. episcopi* as gonopodium length had a low CV (in comparison to other sexually selected traits), a negative allometric relationship and could not be explained by variation in sex ratio. Whilst these observations suggest that sexual selection is unlikely to explain variation in gonopodium length, they do not exclude it. Firstly, little is known about the effects of sexual conflict on allometric relationships (Hosken & Stockley, 2004). Secondly, low CV and negative allometry seem to be common for genital traits in insects (Hosken & Stockley, 2004). Thirdly, sex ratios in *B. episcopi* may show marked temporal variation: Jennions & Kelly reported that sex ratios were more female-biased in high than low predation sites but subsequent data found that sex ratios were more female-biased in low than high predation sites (Chapter 4; also C. Brown & V. A. Braithwaite, unpublished data). If sex ratio exhibits temporal variation, then it may not be a good predictor of levels of sexual selection within a population.

6.5.4 Male melanic markings and body condition

The function of melanic markings in *B. episcopi* is not known, but could be involved in species recognition, camouflage from predators and/or sexual selection. Our preliminary exploration of male markings found that males in better body condition had a greater number of bars and more intensely-coloured bars than males in poorer body condition. The relationship between melanic markings and body condition suggests that melanic markings may be costly to produce and could potentially be an honest indicator of male quality. In general, melanin-based ornaments are not thought to be as costly as carotenoid-based ornaments and are usually associated with intrasexual competition rather than mate choice (Badyaev & Hill, 2000), but recent work suggests that melanin-based ornaments may function as signals in both intra- and intersexual selection (Jawor & Breitwisch, 2003; Tarof et al., 2005). Male swordtails have vertical pigmented bars, which are involved in intrasexual competition in *Xiphophorus multilineatus* (Morris et al., 1995) and in female choice in *X. cortezi* (preference for bar number and symmetry, Morris, 1998; preference for bar area, Morris et al., 2001). We do not know whether female mate choice is important in *B. episcopi*, or whether the amounts of markings predict the outcome of male-male agonistic interactions as in swordtails. If melanic markings are involved in sexual selection, we would expect high variation in the amount of markings between males and for males to be more highly coloured than females. Previous reports about *B. episcopi* disagree as to whether colouration differs between the sexes (reviewed in Farr, 1989) but with the development of photographic colour analysis, future quantitative examination of colour variation should be straightforward and potentially fruitful.

We also found population variation in male melanic markings. For a given body condition, downstream males have more bars than upstream males. Markings could be important for camouflage against predators (Endler, 1978) and if markings have an anti-predatory role, we would expect similar population differences in female markings. Pigment cells in fish can change with a variety of external and internal stimuli over a short-time period (Bagnara & Hadley, 1973), for example, during

transfer from the field to the laboratory (Barber et al., 2000). Thus, it may be necessary to quantify markings on fish immediately after capture and without anaesthetic to obtain reliable estimates to investigate the above hypotheses.

6.5.5 Summary

To conclude, we found no evidence of compensatory testes investment by small males in two populations of *B. episcopi* although there was a trend for smaller males to have proportionally longer gonopodium. There is evidence of individual variation in melanic markings, with preliminary support for the hypothesis that markings are condition-dependent. We suggest that the role of markings in this species could be a fruitful area for further research. We found differences between populations in melanic markings and potentially relative testes mass, which also warrant further investigation.

6.6 Acknowledgements

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7. Discussion

Behaviour, including reproductive behaviour, is not invariant within species but shows variation among populations, among individuals and also varies on a temporal scale. In this thesis, I have found evidence for variation in mating behaviour in the male Panamanian bishop *Brachyrhaphis episcopi* at each of these levels. My results reveal that male *B. episcopi*, though promiscuous, exhibits mate choice for large females and unfamiliar females. Males show plasticity in their choice of mates over a short time scale as light levels change and also over a longer time scale between seasons. The intensity of male mating behaviour varies between males of different sizes, and between populations. Below, I discuss how these results contribute to our current understanding of within-species variation in mating behaviour and suggest potential fruitful avenues for further study.

As with so many other species, the mating behaviour of male *B. episcopi* varies with male size. Both in the absence and presence of competing males, smaller males usually had the lowest levels of mating activity (small males in Rio Macho upstream populations were perhaps an exception to this generalisation; Chapter 5, Appendix 1). Whilst I expected the effects of male size to hold for *in situ* observations, only aggression to other males showed a positive relationship with male size. I do not attempt to infer much from the null effects of male size on other mating behaviours as I am not confident that my measure of male size was sufficiently accurate (Chapter 4). In several taxa, males of lower status (quality, size, age) adopt alternative tactics (Gross, 1996) but I found that smaller males did not attempt more coercive matings than large males (Chapter 4, Appendix 1) nor did they invest proportionally more into testes size (Chapter 6). However, other poeciliids show plasticity in ejaculate size and sperm priming (Evans et al., 2003a; Aspbury & Gabor, 2004), and variable insemination or fertilisation success in relation to male size and colour (Bisazza & Pilastro, 1997; Evans et al., 2003b). Thus, it is likely that adaptations for sperm competition exist in *B. episcopi* and could be a productive area for future research.

In addition to intrinsic effects of size, male behaviour was affected by the size of a rival male; however, patterns were not consistent among populations making it difficult to make any broad conclusions (Chapter 5). Whilst the results suggested that, within a pair of males, large and small males behaved differently, I could not exclude the possibility that the results might be explained by a non-linear relationship between male size and male behaviour. As discussed in Chapter 5, further work is needed in this area to determine the exact role of competition on male-mating behaviours.

Although male-male competition and accordingly male size seems to be an important determinant of male mating success in *B. episcopi*, males are smaller than females. Reversed-sexual size dimorphism is typical in poeciliid fish (Endler, 1983) and is common in other teleost fish and most taxa, except for higher vertebrates (Bisazza, 1993). Endler (1983) and Bisazza (1993) review hypotheses to explain reverse size dimorphism in poeciliid fish: these include selection for larger females (e.g. greater fecundity or larger offspring that are less at risk of being cannibalised), selection for smaller males (e.g. better insemination ability, shorter generation time, reduced mortality prior to reproduction) and an assumption that different forces are acting on different sexes, keeping each one close to a survival and reproductive optimum. Evidence to date in five tribes of poeciliids shows that, despite advantages of large male size in intrasexual competition and female mate choice, thrust attempts by small males are more likely to result in successful copulation, which may explain the maintenance of small poeciliid males (Bisazza & Pilastro, 1997). Of course, the number of successful copulations, or any other behavioural measure, may not be a reliable indication of a male's fitness. Perhaps more useful would be the application of microsatellite markers to determine the relative number of offspring fathered by males of different sizes to really understand how sexual selection acts on males of different sizes and whether it can explain the degree of reverse sexual size dimorphism in poeciliids.

Light levels have been found to affect the behaviour of male guppies *P. reticulata*, in particular the timing and frequency of courtships (Endler, 1987; Reynolds et al.,

1993), but did not affect overall frequencies of male behaviour in *B. episcopi* (Chapter 4). Rather, light levels appear to affect male mate choice for unfamiliar females (Simcox et al., 2005; Chapter 2 this thesis). Males from high predator density sites showed a courtship preference for unfamiliar females only in dim light, whereas males from low predator density sites showed the same preference in bright light. If bright light is consistent with an increased risk of predation as seen in guppies then my results suggest that males from high predation populations exhibit plasticity in mate choice in response to predation pressure.

There are a number of reasons why males might alter mating preferences under predation risk: (1) courtship may increase the risk of predation; (2) mate searching may increase the risk of predation; (3) male mate choice may change under predation pressure; (4) males make wrong decisions about mate choice under predation pressure. There is no reason to suppose that the hypotheses are exclusive, although I discuss each in turn below.

(1) Courtship display may increase the risk of predation (Endler, 1987). There is no evidence to support this hypothesis in *B. episcopi* as total courtship frequencies did not decrease as light levels increased (Chapter 2).

(2) Mate searching may be costly in the presence of a predator, so males may court females indiscriminately to reduce search time (Gwynne, 1989; Crowley et al., 1991). Under this hypothesis, males should attempt to mate with the first female that they meet (e.g. Grafe, 1997) and the distance that a male would be prepared to swim to reach a preferred female might decrease as predation risk increases. To test these predictions, firstly, data could be collected on the rate at which males reject females under predation pressure. Secondly, male mate choice could be tested under predation risk to see whether males will swim a greater distance to a preferred females or a shorter distance to an unpreferred female. Experimental evidence to date suggests that individuals become less choosy as search costs increase: female three-spined stickleback *Gasterosteus aculeatus* are less likely to reject an unattractive male when the energetic costs of accessing mates increase (Milinski & Bakker,

1992), and when the energy expenditure required to access an attractive mate increases, male Pacific blue-eye *Pseudomugil signifer* choose a previously unattractive mate that requires less energy to access (Wong & Jennions, 2003).

(3) Males may alter their choice of mates in the presence of a predator as associating with certain individuals may increase predation risk (Gong & Gibson, 1996; Johnson & Basolo, 2003). Whilst there is no reason to expect that unfamiliar females should be more attractive to predators than familiar females, males may prefer to associate with familiar females under predation pressure (Farmer et al., 2004; but see Griffiths, 1997; Brown, 2002). However, male *B. episcopi* did not show a preference to associate with familiar females under any conditions. Furthermore, under bright light (which may be associated with increased predation risk. Simcox et al., 2005 Chapter 2 this thesis) males from high predator density populations courted both females equally: rather than reversing their choice, they showed no choice at all.

(4) Males may attempt to choose between females but may make wrong decisions under predation pressure (Forsgren, 1992). To detect that a male is making the wrong decision, we must know what the correct decision would be: male mate choice must be predictable at either the population or individual level in the absence of predation risk. The present hypothesis suggests that, under predation risk, males should still exhibit choosiness but there would be little consistent direction to mate choice. At a population level, among-male variation in mate choice would increase under predation risk. At the individual level, males would be unlikely to show repeatable mate choice decisions under predation pressure, but should show repeatable choices in predator-free trials. It is possible, however, that not all males will exhibit the same mating preferences or that males will make different choices over time, which would make the present hypothesis very difficult to investigate. As far as I am aware, the effects of predation on mistakes in decision making have not been studied, although stress is known to affect cognitive ability (Lupien & McEwen, 1997).

Recent work in guppies *P. reticulata* suggests that females, not males, respond to perceived changes in predation risk (Evans et al., 2002) and potentially female

behaviour rather than male choice could explain the results in Chapter 2 (this thesis; Simcox et al., 2005). Females may have a mating preference for unfamiliar males (Hughes et al., 1999); males might preferentially court unfamiliar females, which are more likely to allow the male to mate. Thus, what appears as male mate choice for unfamiliar females may in fact be a choice for receptive females. Under predation risk, females become less choosy and so males direct courtship at random. To examine whether mate choice for unfamiliar individuals is really based on male or female mating preferences, one could manipulate asymmetry of knowledge of familiarity. Familiarity in fish seems to be based primarily on olfactory cues (reviewed in Ward & Hart, 2003). Using a uni-directional flow of water, it might be possible to manipulate which sex has knowledge of familiarity and test whether female or male knowledge of familiarity affects male mate choice. If important, visual cues could also be incorporated into the experiment using one-way glass. To examine whether females, rather than males, become less choosy under predation pressure, one could manipulate knowledge of predation risk by exposing only one sex to a predator (e.g. Godin & Briggs, 1996; Evans et al., 2002). Lack of knowledge about female choice in *B. episcopi* does place a caveat on the conclusions that can be made about male mate choice and is clearly an important area for future research.

Whilst female mate choice could explain why males seem to have a mating preference for unfamiliar females, it is less likely that it could explain why male mating preference for larger females is stronger in the dry than the wet season (Chapter 3 this thesis). There are good explanations for a female preference for unfamiliar males (e.g. inbreeding avoidance, male novelty, genetic heterozygosity of offspring) but there seems little plausible reason to expect that larger females should be more willing to mate in the dry season than any other time.

I present perhaps the first documented occurrence of courtship in natural populations of *B. episcopi*. However, males perform courtships at much higher frequencies in confined, artificial environments than *in situ*, in both the presence and absence of other males. Why might behavioural discrepancies arise under different experimental designs? Females may find it difficult to avoid males in a confined environment or

become less active in a novel environment, which males might interpret as a signal of female receptivity and willingness to mate. If a novel environment triggers courtship, courtship should become less frequent as time kept in the same environment increases. If a confined environment determines courtship activity, courtship should become less frequent as the size of the confined area increases. These hypotheses could be easily tested in wild-caught individuals housed in the laboratory in different sized enclosures for a long period of time. Although these specific questions are not of broad evolutionary interest, it could be important to determine why differences arise between observations made *in situ* and in artificial environments, and whether these differences matter. I have shown, for example, that males have mating preferences for unfamiliar females (Simcox et al., 2005; Chapter 2 this thesis). If the artificial environment affects female behaviour, it should affect both familiar and unfamiliar females identically and is thus unlikely to alter or create male mating preferences. If male behaviour is affected by the environment, it is of greater concern: we may conclude that males have a mating *preference* for unfamiliar females but we cannot know whether mate *choice* occurs in the wild or whether it is simply an artefact of the environment. These issues are of wider relevance to any laboratory study of behaviour where the researcher intends to infer something about the adaptive significance of behaviour.

In situ observations on the mating behaviour of *B. episcopi* suggested that males alter their behaviour in response to variation in OSR (Chapter 4). Given the effect that OSR has effects on mating behaviour in a wide-range of other species (reviewed in Kvarnemo & Ahnesjö, 1996), this result is not surprising. In agreement with the majority of studies, as OSR became more female-biased, male-male competition decreased (in two of three streams) and males spent more time associating with females. Further work is required to determine whether male behaviour shows plasticity in response to short-term changes in OSR, whether male behaviour is fixed at maturation at a level appropriate for local OSR, or both. The latency to respond to changes in OSR has not been explicitly examined in poeciliid fish and thus information in published literature is somewhat conflicting: Jirotukul (1999) found that male guppies *P. reticulata* responded behaviourally to changes in OSR within 24

hours, but Evans & Magurran (1999) found that male guppy behaviour did not alter within the same time period. Male eastern mosquitofish *Gambusia holbrooki* altered their behaviour within an 8-day period in response to changes in OSR (Evans et al., 2003a). It seems likely that male behaviour may not alter immediately in response to changes in OSR, but rather may change over a few hours or days as males acquire information about their environment.

Males may respond to changes in competition associated with OSR by dispersing to sites where competition is lower. Mark-release-recapture studies have not found any sex differences in philopatry in *B. episcopi* (C. Brown, F. Jones, N. Brydges & V. A. Braithwaite, unpublished data), but if it does occur, it would be interesting to see whether small males are particularly likely to disperse to sites with a more female-biased sex ratio. Male-biased dispersal has been found in brook trout *Salvenius fontinalis* (Hutchings & Gerber, 2002) and has also been suggested for guppies *P. reticulata* (Griffiths & Magurran, 1998). Size at maturation may be a further trait that exhibits plasticity in response to levels of intrasexual competition. Certainly, male-male interactions seem to affect size at maturation in many, but not all, poeciliid males (reviewed in Snelson, 1989; also see Bisazza et al., 1996).

It seems that there are many potential opportunities for further work into the effects of OSR on sexual behaviours, and how OSR interacts with other factors. For example, not all males reduce their mating behaviour to the same extent under predation risk (Reynolds et al., 1993; Godin & Briggs, 1996); under predation pressure, some males may not attempt to mate at all, temporarily altering OSR. Fluctuations in OSR on a temporal as well as geographical scale may allow less competitive males to increase their reproductive success, weakening the strength of intrasexual competition and sexual selection.

Whilst I found potential effects of predation pressure on male mate choice (Simcox et al., 2005; Chapter 2 this thesis), there were few effects on population norms of male mating behaviour (Chapter 4). A slightly, but significantly, higher rate of male thrusts was observed in high predator density sites compared to low predator density

sites in only one stream. Predators were present in all high predator density sites when observations were made, which could promote increased thrust rates and consequently obscure any population comparisons. However, populations of *B. episcopi* that live in high predator density sites may rarely experience a temporarily predator free environment so my observations are likely to be representative of population norms of mating behaviours. It is unlikely that predation pressure has not influenced mating behaviour but perhaps, like guppies, the real difference between high and low predator density sites is the plasticity of the response to predation, which may increase with the level of predation that a population experiences. Although I found no evidence of plasticity in mating behaviour proportional to the number of predatory fish in the immediate environment, the effects of predator communities on their prey may be more complex than simply counting numbers of predators or comparing high and low risk sites (Reznick & Endler, 1982; Sih et al., 2000). Of additional interest is the role of aerial and land-based predators of *B. episcopi*. No study has yet tried to quantify predation pressure associated with non-Piscean predators so it cannot be ruled out. My own personal observations suggested that fish in Quebrada Juan Grande had a greater tendency to hide than fish in other streams when a person approached the stream, which could be an anti-predator response against piscivorous wading birds. In general, Quebrada Juan Grande is also a relatively wide stream with little canopy cover so perhaps attracts more aerial predators.

Of course, it is possible that populations of *B. episcopi* with disparate predator pressures have not diverged in mating behaviours. Whilst this seems unlikely given that other aspects of the biology of this species seem to have diverged under differential predation pressure (Jennions & Telford, 2002; Brown & Braithwaite, 2004; Brown et al., 2004; Brown & Braithwaite, 2005; Brown et al., 2005a; Brown et al., 2005b), a lack of divergence may occur for a number of reasons. High levels of gene flow between populations could prevent the evolution of adaptive responses; insufficient time may have passed for populations to respond to selection, or selection on correlated traits could constrain behavioural evolution (reviewed in Thompson, 1999). Gene flow no doubt occurs at low levels from low to high

predation density populations in *B. episcopi*, but is probably highly restricted in the other direction as fish would have to swim against waterfalls. As other behavioural traits in *B. episcopi* show population differentiation consistent with disparate predation pressure it seems unlikely that mating behaviour could not have had sufficient time to evolve, but whether the evolution of mating behaviour is more constrained than other behaviours is not known.

All work exploring the effects of predation on *B. episcopi* to date have been correlational and no causal relationships have been established. However, consistent effects of predation across a number of replicate streams, which differ in canopy cover, elevation, flow, increase the likelihood that predation itself is responsible for observed population differentiation and not a covarying environmental factor (Foster & Endler, 1999a). Covarying environmental factors within streams can also be minimised by selecting high and low predation sites that are close together (Reznick & Endler, 1982). The streams used in the present study varied in the age of the forest, level of disturbance, elevation and canopy cover; and additionally, high and low predation sites were chosen close to the waterfall barrier to minimise genetic differentiation (e.g. Brown & Braithwaite, 2004) and environmental differences. Additional support for the role of predation arises through comparisons with manipulative studies in other species. In particular, I refer to Reznick & Endler's (1982) measurements on life-history patterns in guppies introduced into sites with different predator communities; the results of which are mimicked by life-history studies of natural populations of *B. episcopi* (Jennions & Telford, 2002) and the congener *B. rhabdophora* (Johnson & Belk, 2001).

Examination of multiple populations is clearly necessary for comparative within-species analyses, but it is also important whenever a study is used to generalise about the behaviour of a species. Lack of replicated populations is a weakness in many behavioural studies, and I myself fall foul of this in Chapters 3 & 6, where I carried out studies in two populations from within a single stream. The need to test populations from multiple streams is highlighted by the number of studies in which I found significant differences among streams. Mating behaviour of *B. episcopi*

differed in both magnitude and direction among streams, making it difficult to generalise across all streams. Other behavioural studies into *B. episcopi* also found significant among-stream differences (Brown & Braithwaite, 2004; Brown & Braithwaite, 2005; Brown et al., 2005b). These differences may of course be adaptive, but they may also arise from founder events, where a small number of individuals colonized each stream. *B. episcopi* are most closely related to *Brachyrhaphis* spp. found on the Pacific rather than the Atlantic side of Panama and thus dispersal from the Pacific side is most likely (Mojica, 1998). Some genetic differentiation seems to be associated with allopatric populations of *B. episcopi*: the sequence of a region of cytochrome *b* gene from the mitochondrial genome differed by 3% between a population from the mainland Parque Nacional Soberania and a nearby isolated population on Isla Barro Colorado in the Panama Canal (Mojica et al., 1997). The levels of genetic differentiation among my study populations are not known but knowledge of the source and dispersal of populations in each stream would be very useful for evolutionary studies of behaviour in *B. episcopi*.

As demonstrated in this thesis and elsewhere (reviewed in Foster & Endler, 1999c), mating behaviour cannot be assumed to be invariant within species. Behavioural variation is no longer considered to be a source of noise and may often be adaptive. There are many potentially fruitful avenues of research that could contribute to a greater understanding of the causation of within-species variation in mating systems. For example, how widespread is temporal and spatial variation in mate choice and what implications does it have for models of sexual selection? How exactly does predation risk affect the strength of mate choice, and what are the effects of predator community on prey behaviour? To what extent do individuals exhibit plasticity in reproductive behaviour in response to environmental fluctuations? Is behavioural variation purely a plastic response to the environment or is there evidence of genetic diversity among populations? With multiple populations subject to variation in environmental factors over a small geographic area, the Panamanian bishop *Brachyrhaphis episcopi* could be an ideal species for further study of within-species variation in a natural setting. The causation of variation of mating and other

behaviours will no doubt be a challenging and exciting area of evolutionary research in coming years.

8. References

- Abrams, P. A. & Rowe, L. 1996. The effects of predation on the age and size of maturity of prey. *Evolution*, **50**, 1052-1061.
- Adler, N. T. 1978. On the mechanisms of sexual behaviour and their evolutionary constraints. In: *Biological determinants of sexual behaviour* (Ed. by Hutchison, J. B.), pp. 657-695. Chichester: John Wiley & Sons.
- Aeschlimann, P. B., Häberli, M. A., Reusch, T. B. H., Boehm, T. & Milinski, M. 2003. Female sticklebacks *Gasterosteus aculeatus* use self-reference to optimize MHC allele number during mate selection. *Behavioural Ecology and Sociobiology*, **54**, 119-126.
- Almada, V. C., Goncalves, E. J., Oliveira, R. F. & Santos, A. J. 1995. Courting females: ecological constraints affect sex roles in a natural population of the blennioid fish *Salaria pavo*. *Animal Behaviour*, **49**, 1125-1127.
- Amundsen, T. & Forsgren, E. 2001. Male mate choice selects for female coloration in a fish. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 13155-13160.
- Amundsen, T. & Forsgren, E. 2003. Male preference for colourful females affected by male size in a marine fish. *Behavioral Ecology and Sociobiology*, **54**, 55-64.
- Amundsen, T., Forsgren, E. & Hansen, L. T. T. 1997. On the function of female ornaments: male bluethroats prefer colourful females. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **264**, 1579-1586.
- Andersson, M. & Iwasa, Y. 1996. Sexual selection. *Trends In Ecology & Evolution*, **11**, A53-A58.
- Angermeier, P. L. & Karr, J. R. 1983. Fish communities along environmental gradients in a system of tropical streams. *Environmental Biology of Fishes*, **9**, 117-135.
- Arnold, K. E. & Owens, I. P. F. 2002. Extra-pair paternity and egg dumping in birds: life history, parental care and the risk of retaliation. *Proceedings Of The Royal Society Of London Series B-Biological Sciences*, **269**, 1263-1269.
- Aspbury, A. S. & Gabor, C. R. 2004. Differential sperm priming by male sailfin mollies (*Poecilia latipinna*): Effects of female and male size. *Ethology*, **110**, 193-202.
- Aubin-Horth, N. & Dodson, J. J. 2004. Influence of individual body size and variable thresholds on the incidence of a sneaker male reproductive tactic in Atlantic salmon. *Evolution*, **58**, 136-144.
- Baatrup, E. & Junge, M. 2001. Antiandrogenic pesticides disrupt sexual characteristics in the adult male guppy (*Poecilia reticulata*). *Environmental Health Perspectives*, **109**, 1063-1070.
- Badyaev, A. V. & Hill, G. E. 2000. Evolution of sexual dichromatism: contribution of carotenoid- versus melanin-based coloration. *Biological Journal Of The Linnean Society*, **69**, 153-172.
- Baerends, G. P., Brouwer, R. & Waterbolck, T. J. 1955. Ethological studies on *Lebistes reticulatus* (Peters). *Behaviour*, **8**, 249-334.

- Bagnara, J. & Hadley, M. 1973. Chromatophores and pigments. In: *Fish Physiology v. III: Reproduction and growth, bioluminescence, pigments, and poisons* (Ed. by Hoar, W. S. & Randall, D. J.). New York: Academic Press.
- Baker, A. J. & Jenkins, P. F. 1987. Founder effect and cultural evolution of songs in an isolated population of chaffinches, *Fringilla coelebs*, in the Chatham Islands. *Animal Behaviour*, **35**, 1793-1803.
- Balmford, A. 1991. Mate choice on leks. *Trends In Ecology & Evolution*, **6**, 87-&.
- Barber, I., Arnott, S. A., Braithwaite, V. A., Andrew, J., Mullen, W. & Huntingford, F. A. 2000. Carotenoid-based sexual coloration and body condition in nesting male sticklebacks. *Journal Of Fish Biology*, **57**, 777-790.
- Barber, I., Nairn, D. & Huntingford, F. A. 2001. Nests as ornaments: revealing construction by male sticklebacks. *Behavioral Ecology*, **12**, 390-396.
- Basolo, A. 2004. Variation between and within the sexes in body size preferences. *Animal Behaviour*, **68**, 75-82.
- Bateman, A. J. 1948. Intrasexual selection in *Drosophila*. *Heredity*, **2**, 349-368.
- Baube, C. L. 1997. Manipulations of signalling environment affect male competitive success in three-spined sticklebacks. *Animal Behaviour*, **53**, 819-833.
- Belwood, J. J. & Morris, G. K. 1987. Bat predation and its influence on calling behavior in Neotropical katydids. *Science*, **238**, 64-67.
- Berger, J. & Cunningham, C. 1987. Influence of familiarity on frequency of inbreeding in wild horses. *Evolution*, **41**, 229-231.
- Berglund, A. 1993. Risky sex: male pipefishes mate at random in the presence of a predator. *Animal Behaviour*, **46**, 169-175.
- Berglund, A. 1994. The operational sex ratio influences choosiness in a pipefish. *Behavioral Ecology*, **5**, 254-258.
- Berglund, A. 1995. Many mates make male pipefish choosy. *Behaviour*, **132**, 213-218.
- Berven, K. A. 1981. Mate choice in the wood frog, *Rana sylvatica*. *Evolution*, **35**, 707-722.
- Billard, R. 1986. Spermatogenesis and spermatology of some teleost fish species. *Reproduction Nutrition Development*, **26**, 877-920.
- Birkhead, T. R., Buchanan, K. L., Devoogd, T. J., Pellatt, E. J., Szekely, T. & Catchpole, C. K. 1997. Song, sperm quality and testes asymmetry in the sedge warbler. *Animal Behaviour*, **53**, 965-971.
- Bisazza, A. 1993. Male competition, female mate choice and sexual size dimorphism in Poeciliid fishes. *Marine Behaviour and Physiology*, **32**, 257-286.
- Bisazza, A. & Marin, G. 1995. Sexual selection and sexual size dimorphism in the eastern mosquitofish *Gambusia holbrooki* (Pisces:Poeciliidae). *Ethology Ecology & Evolution*, **7**, 169-183.
- Bisazza, A., Novarini, N. & Pilastro, A. 1996. Male body size and male-male competition: Interspecific variation in poeciliid fishes. *Italian Journal Of Zoology*, **63**, 365-369.
- Bisazza, A. & Pilastro, A. 1997. Small male mating advantage and reversed size dimorphism in poeciliid fish. *Journal of Fish Biology*, **50**, 397-406.
- Blouin, S. F. & Blouin, M. 1988. Inbreeding avoidance behaviors. *Trends in Ecology & Evolution*, **3**, 230-233.

- Bohonak, A. J. & van der Linde, K. 2004. RMA: Software for Reduced Major Axis regression, Java version.: Website: <http://www.kimvdline.com/professional/rma.html>.
- Bonduriansky, R. 2001. The evolution of male mate choice in insects: a synthesis of ideas and evidence. *Biological Reviews*, **76**, 305-339.
- Borg, A. A., Forsgren, E. & Magnhagen, C. 2002. Plastic sex-roles in the common goby - the effect of nest availability. *Oikos*, **98**, 105-115.
- Boughman, J. W. 2001. Divergent sexual selection enhances reproductive isolation in sticklebacks. *Nature*, **411**, 944-948.
- Braithwaite, V. A. & Barber, I. 2000. Limitations to colour-based sexual preferences in three-spined sticklebacks (*Gasterosteus aculeatus*). *Behavioral Ecology and Sociobiology*, **47**, 413-416.
- Brick, O. 1998. Fighting behaviour, vigilance and predation risk in the cichlid fish *Nannacara anomala*. *Animal Behaviour*, **56**, 309-317.
- Briggs, S. E., Godin, J. G. J. & Dugatkin, L. A. 1996. Mate-choice copying under predation risk in the Trinidadian guppy (*Poecilia reticulata*). *Behavioral Ecology*, **7**, 151-157.
- Brockmann, H. J. & Penn, D. 1992. Male mating tactics in the horseshoe crab, *Limulus polyphemus*. *Animal Behaviour*, **44**, 653-665.
- Brooks, R. 1999. Mate choice copying in guppies: females avoid the place where they saw courtship. *Behaviour*, **136**, 411-421.
- Brown, C. 2002. Do female rainbow fish (*Melanotaenia* spp.) prefer to shoal with familiar individuals under predation pressure? *Journal of Ethology*, **20**, 89-94.
- Brown, C. & Braithwaite, V. A. 2004. Size matters: a test of boldness in eight populations of bishop, *Brachyrhaphis episcopi*. *Animal Behaviour*, **68**, 1325-1329.
- Brown, C. & Braithwaite, V. A. 2005. Effects of predation pressure on the cognitive ability of the poeciliid *Brachyrhaphis episcopi*. *Behavioral Ecology*, **16**, 482-487.
- Brown, C., Gardner, C. & Braithwaite, V. A. 2004. Population variation in lateralized eye use in the poeciliid *Brachyrhaphis episcopi*. *Proceedings of the Royal Society of London Series B-(Supplement)*, **271**, S455-S457.
- Brown, C., Gardner, C. & Braithwaite, V. A. 2005a. Differential stress responses in fish from areas of high and low predation pressure. *Journal of Comparative Physiology*, **175**, 305-312.
- Brown, C., Jones, F. C. & Braithwaite, V. A. 2005b. In situ examination of boldness-shyness traits in the tropical poeciliid *Brachyrhaphis episcopi*. *Animal Behaviour*, **70**, 1003-1009.
- Bruce, K. E. & White, W. G. 1995. Agonistic relationships and sexual behavior patterns in male guppies, *Poecilia reticulata*. *Animal Behaviour*, **50**, 1009-1021.
- Byrne, P. G., Roberts, J. D. & Simmons, L. W. 2002. Sperm competition selects for increased testes mass in Australian frogs. *Journal Of Evolutionary Biology*, **15**, 347-355.
- Candolin, U. 1997. Predation risk affects courtship and attractiveness of competing threespine stickleback males. *Behavioral Ecology And Sociobiology*, **41**, 81-87.

- Candolin, U. 1998. Reproduction under predation risk and the trade-off between current and future reproduction in the threespine stickleback. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **265**, 1171-1175.
- Candolin, U. & Voigt, H. R. 1998. Predator-induced nest site preference: safe nests allow courtship in sticklebacks. *Animal Behaviour*, **56**, 1205-1211.
- Candolin, U. & Voigt, H. R. 2003a. Do changes in risk-taking affect habitat shifts of sticklebacks? *Behavioral Ecology and Sociobiology*, **55**, 42-49.
- Candolin, U. & Voigt, H. R. 2003b. Size-dependent selection on arrival times in sticklebacks: Why small males arrive first. *Evolution*, **57**, 862-871.
- Carroll, S. P. & Salamon, M. H. 1995. Variation in sexual selection on male body size within and between populations of the soapberry bug. *Animal Behaviour*, **50**, 1463-1474.
- Chapman, L. J. & Kramer, D. L. 1991. The consequences of flooding for the dispersal and fate of Poeciliid fish in an intermittent tropical stream. *Oecologia*, **87**, 299-306.
- Cooper, W. E. 1985. Female residency and courtship intensity in a territorial lizard, *Holbrookia propinqua*. *Amphibia-Reptilia*, **6**, 63-69.
- Cooper, W. E. 1999. Tradeoffs between courtship, fighting, and antipredatory behavior by a lizard, *Eumeces laticeps*. *Behavioral Ecology and Sociobiology*, **47**, 54-59.
- Craig, A. S., Herman, L. M. & Pack, A. A. 2002. Male mate choice and male-male competition coexist in the humpback whale (*Megaptera novaeangliae*). *Canadian Journal of Zoology-Revue Canadienne De Zoologie*, **80**, 745-755.
- Crawley, M. J. 2002. *Statistical computing: an introduction to data analysis using S-Plus*. Chichester: John Wiley & Sons, Ltd.
- Crespi, B. J. 1988. Alternative male mating tactics in a thrips: effects of sex ratio variation and body size. *American Midland Naturalist*, **119**, 83-92.
- Croft, D. P., Arrowsmith, B. J., Webster, M. & Krause, J. 2004a. Intra-sexual preferences for familiar fish in male guppies. *Journal of Fish Biology*, **64**, 279-283.
- Croft, D. P., Krause, J. & James, R. 2004b. Social networks in the guppy (*Poecilia reticulata*). *Proceedings Of The Royal Society Of London Series B-Biological Sciences*, **271**, S516-S519.
- Crow, R. T. & Liley, N. R. 1979. A sexual pheromone in the guppy, *Poecilia reticulata* (Peters). *Canadian Journal of Zoology-Revue Canadienne De Zoologie*, **57**, 184-188.
- Crowley, P. H., Travers, S. E., Linton, M. C., Cohn, S. L., Sih, A. & Sargent, R. C. 1991. Mate density, predation risk, and the seasonal sequence of mate choices: a dynamic game. *American Naturalist*, **137**, 567-596.
- Dall, S. R. X., Giraldeau, L. A., Olsson, O., McNamara, J. M. & Stephens, D. W. 2005. Information and its use by animals in evolutionary ecology. *Trends In Ecology & Evolution*, **20**, 187-193.
- Danforth, B. N. 1991. The morphology and behavior of dimorphic males in *Perdita portalis* (Hymenoptera, Andrenidae). *Behavioral Ecology And Sociobiology*, **29**, 235-247.
- Davies, N. B. & Lundberg, A. 1984. Food distribution and a variable mating system in the dunnoek, *Prunella modularis*. *Journal Of Animal Ecology*, **53**, 895-912.

- de Fraipont, M., Fitzgerald, G. J. & Guderley, H. 1993. Age-related differences in reproductive tactics in the three-spined stickleback, *Gasterosteus aculeatus*. *Animal Behaviour*, **46**, 961-968.
- Dewsbury, D. A. 1981. Effects of novelty on copulatory behavior: the Coolidge effect and related phenomena. *Psychological Bulletin*, **89**, 464-482.
- Donovan, A. & Verrell, P. A. 1991. The effect of partner familiarity on courtship success in the salamander *Desmognathus ochrophaeus*. *Journal of Herpetology*, **25**, 93-95.
- Eadie, J. M. & Fryxell, J. M. 1992. Density dependence, frequency-dependence and alternative nesting strategies in Goldeneyes. *American Naturalist*, **140**, 621-641.
- Eberle, M. & Kappeler, P. M. 2004. Sex in the dark: determinants and consequences of mixed male mating tactics in *Microcebus murinus*, a small solitary nocturnal primate. *Behavioral Ecology And Sociobiology*, **57**, 77-90.
- Eggert, C. & Guyétant, R. 2003. Reproductive behaviour of spadefoot toads (*Pelobates fuscus*): daily sex ratios and males' tactics, ages, and physical condition. *Canadian Journal Of Zoology-Revue Canadienne De Zoologie*, **81**, 46-51.
- Emlen, D. J. 1997a. Alternative reproductive tactics and male-dimorphism in the horned beetle *Onthophagus acuminatus* (Coleoptera:Scarabaeidae). *Behavioral Ecology And Sociobiology*, **41**, 335-341.
- Emlen, S. T. 1997b. Predicting family dynamics in social vertebrates. In: *Behavioural Ecology: An Evolutionary Approach (4th Edition)* (Ed. by Krebs, J. R. & Davies, N. B.). Oxford: Blackwell Science Ltd.
- Emlen, S. T. & Oring, L. W. 1977. Ecology, sexual selection and evolution of mating systems. *Science*, **197**, 215-223.
- Enders, M. M. 1993. The effect of male size and operational sex-ratio on male mating success in the common spider mite, *Tetranychus urticae* Koch (Acari, Tetranychidae). *Animal Behaviour*, **46**, 835-846.
- Endler, J. A. 1978. A predator's view of animal color patterns. *Evolutionary Biology*, **11**, 319-364.
- Endler, J. A. 1980. Natural selection on color patterns in *Poecilia reticulata*. *Evolution*, **34**, 76-91.
- Endler, J. A. 1983. Natural and sexual selection on color patterns in Poeciliid fishes. *Environmental Biology of Fishes*, **9**, 173-190.
- Endler, J. A. 1987. Predation, light intensity and courtship behaviour in *Poecilia reticulata* (Pisces: Poeciliidae). *Animal Behaviour*, **35**, 1376-1385.
- Endler, J. A. 1993. The color of light in forests and its implications. *Ecological Monographs*, **63**, 1-27.
- Endler, J. A. & Houde, A. E. 1995. Geographic variation in female preferences for male traits in *Poecilia reticulata*. *Evolution*, **49**, 456-468.
- Endler, J. A. & Théry, M. 1996. Interacting effects of lek placement, display behavior, ambient light, and color patterns in three neotropical forest-dwelling birds. *American Naturalist*, **148**, 421-452.
- Estep, D. Q., Nieuwenhuijsen, K., Bruce, K. E. M., Deneef, K. J., Walters, P. A., Baker, S. C. & Slob, A. K. 1988. Inhibition of sexual behavior among subordinate stump-tail macaques, *Macaca arctoides*. *Animal Behaviour*, **36**, 854-864.

- Evans, J. P., Kelley, J. L., Ramnarine, I. W. & Pilastro, A. 2002. Female behaviour mediates male courtship under predation risk in the guppy (*Poecilia reticulata*). *Behavioral Ecology and Sociobiology*, **52**, 496-502.
- Evans, J. P. & Magurran, A. E. 1999. Male mating behaviour and sperm production characteristics under varying sperm competition risk in guppies. *Animal Behaviour*, **58**, 1001-1006.
- Evans, J. P., Pierotti, M. & Pilastro, A. 2003a. Male mating behavior and ejaculate expenditure under sperm competition risk in the eastern mosquitofish. *Behavioral Ecology*, **14**, 268-273.
- Evans, J. P., Zane, L., Francescato, S. & Pilastro, A. 2003b. Directional postcopulatory sexual selection revealed by artificial insemination. *Nature*, **42**, 360-363.
- Farmer, N. A., Ribble, D. O. & Miller, D. G. 2004. Influence of familiarity on shoaling behaviour in Texas and blacktail shiners. *Journal Of Fish Biology*, **64**, 776-782.
- Farr, J. A. 1975. Role of predation in evolution of social behavior of natural populations of guppy, *Poecilia reticulata* (Pisces: Poeciliidae). *Evolution*, **29**, 151-158.
- Farr, J. A. 1977. Male rarity or novelty, female choice behavior, and sexual selection in guppy, *Poecilia reticulata* Peters (Pisces: Poeciliidae). *Evolution*, **31**, 162-168.
- Farr, J. A. 1989. Sexual selection and secondary sexual differentiation in poeciliids: determinants of male mating success and the evolution of female choice. In: *Ecology and evolution of livebearing fishes (Poeciliidae)* (Ed. by Meffe, G. K. & Snelson, F. F., Jr.). New Jersey: Prentice-Hall.
- Farr, J. A., Travis, J. & Trexler, J. C. 1986. Behavioural allometry and interdemographic variation in sexual behaviour of the sailfin molly, *Poecilia latipinna* (Pisces: Poeciliidae). *Animal Behaviour*, **34**, 497-509.
- Fisher, D. O. & Owens, I. P. F. 2004. The comparative method in conservation biology. *Trends In Ecology & Evolution*, **19**, 391-398.
- Foerster, K., Delhey, K., Johnsen, A., Lifjeld, J. T. & Kempenaers, B. 2003. Females increase offspring heterozygosity and fitness through extra-pair matings. *Nature*, **425**, 714-717.
- Forsgren, E. 1992. Predation risk affects mate choice in a Gobiid fish. *American Naturalist*, **140**, 1041-1049.
- Forsgren, E., Amundsen, T., Borg, A. A. & Bjelvenmark, J. 2004. Unusually dynamic sex roles in a fish. *Nature*, **429**, 551-554.
- Forsgren, E., Kvarnemo, C. & Lindstrom, K. 1996. Mode of sexual selection determined by resource abundance in two sand goby populations. *Evolution*, **50**, 646-654.
- Forstmeier, W. 2002. Factors contributing to male mating success in the polygynous dusky warbler (*Phylloscopus fuscatus*). *Behaviour*, **139**, 1361-1381.
- Foster, S. A. 1999. The geography of behaviour: an evolutionary perspective. *Trends in Ecology & Evolution*, **14**, 190-195.
- Foster, S. A. & Endler, J. A. 1999a. Introduction and aims. In: *Geographic variation in behavior* (Ed. by Foster, S. A. & Endler, J. A.). New York: Oxford University Press.

- Foster, S. A. & Endler, J. A. 1999b. Thoughts on geographic variation in behavior. In: *Geographic variation in behavior* (Ed. by Foster, S. A. & Endler, J. A.). New York: Oxford University Press.
- Foster, S. A. & Endler, J. A. 1999c. *Geographic variation in behavior*. New York: Oxford University Press.
- Fuller, R. & Berglund, A. 1996. Behavioral responses of a sex-role reversed pipefish to a gradient of perceived predation risk. *Behavioral Ecology*, **7**, 69-75.
- Gage, M. J. G., Stockley, P. & Parker, G. A. 1995. Effects of alternative male mating strategies on characteristics of sperm production in the Atlantic salmon (*Salmo salar*): Theoretical and empirical investigations. *Philosophical transactions of the Royal Society of London Series B-Biological Sciences*, **350**, 391-339.
- Galeotti, P., Saino, N., Sacchi, R. & Moller, A. P. 1997. Song correlates with social context, testosterone and body condition in male barn swallows. *Animal Behaviour*, **53**, 687-700.
- Gamble, S., Lindholm, A. K., Endler, J. A. & Brooks, R. 2003. Environmental variation and the maintenance of polymorphism: the effect of ambient light spectrum on mating behaviour and sexual selection in guppies. *Ecology Letters*, **6**, 463-472.
- Godin, J. G. J. 1995. Predation risk and alternative mating tactics in male Trinidadian guppies (*Poecilia reticulata*). *Oecologia*, **103**, 224-229.
- Godin, J. G. J. & Briggs, S. E. 1996. Female mate choice under predation risk in the guppy. *Animal Behaviour*, **51**, 117-130.
- Godin, J. G. J., Herdman, E. J. E. & Dugatkin, L. A. 2005. Social influences on female mate choice in the guppy, *Poecilia reticulata*: generalized and repeatable trait-copying behaviour. *Animal Behaviour*, **69**, 999-1005.
- Gong, A. & Gibson, R. M. 1996. Reversal of a female preference after visual exposure to a predator in the guppy, *Poecilia reticulata*. *Animal Behaviour*, **52**, 1007-1015.
- Grafe, T. U. 1997. Costs and benefits of mate choice in the lek-breeding reed frog, *Hyperolius marmoratus*. *Animal Behaviour*, **53**, 1103-1117.
- Griffiths, S. W. 1997. Preferences for familiar fish do not vary with predation risk in the European minnow. *Journal Of Fish Biology*, **51**, 489-495.
- Griffiths, S. W. & Magurran, A. E. 1997. Familiarity in schooling fish: How long does it take to acquire? *Animal Behaviour*, **53**, 945-949.
- Griffiths, S. W. & Magurran, A. E. 1998. Sex and schooling behaviour in the Trinidadian guppy. *Animal Behaviour*, **56**, 689-693.
- Gross, M. J. 1996. Alternative reproductive strategies and tactics: Diversity within sexes. *Trends in Ecology and Evolution*, **11**, 92-98.
- Gwynne, D. T. 1981. Sexual difference theory: Mormon crickets show role reversal in mate choice. *Science*, **213**, 779-780.
- Gwynne, D. T. 1989. Does copulation increase the risk of predation? *Trends In Ecology & Evolution*, **4**, 54-56.
- Gwynne, D. T. 1993. Food quality controls sexual selection in Mormon crickets by altering male mating investment. *Ecology*, **74**, 1406-1413.
- Gwynne, D. T., Bailey, W. J. & Anells, A. 1998. The sex in short supply for matings varies over small spatial scales in a katydid (*Kawanaphila nartee*,

- Orthoptera: Tettigoniidae). *Behavioral Ecology and Sociobiology*, **42**, 157-162.
- Hagelin, J. C. 2002. The kinds of traits involved in male-male competition: a comparison of plumage, behavior, and body size in quail. *Behavioral Ecology*, **13**, 32-41.
- Halliday, T. R. 1983. The study of mate choice. In: *Mate choice* (Ed. by Bateson, P.), pp. 3-32. Cambridge: Cambridge University Press.
- Herdman, E. J. E., Kelly, C. D. & Godin, J. G. J. 2004. Male mate choice in the guppy (*Poecilia reticulata*): Do males prefer larger females as mates? *Ethology*, **110**, 97-111.
- Higgins, L. A. & Waugaman, R. D. 2004. Sexual selection and variation: a multivariate approach to species-specific calls and preferences. *Animal Behaviour*, **68**, 1139-1153.
- Hosken, D. J. & Stockley, P. 2004. Sexual selection and genital evolution. *Trends In Ecology & Evolution*, **19**, 87-93.
- Houde, A. E. 1992. Sex-linked heritability of a sexually selected character in a natural population of *Poecilia reticulata* (Pisces:Poeciliidae) (guppies). *Heredity*, **69**, 229-235.
- Houde, A. E. 1997. *Sex, color, and mate choice in guppies*. Princeton, New Jersey: Princeton University Press.
- Hubbell, S. P. & Johnson, L. K. 1987. Environmental variance in lifetime mating success, mate choice, and sexual selection. *American Naturalist*, **130**, 91-112.
- Hughes, A. L. 1985. Male size, mating success, and mating strategy in the mosquitofish *Gambusia affinis* (Poeciliidae). *Behavioral Ecology And Sociobiology*, **17**, 271-278.
- Hughes, K. A., Du, L., Rodd, F. H. & Reznick, D. N. 1999. Familiarity leads to female mate preference for novel males in the guppy, *Poecilia reticulata*. *Animal Behaviour*, **58**, 907-916.
- Hunt, J. & Simmons, L. W. 2001. Status-dependent selection in the dimorphic beetle *Onthophagus taurus*. *Proceedings Of The Royal Society Of London Series B-Biological Sciences*, **268**, 2409-2414.
- Hutchings, J. A., Bishop, T. D. & McGregor-Shaw, C. R. 1999. Spawning behaviour of Atlantic cod, *Gadus morhua*: evidence of mate competition and mate choice in a broadcast spawner. *Canadian Journal Of Fisheries And Aquatic Sciences*, **56**, 97-104.
- Hutchings, J. A. & Gerber, L. 2002. Sex-biased dispersal in a salmonid fish. *Proceedings Of The Royal Society Of London Series B-Biological Sciences*, **269**, 2487-2493.
- Jakobsson, S., Brick, O. & Kullberg, C. 1995. Escalated fighting behavior incurs increased predation risk. *Animal Behaviour*, **49**, 235-239.
- Janzen, D. H. 1973. Sweep samples of tropical foliage insects: effects of seasons, vegetation types, elevation, time of day, and insularity. *Ecology*, **54**, 687-708.
- Jawor, J. M. & Breitwisch, R. 2003. Melanin ornaments, honesty, and sexual selection. *Auk*, **120**, 249-265.
- Jennions, M. D. & Kelly, C. D. 2002. Geographical variation in male genitalia in *Brachyrhaphis episcopi* (Poeciliidae): is it sexually or naturally selected? *Oikos*, **97**, 76-86.

- Jennions, M. D. & Petrie, M. 1997. Variation in mate choice and mating preferences: A review of causes and consequences. *Biological Reviews Of The Cambridge Philosophical Society*, **72**, 283-327.
- Jennions, M. D. & Telford, S. R. 2002. Life-history phenotypes in populations of *Brachyrhaphis episcopi* (Poeciliidae) with different predator communities. *Oecologia*, **132**, 44-50.
- Jenssen, T. A., Decourcy, K. R. & Congdon, J. D. 2005. Assessment in contests of male lizards (*Anolis carolinensis*): how should smaller males respond when size matters? *Animal Behaviour*, **69**, 1325-1336.
- Jirotkul, M. 1999. Operational sex ratio influences female preference and male-male competition in guppies. *Animal Behaviour*, **58**, 287-294.
- Johnson, J. B. & Basolo, A. L. 2003. Predator exposure alters female mate choice in the green swordtail. *Behavioral Ecology*, **14**, 619-625.
- Johnson, J. B. & Belk, M. C. 2001. Predation environment predicts divergent life-history phenotypes among populations of the livebearing fish *Brachyrhaphis rhabdophora*. *Oecologia*, **126**, 142-149.
- Johnson, L. K. 1982. Sexual selection in a brentid weevil. *Evolution*, **36**, 251-262.
- Johnsson, J. I., Nobbelin, F. & Bohlin, T. 1999. Territorial competition among wild brown trout fry: effects of ownership and body size. *Journal Of Fish Biology*, **54**, 469-472.
- Jones, A. G., Walker, D., Lindstrom, K., Kvarnemo, C. & Avise, J. C. 2001. Surprising similarity of sneaking rates and genetic mating patterns in two populations of sand goby experiencing disparate sexual selection regimes. *Molecular Ecology*, **10**, 461-469.
- Jones, G., Barabas, A., Elliott, W. & Parsons, S. 2002. Female greater wax moths reduce sexual display behavior in relation to the potential risk of predation by echolocating bats. *Behavioral Ecology*, **13**, 375-380.
- Jones, J. C. & Reynolds, J. D. 1999. The influence of oxygen stress on female choice for male nest structure in the common goby. *Animal Behaviour*, **57**, 189-196.
- Kelley, J. L., Graves, J. A. & Magurran, A. E. 1999. Familiarity breeds contempt in guppies. *Nature*, **401**, 661-662.
- Kelley, J. L. & Magurran, A. E. 2003. Effects of relaxed predation pressure on visual predator recognition in the guppy. *Behavioral Ecology and Sociobiology*, **54**, 225-232.
- Kelly, C. D. & Godin, J. G. J. 2001. Predation risk reduces male-male sexual competition in the Trinidadian guppy (*Poecilia reticulata*). *Behavioral Ecology And Sociobiology*, **51**, 95-100.
- Kelly, C. D., Godin, J. G. J. & Abdallah, G. 2000. Geographical variation in the male intromittent organ of the Trinidadian guppy (*Poecilia reticulata*). *Canadian Journal Of Zoology-Revue Canadienne De Zoologie*, **78**, 1674-1680.
- Kelly, C. D., Godin, J. G. J. & Wright, J. M. 1999. Geographical variation in multiple paternity within natural populations of the guppy (*Poecilia reticulata*). *Proceedings of the Royal Society of London Series B-Biological Sciences*, **266**, 2403-2408.
- Kempnaers, B., Verheyen, G. R., Vandebroek, M., Burke, T., Vanbroeckhoven, C. & Dhondt, A. A. 1992. Extra-pair paternity results from female preference for high-quality males in the blue tit. *Nature*, **357**, 494-496.

- Kodric-Brown, A. 1993. Female choice of multiple male criteria in guppies: interacting effects of dominance, coloration and courtship. *Behavioral Ecology And Sociobiology*, **32**, 415-420.
- Kokko, H. & Monaghan, P. 2001. Predicting the direction of sexual selection. *Ecology Letters*, **4**, 159-165.
- Kramer, D. L. 1978. Reproductive seasonality in the fishes of a tropical stream. *Ecology*, **59**, 976-985.
- Kramer, D. L. & Bryant, M. J. 1995. Intestine length in the fishes of a tropical stream.2. Relationships to diet - the long and short of a convoluted issue. *Environmental Biology of Fishes*, **42**, 129-141.
- Krebs, J. R. & Davies, N. B. 1987. *An introduction to behavioural ecology*. Blackwell Scientific Publications.
- Kvarnemo, C. & Ahnesjö, I. 1996. The dynamics of operational sex ratios and competition for mates. *Trends In Ecology & Evolution*, **11**, 404-408.
- Kvarnemo, C., Forsgren, E. & Magnhagen, C. 1995. Effects of sex ratio on intra- and inter-sexual behaviour in sand gobies. *Animal Behaviour*, **50**, 1455-1461.
- Kvarnemo, C. & Simmons, L. W. 2004. Testes investment and spawning mode in pipefishes and seahorses (Syngnathidae). *Biological Journal Of The Linnean Society*, **83**, 369-376.
- Lailvaux, S. P., Herrel, A., VanHooydonck, B., Meyers, J. J. & Irschick, D. J. 2004. Performance capacity, fighting tactics and the evolution of life-stage male morphs in the green anole lizard (*Anolis carolinensis*). *Proceedings Of The Royal Society Of London Series B-Biological Sciences*, **271**, 2501-2508.
- Lande, R. 1980. The genetic covariance between characters maintained by pleiotropic mutations. *Genetics*, **94**, 203-215.
- Landry, C., Garant, D., Duchesne, P. & Bernatchez, L. 2001. 'Good genes as heterozygosity': the major histocompatibility complex and mate choice in Atlantic salmon (*Salmo salar*). *Proceedings of the Royal Society of London Series B-Biological Sciences*, **268**, 1279-1285.
- Langerhans, R. B., Layman, C. A. & DeWitt, T. J. 2005. Male genital size reflects a tradeoff between attracting mates and avoiding predators in two live-bearing fish species. *Proceedings Of The National Academy Of Sciences Of The United States Of America*, **102**, 7618-7623.
- Lank, D. B., Smith, C. M., Hanotte, O., Burke, T. & Cooke, F. 1995. Genetic polymorphism for alternative mating behavior in lekking male ruff *Philomachus pugnax*. *Nature*, **378**, 59-62.
- Lehtonen, T. & Lindstrom, K. 2004. Changes in sexual selection resulting from novel habitat use in the sand goby. *Oikos*, **104**, 327-335.
- Lesna, I. & Sabelis, M. W. 1999. Diet-dependent female choice for males with 'good genes' in a soil predatory mite. *Nature*, **401**, 581-584.
- Lessells, C. M. 1991. The evolution of life histories. In: *Behavioural Ecology: An Evolutionary Approach (3rd Edition)* (Ed. by Krebs, J. R. & Davies, N. B.). Oxford: Blackwell Scientific Publications.
- Lessells, C. M. & Boag, P. T. 1987. Unrepeatable repeatabilities: a common mistake. *Auk*, **104**, 116-121.
- Liley, N. R. 1966. Ethological isolating mechanisms in four sympatric species of Poeciliid fishes. *Behaviour*, **Supplement 13**, 1-197.

- Liljedal, S. & Folstad, I. 2003. Milt quality, parasites, and immune function in dominant and subordinate Arctic charr. *Canadian Journal Of Zoology-Revue Canadienne De Zoologie*, **81**, 221-227.
- Lima, S. L. & Bednekoff, P. A. 1999. Temporal variation in danger drives antipredator behavior: The predation risk allocation hypothesis. *American Naturalist*, **153**, 649-659.
- Lima, S. L. & Dill, L. M. 1990. Behavioral decisions made under the risk of predation: A review and prospectus. *Canadian Journal Of Zoology-Revue Canadienne De Zoologie*, **68**, 619-640.
- Lindström, J., Metcalfe, N. B. & Royle, N. J. 2005. How are animals with ornaments predicted to compensate for a bad start in life? A dynamic optimization model approach. *Functional Ecology*, **19**, 421-428.
- Long, K. D. & Rosenqvist, G. 1998. Changes in male guppy courting distance in response to a fluctuating light environment. *Behavioral Ecology and Sociobiology*, **44**, 77-83.
- Lupien, S. J. & McEwen, B. S. 1997. The acute effects of corticosteroids on cognition: Integration of animal and human model studies. *Brain Research Reviews*, **24**, 1-27.
- Luyten, P. H. & Liley, N. R. 1985. Geographic variation in the sexual behaviour of the guppy, *Poecilia reticulata* (Peters). *Behaviour*, **95**, 164-179.
- Madsen, T. & Shine, R. 1993. Temporal variability in sexual selection acting on reproductive tactics and body size in male snakes. *American Naturalist*, **141**, 167-171.
- Magellan, K., Pettersson, L. B. & Magurran, A. E. 2005. Quantifying male attractiveness and mating behaviour through phenotypic size manipulation in the Trinidadian guppy, *Poecilia reticulata*. *Behavioral Ecology and Sociobiology*, **58**, 366-374.
- Magnhagen, C. 1990. Reproduction under predation risk in the sand goby, *Pomatoschistus minutus*, and the black goby, *Gobius niger*: the effect of age and longevity. *Behavioral Ecology And Sociobiology*, **26**, 331-335.
- Magnhagen, C. 1991. Predation risk as a cost of reproduction. *Trends In Ecology & Evolution*, **6**, 183-185.
- Magnhagen, C. 1995. Sneaking behavior and nest defense are affected by predation risk in the common goby. *Animal Behaviour*, **50**, 1123-1128.
- Magurran, A. E. 1993. Individual differences and alternative behaviours. In: *Behaviour of Teleost Fishes* (Ed. by Pitcher, T. J.), pp. 441-477. London: Chapman & Hall.
- Magurran, A. E. 1998. Population differentiation without speciation. *Philosophical Transactions: Biological Sciences*, **353**, 275-286.
- Magurran, A. E. 2001. Sexual conflict and evolution in Trinidadian guppies. *Genetica*, **112-113**, 474.
- Magurran, A. E. & Nowak, M. A. 1991. Another battle of the sexes - the consequences of sexual asymmetry in mating costs and predation risk in the guppy, *Poecilia reticulata*. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **246**, 31-38.
- Magurran, A. E. & Seghers, B. H. 1990. Risk sensitive courtship in the guppy (*Poecilia reticulata*). *Behaviour*, **112**, 194-201.

- Magurran, A. E. & Seghers, B. H. 1994a. Sexual conflict as a consequence of ecology- evidence from guppy, *Poecilia reticulata*, populations in Trinidad. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **255**, 31-36.
- Magurran, A. E. & Seghers, B. H. 1994b. A cost of sexual harassment in the guppy, *Poecilia reticulata*. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **258**, 89-92.
- Malo, A. F., Roldan, E. R. S., Garde, J., Soler, A. J. & Gomendio, M. 2005. Antlers honestly advertise sperm production and quality. *Proceedings Of The Royal Society B-Biological Sciences*, **272**, 149-157.
- Matthews, M., Evans, J. P. & Magurran, A. E. 1997. Male display rate reveals ejaculate characteristics in the Trinidadian guppy *Poecilia reticulata*. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **264**, 695-700.
- Mays, H. L. & Ritchison, G. 2004. The effect of vegetation density on male mate guarding and extra-territorial forays in the yellow-breasted chat (*Icteria virens*). *Naturwissenschaften*, **91**, 195-198.
- Mazzoldi, C. & Rasotto, M. B. 2002. Alternative male mating tactics in *Gobius niger*. *Journal Of Fish Biology*, **61**, 157-172.
- McLaughlin, M. E. & Bruce, K. E. 2001. The effect of male familiarity on proximity time in female eastern mosquitofish (*Gambusia holbrooki*). *The Psychological Record*, **51**, 237-250.
- McLennan, D. A. & Ryan, M. J. 1997. Responses to conspecific and heterospecific olfactory cues in the swordtail *Xiphophorus cortezi*. *Animal Behaviour*, **54**, 1077-1088.
- McPeck, M. A. 1992. Mechanisms of sexual selection operating on body size in the mosquitofish (*Gambusia holbrooki*). *Behavioral Ecology*, **3**, 1-12.
- Merilä, J. & Sheldon, B. C. 1999. Testis size variation in the greenfinch *Carduelis chloris*: relevance for some recent models of sexual selection. *Behavioral Ecology And Sociobiology*, **45**, 115-123.
- Milinski, M. & Bakker, T. C. M. 1990. Female sticklebacks use male coloration in mate choice and hence avoid parasitized males. *Nature*, **344**, 330-333.
- Milinski, M. & Bakker, T. C. M. 1992. Costs influence sequential mate choice in sticklebacks, *Gasterosteus aculeatus*. *Proceedings Of The Royal Society Of London Series B-Biological Sciences*, **250**, 229-233.
- Mills, S. C. & Reynolds, J. D. 2003. Operational sex ratio and alternative reproductive behaviours in the European bitterling, *Rhodeus sericeus*. *Behavioral Ecology And Sociobiology*, **54**, 98-104.
- Mitchell, S. E., Read, A. F. & Little, T. J. 2004. The effect of a pathogen epidemic on the genetic structure and reproductive strategy of the crustacean *Daphnia magna*. *Ecology Letters*, **7**, 848-858.
- Mojica, C. L. 1998. Comparative ecology and historical biogeography of the genus *Brachyrhaphis*. *Environmental Biology of Fishes*, **51**, 117-127.
- Mojica, C. L., Meyer, A. & Barlow, G. W. 1997. Phylogenetic relationships of species of the genus *Brachyrhaphis* (Poeciliidae) inferred from partial mitochondrial DNA sequences. *Copeia*, 298-305.
- Moller, A. P. 1988. Testes size, ejaculate quality and sperm competition in birds. *Biological Journal Of The Linnean Society*, **33**, 273-283.

- Moller, A. P. 1989. Ejaculate quality, testes size and sperm production in mammals. *Functional Ecology*, **3**, 91-96.
- Morris, M. R. 1998. Female preference for trait symmetry in addition to trait size in swordtail fish. *Proceedings Of The Royal Society Of London Series B-Biological Sciences*, **265**, 907-911.
- Morris, M. R., Batra, P. & Ryan, M. J. 1992. Male-male competition and access to females in the swordtail *Xiphophorus nigrensis*. *Copeia*, 980-986.
- Morris, M. R., Elias, J. A. & Moretz, J. A. 2001. Defining vertical bars in relation to female preference in the swordtail fish *Xiphophorus cortezi* (Cyprinodontiformes, Poeciliidae). *Ethology*, **107**, 827-837.
- Morris, M. R., Moretz, J. A., Farley, K. & Nicoletto, P. 2005. The role of sexual selection in the loss of sexually selected traits in the swordtail fish *Xiphophorus continens*. *Animal Behaviour*, **69**, 1415-1424.
- Morris, M. R., Mussel, M. & Ryan, M. J. 1995. Vertical bars on male *Xiphophorus multilineatus*: a signal that deters rival males and attracts females. *Behavioral Ecology*, **6**, 274-279.
- Nakatsuru, K. & Kramer, D. L. 1982. Is sperm cheap? Limited male fertility and female choice in the lemon tetra (Pisces, Characidae). *Science*, **216**, 753-755.
- Neff, B. D., Cargnelli, L. M. & Cote, I. M. 2004. Solitary nesting as an alternative breeding tactic in colonial nesting bluegill sunfish (*Lepomis macrochirus*). *Behavioral Ecology And Sociobiology*, **56**, 381-387.
- Orrell, K. S. & Jenssen, T. A. 2002. Male mate choice by the lizard *Anolis carolinensis*: a preference for novel females. *Animal Behaviour*, **63**, 1091-1102.
- Palokangas, P., Alatalo, R. V. & Korpimaki, E. 1992. Female choice in the kestrel under different availability of mating options. *Animal Behaviour*, **43**, 659-665.
- Parker, G. A. 1982. Why are there so many tiny sperm? Sperm competition and the maintenance of two sexes. *Journal Of Theoretical Biology*, **96**, 281-294.
- Parker, G. A. 1983. Mate quality and mating decisions. In: *Mate Choice* (Ed. by Bateson, P.), pp. 141-164. Cambridge: Cambridge University Press.
- Parker, G. A. 1990. Sperm competition games: raffles and roles. *Proceedings Of The Royal Society Of London Series B-Biological Sciences*, **242**, 120-126.
- Partridge, L. & Green, P. 1985. Intraspecific feeding specializations and population dynamics. In: *Behavioural Ecology. Ecological Consequences of Adaptive Behaviour* (Ed. by Sibly, R. M. & Smith, R. H.), pp. 207-266. Oxford: Blackwell Scientific Publications, Inc.
- Partridge, L. & Halliday, T. 1984. Mating patterns and mate choice. In: *Behavioural Ecology: An Evolutionary Approach (2nd Edition)* (Ed. by Krebs, J. R. & Davies, N. B.), pp. 222-250. Oxford: Blackwell Scientific Publications.
- Peeke, H. V. S. & Morgan, L. E. 2000. Behavioral, differentiation of adjacent marine and fluvial populations of threespine stickleback in California: A laboratory study. *Behaviour*, **137**, 1011-1027.
- Peer, K., Robertson, R. J. & Kempnaers, B. 2000. Reproductive anatomy and indices of quality in male tree swallows: The potential reproductive role of floaters. *Auk*, **117**, 74-81.

- Perrin, W. F. & Mesnick, S. L. 2003. Sexual ecology of the spinner dolphin, *Stenella longirostris*: Geographic variation in mating system. *Marine Mammal Science*, **19**, 462-483.
- Pettersson, J., Johnsson, J. I. & Bohlin, T. 1996. The competitive advantage of large body size declines with increasing group size in rainbow trout. *Journal Of Fish Biology*, **49**, 370-372.
- Pettersson, L. B., Ramnarine, I. W., Becher, S. A., Mahabir, R. & Magurran, A. E. 2004. Sex ratio dynamics and fluctuating selection pressures in natural populations of the Trinidadian guppy, *Poecilia reticulata*. *Behavioral Ecology And Sociobiology*, **55**, 461-468.
- Pilastro, A., Benetton, S. & Bisazza, A. 2003. Female aggregation and male competition reduce costs of sexual harassment in the mosquitofish *Gambusia holbrooki*. *Animal Behaviour*, **65**, 1161-1167.
- Pilastro, A., Giacomello, E. & Bisazza, A. 1997. Sexual selection for small size in male mosquitofish (*Gambusia holbrooki*). *Proceedings Of The Royal Society Of London Series B-Biological Sciences*, **264**, 1125-1129.
- Pilastro, A., Scaggiante, M. & Rasotto, M. B. 2002. Individual adjustment of sperm expenditure accords with sperm competition theory. *Proceedings Of The National Academy Of Sciences Of The United States Of America*, **99**, 9913-9915.
- Plaistow, S. J., Johnstone, R. A., Colegrave, N. & Spencer, M. 2004. Evolution of alternative mating tactics: conditional versus mixed strategies. *Behavioral Ecology*, **15**, 534-542.
- Pruett-Jones, S. 1992. Independent versus nonindependent mate choice: do females copy each other? *American Naturalist*, **140**, 1000-1009.
- Ptacek, M. B. & Travis, J. 1997. Mate choice in the sailfin molly, *Poecilia latipinna*. *Evolution*, **51**, 1217-1231.
- Qvarnström, A. 2001. Context-dependent genetic benefits from mate choice. *Trends in Ecology & Evolution*, **16**, 5-7.
- Ramm, S. A., Parker, G. A. & Stockley, P. 2005. Sperm competition and the evolution of male reproductive anatomy in rodents. *Proceedings Of The Royal Society B-Biological Sciences*, **272**, 949-955.
- Ramsey, F. L. & Schafer, D. W. 1997. *The statistical sleuth: a course in methods of data analysis*. Belmont, California: London: Duxbury Press.
- Regan, C. T. 1913. A revision of the Cyprinodont fishes of the subfamily Poeciliinae. *Proceedings of the Zoological Society of London*, 997-1018.
- Reichard, M., Bryja, J., Ondračková, M., Dávidová, M., Kaniewska, P. & Smith, C. 2005. Sexual selection for male dominance reduces opportunities for female mate choice in the European bitterling (*Rhodeus sericeus*). *Molecular Ecology*, **14**, 1533-1542.
- Reimchen, T. E. 1989. Loss of nuptial color in threespine sticklebacks (*Gasterosteus aculeatus*). *Evolution*, **43**, 450-460.
- Reynolds, J. D. 1993. Should attractive individuals court more? Theory and a test. *American Naturalist*, **141**, 914-927.
- Reynolds, J. D. 1996. Animal breeding systems. *Trends In Ecology & Evolution*, **11**, A68-A72.

- Reynolds, J. D. & Gross, M. R. 1992. Female mate preference enhances offspring growth and reproduction in a fish; *Poecilia reticulata*. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **250**, 57-62.
- Reynolds, J. D., Gross, M. R. & Coombs, M. J. 1993. Environmental conditions and male morphology determine alternative mating behavior in Trinidadian guppies. *Animal Behaviour*, **45**, 145-152.
- Reznick, D. 1983. The structure of guppy life histories: the trade-off between growth and reproduction. *Ecology*, **64**, 862-873.
- Reznick, D. & Endler, J. A. 1982. The impact of predation on life-history evolution in Trinidadian guppies (*Poecilia reticulata*). *Evolution*, **36**, 160-177.
- Reznick, D., Meyer, A. & Frear, D. 1993. Life history of *Brachyrhaphis rhabdophora* (Pisces: Poeciliidae). *Copeia*, **1**, 103-111.
- Reznick, D. & Yang, A. P. 1993. The influence of fluctuating resources on life history: patterns of allocation and plasticity in female guppies. *Ecology*, **74**, 2011-2019.
- Reznick, D. N. 1989. Life history evolution in guppies. 2. Repeatability of field observations and the effects of season on life histories. *Evolution*, **43**, 1285-1297.
- Riechert, S. E. 1998. Game theory and animal contests. In: *Game theory and animal behavior* (Ed. by Dugatkin, L. A. & Reeve, H. K.), pp. 64-93. New York: Oxford University Press.
- Roberts, S. C., Gosling, L. M., Thornton, E. A. & McClung, J. 2001. Scent-marking by male mice under the risk of predation. *Behavioral Ecology*, **12**, 698-705.
- Rodd, F. H. & Sokolowski, M. B. 1995. Complex origins of variation in the sexual behaviour of male Trinidadian guppies, *Poecilia reticulata*: interactions between social environment, heredity, body size and age. *Animal Behaviour*, **49**, 1139-1159.
- Rosen, D. E. & Gordon, M. 1953. Functional anatomy and evolution of male genitalia in Poeciliid fishes. *Zoologica (New York Zoological Society)*, **25**, 1-48.
- Ruchon, F., Laugier, T. & Quignard, J. P. 1995. Alternative male reproductive strategies in the peacock blenny. *Journal Of Fish Biology*, **47**, 826-840.
- Ryan, K. K. & Altmann, J. 2001. Selection for male choice based primarily on mate compatibility in the oldfield mouse, *Peromyscus polionotus rhoadsi*. *Behavioral Ecology and Sociobiology*, **50**, 436-440.
- Ryan, M. J. & Causey, B. A. 1989. "Alternative" mating behavior in the swordtails *Xiphophorus nigrensis* and *Xiphophorus pygmaeus* (Pisces: Poeciliidae). *Behavioral Ecology And Sociobiology*, **24**, 341-348.
- Ryan, M. J., Cocroft, R. B. & Wilczynski, W. 1990. The role of environmental selection in intraspecific divergence of mate recognition signals in the cricket frog, *Acris crepitans*. *Evolution*, **44**, 1869-1872.
- Ryan, M. J., Pease, C. M. & Morris, M. R. 1992. A genetic polymorphism in the swordtail *Xiphophorus nigrensis*: testing the prediction of equal fitnesses. *American Naturalist*, **139**, 21-31.
- Ryan, M. J., Rand, A. S. & Weigt, L. A. 1996. Allozyme and advertisement call variation in the tungara frog, *Physalaemus pustulosus*. *Evolution*, **50**, 2435-2453.

- Ryan, M. J. & Wilczynski, W. 1988. Coevolution of sender and receiver: effect on local mate preference in cricket frogs. *Science*, **240**, 1786-1788.
- Saarikettu, M., Liimatainen, J. O. & Hoikkala, A. 2005. Intraspecific variation in mating behaviour does not cause sexual isolation between *Drosophila virilis* strains. *Animal Behaviour*, **70**, 417-426.
- Sargent, R. C., Gross, M. R. & Vandenberghe, E. P. 1986. Male mate choice in fishes. *Animal Behaviour*, **34**, 545-550.
- Scaggiante, M., Grober, M. S., Lorenzi, V. & Rasotto, M. B. 2004. Changes along the male reproductive axis in response to social context in a gonochoristic gobiid, *Zosterisessor ophiocephalus* (Teleostei, Gobiidae), with alternative mating tactics. *Hormones And Behavior*, **46**, 607-617.
- Scaggiante, M., Mazzoldi, C., Petersen, C. W. & Rasotto, M. B. 1999. Sperm competition and mode of fertilization in the grass goby *Zosterisessor ophiocephalus* (Teleostei: Gobiidae). *Journal Of Experimental Zoology*, **283**, 81-90.
- Schlupp, I., McKnab, R. & Ryan, M. J. 2001. Sexual harassment as a cost for molly females: bigger males cost less. *Behavior*, **138**, 277-286.
- Shaw, P. W., Carvalho, G. R., Magurran, A. E. & Seghers, B. H. 1994. Factors affecting the distribution of genetic variability in the guppy, *Poecilia reticulata*. *Journal Of Fish Biology*, **45**, 875-888.
- Sih, A. 1988. The effects of predators on habitat use, activity and mating behavior of a semi-aquatic bug. *Animal Behaviour*, **36**, 1846-1848.
- Sih, A. 1994. Predation risk and the evolutionary ecology of reproductive behaviour. *Journal Of Fish Biology*, **45**, 111-130.
- Sih, A., Bell, A. & Johnson, J. C. 2004. Behavioral syndromes: an ecological and evolutionary overview. *Trends in Ecology & Evolution*, **19**, 372-378.
- Sih, A., Krupa, J. & Travers, S. 1990. An experimental study on the effects of predation risk and feeding regime on the mating behavior of the water strider. *American Naturalist*, **135**, 284-290.
- Sih, A. & Krupa, J. J. 1992. Predation risk, food deprivation and nonrandom mating by size in the stream water strider, *Aquarius remigis*. *Behavioral Ecology And Sociobiology*, **31**, 51-56.
- Sih, A., Ziemba, R. & Harding, K. C. 2000. New insights on how temporal variation in predation risk shapes prey behavior. *Trends In Ecology & Evolution*, **15**, 3-4.
- Simcox, H., Colegrave, N., Heenan, A., Howard, C. & Braithwaite, V. A. 2005. Context-dependent male mating preferences for unfamiliar females. *Animal Behaviour*, **70**, 1429-1437.
- Simmons, L. W. 1987. Heritability of a male character chosen by females of the field cricket, *Gryllus bimaculatus*. *Behavioral Ecology And Sociobiology*, **21**, 129-133.
- Simmons, L. W. & Bailey, W. J. 1990. Resource influenced sex roles of zaprochiline tettigoniids (Orthoptera:Tettigoniidae). *Evolution*, **44**, 1853-1868.
- Slabbekoorn, H. & Smith, T. B. 2002. Habitat-dependent song divergence in the little greenbul: An analysis of environmental selection pressures on acoustic signals. *Evolution*, **56**, 1849-1858.

- Slagsvold, T. & Saetre, G. P. 1991. Evolution of plumage color in male pied flycatchers (*Ficedula hypoleuca*): evidence for female mimicry. *Evolution*, **45**, 910-917.
- Smith, E. J., Partridge, J. C., Parsons, K. N., White, E. M., Cuthill, I. C., Bennett, A. T. D. & Church, S. C. 2002. Ultraviolet vision and mate choice in the guppy (*Poecilia reticulata*). *Behavioral Ecology*, **13**, 11-19.
- Smith, M. E. & Belk, M. C. 2001. Risk assessment in western mosquitofish (*Gambusia affinis*): do multiple cues have additive effects? *Behavioral Ecology And Sociobiology*, **51**, 101-107.
- Snelson, F. F., Jr. 1989. Social and environmental control of life history traits in Poeciliid fishes. In: *Ecology and evolution of livebearing fishes (Poeciliidae)* (Ed. by Meffe, G. K. & Snelson, F. F., Jr.). New Jersey: Prentice-Hall.
- Snook, R. R. 2005. Sperm in competition: not playing by the numbers. *Trends In Ecology & Evolution*, **20**, 46-53.
- Sokal, R. R. & Rohlf, F. J. 1969. *Biometry*. San Francisco: W. H. Freeman and Company.
- Stearns, S. C. 1989. Trade-offs in life-history evolution. *Functional Ecology*, **3**, 259-268.
- Stockley, P., Gage, M. G. J., Parker, G. A. & Moller, A. P. 1997. Sperm competition in fishes: The evolution of testis size and ejaculate characteristics. *American Naturalist*, **149**, 933-954.
- Strong, D. R. 1973. Amphipod amplexus: significance of ecotypic variation. *Ecology*, **54**, 1383-1388.
- Sutherland, W. J. 1985. Measures of sexual selection. In: *Oxford surveys in evolutionary biology* (Ed. by Dawkins, R. & Ridley, M.), pp. 90-101. Oxford: Oxford University Press.
- Taborsky, M. 1998. Sperm competition in fish: 'bourgeois' males and parasitic spawning. *Trends In Ecology & Evolution*, **13**, 222-227.
- Taborsky, M. 2001. The evolution of bourgeois, parasitic, and cooperative reproductive behaviors in fishes. *Journal of Heredity*, **92**, 100-110.
- Tarof, S. A., Dunn, P. O. & Whittingham, L. A. 2005. Dual functions of a melanin-based ornament in the common yellowthroat. *Proceedings Of The Royal Society B-Biological Sciences*, **272**, 1121-1127.
- Thompson, D. B. 1999. Different spatial scales of natural selection and gene flow: the evolution of behavioral geographic variation and phenotypic plasticity. In: *Geographic variation in behavior* (Ed. by Foster, S. A. & Endler, J. A.). New York: Oxford University Press.
- Toft, G., Edwards, T. M., Baatrup, E. & Guillette, L. J. 2003. Disturbed sexual characteristics in male mosquitofish (*Gambusia holbrooki*) from a lake contaminated with endocrine disruptors. *Environmental Health Perspectives*, **111**, 695-701.
- Tokarz, R. R. 1992. Male mating preference for unfamiliar females in the lizard, *Anolis sagrei*. *Animal Behaviour*, **44**, 843-849.
- Tomkins, J. L. & Simmons, L. W. 2002. Measuring relative investment: a case study of testes investment in species with alternative male reproductive tactics. *Animal Behaviour*, **63**, 1009-1016.

- Travis, J. & Woodward, B. D. 1989. Social context and courtship flexibility in male sailfin mollies, *Poecilia latipinna* (Pisces: Poeciliidae). *Animal Behaviour*, **38**, 1001-1011.
- Tregenza, T. & Wedell, N. 2000. Genetic compatibility, mate choice and patterns of parentage: Invited review. *Molecular Ecology*, **9**, 1013-1027.
- Trivers, R. L. 1972. Parental investment and sexual selection. In: *Sexual selection and the descent of man, 1871-1971* (Ed. by Campbell, B.), pp. 136-179. London: Heinemann.
- Trumbo, S. T. 1999. Using integrative biology to explore constraints on evolution. *Trends In Ecology & Evolution*, **14**, 5-6.
- Tsukamoto, L., Kuki, K. & Tojo, S. 1994. Mating tactics and constraints in the gregarious insect *Parastrachia japonensis* (Hemiptera, Cydnidae). *Annals Of The Entomological Society Of America*, **87**, 962-971.
- Turner, C. L. 1937. Reproductive cycles and superfetation in poeciliid fishes. *Biological Bulletin*, **72**, 145-164.
- Turner, C. L. 1938. The reproductive cycle of *Brachyrhaphis episcopi*, an ovoviviparous poeciliid fish in the natural tropical habitat. *Biological Bulletin*, **75**, 56-65.
- Tuttle, M. D. & Ryan, M. J. 1981. Bat predation and the evolution of frog vocalizations in the Neotropics. *Science*, **214**, 677-678.
- Tuttle, M. D. & Ryan, M. J. 1982. The role of synchronized calling, ambient light, and ambient noise, in anti-bat-predator behavior of a treefrog. *Behavioral Ecology And Sociobiology*, **11**, 125-131.
- Tuttle, M. D., Taft, L. K. & Ryan, M. J. 1982. Evasive behavior of a frog in response to bat predation. *Animal Behaviour*, **30**, 393-397.
- Uzendoski, K., Maksymovitch, E. & Verrell, P. 1993. Do the risks of predation and intermale competition affect courtship behavior in the salamander *Desmognathus ochrophaeus*? *Behavioral Ecology And Sociobiology*, **32**, 421-427.
- Vehrencamp, S. L. & Bradbury, J. W. 1984. Mating systems and ecology. In: *Behavioural Ecology: An Evolutionary Approach (2nd Edition)* (Ed. by Krebs, J. R. & Davies, N. B.). Oxford: Blackwell Scientific Publications.
- Verrell, P. A. 1999. Geographic variation in sexual behavior. In: *Geographic variation in behavior* (Ed. by Foster, S. A. & Endler, J. A.). New York: Oxford University Press.
- Verrell, P. A. & Krenz, J. D. 1998. Competition for mates in the mole salamander, *Ambystoma talpoideum*: Tactics that may maximize male mating success. *Behaviour*, **135**, 121-138.
- Ward, A. J. W. & Hart, P. J. B. 2003. The effects of kin and familiarity on interactions between fish. *Fish and Fisheries*, **4**, 348-358.
- West-Eberhard, M. J. 1983. Sexual selection, social competition, and speciation. *Quarterly Review Of Biology*, **58**, 155-183.
- Westneat, D. F. 1994. To guard mates or go forage - Conflicting demands affect the paternity of male red-winged blackbirds. *American Naturalist*, **144**, 343-354.
- Witte, K., Farris, H. E., Ryan, M. J. & Wilczynski, W. 2005. How cricket frog females deal with a noisy world: habitat-related differences in auditory tuning. *Behavioral Ecology*, **16**, 571-579.

- Wong, B. B. M. & Jennions, M. D. 2003. Costs influence male mate choice in a freshwater fish. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **270**, S36-S38.
- Yezerinac, S. M., Loughheed, S. C. & Handford, P. 1992. Measurement error and morphometric studies: statistical power and observer experience. *Systematic Biology*, **41**, 471-482.
- Zaret, T. M. & Rand, A. S. 1971. Competition in tropical stream fishes: support for the competitive exclusion principle. *Ecology*, **52**, 336-342.
- Zbinden, M., Mazzi, D., Kunzler, R., Largiader, C. R. & Bakker, T. C. M. 2003. Courting virtual rivals increase ejaculate size in sticklebacks (*Gasterosteus aculeatus*). *Behavioral Ecology and Sociobiology*, **54**, 205-209.
- Zimmerer, E. J. & Kallman, K. D. 1989. Genetic basis for alternative reproductive tactics in the pygmy swordtail, *Xiphophorus nigrensis*. *Evolution*, **43**, 1298-1307.
- Zuk, M., Simmons, L. W. & Cupp, L. 1993. Calling characteristics of parasitized and unparasitized populations of the field cricket *Teleogryllus oceanicus*. *Behavioral Ecology And Sociobiology*, **33**, 339-343.

Appendix 1. Preliminary laboratory studies of male mating behaviour in *Brachyrhaphis episcopi*

A1.1 Aims

1. To characterize and quantify male reproductive behaviour of the poeciliid fish *Brachyrhaphis episcopi*.
2. To investigate relationships between fish size (male standard length, female standard length, male:female length ratio) and reproductive behaviours

A1.2 Methods

A1.2.1 Experimental procedure

Table A1 Numbers of female-deprived and non-deprived males tested from each population

Stream	Upstream		Downstream	
	Not deprived	Female deprived	Not deprived	Female deprived
Agua Salud (AS)	6	0	0	0
Quebrada Juan Grande (QJG)	0	2	2	5
Rio Macho (RM)	6	5	4	4
ALL	12	7	6	9

I tested 35 wild-caught male *B. episcopi* that had been housed in the laboratory for between 2 and 7 months (Table A1). 16 of these males had been housed without access to females for at least 1 month, so I also examined whether female-deprived males differed in behaviours from non-deprived males.

I placed the test male in a 12L aquarium that was divided into two halves by a clear partition. I transferred a female from the same population but different stock tank (unfamiliar) to the other half of the aquarium. Each half of the aquarium contained a small plant pot for cover, and was filled with mature water. I allowed fish to settle overnight before testing the following day. I did not know the stage of the reproductive cycle for females.

Prior to testing, I fed both fish with a small pinch of standard flake food and arranged the aquarium for observations. I placed a white shroud around the front of the tank, and set up a digital video recorder to record fish behaviour through a small opening in the shroud. After sixty minutes, I lifted the clear partition via a pulley system. For 10 minutes, I recorded behaviour of the male towards the female onto a digital video tape. At the end of the trial, I took mass and standard length measurements of both

fish. Fish were retained in the test aquarium, separated by a partition and returned to their stock tanks the following day.

From the video analysis, I recorded the following behaviours:

1. Time spent associating with female (within one body length and orientated towards the female)
2. Number of attempted thrusts
3. Number of courtships
4. Number of turns in courtship
5. Number of mating attempts following courtship
6. Number of displays (body in similar position to courtship, but without turns or forward movement)
7. Total duration of displays
8. Number of nips or lunges by the female to the male
9. Total duration of chases by the female to the male
10. Time spent in gonoporal nibbling
11. Number of gonopodial jerks during gonoporal nibbling.

A1.2.2 Statistical analyses

I considered that males that had been housed in the same tank to be non-independent of each other. Thus analyses were carried out on mean behaviours and fish sizes per tank (N=16). Males were sampled from a number of populations (table A1). There were insufficient independent replicates to investigate individual population differences (i.e. interaction between stream and upstream/downstream), but stream and upstream/downstream were included in general linear models to control for any variation between streams or between upstream and downstream populations.

Firstly, I examined how behaviours correlated with each other. To examine characteristics of courtship attempts (mean number of turns and proportion of courtships followed by mating attempts) I only used data from thirteen tanks where courtship was observed.

Secondly, I examined variation between tank means for the four most common male behaviours (table A2). I looked to see whether males deprived of females differed in behaviours to males housed with females, whether males from each stream or from upstream and downstream differed in behaviours and whether behaviours were related to male size, female size or the ratio of male to female size. I used general linear models (GLM) in Minitab (Minitab 14.1, 2003, Minitab Inc., Six Sigma Academy International) to examine these relationships. Time data were converted to proportions and transformed with the arcsine square-root transformation; count data were square-root transformed.

A1.3 Results

Table A2 Percentage of trials in which behaviours were observed

Behaviour:	Observed in % of trials (N=34)
Associating with females	82.4
Attempted thrusts	76.5
Courtship	61.8
Post-courtship mating attempts	52.9
Displays	67.6
Gonoporal nibbling	32.4
Gonopodial jerks	20.6
Nips or lunges from female	58.8
Chases from female	50.0

The most common behaviours carried out by males were associating with females, thrust attempts, courtship and displays (table A2).

A1.3.1 Relationships among mean behaviours of males from each tank

The mean number of thrusts by males from a tank was positively correlated with mean duration of gonoporal nibbling and mean number of gonopodial jerks (Spearman's rank correlations: $R_s = 0.568$, $P = 0.022$; $R_s = 0.562$, $P = 0.023$, $N = 16$). There was also a trend for mean number of thrusts by males to increase with mean duration of association time with females (Spearman's rank correlation: $R_s = 0.466$, $P = 0.069$; $N = 16$).

Males from tanks that carried out more courtships did not spend a greater amount of time associating with females, and did not carry out more thrust attempts or more turns per courtship ($P > 0.209$). However, males from tanks that attempted to mate after a greater proportion of courtships carried out more gonopodial jerks ($R_s = 0.770$, $P = 0.002$, $N = 13$).

Males from tanks that received more aggression from females (number of nips and duration of chases) attempted more thrusts ($R_s = 0.611$, $P = 0.012$; $R_s = 0.567$, $P = 0.022$, $N = 16$). Males that received a greater mean number of nips from females also spent more time on gonoporal nibbles ($R_s = 0.579$, $P = 0.019$, $N = 16$) and males that spent longer being chased by females attempted more gonopodial jerks ($R_s = 0.586$, $P = 0.016$, $N = 16$).

Mean number and duration of displays did not correlate with any other behavioural variables except each other ($R_s = 0.811$, $P < 0.001$, $N = 16$).

A1.3.2 Fish sizes and effects of female-deprivation

The mean number of courtships by males had a tendency to increase as the mean size of males in a tank increased ($R_s = 0.466$, $P = 0.069$, $N = 16$). No other behavioural variables correlated with mean male size, mean female size or mean male: female size ratio ($P > 0.18$).

Examination of mean courtship number using GLMs revealed a positive relationship with mean male size in only males from Rio Macho tanks (significant stream and mean male size interaction: GLM: $F_{2,8} = 6.07$, $P = 0.025$). There were no significant relationships between mean courtship number and male size in QJG and AS, but this is not surprising as I tested 4 tanks from QJG and only 2 from AS. Males from tanks that had been deprived of females attempted more courtships than males that had not been female-deprived prior to testing ($F_{1,8} = 9.63$, $P = 0.015$).

The mean proportion of time spent associating with females increased with the mean ratio between male and female size in males that had not been female-deprived, but there was no relationship with males that were female-deprived ($F_{1,9} = 7.66$, $P = 0.022$). This implies that as mean male size increases relative to mean female size, males spent a greater amount of time associating with females.

Males from tanks that had been isolated from females carried out more displays ($F_{1,11} = 9.62$, $P = 0.010$), as did males from downstream populations ($F_{1,11} = 6.33$, $P = 0.029$).

A1.4 Summary

1. There were no correlations between the four most common male behaviours (associating with females, thrusts, courtship and displays) except for a tendency for the number of thrust attempts to increase with association time.
2. There were little effects of fish sizes on any measured behaviours. In the stream with the greatest number of independent replicates (RM), the number of courtship attempts increased with male size.
3. Males from tanks that were female-deprived prior to testing attempted more courtships and performed more displays than non-deprived males.
4. As the mean of male size, relative to female size increased, non-deprived males spent increasingly more time associating with females but association times of female-deprived males did not vary with male: female size ratio.



Context-dependent male mating preferences for unfamiliar females

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We used laboratory and field-based experiments to examine male mate choice in the promiscuous Panamanian bishop *Brachyrhaphis episcopi* (Poeciliidae) (also referred to as *Brachyrhaphis episcopi*). As females of this species can store sperm, males could increase their reproductive success by selectively mating with different females. To test this, males in both the laboratory and field were allowed to choose between a familiar (same tank or pool) and an unfamiliar female (different tank or pool). We compared males from streams and seasonally occurring pools to see whether lack of access to new females in pools promotes male mate choice for unfamiliarity. In addition, we chose streams and pools both with and without predatory fish to examine the influence of predation risk. In both the laboratory and the field, males attempted more matings with unfamiliar than familiar females. Field experiments showed that courtship preferences differed between males from populations with and without predatory fish: males from populations with predators were choosy only when light levels were dim, whereas males from populations without predators were choosy when light levels were brighter. Males from both streams and pools discriminated between familiar and unfamiliar females, but there were no differences in mating preferences between males from each habitat. Although the reasons for a preference for unfamiliar females remain unclear, the plasticity of this behaviour is evident. Differences in male mate choice between populations with and without predators suggest that males may face a trade-off between the costs and benefits of being choosy.

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An individual's choice of mate or mates may have profound consequences for their subsequent fitness (Halliday 1983) and thus has an important evolutionary role (Partridge & Halliday 1984). As females usually invest more in each offspring, they tend to be more discriminating than males in their choice of mates (Bateman 1948; Trivers 1972). If there is variation in female quality, however, and the costs of being choosy are sufficiently low, males should preferentially mate with females that will maximize their reproductive success (Parker 1983). Male mate choice has been documented repeatedly, with selection for more colourful females (e.g. Amundsen et al. 1997; Amundsen & Forsgren 2003), larger females (e.g. Ptacek & Travis 1997; Herdman et al. 2004; but see Basolo 2004) and genetic compatibility (e.g. Ryan & Altmann 2001). A female-biased operational sex ratio may also promote choosiness in males (Hubbell & Johnson 1987).

Even the most promiscuous male may be choosy. If male reproductive success increases with the number of female partners, a male should benefit from selectively mating with different females (Adler 1978). Such a benefit is invoked to explain the Coolidge effect, where satiated polygamous males show a heightened sexual interest in new females (Dewsbury 1981). A preference for new or unfamiliar females has also been shown in males that were not satiated, in lizards (*Holbrookia propinqua*: Cooper 1985; *Anolis sagrei*: Tokarz 1992; *A. carolinensis*: Orrell & Jenssen 2002), salamanders, *Desmognathus ochrophaeus* (Donovan & Verrell 1991), wild horses, *Equus caballus* (Berger & Cunningham 1987) and guppies, *Poecilia reticulata* (Kelley et al. 1999). Guppies show population variation in this behaviour (Kelley et al. 1999). Male guppies confined in seasonal pools preferentially court unfamiliar over familiar females, but males from rivers show no preference. Kelley et al. (1999) suggested that males from rivers move between schools of females to maximize their mating opportunities and do not need to, or cannot, discriminate between familiar and unfamiliar females. Males trapped in pools, however, may benefit from identifying and mating with unfamiliar females.

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Mate choice behaviour varies not only between populations, but also with environmental conditions (e.g. Endler & Théry 1996; Houde 1997). Predation risk, in particular, may affect mate choice: models predict that choosiness is reduced or absent as the costs of choice increase (Sutherland 1985; Hubbell & Johnson 1987; Crowley et al. 1991). In a number of fish species, female mate choice is absent or altered when a predator is present (e.g. Forsgren 1992; Briggs et al. 1996; Godin & Briggs 1996; Johnson & Basolo 2003). Predation risk affects male mating behaviour (e.g. Endler 1987; Magurran & Seghers 1990; but see Evans et al. 2002) and may also affect male mate choice (Houde 1997). A recent study supports the theory that male mate choice should change as the costs of choosing increase (Wong & Jennions 2003), but the relation between predation risk and male mate choice has been specifically addressed only in pipefish, which have a role-reversed mating system (Berglund 1993).

We examined male mate choice in the Panamanian bishop *Brachyrhaphis episcopi* (Poeciliidae) (also referred to as *Brachyrhaphis episcopi*), a promiscuous live-bearing fish native to central Panama. In the wild, males occasionally court females, but the majority of attempted matings are coercive (H. Simcox, unpublished data). The operational sex ratio is highly skewed in favour of females, which defend profitable foraging areas within a stream. Larger males attempt to monopolize access to females through aggression towards smaller males (H. Simcox, personal observation). Lack of paternal care combined with female sperm storage means that males could benefit from mating with as many different females as possible. We predicted that, if males could acquire familiarity with resident females, those males would preferentially mate with unfamiliar females.

We used both laboratory and field-based mate choice experiments to examine whether male *B. episcopi* prefer unfamiliar females, and how this choice varies between populations under different environmental conditions. We compared populations from streams and seasonally occurring pools, since this factor affects male mating preferences in guppies (Kelley et al. 1999). We also examined the possible influence of predation risk on male mate choice by selecting streams and pools with and without predatory fish. We estimated light levels in the field, because these may interact with factors such as predator density to affect male mating behaviour (e.g. Endler 1987).

METHODS

Study System

We sampled *B. episcopi* from five independent freshwater streams that run into the Panama Canal (Pipeline Road, Parque Nacional Soberania, Panama, Fig. 1). Each stream flows across an escarpment creating a waterfall, which acts as a barrier to the upstream movement of larger fish (Jennions & Telford 2002). Above the falls, the fish community is dominated by *B. episcopi* and the killifish

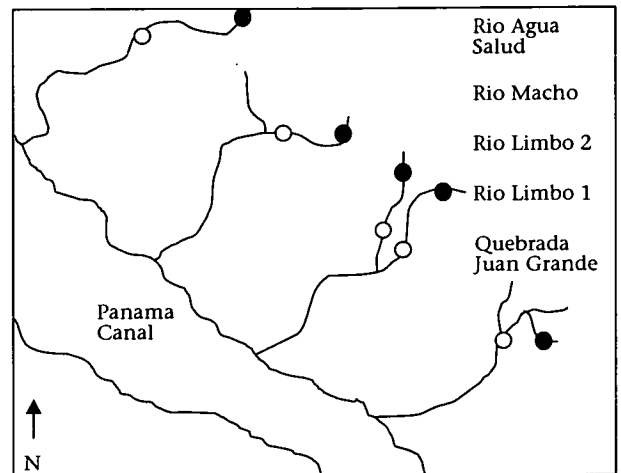


Figure 1. Populations of *Brachyrhaphis episcopi* sampled from the Frijoles drainage basin, Parque Nacional Soberania, Panama. Coordinates of each site are available on request. ●: Low predator density population; ○: high predator density population.

Rivulus brunneus (Cyprinodontidae). Although *R. brunneus* may prey on juvenile *B. episcopi*, it is incapable of eating adults (Angermeier & Karr 1983). Below the falls is a suite of piscivorous fish that live in sympatry with *B. episcopi*, such as the tigerfish, *Hoplias microlepis*, various cichlids and tetras (Brown & Braithwaite 2004).

Brachyrhaphis episcopi inhabits the headwaters of streams and is replaced further downstream by two other poeciliid species, *Brachyrhaphis cascajalensis* (Mojica et al. 1997; Jennions & Telford 2002) and *Neoheterandria tridentiger* (H. Simcox, personal observation). This transition occurs upstream of any junctions where our study streams merge. We therefore consider each stream to be independent and treat sites with high and low predator densities within a stream as independent populations in our analyses (as defined by Johnson & Belk 2001; Jennions & Kelly 2002; Jennions & Telford 2002).

Laboratory Experiment

Fish were collected from the wild with dip nets and were air-freighted to the University of Edinburgh under a collection and export permit from the Autoridad Nacional del Ambiente (ANAM) and an import permit from the Scottish Executive Environment and Rural Affairs Department. A total of 400 fish were transported in July 2002 and February 2003 as part of a larger study. All fish survived transport. Fish were housed for a minimum of 4 months in mixed-sex 90-litre tanks before testing. Between one and four males were housed with around 15 females from the same population, reflecting the highly skewed sex ratio found in the wild. Any offspring from the wild-caught fish were removed and housed separately to the adults. Tanks were maintained at 24–26°C on a 12:12 h light: dark regime and fish received standard flake food daily.

We carried out the laboratory experiment in June–August 2003. We tested 20 males, but because males from each tank were not considered to be independent, we pooled data for each tank ($N = 11$). Three populations were in sites with a high predator density (Rio Agua Salud, $N = 2$; Rio Macho, $N = 2$; Quebrada Juan Grande, $N = 2$) and three in sites with a low predator density (Rio Agua Salud, $N = 2$; Rio Macho, $N = 2$; Rio Limbo 1, $N = 1$). Sampling was not equal for all populations as a result of differing sex ratios in the holding tanks before the experiment. We recorded the standard lengths of all fish ($\bar{X} \pm \text{SD}$; males: 25.3 ± 2.90 mm; familiar females: 35.9 ± 4.36 mm; unfamiliar females: 36.2 ± 4.90 mm) and no fish was used more than once in the experiment.

We ran two tests to examine male mate choice between a 'familiar' female (same tank) and 'unfamiliar' female (different tank, same population). The first was a dichotomous choice test where a female was contained at either end of a tank, and the relative time that a male spent in the 'choice zone' next to each female was used to infer mating preference. This was followed by an open tank test, where the male could interact freely with both females.

We did not know the reproductive status of females, but around 10% of females were probably receptive to males (based on a 3-day receptive period in a 30-day reproductive cycle in other poeciliids, e.g. Houde 1997). There is no reason to suspect that, on average, familiar and unfamiliar females differed in receptive state. As females store sperm, we do not know whether familiar females had successfully reproduced with test males before testing, but it seems unlikely that copulation with the familiar female had not occurred during the 4-month pre-experimental period.

We assembled the test arena inside a 90-litre tank (Fig. 2), which was masked by white fabric. The back and sides of the arena were made from grey plastic, and we made observations via a video camera through the

clear plastic front. The arena was divided into three compartments separated by grey and clear plastic removable screens. The tank was lit from above by a 15-W fluorescent lamp, which had an overall colour output close to that of natural sunlight including components in the ultraviolet (Arcadia, Croyden, U.K.). As UV light affects mate choice in other poeciliids (Smith et al. 2002), we used clear plastic that did not block its transmission.

With all partitions in place, we placed a male into a clear tube in the centre compartment, a familiar female into one side compartment and a size-matched unfamiliar female into the other (sides assigned at random). We allowed the fish to settle for 10 min before we lifted the opaque screens and for a further 3 min before the trial began. We lifted the clear tube to release the male and, for 10 min, we recorded the time that he spent in each choice zone. For a trial to be valid, the male had to visit both choice zones. To minimize the handling stress to the fish, we did not repeat the trial with females positioned on opposite sides. Over all trials males showed no side preference (paired t test: $t_{19} = 0.83$, $P = 0.416$).

Immediately after the dichotomous choice trial, we lifted the clear screens allowing all three fish access to the whole arena. For 10 min, we recorded (1) association time, where the male was oriented towards and within one (female) body length of the focal female (time spent attempting thrusts and courting was included in this measure), (2) number of thrusts (coercive mating attempts) towards the focal female, where the male darted quickly underneath the female, (3) number of courtship attempts, where the male displayed with extended fins and gonopodium in front of the female. Courtship attempts included at least one turn. Each attempt was scored as one courtship until the display stopped (female or male swam away or male attempted to mate). We also counted (4) aggressive acts (nips or chases) by the focal female towards the male, as an indication of female receptivity (Reynolds & Gross 1992). A male may avoid nips or short chases by increasing the distance between himself and the female. Prolonged chase sequences, however, may indicate that the male is unable to avoid aggression from the female. Thus, we stopped any trials where the male was continuously chased for more than 10 s. Under this criterion, one trial was aborted after 8 min 2 s, although the male did not show any physical damage. All fish were returned to their holding tanks for use in future behavioural observations.

Field Experiment

To examine whether males discriminate between familiar and unfamiliar females in the wild, we tested fish, immediately after capture in January–April 2004, in an artificial pool at the side of each stream. The artificial pool (diameter 1 m, mean water height 15 cm) was lined with small pebbles and filled with stream water.

We tested 53 males from six populations. Three populations occurred with high densities of predators (Rio Macho, $N = 10$; Rio Limbo 1, $N = 8$; Rio Limbo 2, $N = 8$) and three with low densities of predators (Rio Macho,

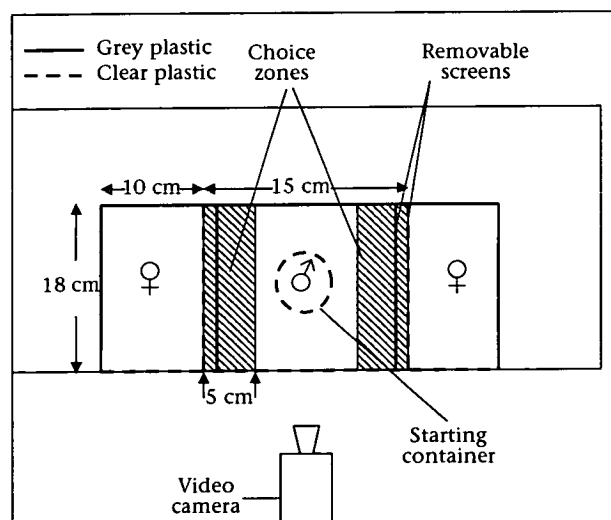


Figure 2. Schematic diagram of view above the holding tank containing the test arena used for the laboratory experiment (water height 26 cm).

$N = 10$; Rio Limbo 1, $N = 8$; Rio Limbo 2, $N = 9$). As a result of low discharge during the dry season, Rio Limbo 1 had dried up into a series of widely separated pools. Rio Macho and Rio Limbo 2, however, were still flowing and could be characterized by defined pool-riffle sequences (Angermeier & Karr 1983). Each male came from a different pool, and we used no fish more than once.

Male mate choice was tested with a familiar female (same pool) and an unfamiliar size-matched female from the same population (different pool, 20–100 m along the river). We captured fish with dip nets under an ANAM permit and returned them to their pool of capture after testing. We use the terms 'familiar' and 'unfamiliar' for consistency with the laboratory experiment, although we cannot be sure that fish caught in the same pool in Rio Macho or Rio Limbo 2 were familiar with each other. As with the laboratory experiment, we did not know the reproductive status of females, but there is no reason to suspect that familiar and unfamiliar females differed, on average, in receptive state. We measured all fish used ($\bar{X} \pm \text{SD}$; males: 22.6 ± 2.55 mm; familiar females: 30.8 ± 4.79 mm; unfamiliar females: 31.1 ± 4.97 mm).

We released a familiar and an unfamiliar female into the artificial pool and allowed them to acclimatize for 10 min. In pilot trials, females swam slowly around the pool and foraged within this time. After 10 min, we released a male into the pool at a point equidistant from both females. Males settled more quickly than females and began to show sexual interest in the females within 3–5 min. Behavioural recording began after the male had been in the pool for 5 min.

Two of us sat at opposite sides of the pool recording male mating behaviour towards the familiar or unfamiliar female. We alternated between females for subsequent trials. Females could be individually identified by small differences in size, shape or markings.

For 10 min, we recorded behaviours as detailed in the open tank laboratory experiment. We also counted displays, where a male positions himself with extended fins and gonopodium in front of a female but does not turn. We interpreted these displays as the beginning of courtship attempts that were terminated by the female (swam away or was aggressive towards male) or by the male (swam away). During one trial, the display was part of an agonistic encounter, rather than courtship, and the counts were not included in our analyses.

Given the importance of light levels for guppy mating behaviour (Endler 1987; Reynolds 1993; Reynolds et al. 1993), we incorporated estimates of ambient light level into our analysis. We estimated the mean light levels on the pool during the trial as dim, dim/medium, medium/bright or bright. As a result of changes in cloud cover, light levels changed quickly during the observation period and across the area of the pool. Thus, it was not appropriate to take a single reading before or after the trial or at any one point over the pool, and we believe that our estimates, although not quantitative, provide a relative measure of mean light intensity differences between trials.

We found no significant differences in light levels between individual sites (Kruskal–Wallis test: $H_5 = 8.00$, $P = 0.156$) or by predator density ($H_1 < 0.01$, $P = 0.965$).

Median light levels were 'dim/medium' in low predator density sites for both tributaries of the Rio Limbo and for the high predator density site in Rio Macho. At the other sites, median light levels were 'medium/bright'.

Irradiance measurements were made by Endler (1993) in March 1989 in a lowland tropical rainforest on Barro Colorado Island, a few kilometres from our study area. In sunny conditions in large forest gaps, mean total light intensity (400–700 nm) was $1195.8 \mu\text{mol}/\text{m}^2$ per s (Endler 1993). In cloudy conditions, total light intensities are likely to be lower than this, although this is highly site specific, depending on the size of the canopy gap (Endler 1993).

Data Analyses

We defined male mating preference as male behaviour towards the unfamiliar female minus behaviour towards the familiar female. Differences in female aggression were calculated in the same way (score for unfamiliar female minus familiar female). Not all males attempted thrusts, courtships or displays, and we analysed variation in male mating preference using subsets of data that excluded these males. To examine whether our measures of mating preference were correlated with each other, we used the whole data set (including those males that did not court, display or thrust). For statistical analyses we used Minitab version 13 (Minitab Inc., State College, PA, U.S.A.) and StatView version 5.01 (SAS Institute Inc., Cary, NC, U.S.A.). Where necessary, data were transformed to meet requirements of parametric analysis.

For the laboratory experiment, we standardized the dichotomous choice data by dividing male preference (time spent in unfamiliar choice zone minus time in familiar choice zone) by the total time spent in both choice zones. Data from the open tank trial that was aborted after just over 8 min were excluded from our analyses. We did not have enough data points to examine predator density, stream and fish size in one analysis, so we tested each variable separately.

For the field experiment, we used GLMs to examine male behavioural differences between streams (random factor), predator density and light levels. There were not enough cross-factor combinations to analyse the full three-way interaction. Male and mean female standard length, time of day and distance between familiar and unfamiliar pools were added as covariates to the maximal GLM.

RESULTS

Laboratory Experiment

Males preferred unfamiliar to familiar females, but only in the open tank trial. Males spent more time associating with and attempted more thrusts with unfamiliar females (association time: \bar{X} difference \pm SE = 49.0 ± 17.7 s, paired t test: $t_9 = 2.76$, $P = 0.022$; thrusts: difference = 2.9 ± 1.0 thrusts, $t_8 = 3.03$, $P = 0.016$; Fig. 3). For one tank, the test male did not attempt any thrusts with either female. There was no significant difference in the

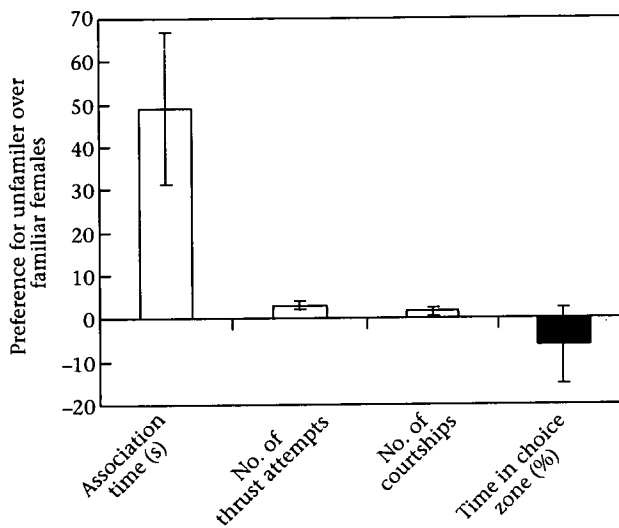


Figure 3. Mean \pm SE male mating preferences for unfamiliar over familiar females in the laboratory. In the open tank trial (\square) the male could interact freely with the females; in the dichotomous choice trial (\blacksquare) he had visual access only. Choice zone preference is presented as a percentage of the total time spent by the male in the choice zone.

number of courtships towards unfamiliar and familiar females (\bar{X} difference \pm SE = 1.7 ± 1.0 courtships, $t_9 = 1.71$, $P = 0.122$). In the dichotomous choice trial, where there was no physical access among fish, males showed no preference for either female ($t_{10} = -0.70$, $P = 0.499$).

Association preference and courtship preference were significantly positively correlated, but thrust preference was not correlated with either of these (Table 1). Although preferred females were sometimes more aggressive to males (Table 1, thrust preference), there were no differences in the number of aggressive acts received by males from familiar and unfamiliar females (paired t test: $t_9 = 0.49$, $P = 0.633$).

Table 1. Pearson correlations between measures of male mating preference, female aggression and standard lengths of fish in the laboratory

Behaviours	Association preference		Thrust preference		Courtship preference	
	r_8	P	r_8	P	r_8	P
Thrust preference	0.576	0.081	—	—	—	—
Courtship preference	0.969	<0.001	0.527	0.117	—	—
Aggression difference	0.589	0.073	0.796	0.009	0.532	0.113
Male size	0.281	0.431	0.582	0.077	0.193	0.592
Female size difference	0.584	0.076	0.381	0.277	0.617	0.057

Mating preferences and aggression were calculated as unfamiliar female score minus familiar female score. Differences in female sizes were calculated as unfamiliar female standard length minus familiar female standard length.

There were no differences in our measures of male mate choice between males from high and low predator density populations (association time: Student's t test: $t_8 = 1.00$, $P = 0.349$; thrust attempts: Mann-Whitney U test: $W = 31.5$, $N_1 = 6$, $N_2 = 3$, $P = 0.792$; courtship attempts: Student's t test: $t_8 = 1.41$, $P = 0.197$) or from different streams (association time: Kruskal-Wallis test: $H_3 = 2.98$, $P = 0.394$; thrust attempts: $H_3 = 2.37$, $P = 0.500$; courtship attempts: $H_3 = 1.82$, $P = 0.610$); however, sample sizes for each stream were very low. Male mate choice was not correlated with male standard length or difference in female standard lengths, but there was a marginally nonsignificant positive correlation between courtship preference and female size difference (Table 1).

Field Experiment

All 53 males spent some time associating with each female: 41 of these males attempted at least one thrust, 29 males displayed and 22 males courted at least once. Three of our measures of male mate choice (association time, thrusts and courtships) were significantly and positively correlated with each other, but not with display preference (Table 2).

Males spent more time associating with unfamiliar than familiar females but only in bright light levels (\bar{X} difference \pm SE = 52.4 ± 14.4 s). In light levels dimmer than this, males showed no preference between females (GLM: $F_{3,49} = 3.27$, $P = 0.029$). There were significant complex interactions involving stream of origin, predator density, light, time of day, mean female size and male size. However, after we removed interactions with size (which may be part of overall population differences), the remaining interactions were not significant.

Male courtship preference varied with light levels, but there was a significant interaction with predator density (GLM: $F_{3,14} = 4.41$, $P = 0.022$; Fig. 4). Males from low predator density populations courted unfamiliar females more than familiar females only in bright light (\bar{X} difference \pm SE = 4.0 ± 1.9 courtships) and medium/bright light (difference = 1.3 ± 0.8 courtships). In light levels dimmer than this, males showed no preference

Table 2. Spearman rank correlations between measures of male mating preference in the field ($N = 53$)

Ranked behaviours	Association preference		Thrust preference		Courtship preference	
	r_5	P	r_5	P	r_5	P
Thrust preference	0.531	<0.001	—	—	—	—
Courtship preference	0.325	0.018	0.570	<0.001	—	—
Display preference	-0.011	0.936	0.010	0.943	0.132	0.348

Mating preferences were calculated as unfamiliar female score minus familiar female score.

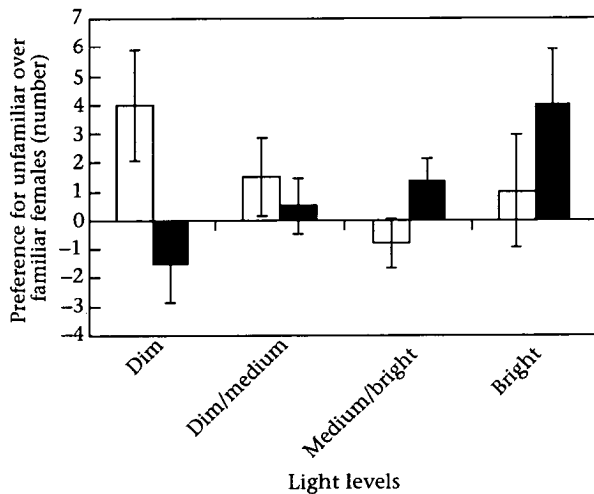


Figure 4. Least square mean \pm SE courtship preference for unfamiliar over familiar females in the field at various light levels. ■: Low predator density population; □: high predator density population.

between females. Conversely, males from high predator density populations preferentially courted unfamiliar females only in dim light levels (\bar{X} difference \pm SE = 4.0 ± 1.9 courtships). In light levels brighter than this, males showed no preference. The total number of courtships by males towards both females did not vary with light level or predator density.

Display preferences also varied, with a significant interaction between predator density and light levels (GLM: $F_{3,13} = 4.89$, $P = 0.017$), but the results differed to those observed for courtship preferences. In dim light levels, males from low and high predator density populations displayed more to unfamiliar than familiar females (\bar{X} difference \pm SE = 2.7 ± 0.8 and 3.2 ± 1.2 displays, respectively), but in intermediate light levels, males showed no preference for either female. In bright light levels, males from high predator density populations displayed more to unfamiliar females (difference = 4.5 ± 0.8 displays) but males from low predator density populations displayed more to familiar females (difference = 12.0 ± 1.5 displays).

Male display preferences also varied, with an interaction between stream and light levels (GLM: $F_{6,13} = 7.09$, $P = 0.002$). In dim light levels, males from Rio Limbo 2 displayed more to unfamiliar females (\bar{X} difference \pm SE = 9.3 ± 1.6 displays), but males from Rio Macho and Rio Limbo 1 showed no preferences. In intermediate light levels, males from all three rivers showed no preference for either female. In bright light levels, males from Rio Macho displayed more to unfamiliar than familiar females (\bar{X} difference \pm SE = 8.8 ± 0.9 displays) but males from Rio Limbo 1 and 2 displayed more to familiar females (difference = 7.8 ± 1.3 and -12.3 ± 1.7 displays, respectively).

Males showed no preferences between females when attempting thrusts, regardless of stream of origin, predator density, light levels or any interactions between these factors (light levels: GLM: $F_{3,32} = 0.38$, $P = 0.766$; predator density: $F_{3,35} = 0.22$, $P = 0.638$; stream: $F_{2,38} = 0.75$, $P = 0.478$). We found no relationships between courtship

or thrust preferences and male standard length, mean female standard length, time of day, distance between familiar and unfamiliar pools and differences in female aggression. In addition, familiar and unfamiliar females did not differ in the number of aggressive acts towards the male (Wilcoxon signed-ranks test: $T = 311.5$, $N = 53$, $P = 0.217$).

DISCUSSION

We found that male *B. episcopi* preferentially mated with unfamiliar females, in both the laboratory and the field. In the open tank laboratory experiment, all males appeared to prefer unfamiliar females, but with visual access only, males showed no mating preference. In the field, patterns of male mate choice were more complex than in the laboratory and depended on context. Males from high and low predator density populations showed variation in courtship and display preferences under increasing light levels. Association preferences also varied with light levels, but there were no population differences in this behaviour. In the laboratory, males attempted more thrusts with unfamiliar females, but this preference was not repeated in the field. Our results highlight the importance of testing animal behaviour in both laboratory and field-based experiments.

Populations with high and low predator densities showed striking differences in male courtship preferences as light levels changed in the field. Males from high predator density populations preferentially courted unfamiliar females but only when light levels were dim. In brighter light, males showed no courtship preference. Conversely, males from low predator density populations preferentially courted unfamiliar females in brighter light levels, but this preference decreased in dimmer light levels. Given the number of potential interactions, a type I error is possible. There are, however, good biological reasons to suspect that these patterns are real. Guppies experience an increased risk of predation in bright light (Endler 1987); we suggest that the risk is similar for *B. episcopi*. Certainly, predation pressure affects life history variables (Jennions & Telford 2002), boldness (Brown & Braithwaite 2004), cognitive abilities (Brown & Braithwaite 2005) and cerebral lateralization (Brown et al. 2004) in this species, so there is ample cause to suspect it also influences mate choice. If so, the response of the high predator density population is consistent with an evolutionary trade-off between the benefits and costs of being choosy (Fuller & Berglund 1996).

Predation pressure offers an additional explanation for the results reported by Kelley et al. (1999). Both river populations, where males did not discriminate between females, came from sites with high predator densities. In contrast, the pool populations, where males were choosy, came from a site with low predator density and a laboratory stock bred from a site with high predator density (J. L. Kelley, personal communication). As responses to predators may decline in laboratory-reared fish (e.g. Kelley & Magurran 2003), these results are consistent with the idea that males are choosy only when the risk of predation is low.

It is less clear why courtship preferences in males from low predator density populations varied with light levels. Long & Rosenqvist (1998) showed that male guppies from low predator density populations courted females at greater distances in bright light than in dim light. Male courtship may be more efficient in bright light conditions, and, in the absence of predators, males from low-predation populations are free to use this efficiency to their advantage. In turn, doing so may select for more conspicuous colour patterns in *B. episcopi* from low predator density areas, as seen in guppies (e.g. Endler 1978).

We found no evidence that overall light level differences covaried with predator regimes, supporting the idea that a common factor such as predator density may explain population differences. Other variables may correlate with fish predator density, however, including ecological (e.g. resource competition), physical (e.g. elevation, stream width) and biological (e.g. stress responses, sex ratio, differential cue use) factors. One or a combination of these factors could interact with light levels to create the observed patterns of male mate choice. Further experiments are required to test the role of predation, perhaps by conducting open tank mate choice experiments with wild-caught fish, while manipulating the risk of predation.

Unlike the study on guppies by Kelley et al. (1999), we found no differences in mating preferences between males from pools and males from rivers: males from both habitats discriminated between familiar and unfamiliar females. This result suggests that all males acquire familiarity with individuals in the same pool, and use this information during mate choice decisions. Why might *B. episcopi* differ to guppies? Male guppies move between schools of females (Griffiths & Magurran 1998), but perhaps male *B. episcopi* are much more sedentary. Pilot mark-release-recapture data suggest that *B. episcopi* move relatively little over a 3-week period (C. Brown & H. Simcox, unpublished data); females defend profitable foraging areas and males defend access to females (H. Simcox, personal observation). This sedentary behaviour could promote the acquisition of familiarity in streams as well as pools and explain why males from both habitats showed a preference for unfamiliar females.

We expected males from pools to show more interest than males from streams in unfamiliar females, but perhaps the pools had not been isolated for sufficient time for us to detect this difference. In guppies, familiarity (indicated by schooling preferences) takes 12 days to develop (Griffiths & Magurran 1997; Croft et al. 2004a). We tested pools 2–4 weeks after isolation, which should have been enough time to acquire familiarity, but the strength of individual recognition may increase further after this time. The development of familiarity may also be species-specific and depend on the context in which it is used (Ward & Hart 2003).

The numbers of adult *B. episcopi* in our sample pools (mean = 11.8, range 4–29) were not noticeably different to those tested by Kelley et al. (1999) (range 11–24). Our small group sizes could promote familiarity (Griffiths & Magurran 1997), but do not appear to explain differences between our results and those of Kelley et al.

It would also be interesting to know how male status affects male mate choice, as optimal choice may depend on the male's own quality or phenotype (Parker 1983). Evidence in the two-spotted goby, *Gobiusculus flavescens* (Amundsen & Forsgren 2003) and in the sailfin molly, *Poecilia latipinna* (Ptacek & Travis 1997) shows that smaller males are less choosy than larger males, presumably because smaller males usually have fewer mating opportunities and thus a greater cost to being choosy. Although we found no relation between male size and choosiness, we do not know the relative size and dominance status of each of our males within their home pool. More extensive mark-release-recapture studies are required, particularly examining differences in site fidelity between the sexes and between dominant and subordinate males. If combined with a measure of social networks (e.g. Croft et al. 2004b), this approach could be a powerful way to measure inter- and intrapopulation variation in familiarity before testing mate choice.

Kelley et al. (1999) found that only confined male guppies preferentially courted unfamiliar females, presumably as a mechanism to increase the number of different mating partners. In contrast, we found that both confined and nonconfined male *B. episcopi* could choose on the basis of familiarity, and we suggest that males in this species may be confined to a site by their behaviour even if they are not physically confined. Thus, males could benefit from mating with as many different partners as possible to increase their reproductive success (Adler 1978). We consider below other possible explanations that could be driving a preference for new unfamiliar females.

A mating preference for unfamiliar individuals may evolve to promote outbreeding, as well as to increase the number of partners (Farr 1977). Mate choice is thought to be cheaper than dispersal as a way of avoiding inbreeding (Blouin & Blouin 1988). We do not know whether *B. episcopi* are inbred or suffer inbreeding depression; fine-scale resolution of genetic relatedness would be required to examine this.

Alternatively, male preference for unfamiliar females may result from a sensory bias for novelty that has arisen from some purpose other than mate choice (Hughes et al. 1999). *Brachyrhaphis episcopi* are opportunistic omnivores (Angermeier & Karr 1983), so an attraction to novelty may be an adaptation for competitive foraging rather than optimal mate choice.

We used aggression from the female to the male as an indication of her unwillingness to mate. Familiar and unfamiliar females did not differ in how aggressive they were to the male, suggesting that our results were affected by male mate choice rather than female choice. We cannot be sure, however, that our results are not confounded by female mating preferences. Females rarely approached males and usually responded to male association by swimming away, but responses to males might have been more subtle than we could observe. Female poeciliid fish also prefer to mate with unfamiliar or novel males (Farr 1977; Hughes et al. 1999; McLaughlin & Bruce 2001), and responsiveness to ambient light spectrum and predation risk are not inconsistent with female guppy mating behaviour (Evans et al. 2002; Gamble et al. 2003).

A further potential measure of female receptivity could be gained by examining the number of displays. Displays are failed courtships that may result from female aggression as well as a decision by the male not to continue courtship. Display preference was not correlated with any other male behaviour and we suggest that it may not be a reliable indicator of male mating preference. Hence, we do not attempt to explain why male display preferences varied with light level, stream and predator density. We did not collect data on why courtships were terminated and collecting this information would be useful in future studies.

Finally, we consider the cues that males use to discriminate between females. Olfactory cues are thought to be important in recognition and mate choice in other fish (e.g. Crow & Liley 1979; McLennan & Ryan 1997), and the failure of our dichotomous choice test to predict mating preferences suggests that visual cues alone are not sufficient for recognition. The dichotomous choice test might not have been suitable to elicit a mating response, however, because males did not court females through the clear partition. Further investigation of cue use in recognition in *B. episcopi* is required, with emphasis on potential population variation.

To conclude, we found that *B. episcopi* can discriminate between mates on the basis of familiarity and we suggest that this ability represents an intrinsic preference to mate with unfamiliar females. Although the reasons for this preference remain unclear, the plasticity of this response is evident and may represent a cost-benefit trade-off in male mate choice.

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References

- Adler, N. T. 1978. On the mechanisms of sexual behaviour and their evolutionary constraints. In: *Biological Determinants of Sexual Behaviour* (Ed. by J. B. Hutchison), pp. 657–695. Chichester: J. Wiley.
- Amundsen, T. & Forsgren, E. 2003. Male preference for colourful females affected by male size in a marine fish. *Behavioral Ecology and Sociobiology*, **54**, 55–64. doi:10.1007/s00265-003-0593-4.
- Amundsen, T., Forsgren, E. & Hansen, L. T. T. 1997. On the function of female ornaments: male bluethroats prefer colourful females. *Proceedings of the Royal Society of London, Series B*, **264**, 1579–1586.
- Angermeier, P. L. & Karr, J. R. 1983. Fish communities along environmental gradients in a system of tropical streams. *Environmental Biology of Fishes*, **9**, 117–135.
- Basolo, A. 2004. Variation between and within the sexes in body size preferences. *Animal Behaviour*, **68**, 75–82. doi:10.1016/j.anbehav.2003.07.019.
- Bateman, A. J. 1948. Intrasexual selection in *Drosophila*. *Heredity*, **2**, 349–368.
- Berger, J. & Cunningham, C. 1987. Influence of familiarity on frequency of inbreeding in wild horses. *Evolution*, **41**, 229–231.
- Berglund, A. 1993. Risky sex: male pipefishes mate at random in the presence of a predator. *Animal Behaviour*, **46**, 169–175.
- Blouin, S. F. & Blouin, M. 1988. Inbreeding avoidance behaviors. *Trends in Ecology and Evolution*, **3**, 230–233.
- Briggs, S. E., Godin, J.-G. J. & Dugatkin, L. A. 1996. Mate-choice copying under predation risk in the Trinidadian guppy (*Poecilia reticulata*). *Behavioral Ecology*, **7**, 151–157.
- Brown, C. & Braithwaite, V. A. 2004. Size matters: a test of boldness in eight populations of the poeciliid *Brachyrhaphis episcopi*. *Animal Behaviour*, **68**, 1325–1329. doi:10.1016/j.anbehav.2004.04.004.
- Brown, C. & Braithwaite, V. A. 2005. Effects of predation pressure on the cognitive ability of the poeciliid *Brachyrhaphis episcopi*. *Behavioral Ecology*, **16**, 482–487. doi:10.1093/beheco/ari016.
- Brown, C., Gardner, C. & Braithwaite, V. A. 2004. Population variation in lateralized eye use in the poeciliid *Brachyrhaphis episcopi*. *Proceedings of the Royal Society of London, Series B (Supplement)*, **271**, S455–S457. doi:10.1098/rsbl.2004.0222.
- Cooper, W. E. 1985. Female residency and courtship intensity in a territorial lizard, *Holbrookia propinqua*. *Amphibia-Reptilia*, **6**, 63–69.
- Croft, D. P., Arrowsmith, B. J., Webster, M. & Krause, J. 2004a. Intra-sexual preferences for familiar fish in male guppies. *Journal of Fish Biology*, **64**, 279–283.
- Croft, D. P., Krause, J. & James, R. 2004b. Social networks in the guppy (*Poecilia reticulata*). *Proceedings of the Royal Society of London, Series B (Supplement)*, **271**, S516–S519. doi:10.1098/rsbl.2004.0206.
- Crow, R. T. & Liley, N. R. 1979. A sexual pheromone in the guppy, *Poecilia reticulata* (Peters). *Canadian Journal of Zoology*, **57**, 184–188.
- Crowley, P. H., Travers, S. E., Linton, M. C., Cohn, S. L., Sih, A. & Sargent, R. C. 1991. Mate density, predation risk, and the seasonal sequence of mate choices: a dynamic game. *American Naturalist*, **137**, 567–596.
- Dewsbury, D. A. 1981. Effects of novelty on copulatory behavior: the Coolidge effect and related phenomena. *Psychological Bulletin*, **89**, 464–482.
- Donovan, A. & Verrell, P. A. 1991. The effect of partner familiarity on courtship success in the salamander *Desmognathus ochrophaeus*. *Journal of Herpetology*, **25**, 93–95.
- Endler, J. A. 1978. A predator's view of animal color patterns. *Evolutionary Biology*, **11**, 319–364.
- Endler, J. A. 1987. Predation, light intensity and courtship behaviour in *Poecilia reticulata* (Pisces: Poeciliidae). *Animal Behaviour*, **35**, 1376–1385.
- Endler, J. A. 1993. The color of light in forests and its implications. *Ecological Monographs*, **63**, 1–27.
- Endler, J. A. & Théry, M. 1996. Interacting effects of lek placement, display behavior, ambient light, and color patterns in three neotropical forest-dwelling birds. *American Naturalist*, **148**, 421–452.
- Evans, J. P., Kelley, J. L., Ramnarine, I. W. & Pilastro, A. 2002. Female behaviour mediates male courtship under predation risk in the guppy (*Poecilia reticulata*). *Behavioral Ecology and Sociobiology*, **52**, 496–502. doi:10.1007/s00265-002-0535-6.
- Farr, J. A. 1977. Male rarity or novelty, female choice behavior, and sexual selection in guppy, *Poecilia reticulata* Peters (Pisces: Poeciliidae). *Evolution*, **31**, 162–168.
- Forsgren, E. 1992. Predation risk affects mate choice in a Gobiid fish. *American Naturalist*, **140**, 1041–1049.
- Fuller, R. & Berglund, A. 1996. Behavioral responses of a sex-role reversed pipefish to a gradient of perceived predation risk. *Behavioral Ecology*, **7**, 69–75.

- Gamble, S., Lindholm, A. K., Endler, J. A. & Brooks, R. 2003. Environmental variation and the maintenance of polymorphism: the effect of ambient light spectrum on mating behaviour and sexual selection in guppies. *Ecology Letters*, **6**, 463–472.
- Godin, J.-G. J. & Briggs, S. E. 1996. Female mate choice under predation risk in the guppy. *Animal Behaviour*, **51**, 117–130.
- Griffiths, S. W. & Magurran, A. E. 1997. Schooling preferences for familiar fish vary with group size in a wild guppy population. *Proceedings of the Royal Society of London, Series B*, **264**, 547–551.
- Griffiths, S. W. & Magurran, A. E. 1998. Sex and schooling behaviour in the Trinidadian guppy. *Animal Behaviour*, **56**, 689–693.
- Halliday, T. R. 1983. The study of mate choice. In: *Mate Choice* (Ed. by P. Bateson), pp. 3–32. Cambridge: Cambridge University Press.
- Herdman, E. J. E., Kelly, C. D. & Godin, J.-G. J. 2004. Male mate choice in the guppy (*Poecilia reticulata*): do males prefer larger females as mates? *Ethology*, **110**, 97–111.
- Houde, A. E. 1997. *Sex, Color, and Mate Choice in Guppies*. Princeton, New Jersey: Princeton University Press.
- Hubbell, S. P. & Johnson, L. K. 1987. Environmental variance in lifetime mating success, mate choice, and sexual selection. *American Naturalist*, **130**, 91–112.
- Hughes, K. A., Du, L., Rodd, F. H. & Reznick, D. N. 1999. Familiarity leads to female mate preference for novel males in the guppy, *Poecilia reticulata*. *Animal Behaviour*, **58**, 907–916.
- Jennions, M. D. & Kelly, C. D. 2002. Geographical variation in male genitalia in *Brachyrhaphis episcopi* (Poeciliidae): is it sexually or naturally selected? *Oikos*, **97**, 76–86.
- Jennions, M. D. & Telford, S. R. 2002. Life-history phenotypes in populations of *Brachyrhaphis episcopi* (Poeciliidae) with different predator communities. *Oecologia*, **132**, 44–50. doi:10.1007/s00442-002-0942-4.
- Johnson, J. B. & Basolo, A. L. 2003. Predator exposure alters female mate choice in the green swordtail. *Behavioral Ecology*, **14**, 619–625. doi:10.1093/beheco/arg046.
- Johnson, J. B. & Belk, M. C. 2001. Predation environment predicts divergent life-history phenotypes among populations of the live-bearing fish *Brachyrhaphis rhabdophora*. *Oecologia*, **126**, 142–149. doi:10.1093/s004420000504.
- Kelley, J. L. & Magurran, A. E. 2003. Effects of relaxed predation pressure on visual predator recognition in the guppy. *Behavioral Ecology and Sociobiology*, **54**, 225–232. doi:10.1007/s00265-003-0621-4.
- Kelley, J. L., Graves, J. A. & Magurran, A. E. 1999. Familiarity breeds contempt in guppies. *Nature*, **401**, 661–662.
- Long, K. D. & Rosenqvist, G. 1998. Changes in male guppy courtship distance in response to a fluctuating light environment. *Behavioral Ecology and Sociobiology*, **44**, 77–83.
- McLaughlin, M. E. & Bruce, K. E. 2001. The effect of male familiarity on proximity time in female eastern mosquitofish (*Gambusia holbrooki*). *The Psychological Record*, **51**, 237–250.
- McLennan, D. A. & Ryan, M. J. 1997. Responses to conspecific and heterospecific olfactory cues in the swordtail *Xiphophorus cortezi*. *Animal Behaviour*, **54**, 1077–1088.
- Magurran, A. E. & Seghers, B. H. 1990. Risk sensitive courtship in the guppy (*Poecilia reticulata*). *Behaviour*, **112**, 194–201.
- Mojica, C. L., Meyer, A. & Barlow, G. W. 1997. Phylogenetic relationships of species of the genus *Brachyrhaphis* (Poeciliidae) inferred from partial mitochondrial DNA sequences. *Copeia*, 298–305.
- Orrell, K. S. & Jenssen, T. A. 2002. Male mate choice by the lizard *Anolis carolinensis*: a preference for novel females. *Animal Behaviour*, **63**, 1091–1102. doi:10.1006/ande.2002.3013.
- Parker, G. A. 1983. Mate quality and mating decisions. In: *Mate Choice* (Ed. by P. Bateson), pp. 141–164. Cambridge: Cambridge University Press.
- Partridge, L. & Halliday, T. 1984. Mating patterns and mate choice. In: *Behavioural Ecology: an Evolutionary Approach* (Ed. by J. R. Krebs & N. B. Davies), pp. 222–250. Oxford: Blackwell Scientific.
- Ptacek, M. B. & Travis, J. 1997. Mate choice in the sailfin molly, *Poecilia latipinna*. *Evolution*, **51**, 1217–1231.
- Reynolds, J. D. 1993. Should attractive individuals court more? Theory and a test. *American Naturalist*, **141**, 914–927.
- Reynolds, J. D. & Gross, M. R. 1992. Female mate preference enhances offspring growth and reproduction in a fish, *Poecilia reticulata*. *Proceedings of the Royal Society of London, Series B*, **250**, 57–62.
- Reynolds, J. D., Gross, M. R. & Coombs, M. J. 1993. Environmental conditions and male morphology determine alternative mating behavior in Trinidadian guppies. *Animal Behaviour*, **45**, 145–152.
- Ryan, K. K. & Altmann, J. 2001. Selection for male choice based primarily on mate compatibility in the oldfield mouse, *Peromyscus polionotus rhoadsi*. *Behavioral Ecology and Sociobiology*, **50**, 436–440. doi:10.1007/s002650100385.
- Smith, E. J., Partridge, J. C., Parsons, K. N., White, E. M., Cuthill, I. C., Bennett, A. T. D. & Church, S. C. 2002. Ultraviolet vision and mate choice in the guppy (*Poecilia reticulata*). *Behavioral Ecology*, **13**, 11–19.
- Sutherland, W. J. 1985. Measures of sexual selection. In: *Oxford Surveys in Evolutionary Biology* (Ed. by R. Dawkins & M. Ridley), pp. 90–101. Oxford: Oxford University Press.
- Tokarz, R. R. 1992. Male mating preference for unfamiliar females in the lizard, *Anolis sagrei*. *Animal Behaviour*, **44**, 843–849.
- Trivers, R. L. 1972. Parental investment and sexual selection. In: *Sexual Selection and the Descent of Man, 1871–1971* (Ed. by B. Campbell), pp. 136–179. London: Heinemann.
- Ward, A. J. W. & Hart, P. J. B. 2003. The effects of kin and familiarity on interactions between fish. *Fish and Fisheries*, **4**, 348–358.
- Wong, B. B. M. & Jennions, M. D. 2003. Costs influence male mate choice in a freshwater fish. *Proceedings of the Royal Society of London, Series B*, **270**, S36–S38. doi:10.1098/rsbl.2003.0003.